The health effect of probiotics on high-fat diet-induced cognitive impairment, depression and anxiety: A cross-species systematic review

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ABSTRACT

Obesity is a complex disease with many co-morbidities, including impaired cognitive functions. Obese individuals often contain an aberrant microbiota. Via the microbiota-gut-brain axis, the altered microbiota composition can affect cognition or induce anxiety- or depressive-like behavior. Probiotics have been shown to alleviate both obesity- and neurobehavioral disorder-related symptoms. Here, we evaluated previously published results on the effectiveness of probiotic intervention in alleviating obesity- or high-fat diet (HFD)-related cognitive impairment, depression and anxiety. A systematic search was performed in PubMed, Embase, and Google Scholar until June 2021 to identify relevant articles. Seventeen studies were included: one human and sixteen animal studies. Overall, the findings support the beneficial health effect of probiotics on HFD-induced cognitive impairment and anxiety. However, the results suggest that multi-strain probiotic treatments should be used with caution, especially in the absence of HFD-induced impairment. Future studies should overcome the large variation in study design and high risk of bias found in the current evidence. Nevertheless, probiotic treatment, in particular using the Lactobacillus genus, seems promising.

1. Introduction

The worldwide prevalence of obesity increases rapidly. The World Health Organization (WHO) reported that between 1975 and 2016 the prevalence of overweight and obesity nearly tripled, resulting in 1.9 billion cases of adult overweight (BMI ≥ 25) in 2016 of which 650 million people were obese (BMI ≥ 30)(WHO, 2021). The impact of this disease on quality of life is significant, especially because obesity is associated with co-morbidities that do not only affect metabolic function but also a wide range of cognitive functions (Agusti et al., 2018a; Monda et al., 2017; Sellbom and Gunstad, 2012). For example, learning and memory are impaired in both obese humans and diet-induced obese animals (Almeida-Suhett et al., 2017; Bocarsly et al., 2015; Cordner and Tamashiro, 2015; Fazzari et al., 2018; Greenwood and Winocur, 1990). Furthermore, obese people or diet-induced obese animals show enhanced anxiety- and depressive-like behavior (Alonso-Caraballo et al., 2019; de Noronha et al., 2017; Foster and McVey Neufeld, 2013; Le Port et al., 2012; Luppino et al., 2010). The gut microbiota has been suggested to play an important role in this interaction between obesity and cognitive impairment.

First of all, changes in the gut microbiota and obesity are tightly linked. For example, obese people have a deregulated gut microbiota composition that differs from that of non-obese people, which is called gut dysbiosis (Abenavoli et al., 2019; Aggarwal et al., 2013). Moreover, gut dysbiosis is induced by a high-fat diet (HFD) or Western diet aimed to model obesity in animals (Hildebrandt et al., 2009; Li et al., 2009; Turnbaugh et al., 2008). Although the exact mechanisms are not yet fully clear, gut dysbiosis was found to induce obesity by influencing weight gain via several pathways, among which insulin sensitivity, fat, and cholesterol metabolism, and inflammatory pathways (Baohman et al., 2016; Bliss and Whiteside, 2018; Cani et al., 2008; Rogers et al., 2016). On the contrary, intervention at the level of the microbiome using probiotics not only changes the composition of the gut microbiota (Wang et al., 2015), but also reduces obesity-related symptoms like weight gain, fat and cholesterol levels, and several liver toxicity biomarkers (Ji et al., 2012; Park et al., 2013). Secondly, the gut microbiota influences cognitive behavior like learning and memory, anxiety-like and depressive-like behavior via the microbiota-gut-brain axis (Cryan et al., 2019; Noble et al., 2017). The gut microbiota exerts this influence partially via the same pathways with...
which it influences obesity-related gut- and intestinal-based metabolic factors, for example, via insulin sensitivity and the inflammatory pathways (Pistell et al., 2010). This overlap in pathways could explain part of the co-morbidity of obesity and cognitive deficits, anxiety-like and depressive-like behavior. In fact, it was demonstrated that transplantation of obesity-type gut microbiota induced neurobehavioral changes even in the absence of obesity (Bruce-Keller et al., 2015). Indeed, the microbiota-gut-brain axis includes other important pathways as well, for example, neurotransmitters and hormonal pathways (Freeman et al., 2014; Mohanta et al., 2020). Together, the extensive influence of the microbiota on both metabolic and cognitive obesity-related symptoms makes intervention at the level of the gut microbiota, for example with probiotics, a promising way to prevent or treat obesity-related cognitive deficits, anxiety and depression.

Regarding the metabolic obesity symptoms, the beneficial health influence of probiotic interventions has already been reviewed numerous times (Azad et al., 2018; Kobyliak et al., 2016; Million et al., 2012), the ability of probiotics to ameliorate neurobehavioral symptoms in primarily cognitive disorders has been demonstrated and reviewed extensively. For example, disorders such as Alzheimer’s Disease (AD), Major Depressive Disorder (MDD), Anxiety, Parkinson’s Disease (PD), and Autism Spectrum Disorders (ASD) are associated with gut dysbiosis (Leblhuber et al., 2021; Luna and Foster, 2015). Intervention at the level of the microbiome in these disorders, using different probiotic strains or combinations of strains, has proven beneficial in both animal models and corresponding human trials (Ansari et al., 2020). In short, probiotic treatment reduces biomarkers, for example, amyloid-β in AD and pro-inflammatory markers in general, hypothalamic-pituitary-adrenal axis-related stress responses that influence anxiety and depression, dopaminergic neuronal degeneration in PD, and gastrointestinal symptoms that often co-occur in patients with ASD. Most importantly, these alleviations of disease parameters are paired with either relief of cognitive impairment or depressive and anxious behavior, or improvement in communicational and social skills (Kesika et al., 2021; Ng et al., 2019; Pirbaglou et al., 2016; Slyepchenko et al., 2015; Tan et al., 2021). However, a comprehensive systematic review focusing on the effect of probiotic treatment for obesity-related neurobehavioral symptoms is not yet published.

To determine whether probiotics exert the same beneficial effect on obesity-related cognitive symptoms, anxiety and depression, we systematically reviewed and outlined the evidence on the effect of probiotic treatment on behavioral outcome assessments in obese people and HFD-induced cognitive impairment, anxiety-like and depressive-like behavior in animal studies. This review is an important step towards our understanding of the effect of probiotic treatment on obesity-related learning and memory impairment and anxiety-like and depressive-like behavior.

2. Methods

2.1. Search strategy

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021). Three electronic databases (PubMed, Embase, and Google Scholar) were searched using the following keywords: (1) “Probiotic *” AND (2) “obes * OR high-fat OR high fat” AND (3) “cogni * OR memory OR learning”. No filters were applied. The search was completed up to June 2021. Since in Google Scholar these keywords also yielded several articles including depressive-like and anxiety-like behavior in animal studies, the following keywords were added to the third search term in a second search round in PubMed and Embase: (3) “depress * OR anxiety”.

The specific search terms, per database, and per search round are reported in Table S1. Subsequently, the reference lists of eligible articles
Table 1
Characteristics of included studies in animals. (Continued).

| Reference | Species | Strain | Intervention protocol | Sample sizes | Sex, Age at experiment week 0 | Type of intervention (HFD + Pro) | Intervention characteristics | Aimed model |
|-----------|---------|--------|-----------------------|--------------|-----------------------------|---------------------------------|-------------------------------|--------------|
| (Agusti et al., 2018b) | Mice | C57BL-6 | ND + VEH | 10 | male, 7–9 weeks old | ND (12.4%-kcal fat) HFD (60.3%-kcal fat) VEH: 10% skimmed milk by gavage in 10% skimmed milk | week 1-13/14, 1 x 10^7 CFU/day, | Obesity, Sign. weight gain, Prevention of phenotype |
| | | | ND + Pro | 10 | | | week 1-13/14, | |
| | | | HFD + VEH | 10 | | | by gavage | |
| | | | HFD + Pro | 10 | | | in 10% skimmed milk | |
| (Forooshan et al., 2021) | Mice | C57BL/6 | ND + VEH | 6 | male, 7 ± 1 weeks old | ND (10.60%-kcal fat) HFD (45%-kcal fat) VEH: PBS | week 1-8, 1 x 10^6 CFU/day, by gavage | Obesity, Sign. weight gain, Rescue of phenotype |
| | | | HFD + VEH | 6 | | | week 8-10, | |
| | | | HFD + Pro | 6 | | | week 9-11, | |
| (Jang et al., 2019) | Mice | C57BL/6 | ND | 8 | male, 7 weeks old | ND | week 1-8 | Obesity, Sign. weight gain, Rescue of phenotype |
| | | | HFD + VEH | 8 | | | week 5-8, | |
| | | | HFD + OK67 | 8 | | | week 6-8, | |
| | | | HFD + PK16 | 8 | | | week 7-9, | |
| | | | HFD + Mix | 8 | | | week 8-10, | |
| (Ohland et al., 2013) | Mice | 129/SvEv | ND + VEH | 5/6 | sex unspecified, start experiment | ND (13%-kcal fat) HFD (Western-style diet) VEH: PBS | week 1-3, 1 x 10^5 CFU/day, by oral gavage | Co-administration of western-style diet and probiotics, Prevention of phenotype |
| | | | ND + Pro | 5/6 | | | week 1-5, | |
| | | | HFD + VEH | 5/6 | after weaning | VEH: PBS | week 2-4, | |
| | | | HFD + Pro | 5/6 | (3 weeks old) | | week 3-5, | |
| (Patterson et al., 2019) | Mice | C57BL/6 | ND | 13 | male, 8 weeks old | ND (Low-fat diet; 10%-kcal fat) | week 1-24, 1 x 10^10 CFU/day, in drinking water | Metabolic dysfunction, Sign. weight gain, Rescue of phenotype |
| | | | HFD | 13 | | | week 13-24, | |
| | | | HFD + Pro1 | 14 | | | | |
| | | | HFD + Pro2 | 14 | | | | |
| (Wang et al., 2020) | Mice | C57BL/6 | HFD | 5–8 | male, 80-82 weeks old | HFD (60% kcal fat) | duration not specified | Obesity & aging, No control for weight gain, Prevention of phenotype |
| | | | HFD + Pro | 5–8 | | | week 1-10, 1 x 10^5 CFU/mL/day, in drinking water | |
| (Yang et al., 2019) | Mice | C57BL/6 | ND | 8 | male, 3 weeks old | ND (standard chow) HFD (60% kcal from fat) VEH: PBS | week 1-6, duration unclear, week 1-6 or shorter period herein, 5 x 10^5 CFU/mL, by gavage in PBS | Obesity, Sign. weight gain, Prevention/ rescue of phenotype |
| | | | HFD | 8 | | | week 8-10, | |
| | | | HFD + VEH | 8 | | | week 9-11, | |
| | | | HFD + AKK | 8 | | | week 10-12, | |
| | | | HFD + hk-AKK | 6 | | | week 11-13, | |
| | | | HFD + Lac | 6 | | | week 12-14, | |
| (Abildgaard et al., 2017) | Rats | Sprague-Dawley | ND + VEH | 10 | male, 4 weeks old | ND (11 kJ%-fat) HFD (60 kJ%-fat) VEH: carrier matrix of maize starch, maltodextrins, and vegetable protein | week 1-10, 2.5 x 10^10 CFU, 4.5 g freeze-dried powder dissolved in 30 mL tap water | Co-administration of western-style diet and probiotics, Sign. weight gain, Rescue of phenotype |
| | | | ND + Pro | 10 | | | week 6-10, | |
| | | | HFD + VEH | 10 | | | week 7-11, | |
| | | | HFD + Pro | 10 | | | week 8-12, | |

(continued on next page)
| Reference | Species | Strain | Intervention protocol | Sample sizes | Sex, Age at experiment week 0 | Type of intervention (HFD + Pro) | Intervention characteristics | Aimed model |
|-----------|---------|--------|-----------------------|--------------|--------------------------------|---------------------------------|----------------------------|-------------|
| (Beilharz et al., 2018) | Rats | Sprague-Dawley | ND + VEH | 10 | male, 200 g + 10 days old | ND (standard chow) | Week 3-6.5, in 0.3 mL of maple syrup | Obesity/ Western-style diet, Sign. weight gain |
| | | | ND + Low Pro | 10 |  | Caf (cafeteria diet) | week 1–6.5, Low: 2,5 × 10^6 CFU/day; Pro: VSL#3 | Prevention of phenotype |
| | | | ND + High Pro | 10 |  | VEE unspecified | High: 2,5 × 10^8 CFU/day, via oral feeding | |
| | | | Caf + VEH | 10 |  |  |  | |
| | | | Caf + Low Pro | 10 |  |  |  | |
| | | | Caf + High Pro | 10 |  |  |  | |
| (Chunchai et al., 2018) | Rats | Wistar | ND + VEH | 6 | male, 180-200 g + 7 days old | ND (19.77% energy from fat) | week 1–12, week 13–24, 1 × 10^6 CFU/day, by gavage resuspended in PBS | Obesity, Sign. weight gain, Rescue of phenotype |
| | | | ND + Pro | 6 |  | HFD (59.28% energy from fat) |  | |
| | | | HFD + VEH | 6 |  | VEH: PBS |  | |
| | | | HFD + Pro | 6 |  | Pro: VSL#3 |  | |
| (Ilgarza et al., 2021) | Rats | Sprague-Dawley | ND | 8 | male, 220 g weighted | ND (13% kcal fat) | week 1–14, week 8–11, 1 × 10^6 CFU/day, by gavage resuspended in PBS | NASH, Sign. weight gain, Rescue of phenotype |
| | | | HFHC | 8 |  | HFHC (65% kcal fat, 2% kcal cholesterol) |  | |
| | | | HFHC + VEH | 8 |  | VEH: PBS |  | |
| | | | HFHC + LGG | 8 |  | LGG: Lactobacillus rhamnosus GG |  | |
| | | | HFHC + AKK | 8 |  | AKK: Akkermansia muciniphila CIP107961 |  | |
| (Mohammed et al., 2020) | Rats | Wistar | ND | 6 | male, 150 g + 7 days old | ND (10% kcal fat) | week 1–12, week 11–12, 1.2 × 10^6 CFU/mL, by oral gavage | NASH, Sign. weight gain, Rescue of phenotype |
| | | | ND + Pro | 6 |  | HFD (40% kcal fat) |  | |
| | | | ND + VEH | 6 |  | VEH: malic acid, xyitol, and maloadextrin |  | |
| | | | HFD + VEH | 6 |  | Pro: Bifidobacterium longum R0175 and Lactobacillus helveticus R0052 |  | |
| | | | HFD + Pro | 6 |  |  |  | |
| (Myles et al., 2020) | Rats | Long-Evans | ND + VEH | m + f – 32 female, 30 male, 3 weeks old | ND (10% kcal fat) | week 1–7/7.5, dams: during pregnancy and lactation; offspring: week 1–7/7.5, 1 × 10^6 CFU/day, via syringe feeding | Lifelong probiotic administration, Sign. weight gain, Prevention of phenotype |
| | | | ND + Pro | 7 + 8 |  | HFD (10% kcal fat) |  | |
| | | | HFD + VEH | 8 + 8 |  | VEH: PBS |  | |
| | | | HFD + Pro | 8 + 8 |  | Pro: Lactobacillus plantarum EMCC1039 |  | |
| (Zaydi et al., 2020) | Rats | Sprague-Dawley | ND | 6 | male, 9 weeks old | ND (10% kcal fat) | week 1–12, week 1–12, 1 × 10^6 CFU/day, mixed into 1 g of food pellet | Obesity & aging, HFD did not induce weight gain, Prevention of phenotype |
| | | | HFD | 6 |  | HFD (ND + 25% animal fat) |  | |
| | | | HFD + Statin | 6 |  | Pro: Lactobacillus plantarum DR7 Statin: Lovastatin (2 mg/kg/day) |  | |
| | | | HFD + Pro | 6 |  | Biocult Strong/POS: Three g of probiotics contained: Streptococcus thermophilus SGS01 (1.5 × 1010 colony-forming unit CFU), Bifidobacterium animalis subsp. Lactis SGB06 (1.5 × 1010 colony-forming unit CFU), Streptococcus thermophilis (1.5 × 1010 colony-forming unit CFU), Lactobacillus delbrueckii spp. Bulgaricus DSM 20081 (1.5 × 1010 colony-forming unit CFU), Lactococcus lactis subsp. Lactis SGLc01 (1.5 × 1010 colony-forming unit CFU), Lactobacillus acidophilus SGL11 (1.5 × 1010 colony-forming unit CFU), Lactobacillus paracasei paracasei HH01 (Chunchai et al., 2018) Rats Wistar |  | |
| (Avolio et al., 2019) | Hamsters | Syrian golden hamster | ND + Pro | 6 | male, 28 weeks old | ND (60% energy from fat) | week 1–4, 3 g/200 mL/day, in water bottle | Obesity, Sign. weight gain, Prevention of phenotype |
| | | | HFD | 6 |  |  |  | |
| | | | HFD + Pro | 6 |  | Pro: Biocult Strong (Avolio et al., 2019) Hamsters Syrian golden hamster ND + Pro |  | |
| (Zeitser et al., 2020) | Pigs | Iberian pig | ND | 4 | sex unspecified, 13 days old | ND (11.2 g fat, 0 g fructose) | week 1–10, 6.2 × 10^5 CFU/mL, 45 mL/kg body weight at 6 h intervals | NAFLD, Weight gain not reported, Prevention of phenotype |
| | | | ND + Pro | 4 |  | HFF (20.6 g fat, 10 g fructose) |  | |
| | | | HFF + Pro | 6 |  | Pro: Maltibio 3PS |  | |

Cafeteria diet: standard rat chow supplemented with a selection of cakes (for example, chocolate mud cake, jam roll, lamingtons), biscuits (e.g., chocolate chip, monte carlo, scotch fingers), and a protein source (for example, party pie, dim sims, dog roll). Composition of probiotic mixtures: Biocult Strong/POS: Three g of probiotics contained: Streptococcus thermophilus SGS01 (1.5 × 1010 colony-forming unit CFU), Bifidobacterium animalis subsp. Lactis SGB06 (1.5 × 1010 colony-forming unit CFU), Streptococcus thermophilis (1.5 × 1010 colony-forming unit CFU), Bifidobacterium bifidum SGB02 (1.5 × 1010 colony-forming unit CFU), Lactobacillus delbrueckii spp. Bulgaricus DSM 20081 (1.5 × 1010 colony-forming unit CFU), Lactococcus lactis subsp. Lactis SGLc01 (1.5 × 1010 colony-forming unit CFU), Lactobacillus acidophilus SGL11 (1.5 × 1010 colony-forming unit CFU), Lactobacillus paracasei paracasei HH01 (Chunchai et al., 2018) Rats Wistar ND + VEH 6 220 g weighted ND (13% kcal fat) week 1–14, week 8–11, 1 × 10^6 CFU/day, by gavage resuspended in PBS NASH, Sign. weight gain, Rescue of phenotype LFHC |  | |

ND + Pro | 6 |  | HFD (59.28% energy from fat) |  | |
| NFHC + Pro | 6 |  | VEH: PBS |  | |
| NFHC + LGG | 8 |  | LGG: Lactobacillus rhamnosus GG |  | |
| NFHC + AKK | 8 |  | AKK: Akkermansia muciniphila CIP107961 |  | |
Lactobacillus plantarum SGL07 (1.5 × 1010 colony-forming unit CFU), Lactobacillus reuteri SGL01 (1.5 × 1010 colony-forming unit CFU), maltodextrin, and vegetable protein.

Bifidobacterium W23, B. lactis W52, L. acidophilus W37, L. brevis W63, L. casei W56, L. salivarius W24, Lc. Lactis W19, Lc. Lactis W58) in a carrier matrix of maize starch, and

criteria were: (1) publications without original data, for example, reviews, commentaries, editorial notes or book chapters, (2) diseased-model organisms other than those induced by HFD treatment, for example, diabetes, metabolic diseases, dementia, depression, (3) treatment. Moreover, SYRCLE's RoB tool was supplemented with five additional entries aimed to treat obesity-related symptoms (Fig. 1).

2.2. Eligibility criteria

Only original data articles (research articles) were included. Inclusion criteria were: (1) obesity diagnosis (BMI ≥ 30) or administration of a high-fat, Western or similar diet, (2) administration of a probiotic (compound), (3) at least one outcome measurement that measured learning, memory, depressive-like or anxiety-like behavior. Exclusion criteria were: (1) publications without original data, for example, reviews, commentaries, editorial notes or book chapters, (2) diseased-model organisms other than those induced by HFD treatment, for example, diabetes, metabolic diseases, dementia, depression, (3) treatment groups in which probiotics were simultaneously combined with another intervention aimed to treat obesity-related symptoms (Fig. 1).

2.3. Data extraction

Two investigators (JL and LK) independently extracted data from eligible papers and summarized relevant data in a pre-formatted table, which included the following information: model organism, sample size, age at the start of the experiment and age at read-outs, sex, dietary and probiotic interventions with their relevant characteristics, included control groups, and behavioral and other relevant outcome measures.

2.4. Risk-of-bias and quality assessment

Two investigators (JL and LK) independently assessed the study quality of all eligible articles. Quality was assessed using SYRCLE’s risk of bias (RoB) tool for animal studies (Hooijmans et al., 2014), as most included articles reported on animal studies. SYRCLE’s RoB tool is based on the Cochrane RoB tool for randomized controlled trials and adjusted taking into account the differences between animal and human studies. SYRCLE’s RoB tool consists of ten entries, together covering six different bias domains (Fig. 4A).

The second entry in SYRCLE’s RoB tool (Selection bias – Baseline characteristics) was divided into two separate entries for the baseline characteristics at the start of HFD treatment and the start of probiotics treatment. Moreover, SYRCLE’s RoB tool was supplemented with five items specifically related to probiotic interventions in animal studies, taken from (Joseph and Law, 2019). To keep the original numbering of the tool intact, supplemented entries were classified into one of the predefined bias domains in SYRCLE’s RoB tool and numbered using small letters (Fig. 4A).

3. Results

3.1. Study selection

The process of study selection for the first and second search together is illustrated in Fig. 1. The initial search strategy identified 631 records: 40 from PubMed, 131 from Embase, and 460 from Google Scholar. Duplicates were removed (n = 61) and studies that did not include original data were excluded based on keywords (review, commentary, editorial, note, book chapter; n = 442). Next, titles, abstracts, and if necessary, methods sections were screened to remove articles based on an inappropriate study protocol (n = 78), for example, because they lacked the correct combination of interventions or included non-relevant interventions or disease models. Furthermore, articles were removed because they did not report a cognitive outcome measure (n = 29) or because the subject they reported on was not relevant (n = 7). This left 16 articles for full-text investigation (Fig. 1).

The additional search strategy identified 237 records: 56 from PubMed and 181 from Embase. Duplicates overlapping with the first search were removed (n = 74) leaving 39 new records from PubMed and 124 from Embase. Duplicates within this selection were removed (n = 33), as well as articles without original data (n = 108). After screening of titles, abstracts, and methods sections articles were removed due to inappropriate study protocols (n = 14), the lack of a cognitive outcome measure (n = 2), and a non-relevant subject (n = 3). This left an additional 4 papers for full-text analysis.

After a full-text investigation, 3 more papers were removed because they lacked the appropriate intervention, leaving 17 articles for systematic review. No additional articles were found after manually reviewing the references of eligible articles.

3.2. Human study on the effect of probiotics on obesity-related depression and anxiety

One study reported on a human randomized double-blind controlled trial. In this trial, the effectiveness of a 3-week multi-strain probiotic intervention (psychobiotics oral suspension; POS; see Biocult Strong in Table 1 legend for ingredients) on obesity-related symptoms in women between the age of 28 and 56 was investigated (De Lorenzo et al., 2017). The study population consisted of a ‘normal weight lean’ group (NWL), a ‘normal weight obese’ group (NWO), and a ‘pre-obese/obese’ group (Preob/OB). The division of the subjects over these three groups was not specified. In total, 48 women were included in the study, who were equally divided over two experimental groups. The first group received a 3-week POS treatment followed by a 3-week wash-out period and subsequently a 3-week placebo treatment. The other group started with the placebo treatment and received POS treatment after the 3-week wash-out period. Subjects took one bag of 3 g POS or placebo per day via self-administration.

At baseline and after treatment, general psychopathology was assessed with the symptom checklist 90 (SCL90R), which includes the domains depression and anxiety. After three weeks of POS administration, the NWL group showed a significant decrease in the depression domain of the SCL90R, whereas the Preob/OB group showed a significant decrease in the anxiety domain. None of the other group-domain combinations was found to be significantly altered by POS treatment.

3.3. Animal studies on the effect of probiotics on HFD-induced cognitive impairment, anxiety and depression

3.3.1. Study characteristics

The study characteristics of all eligible articles reporting on animal studies are summarized in Table 1 by model organism.

Of sixteen animal studies, seven were conducted in mice, seven in rats, one in hamsters, and one in pigs. Only one study explicitly stated...
### Table 2
Behavioral outcomes associated with HFD and probiotics. (Continued.)

| Reference          | Behavioral read-out | Effect HFD vs Control | Effect Pro vs HFD | Comments |
|--------------------|---------------------|-----------------------|-------------------|----------|
| Agusti et al.      | LDT                 | ↓ latency in moving from dark to light box (↑ anxiety-like behavior) | No sign. effect on locomotor activity | Partial rescue; No sign. difference between HFD + Pro with both HFD + VEH and ND + VEH interpreted by authors as anxiety-like behavior and hyperactivity |
|                    | OPT                 | ↑ wall time, ↑ corner time (↑ anxiety-like behavior) | No sign. effect on locomotor activity | No sign. effect on anxiety-like depression, ↑ time to reach platform (↑ spatial memory) |
|                    | SSPT                | ↑ total distance, ↑ overall speed (↑ locomotor activity) | No sign. effect on locomotor activity | ↑ rate of rearing, ↑ time in center (↑ anxiety-like behavior) |
| Jang et al.        | EPM                 | ↑ latency to immobility (↑ depressive-like behavior) | ↑ depressive-like behavior (complete rescue) | ↑ ambulatory + total motor activity, ↑ muscle strength (↑ physical function) |
|                    | OPT                 | ↑ anhedonia (↑ depressive-like behavior) | ↑ depressive-like behavior (complete rescue) | ↑ activity |
| Foroozian et al.   | EPM                 | ↓ anxiety-like behavior (complete rescue) | ↓ anxiety-like behavior (complete rescue, including ↑ center time) | The difference between control and HFD in the EPM is not explicitly stated by the authors |
|                    | OPT                 | ↓ wall time, ↓ corner time (↑ anxiety-like behavior) | ↓ anxiety-like behavior (complete rescue) | ↓ time in target quadrant (↑ spatial memory) |
|                    | OP                 | ↑ total distance, ↑ overall speed (↑ locomotor activity) | ↑ locomotor activity (complete rescue) | ↓ freezing in training, 30 min and 24 h contextual fear test (↑ hippocampus-dependent contextual short- and long-term memory) |
| Ohland et al.      | BM                  | ↑ latency in immobility time, ↑ fecal pellets produced (↑ depressive-like behavior, ↑ anxiety-like behavior) | No sign. effect on spatial memory | ↑ hippocampus-dependent memory; rescue level unclear |
|                    | NORT                | ↑ total distance (↑ physical function) | ↑ hippocampus-dependent spatial learning | No sign. effect |
| Patterson et al.   | BM                  | No sign. effect on spatial memory | ↑ latency in immobility time, ↑ fecal pellets produced (↑ depressive-like behavior, ↑ anxiety-like behavior) | ↑ hippocampus-dependent memory (complete reversal of improvement) |
|                    | EPM                 | ↑ latency in immobility time, ↑ fecal pellets produced (↑ depressive-like behavior, ↑ anxiety-like behavior) | No sign. effect on locomotor activity | ↑ immobility time (↑ depressive-like behavior) |
|                    | NORT                | ↑ total distance (↑ physical function) | ↑ total distance (↑ physical function); complete | Probiotic effect on NORT independent of diet |
| Beilharz et al.    | NORT                | No sign. effect on anxiety-like behavior | No sign. effect on anxiety-like behavior | No sign. effect |
|                    | EPM                 | No sign. effect on anxiety-like behavior | No sign. effect on anxiety-like behavior | No sign. effect |
|                   |                     | ↑ total distance (↑ physical function) | ↑ total distance (↑ physical function); complete | Probiotic effect on NORT independent of diet |

### Table 2 (continued)

| Reference          | Behavioral read-out | Effect HFD vs Control | Effect Pro vs HFD | Comments |
|--------------------|---------------------|-----------------------|-------------------|----------|
| Wang et al.        | MWM                 | ↑ number of errors, ↑ escape latency to find target hole (↑ spatial learning) | ↑ number of errors, ↑ escape latency to find target hole (↑ spatial learning) | No sign. effect on anxiety-like behavior |
|                    | BM                  | ↑ latency in EPM; No sign. effect on anxiety-like behavior and hyperactivity | ↑ latency in EPM; No sign. effect on anxiety-like behavior and hyperactivity | ↑ latency in EPM; No sign. effect on anxiety-like behavior and hyperactivity |
| Abildgaard et al.  | BM                  | ↑ time to complete last training trial (↑ hippocampus-dependent memory) | ↑ time to complete last training trial (↑ hippocampus-dependent memory) | ↑ time to complete last training trial (↑ hippocampus-dependent memory) |
|                    | FST                 | ↓ distance and time before locating escape box in recall session (↑ hippocampus-dependent spatial memory) | ↓ distance and time before locating escape box in recall session (↑ hippocampus-dependent spatial memory) | ↓ distance and time before locating escape box in recall session (↑ hippocampus-dependent spatial memory) |
|                    | OPT                 | ↑ exploration ratio (N/N + F) (↑ object memory) | ↑ exploration ratio (N/N + F) (↑ object memory) | ↑ exploration ratio (N/N + F) (↑ object memory) |
|                    | (locomotor activity) | No sign. effect | No sign. effect | No sign. effect |
|                   |                     |                       |                   |           |

(continued on next page)
\textbf{Table 2 (continued))}

| Reference                  | Behavioral read-out | Effect HFD vs Control | Effect Pro vs HFD | Comments |
|----------------------------|---------------------|-----------------------|-------------------|----------|
| Chunchai et al. (2018)     | MWM                 | ↑ time to reach        | ↑ short- and       | Rescue beyond control; No sign. effect |
|                            | NORT                | target quadrant       | long-term memory  | (partial recovery) |
|                            | LA                  | (↓ short-term memory, long-term memory) | (complete rescue) | No sign. effect |
| Higranza et al. (2021)     | MWM                 | ↑ retention            | AKK: ↑ spatial working memory | No sign. effect |
|                            | NORT                | latencies              | (↓ spatial working memory) | (complete rescue) |
|                            | LA                  | target quadrant        | LGG: No sign. effect | No sign. effect |
|                            |                      | (↓ object memory)      | AKK: ↑ object memory | No sign. effect |
| Mohammed et al. (2020)     | MWM                 | ↑ time to reach        | ↑ spatial memory   | rescue |
|                            | NORT                | target quadrant        | (almost complete rescue) | No sign. effect |
|                            |                      | (↓ object memory)      | ↑ object memory | (complete rescue) |
|                            |                      | recognition index (N – F/N + F) | LGG: No sign. effect | No sign. effect |
|                            |                      | (↓ N index)            | AKK: ↑ object memory | No sign. effect |
| Myles et al. (2020)        | LDT                 | No sign. effect        | ↓ supported rears | All probiotic effects independent of diet |
|                            | OFT                 |                       | ↓ transitions light and dark areas | The authors note that supported rears should be interpreted with caution |
| Zaydi et al. (2020)        | MWM                 | Delayed ↓ in latency time over subsequent sessions | ↓ spatial learning and memory; complete rescue beyond control level | Accelerated ↓ in latency time over subsequent sessions (↓ anxiety-like behavior) |
|                            | NORT                | (↓ spatial learning and memory) | No sign. effect | Between-group differences not reported for both tests |
| Avolio et al. (2019)       | CPPT                | ↓ mean time on         | ↑ place preference/ memory | All probiotic effects independent of diet |
|                            | NORT                | grid floor             | (rescue level unclear) | The authors note that supported rears should be interpreted with caution |
|                            | EPM                 | (↓ place preference/memory) | ↑ recognition index (N – F/N + F) | No sign. effect |
|                            | LDT                 |                       | ↓ short- and long-term memory | (partial recovery, increase) |

“↑” indicates increase/improvement by the stated intervention, “↓” indicates decrease/impairment by the stated intervention. A complete rescue means that the outcome assessment of HFD + Pro is significantly different from that of HFD (+ VEH) and not significantly different from the control group. A partial rescue means that the outcome assessment of HFD + Pro is either significantly different from both HFD (+ VEH) and the control group or not significantly different from both when HFD (+ VEH) does differ significantly from the control group. Abbreviations: BM: Barnes maze, CFCT: contextual fear conditioning test, CPP: conditioned place preference, EPM: elevated plus maze, F: familiar object/place, FST: forced swim test, HFD: high-fat diet, LA: locomotor activity, LDT: light-dark box test, MWM: Morris water maze, N: novel object/place, NORT: novel object recognition test, NPRT: novel place recognition test, OFT: open field test, Pro: probiotic, sign: significant, SSPT: sucrose and saccharin preference test.
inclusion of both male and female animals, thirteen studies only investigated males and two studies did not specify sex. The age of experimental animals at the start of the experiment was 3–9 weeks in most studies. Four studies using rats did not specify age, but specified weights between 150 and 220 g upon purchase, which could represent an age between 27 and 52 days (McCutcheon and Marinelli, 2009). Two studies started experiments when animals were aged 28 or 80–82 weeks-old (hamsters and mice, respectively).

All studies but one compared standard chow (ND) with a variation of a HFD with percentages kcal from fat ranging between 33% and 65%, with most HFDs containing 60% kcal from fat. The duration of the diets varied greatly (range: 3–24 weeks). The models aimed to induce by HFD or Western-style diet included obesity, general metabolic dysfunction, non-alcoholic steatohepatitis (NASH), and non-alcoholic fatty liver disease (NAFLD). The diets in the majority of animal studies induced weight gain except for the study of Zaydi et al. (2020), which failed to detect weight gain after 12 weeks of HFD and the study of Zeltser et al. (2020) that did not report changes in body weight after 10 weeks high-fat high-fructose diet. There was one study that did not include a normal diet control group and therefore diet-induced weight gain could not be measured (Wang et al., 2020).

The sixteen studies included a total of 22 different probiotic interventions (two studies included two different probiotic interventions, while another two studies included three). Four of these interventions consisted of multi-strain treatments (see Table 1 legend). Two studies investigated double-strain treatments: one combining Bifidobacterium longum R0175 and Lactobacillus helveticus R0052, and the other combining Lactobacillus sakei OK67 and PK16. Single-strain treatments comprised three different probiotic genera: (1) Lactobacillus (twelve different interventions with species and strains brevis DPC6108 and DSM32386, helveticus R0052, paracasei H1101 and heat-killed paracasei D3–5, plantarum EMCC-1039 and DR7, reuteri MM4–1A (ATCC-PTA-6475), two times Lactobacillus rhamnosus GG, and sakei OK67 and PK16), (2) Akkermansia (two interventions with strains muciniphila CIP107961 and both viable and heat-killed muciniphila (ATCC BAA845)) and (3) Bifidobacterium (one intervention with strain pseudocatenulatum CECT 7765). For the probiotic treatments, a dosage between $1 \times 10^8$ and $1 \times 10^{10}$ CFU/day was used, except for one study that used a lower dosage of $6.2 \times 10^4$ CFU/day (Zeltser et al., 2020). A great variation existed in the duration of the probiotic intervention (range: 2–14 weeks).

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Fig. 3. Behavioral outcomes found in animal studies. A) Effect of HFD on behavioral outcomes. The inner circle represents the total number of assessments per cognitive domain. The outer circle represents the percentage of assessments that show HFD-induced impairment, HFD-induced improvement, or no significant HFD effect per cognitive domain. B) Effect of probiotics on behavioral outcomes for learning and memory, C) anxiety-like behavior and D) depressive-like behavior. The inner circles represent the same percentages as the domain-specific parts of the outer circle of (A). The outer circles represent the subsequent effect of probiotic treatment per HFD-induced effect. Abbreviations: Anx: anxiety-like behavior, Dep: depressive-like behavior, HFD: high-fat diet, L&M: Learning and memory, NS: No significant effect, Pro: probiotic intervention. ↑ indicates improvement by the stated intervention, ↓ indicates impairment by the stated intervention.
weeks). Moreover, the probiotic treatment commenced either before, simultaneously with, or after HFD treatment. This resulted in either a rescue paradigm of HFD-induced cognitive impairment, anxiety or depression (n = 7) or a prevention paradigm (n = 9) (Table 1).

3.3.2. Behavioral outcomes associated with HFD and probiotic treatment

The behavioral outcome assessments and HFD- and probiotic-related results of all included articles on animal studies are reported in Table 2 by model organism and are discussed in the next paragraphs.

3.3.2.1. Outcome assessments for learning and memory, anxiety-like and depressive-like behavior

A wide range of outcome assessments was used across the sixteen studies (Fig. 2). Learning and memory was assessed with the Novel Object Recognition Test (NORT; n = 6), the Morris Water Maze (MWM; n = 5), the Barnes Maze (BM; n = 3), the Novel Place Recognition Test (NPR; n = 1), the Contextual Fear Conditioning Test (CFCT; n = 1) and the Conditioned Place Preference Test (CPP; n = 1). Anxiety-like behavior was assessed with the Open Field Test (OFT; n = 6), the Elevated Plus Maze (EPM; n = 5), the Light-Dark Box Test (LDT; n = 3), a marble-burying task (n = 1), the BM (n = 1) and the Forced Swim Test (FST; n = 1). Lastly, depressive-like behavior was assessed with the FST (n = 3) and the Sucrose and Saccharin Preference Test (SSPT; n = 1).

3.3.2.2. Effect of HFD on behavioral outcomes

The effects of HFD on behavioral assessments are summarized in Fig. 3A. Of a total of seventeen assessments for learning and memory, HFD-induced cognitive impairment was found in eleven tests (64.7%) (Avolio et al., 2019; Beilharz et al., 2018; Chunchai et al., 2018; Higarza et al., 2021; Mohammed et al., 2020; Yang et al., 2019; Zaydi et al., 2020), while HFD-induced improvement was found in only one test (5.9%) (Abildgaard et al., 2017). The remaining five tests showed no significant effect of dietary treatment (29.4%) (Beilharz et al., 2018; Ohland et al., 2013; Patterson et al., 2019; Wang et al., 2020; Zeltser et al., 2020). Anxiety-like behavior was also assessed seventeen times in total, of which ten tests (58.8%) showed HFD-induced anxiety-like behavior (Agusti et al., 2018b; Avolio et al., 2019; Foroozan et al., 2021; Jang et al., 2019; Ohland et al., 2013; Patterson et al., 2019), while HFD-induced anxiety-like behavior (23.5%) (Myles et al., 2020; Wang et al., 2020; Zaydi et al., 2020), once for learning and memory (5.9%) (Wang et al., 2020) and once for depressive-like behavior (25%) (Abildgaard et al., 2017), or negative: twice for learning and memory (11.8%) (Beilharz et al., 2018; Zeltser et al., 2020). Notably, one study that found a probiotic alleviation of HFD-induced anxiety-like behavior, on the contrary, found an increase in anxiety-like behavior by probiotics in the absence of HFD treatment (not included in Fig. 3) (Ohland et al., 2013).

Some studies also assessed physical function, either with the OPT (n = 5), the EPM (n = 1), preceding a separate locomotor activity test (n = 2) or pen activity (n = 1). Out of the nine assessments for physical function, five (55.6%) showed a non-significant result for both HFD and probiotic intervention (Abildgaard et al., 2017; Agusti et al., 2018b; Chunchai et al., 2018; Higarza et al., 2021; Zeltser et al., 2020). Two tests showed impairment by HFD (22.2%) (Foroozan et al., 2021; Patterson et al., 2019), which was rescued by probiotics in only one test (11.1%) (Foroozan et al., 2021). One test showed probiotic reversal of HFD-induced improvement (11.1%) (Avolio et al., 2019) and the last test showed improvement by probiotic treatment independent of diet (11.1%) (Wang et al., 2020).

3.3.2.3. Effect of probiotic strains on HFD-influenced behavioral outcomes

In all eleven tests (64.7%) where HFD-induced cognitive impairment (learning and memory) was observed, probiotic intervention was able to reverse or prevent HFD-induced cognitive impairment (100% rescue; Fig. 3B) (Avolio et al., 2019; Beilharz et al., 2018; Chunchai et al., 2018; Higarza et al., 2021; Mohammed et al., 2020; Yang et al., 2019; Zaydi et al., 2020). Lastly, depressive-like behavior was assessed four times, of which three tests (75%) showed significant HFD-induced depressive-like behavior (Agusti et al., 2018b; Patterson et al., 2019) and one lacked a significant HFD effect (25%) (Abildgaard et al., 2017).

3.3.2.4. Effect of probiotic strains on HFD-influenced behavioral outcomes

The studies included in this systematic review that used single-strain treatment included three genera: Akkermansia, Lactobacillus, and Bifidobacterium. Lactobacillus was most commonly used and resulted in positive effects in all five studies measuring anxiety (Foroozan et al., 2021; Jang et al., 2019; Ohland et al., 2013; Patterson et al., 2019; Wang et al., 2020). For learning and memory Lactobacillus exerted positive effects (Chunchai et al., 2018; Mohammed et al., 2020; Zaydi et al., 2020), but two studies failed to show significant improvement in any of the cognitive test (Higarza et al., 2021; Yang et al., 2019). No negative effects on HFD-induced cognitive impairment have been observed for the use of this genus and for two studies positive effects have been observed without HFD (Wang et al., 2020; Zaydi et al., 2020). Only one study showed that on normal chow diet, Lactobacillus decreased exploratory behavior in the Barnes maze (Ohland et al., 2013).

Two studies investigated two genera separately and found that probiotic improvement of HFD-induced cognitive impairment (learning and memory) was obtained using Akkermansia, but failed to be detected upon using Lactobacillus (Higarza et al., 2021; Yang et al., 2019). One study showed strain-specific effects within the Lactobacillus brevis species (Patterson et al., 2019). Four studies investigated multi-strain treatments, where three of the four studies showed a negative effect of these treatments on HFD-induced cognitive impairment (learning and memory) (Abildgaard et al., 2017), or learning and memory in the absence of HFD (Beilharz et al., 2018; Zeltser et al., 2020). Whereas only one study, using the Biocult Strong mix, showed positive effects on HFD-induced cognitive impairment (learning and memory) and anxiety (Avolio et al., 2019).

3.4. Study quality

The assessment of study quality using SYRCLE’s RoB tool (Hooijmans et al., 2014) complemented with several items from Joseph and Law (2019) is presented in Fig. 4. A high risk of selection bias was seen for baseline characteristics at the start of HFD (item 2a: n = 3; 17.6%) or probiotic treatment (item 2b: n = 8; 47.1%). However, a high risk of bias is inevitable here due to the prevention- or rescue-based experimental setup of most studies. For this setup, probiotics were administered either before or after the start of HFD, meaning that baseline characteristics at the start of the latter intervention automatically differed for experimental and control groups.

Regarding performance bias, many studies had a high risk of bias for random housing (item 4a: n = 8; 47.1%) as animals were housed in pairs or larger groups. On the other hand, most studies tested probiotic...
Fig. 4. Risk bias assessment. A) Risk bias, per bias item, per article. B) Sum of risk bias, per bias domain. *: Items in agreement with the items in the Cochrane Risk of Bias tool. #: Items supplemented from (Joseph and Law, 2019). a: Control values were not displayed in figures for the CPPT and the NORT. b: Discrepancies between figures and accompanying text. c: Unclear distinction between different experimental groups and corresponding experimental designs within the article. Abbreviations: assess.: assessment, CPPT: conditioned place preference test, exp: experimental, HFD: high-fat diet, NORT: novel object recognition test, Pro: probiotics.
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2016; Slyepchenko et al., 2015; Tan et al., 2021). The included studies

Notably, a relatively high number of studies had a high risk of

reporting bias (items 9a: n = 7; 41.2%, and 9b: n = 3; 17.6%). These

scorings mainly resulted from studies reporting incomplete or unclear

study protocols, which were considered insufficient for a precise inter-

pretation of data or reproducibility.

Overall, most studies obtained one to four negative RoB scores for a

total of fifteen items, however, one study stood out by seven negative RoB scores. The main reason for this high number of categories scoring a

high risk of bias was this study its experimental setup, as probiotics were not

only not administered to the experimental animals but also their dams

during pregnancy and lactation (Myles et al., 2020). Therefore, the

allocation of animals in experimental groups was already restricted

before birth and baseline characteristics were not similar for different

experimental groups.

Finally, many items and bias domains could not be assessed due to

missing information in the publication. For example, methodological
details about concealment methods for allocation of animals to different
treatment groups (item 3: n = 13; 76.5%), blinding of animal caretakers
(item 5: n = 14; 82.4%), and randomization of animal selection for
outcome assessment (items 6a and 6b: n = 14; 82.4% and n = 7; 41.2%
respectively) were not reported in most studies, which made it impossible
to estimate the risk of bias for these items.

4. Discussion

4.1. Probiotic treatment ameliorates HFD-induced cognitive impairment

and anxiety

In this review, we gave a comprehensive overview of the present
literature on the effect of probiotic treatment on HFD-induced cognitive
improvement, anxiety or depression. One human study and sixteen animal
studies were included. The human randomized controlled trial showed a
decrease in (pre-)obese women in anxiety but not in depressive-like
behavior. Most behavioral outcome assessments that were impaired in
HFD-treated animals (66.2% in total) could be prevented or rescued by the
probiotic intervention (82.2% of impaired tests or 53.9% in total),
especially in the category learning and memory (100% of impaired tests or
64.7% in total). Moreover, the three cases in which HFD-induced
anxiety-like and depressive-like behavior were not reversed by probiotics
still showed a partial rescue as the behavior was neither significantly
different from the HFD- and vehicle-treated group nor from the
control group (Abildgaard et al., 2018b). These findings are in line with the
results of other systematic reviews reporting on the health effect of probiotics on neurobehavioral impairments in different disorders
(Ansari et al., 2020; Kesika et al., 2021; Ng et al., 2019; Pirbaglou et al.,
2016; Slyepchenko et al., 2015; Tan et al., 2021). The included studies
used either a prevention or rescue paradigm, which both resulted in
positive effects of probiotics in multiple studies suggesting that the
timing of starting the probiotic treatment does not have major effects on
its effectiveness. Moreover, Mohammed and colleagues (2020) found
improvement of both spatial and object memory after only two weeks of
probiotic treatment following HFD, suggesting a relatively short treat-
ment period is sufficient to exert a positive effect of probiotics.

A substantial number of studies did not report statistically significant
effects of HFD and probiotics on cognition, anxiety-like or depressive-
like behavior, while four studies even reported opposite effects to the
expected HFD-induced impairment and probiotic-induced improvement
(Abildgaard et al., 2017; Beilharz et al., 2018; Ohland et al., 2013;
Zeltser et al., 2020). One of these studies showed a negative effect of
probiotic treatment in the absence of HFD treatment, whereas probiotics
in combination with HFD exerted an ameliorating effect (Ohland et al.,
2013), two studies showed a negative effect of probiotics independent of
diet (Beilharz et al., 2018; Zeltser et al., 2020), and the last showed
impairment by probiotics of HFD-induced improvement (Abildgaard
et al., 2017). According to Abildgaard and colleagues, this last result
could be explained by their experimental setup to measure spatial
memory (2017). As they used an aversive cognitive impairment- or negative effect of probiotics, could result from many factors, or
combinations thereof as explained below.

4.1.1. The effects of HFD dietary composition, age and treatment duration

on weight gain and biobehavioral readouts

The dietary composition and treatment duration varied across
studies. Regarding the composition, the percentage kcal from fat in
HFDs varied between twice to six times as much as compared to normal
diets given to the control groups. One study reported that HFD did not
result in significant weight gain after 12 weeks of HFD (Zaydi et al.,
2020). This study had a relatively low percentage of fat added in their
HFD, namely 25% animal fat. Whereas most other studies added at least
30% of additional fat to the normal chow. Although the study of Zaydi
and colleagues failed to show weight gain, effects on learning and
memory were found and were restored upon probiotic treatment (2020).
This suggests that significant weight gain might not always be required
to induce cognitive impairment. The study of Ohland et al. (2013) only
gave 20% additional fat to the diet but did show significant weight gain.
They used a Western-style diet which in addition to an increased fat
percentage contained refined carbohydrates instead of a combination of
different types of carbohydrates which could explain the significant
weight gain in that study with limited amount of added fat (Ohland et al.,
2013). However, it is still unclear what the influence is of differ-
ences in the composition of other nutrients than fat, specifically carbo-
hydrates on the development of obesity (Sartorius et al., 2018).

HFD-induced effects were found to be influenced by age, which
varied highly among the included studies in this review. For example,
the severity of HFD-induced metabolic symptoms increases with age
(Nunes-Souza et al., 2016). On the other hand, relational memory
impairment by HFD was reported to be most severe in young animals
(Boitard et al., 2012) and, likewise, age decreases the risk of
obesity-related anxiety (DeJesus et al., 2016). However, due to the
limited number of studies tackling this issue no consensus is yet estab-
lished as can be observed in Table 1 showing that the majority of studies
included young animals.

Moreover, the range of HFD treatment duration of the included
studies was 3–24 weeks, whereas previous studies found that behavioral
read-outs are differently affected depending on HFD duration (Beilharz
et al., 2014; Kanoski and Davidson, 2010). For example, spatial memory
is affected already after short-term dietary treatment, while object
memory only after long-term treatment (Beilharz et al., 2014; Kanoski
and Davidson, 2010). Notably, object memory was precisely the
behavioral read-out that was impaired by probiotics instead of HFD in
Beilharz et al. (2014) and Zeltser et al. (2020). An insufficiently long
HFD treatment in these studies could therefore be a possible explanation
for the lack of an HFD effect, which in turn could have facilitated the
negative probiotic effect that was found. The study of Ohland et al. used
a western-style diet of only three weeks that did not impair learning and
memory but affected solely exploratory behavior, suggesting that three
weeks of western-style diet is insufficient to affect cognitive function
(Ohland et al., 2015).

4.1.2. The influences of biobehavioral outcome measures

A wide range of behavioral outcome assessments was used to mea-
sure learning and memory, anxiety- and depressive-like behavior. All
these tests measure a more or less different behavior, depending on
specific pathways and brain regions. Therefore, both HFD and probiotic
treatment likely affect these behaviors in distinct ways. Although most
different tests for a certain cognitive domain showed similar results,
some variation is almost inevitable as some tests are more suitable than
others. For example, Ohland and colleagues, who showed a negative effect of probiotics on anxiety-like behavior only in the absence of HFD, uniquely used an additional read-out in the BM to measure this kind of behavior (Ohland et al., 2013). However, the behavior that is measured with this test must primarily be seen as exploratory behavior and must be interpreted with caution (Sharma et al., 2010). The BM test also presented with varying effects measuring learning and memory impairment upon HFD and in combination with probiotic treatment (Ablidgaard et al., 2017; Yang et al., 2019), suggesting that the outcomes of this test might be too variable to draw overall conclusions. The MWM for example showed consistent effects regarding the effects of HFD-induced cognitive impairment and the positive effects of probiotic treatment across studies (Chunchai et al., 2018; Higarza et al., 2021; Mohammed et al., 2020; Wang et al., 2020; Zaydi et al., 2020). Therefore, this test seems to be a robust assessment for learning in memory for this type of studies. All other tests used showed either varying effects between studies, or were only used in one study, which made it impossible to assess robustness.

4.1.3. Probiotic genus, strain and multi-strain treatments affect neurobehavioral readouts differently

The main factor influencing probiotic efficacy in these studies was probiotic genus and strain. The sixteen single-strain treatments reviewed here included three different probiotic genera, nine different species, and fourteen different strains. Due to the variation in species and strains used no conclusions can be drawn about species- or strain-specific effects. Single-strain treatment included the genera Akkermania, Lactobacillus, and Bifidobacterium. Lactobacillus was most commonly used and resulted in positive effects in all five studies measuring anxiety (Foroozan et al., 2021; Jang et al., 2019; Ohland et al., 2013; Patterson et al., 2019; Wang et al., 2020). Therefore, this gives a clear indication that the use of Lactobacillus has a positive effect on HFD-induced anxiety. For cognitive impairment the effects of Lactobacillus are less clear since three studies reported positive effects (Chunchai et al., 2018; Mohammed et al., 2020; Zaydi et al., 2020), but two studies failed to show significant improvement (Higarza et al., 2021; Yang et al., 2019). It is important to note that, except for reduced exploratory behavior in normal chow conditions in one study (Ohland et al., 2013), no negative effects on HFD-induced cognitive function have been observed for the use of this genus. This negative effect should be interpreted with caution as mentioned earlier, since this readout measures merely exploratory behavior. Interestingly, the included studies that investigated multiple genera separately found that probiotic improvement of HFD-induced cognitive impairment (learning and memory) was genus-specific (Higarza et al., 2021; Yang et al., 2019), showing positive effects of Akkermania over Lactobacillus. One study showed strain-specific effects within the Lactobacillus brevis species (Patterson et al., 2019). Furthermore, reviews comparing single-strain and multi-strain probiotic treatments suggest an increased health benefit of multi-strain combinations (Chapman et al., 2011; Timmerman et al., 2004). On the contrary, in the current review three of the four studies investigating multi-strain treatments showed a negative effect of these treatments (Ablidgaard et al., 2017; Beilharz et al., 2018; Zeltser et al., 2020). Whereas only one study using the Biocult Strong mix showed positive effects (Avolio et al., 2019). This warrants a careful trade-off between the enhanced efficacy of multi-strain treatments on health on the one hand, and their possible adverse effects on cognitive function and anxiety on the other hand for future studies. Taken together, we can conclude that Lactobacillus is the most well-studied and promising probiotic to use to improve HFD-induced anxiety and cognitive impairment in rodents and multi-strain treatments should be used with caution.

4.2. Limitations

We already pointed out the wide variety in study characteristics of the included studies. Since the number of eligible articles was also quite small, the evidence was too limited to conduct a meta-analysis. In general, a systematic search of the literature showed that probiotics as a treatment of neurobehavioral symptoms in obesity models are still underrepresented. This trend is especially clear regarding human studies, as only one of the seventeen articles eligible for this review reported on a human trial. Any conclusions about the health effect of probiotics on obesity-related cognitive impairment, anxiety or depression in humans is therefore impossible at this point. In addition, the number of behavioral read-outs on depressive-like behavior in animal studies was very low. Therefore, general statements about the health effect of probiotics on HFD-related depressive-like behavior cannot be made.

The lack of studies combining obesity, cognition, and probiotics could result from the involvement of multiple research fields in addressing this topic, mainly gastroenterology and neuroscience. Although interdisciplinarity in science, especially concerning microbiota-gut-brain axis research, is required, and appreciated, most research papers still focus on a relatively small field of expertise. As a result, most researchers investigating obesity models use metabolic read-outs rather than neurobehavioral ones. More interdisciplinary collaborations between researchers of different research fields could therefore boost our knowledge on probiotic health effects on neurobehavioral symptoms in metabolic diseases such as obesity.

What also complicates the field is the translatability between animal studies and human studies. Human studies often use questionnaires (self-assessments) to assess neurobehavioral symptoms and are more difficult to compare between subjects than behavioral tasks and read-outs used in animal studies. Besides, in animal studies, HFD is used as a model for obesity, although the same treatment is also used to induce other disorders like NASH or NAFLD (Higarza et al., 2021; Mohammed et al., 2020; Zeltser et al., 2020). This raises the question of how specific the resulting obesity model is and what the level of similarity is in symptoms between animal and human studies. In addition, it is much harder to control the composition of the microbiome in human subjects than in animals as it is influenced by many factors, including diet (Allenberg and Wu, 2014) and exercise (Mach and Fuster-Botella, 2017; Zeppa et al., 2020). Therefore, the mechanisms by which different probiotic strains influence cognitive symptoms must be elucidated. In this way, specific strains or combinations of strains can be more accurately assigned to specific symptoms and biomarker read-outs.

A major limitation of the included animal articles is the underrepresentation of female subjects. Most animal studies only looked at males, although several publications reported sex differences in HFD-induced symptoms (Bridgewater et al., 2017; DeJesus et al., 2016; Taraschenko et al., 2011). Moreover, the only study in this review that included both males and females reported several sex differences in the probiotic effect on obesity-related cognitive symptoms, most notably an increase in anxiety-like behavior in males compared to females as well as a sex x probiotic treatment interaction effect regarding anxiety-like behavior (Myles et al., 2020). Therefore, the inclusion of both males and females in future studies on probiotic treatment is warranted, especially in view of the translation to human trials.

Furthermore, only a small number of studies included locomotor activity in their behavioral assessments. The included studies reported varying effects on locomotion, including both HFD- and probiotic-induced improvement and impairment. A general statement of how locomotion is influenced in the included studies is thus impossible. Still, locomotor activity is needed in most behavioral assessments that were used in these studies. Therefore, the effect of possible locomotion alterations by HFD and/or probiotics in the results included in this review remains unclear.

From the risk of bias assessment, it became clear that details regarding several bias categories were not or insufficiently described in many articles on animal studies. This not only increases the risk of bias in these studies but sometimes makes the data difficult to interpret correctly. An important factor in this is that many reports lacked exact values of the behavioral outcome assessments. The quality of
publications would therefore benefit if SYRCLE’s risk of bias tool (Hooijmans et al., 2014) is not only used as a quality check after publication, but, as recommended by the authors, also as a checklist when writing an article, during peer-review, or when accepting articles for publication in a journal.

This review did not include other interventions than probiotics. However, several studies have shown the beneficial health effect of, for example, prebiotics (Marx et al., 2020; Paiva et al., 2020), or exercise (Burghard et al., 2004; Foroozan et al., 2021; Salam et al., 2009) on cognitive function. Additional research is needed to investigate whether this health effect is also exerted on obesity-related symptoms. Furthermore, when mechanisms of probiotics have become clearer, it would be interesting to investigate whether the combination of probiotics and prebiotics, exercise, or other factors can enhance the health effects.

5. Conclusion

In sum, we found seventeen articles reporting the effect of probiotic treatment on obesity-related neurobehavioral symptoms. Most studies suggested that probiotics were able to successfully prevent or rescue HFD-induced cognitive impairment and anxiety. However, in the absence of HFD-induced impairment, probiotics could in some cases exert a negative effect on cognitive function. This seems especially true for multi-strain combinations. The results of this review suggest that probiotic effects are genus- and strain-specific, with Lactobacillus being the most well-studied and promising genus to use to improve HFD-induced anxiety and cognitive impairment.

With the current evidence, the future for probiotic treatment of HFD-induced cognitive impairment and anxiety seems promising if certain obstacles will be overcome in future studies. First of all, the evidence for the inclusion of female animals in future studies is essential for the translation of these findings to humans.

Data availability statement

No new data was generated in this study.

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Declaration of interest

Declarations of interest: none.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neubiorev.2022.104634.

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