Short-term probiotic augmentation in depression treatment: study protocol for a randomised controlled trial

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Study protocol

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Abstract

Background There is urgent need to develop novel augmentation options for improving inadequate response to antidepressants. Emerging evidence showed that the composition of the gut microbiota is altered in patients with depression and related to their symptoms, suggesting that restoring microbial composition may constitute a promising strategy in depression treatment. This protocol introduces a study design to test the effectiveness of a short-term probiotic augmentation therapy in patients with a current depressive episode. Methods Using a randomised, double-blind, placebo-controlled design, the effectiveness of probiotic supplementation will be examined in 60 patients with current severe depressive episodes (Hamilton Depression Rating Scale > 24). Patients will be randomly assigned either to a 4-weeks probiotic or placebo treatment. Assessments will take place at baseline (pre-intervention), at 4 weeks (post-intervention) and at 8 weeks (follow-up) of the study program. The primary study outcome will be depressive symptoms as measured with the Hamilton Depression Rating Scale (experts' ratings), while secondary outcomes include immune signalling and inflammatory processes, hypothalamic-pituitary-adrenal axis responses, neurogenesis, the release of appetite-regulating hormones, the composition of gut microbiota, sleep quality, physical activity and multimodal neuroimaging. Discussion In search for augmentation strategies in depression treatment, this protocol describes a study design to test the effectiveness of a short-term probiotic augmentation therapy in patients with major depressive disorders. We also suggest the assessment of comprehensive secondary outcomes to determine biomarkers for personalized treatment responses and the development of more efficient microbial interventions in major depressive disorders.

Background

Almost two thirds of patients with major depressive disorder (MDD) do not experience adequate response to their initial antidepressant medication [1]. Augmentation strategies to complement insufficient responses to current treatment strategies are therefore urgently needed [2, 3]. Such augmentation approaches may involve compounds that work synergistically with antidepressants [4]. In the past few years, it has become increasingly evident that resident gut bacteria are an important contributor to healthy metabolism, and there is significant evidence linking altered composition of the gut microbiota with disorders such as MDD [5-8]. Preclinical work in animals have reported associations between altered microbial composition and anxiety-like behaviour [9-11], depressive-like symptoms [12] and stress responsiveness [10, 13]. In line with these preclinical findings, recent studies found an altered composition of faecal microbiota in patients with MDD [11, 14, 15]. Changes in gut microbiota can influence cognitive and emotional stress processes through interactions with the brain [16], and dysfunction of the gut microbiota-brain axis has been implicated in stress-related disorders such as MDD [17]. Brain-gut interactions could occur in various ways: 1) microbial compounds communicate via the vagus nerve, which connects the brain and the digestive tract, and 2) microbially derived metabolites interact with the immune system, which maintains its own communication with the brain [18]. Although the pathways linking gut bacteria with the brain are incompletely understood, one of the principal mechanisms proposed to underlie stress-induced alterations is the so-called leaky gut phenomenon. The leaky gut phenomenon points to the fact that increased translocation of bacterial products, due to a compromised gut barrier, has been linked to activation of the immune system and hypothalamic-pituitary-adrenal (HPA) axis [19, 20]. Indeed, human studies have demonstrated a stress-induced increase in bacterial translocation in depression [19, 21]. The stress-induced interactions between the gut microbiota and the brain are further mediated via central processes such as neurotransmission and neurogenesis [22-25]. For instance, there is substantial evidence to demonstrate a role for the gut commensals in the regulation and development of the serotonin (5-hydroxytryptamine; 5-HT) system [26] and the expression of brain-derived neurotrophic factor (BDNF) [27]. Notably, BDNF may contribute to the modulation of neurogenesis in response to both stress and antidepressants, as hippocampal BDNF levels decrease in response to chronic stress [28, 29] and increase in response to antidepressant treatments [30, 31].

Compelling preclinical data demonstrated the beneficial effect of probiotics in normalizing HPA axis functioning, BDNF levels and 5-HT neurotransmission [5, 7, 25, 32]. In particular, certain probiotics such as lactobacilli and bifidobacteria can reverse psychological stress-induced HPA axis activation [33] and possess antidepressant or anxiolytic activity in rats [7, 32].

A seminal work in experimental animals showed that altered stress responsiveness has been partially reversed by colonization of the gut [13]. Importantly, a recent and innovative study showed that short-term consumption of mostly animal or mostly plant diet rapidly and reproducibly altered the human gut microbiome [34], suggesting that the development of dietary supplements may provide a novel promising adjuvant therapy in addition to pharmacological antidepressant treatments in depression [3, 32, 35-37]. Recent reports of trials administrating a combination of probiotics to healthy subjects demonstrated improvements in depression or anxiety measures [38, 39]. Moreover, recent meta-analytical evidence has already suggested the putative beneficial effect of probiotic treatment in individuals with pre-existing mood symptoms [40].

In summary, there is substantial evidence to demonstrate that the composition of the gut microbiota is a master regulator of key neurophysiological processes that are affected in depression. Accordingly, restoring disturbed gut microbiota-brain interactions via probiotic bacteria might be a new and promising treatment strategy for depression [7, 10, 41, 42]. Although these results are promising, evidence is still sparse. Accordingly, more randomised controlled trials (RCT) in clinical populations are needed, especially using probiotics as adjuvant therapy rather than primary treatment [40, 43, 44]. This protocol introduces a study design to test the effectiveness of a four-week, high-dosage probiotic adjuvant therapy in patients with current severe depressive episodes with the aim to achieve a fast antidepressant response and thereby complement effects of standard antidepressant medication, whose efficacy should be awaited al least six weeks before declaring nonresponse [45, 46]. Furthermore, this protocol also presents a comprehensive assessment of secondary (mostly biological) outcomes to investigate the effect of probiotic augmentation on brain-gut interactions, and whether these effects are associated with the probiotic-induced clinical effect. These secondary outcomes serve both to detect biomarkers for predicting personalized treatment response and the development of novel microbial intervention in depression treatment.

Methods / Design

Objectives

Primary objective: To test whether the effect of a 4-weeks lasting probiotic augmentation therapy on symptoms of depression in patients with current moderate to severe MDD is superior relative to a placebo-controlled standard antidepressant treatment.

Secondary objective: To elucidate the effect of the probiotic augmentation therapy on brain-gut interactions with the aim to identify biomarkers for personalized treatment responses and the development of more efficient and microbial treatments.

Safety objective: To monitor adverse events and side effects induced by the probiotic augmentation therapy. Specific study aims and corresponding outcomes are listed in Table 1.

Insert Table 1 about here

Participant characteristics

After entering the service of our clinic (Universitäre Psychiatrische Kliniken (UPK) Basel, Switzerland), patients will be assessed with different clinical and psychosocial questionnaires to examine eligibility criteria. Female and male patients fulfilling the following inclusion criteria will be eligible for the study:

· Age ≥18

· Current severe depressive episodes (Hamilton Depression Rating Scale (HADRS) > 24 [47])

· Inpatient in the adult psychiatry department of the UPK Basel

· Treated with standard antidepressant medication

· Able to read and understand the study protocol and participant's information, and to comply with the study conditions

· Signed written informed consent

The presence of any of the following exclusion criteria will lead to exclusion of the participant:

· Comorbid psychiatric disturbances such as addiction, bipolar disorder, schizophrenia

· Current medical conditions such as acute infectious disease, dietary restrictions (not taking meals from the hospital restaurant)

· Immunosuppressed patients

· Pregnancy, breast-feeding

· Inability to read and understand the participant's information

· Body Mass Index (BMI) > 30

Study design

This study is designed as a single-site, randomised, double-blind, placebo-controlled, parallel group clinical trial and fulfils the requirements of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist [48] (Additional file 1). Relative to a placebo condition, it will test the superiority of a 4-week probiotic augmentation therapy in patients with a current episode of moderate to severe depression. After signed written informed consent, each subject will be assigned to a subject number and allocated either to the placebo or probiotic group using a computer-based block randomization algorithm (allocation ration 1:1). Both groups will be matched for age, gender, BMI and antidepressant medication. Each package consisting of 2 placebo or probiotic sachets will be labelled with a randomization number, which is listed in the electronic case report forms (CRF) and in the Drug Accountability Log. The nursing personnel will hand over the daily package of sachets to the patient every morning after breakfast during 28 days and stay with the patient until the sachets have been finished. In this way both the personnel (study nurse, outcome assessor) and the patient will be blinded. Any non-compliance will be reported. Patients who will miss more than one daily dose will be excluded from the final statistical analyses. Besides patients' right to withdraw from the study at any time for any reason without being obliged to give reason, the investigator also has the right to withdraw subjects from the study for the following reasons: Adverse events (AE) challenging the health of the subject, if continuing the study (e.g. acute disease) or severe protocol violations such as refusal of measurements, consumption of psychotropic substances, intake of immunosuppressive agents or significant changes in diet. Withdrawal in case of AE, the participant will be told which test substance he/she had taken to avoid future discomfort (unblinding). In case of an acute allergic reaction, the participant is referred to our emergency department and the study is discontinued. The participant is informed about the applied test substance (unblinding) and will eventually be referred to an allergologist to verify allergic reaction to one of the test substances in order to avoid future exposure. In case a participant wishes to discontinue study participation, the data collected to this time point is included in the analysis. A follow-up visit is not planned in this case.

Study interventions

Probiotic: The probiotic preparation selected for this study is a multi-strain probiotic (Vivomixx®, Mendes SA, Switzerland) consisting of the following eight strains: Streptococcus thermophiles DSM 24731, bifidobacteria (B. breve DSM 24732, B. longum DSM 24736, B. infantis DSM 24737) lactobacilli (L. acidophilus DSM 24735, L. plantarum DSM 24730, L. paracasei DSM 24733, L. delbrueckii subsp. Bulgaricus DSM 24734). The strains contained in Vivomixx® have been selected not only for their specific individual characteristics, but also for their synergistic activities. Vivomixx® will be provided in sachets (two sachets per day, total of 900 billion CFU/day). Sachets can be mixed with any cold, non-carbonated drink or with food such

as yogurt. This is the recommended daily dose to support a balanced digestive system from the distributor (MENDES S.A., Lugano, Switzerland). To ensure gut mucosal colonization by the probiotic [49], the intervention phase will last four weeks. Vivomixx® sachets will keep refrigerated at 2-8°C and away from light all times.

Placebo: To allow comparison with previous probiotic trials in depression [50], subjects in the placebo group will receive a placebo that contains maltose but no bacteria. The appearance of the placebo will be indistinguishable in color, shape, size, packaging, smell, and taste from that of the probiotic supplement.

Study procedure

The study comprises an enrolment / allocation, post-allocation (pre-intervention, intervention and post-intervention) and follow-up period (Figure 1).

Insert Figure 1 about here

Including the follow-up assessment, duration for an individual study participant will be approximately 9 weeks. During the enrolment / allocation day, study personnel (NS, CK) will conduct an eligibility screen including the following: Sociodemographic information, personal and family medical history, physical examination, structured diagnostic interview, tox screening, breathalyser and pregnancy test. If inclusion criteria are fulfilled, patients will be informed about the study aims (by NS or CK) and provide written informed consent. All patients have to be able to read and understand the study information and to sign the written informed consent form. They will also be asked for consent to use biological material (MRI, blood/saliva/stool samples) in ancillary studies. For instance, we will use the MRI data from this study in future transdiagnostic analyses with datasets from samples of schizophrenia or heroin-addicted patients. Patients will then be randomly allocated either to the placebo or probiotic group (each n=30, see below for more details). Within one week, a pre-intervention test battery consisting of clinical, physiological, microbial and neural assessments will then be conducted. Afterwards, patients receive the intervention (probiotic or placebo) for 4 weeks. In the UPK most of the patients suffering from severe depression stay in the hospital for at least 44 days, what makes a 4-week adjuvant therapy under controlled conditions feasible. During this period, patients' depressive symptoms will be evaluated weekly, while eating behaviour, weight, gastrointestinal side effects, adverse events and physical activity will be assessed at a daily base. After this 4-week period a post-intervention test battery will be conducted within one week using exactly the same assessment as in the pre-intervention assessment. To avoid inter-rater variability, the assessor for the pre / post-intervention and follow-up assessments will be the same person. One month after the intervention (follow-up), we will assess patients' depressive symptoms, adverse events and gastrointestinal side effects and collect blood and stool samples. Patients will receive a box Vivomixx® sachets free of charge to encourage them to complete the follow-up assessments. A detailed assessment schedule is provided in Table 2.

Insert Table 2 about here

Determination of sample size

The primary aim of this RCT is to demonstrate a significant reduction in HADRS score after probiotic supplementation relative to the placebo treatment. According the 2004 National Institute of Health and Clinical Excellence (NICE) guidelines [51], a difference of three points on the HADRS and standardized mean difference of 0.50 can be considered as clinically significant. Supposing the use of a 2-sample t-test, a power of 95% at a significance level of p=0.05, sample size was estimated to be 26 per group. To ensure 26 evaluable patients per group, 60 patients in total need to be included considering a dropout rate of approximately 10 %.

Data collection and management

Study data is recorded with paper case report form (CRF). For each enrolled study participant CRFs are maintained. Participants are not identified in the CRF by name or initials and birth date; instead, the participant number is used.

secuTrial®, an entirely browser-based GCP-compliant system for collecting patient data in clinical studies will be used during the trial to enter data from the paper CRF to generate an electronic CRF. All data will be validated when entering them into the database and a quality check will be conducted before analyses. Upon conclusion, the database is secured and cannot be changed anymore. All study data will be archived for a minimum of 10 years after study termination or premature termination of the clinical trial. All data is archived in folders at the study site.

Confidentiality and data protection

Participant's confidentiality will be maintained at all times. The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Individual subject medical information obtained as a result of this study is considered confidential, and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files. Personnel from the sponsor, from the Psychiatric University Hospital Basel and members of the Independent Ethics Committee (IEC) are obliged to respect medical secrecy and to refrain from divulging the participant's identity or any other personal information they might fortuitously be aware of.

Direct access to source documents will be permitted for purposes of monitoring, audits and inspections. Demographic data and personal data will be kept in the electronic database. Subjects will receive a study number upon inclusion, and in the data base only the study number will appear. Only the principal investigator (PI: AS) not otherwise involved in patients' assessments and study condition assignments will have the key. Data generation, transmission, storage and analysis of health-related personal data and the storage of biological samples within this project will follow strictly the current Swiss legal requirements for data protection and will be performed according to the Ordinance HRO Art. 5. Health related personal data captured during this project and biological samples from participants are strictly confidential and disclosure to third parties is prohibited; coding will safeguard participants' confidentiality. Project data will be handled with uttermost discretion and only be accessible to authorised personnel.

Monitoring

The aim of monitoring is to evaluate the progress of the study, to verify the accuracy and completeness of CRFs, to ensure that all protocol requirements, applicable local authority regulations and investigator's obligations are being fulfilled, and to resolve any inconsistencies in the study records. Regular monitoring visits at the investigator's site prior to the start and during the course of the study will be performed by independent monitors (not included in the research group). The source data/documents are accessible to monitors, and questions are answered during monitoring. A data monitoring committee is not needed in a risk category A trial without drugs/medical devices. No interim analysis is foreseen. Audits or inspections by regulatory authorities during study or after study closure may be performed to ensure proper study conduct and data handling procedures according to ICH-GCP guidelines and regulatory requirements. Audits and inspections may include verification of all source documents, check of CRFs and site files and a visual inspection of the study site.

Primary statistical analysis

The primary aim of this study is to investigate whether a four-week probiotic augmentation therapy significantly reduced depressive symptoms (HADRS score) compared to the placebo intervention in patients with current moderate to severe. The HADRS change score from the pre- to the post-intervention assessment will be compared between the placebo and the probiotic group using a 2-sample t-test at a significance level $\alpha=0.05$. T

Effect sizes will be expressed with Cohen's d.

Secondary statistical analysis

We do not propose concrete analyses for the secondary outcomes but suggest performing multidimensional approaches. In addition to conventional analyses exploring the probiotic effect on isolated measures, our group will combine data across

the different modalities to construct comprehensive predictive models. In particular, using machine learning we are going to test whether baseline multidimensional data can predict individual treatment responses (responder vs. non-responder; remission vs. non-remission).

Handling of missing data and drop-outs

If less than 10% data of a specific measure are missing, multiple imputation techniques can be applied to replace them. However, in some cases (e.g. MRI data), data cannot be imputed and will be therefore removed from further analyses. Data from dropouts will be included in the analysis if feasible (e.g. baseline values can be of value to characterise the study population). Data are analysed both per protocol and by intent-to-treat (ITT), with the last observation carried forward (LOCF).

Discussion

Testing the effectiveness of short-term probiotic treatment as an augmentation therapy

Current options for the treatment of depression have not reached optimal efficacy [1, 52], indicating the urgent need for novel augmentation therapies. A very recent RCT in patients with mild to moderate MDD already showed that probiotic supplementation over 8 weeks along with standard antidepressant medication significantly decreased depressive symptoms [53]. This evidence indicates that probiotic treatment may be a promising augmentation therapy to standard antidepressant medication [43, 44], which is in line with meta-analytical evidence supporting the adjunctive use of nutraceuticals for depression [3]. To further support and expand upon this preliminary evidence, this protocol provides a study design to test the effectiveness of a high dosage probiotic augmentation therapy over four weeks in patients with current severe depressive episodes. A four-weeks lasting probiotic augmentation should be sufficient to ensure colonisation in the lower gastrointestinal tract [49], ensure optimal therapy adherence and accelerate clinical response relative to antidepressant monotherapy, which efficacy should be assessed at least up to 6 weeks [45, 46]. If effective, short-term probiotic augmentation may be a pragmatical, non-stigmatizing and low-cost option that is worthy of clinical consideration.

Biomarkers search for developing more efficient and personalized microbial interventions

Besides testing the effectiveness of a short-term probiotic augmentation therapy in ameliorating depressive symptoms, this protocol also outlines a comprehensive assessment of physiological outcomes. These secondary outcomes will either help to predict individual responsiveness to currently available treatments or develop novel and more efficient microbial interventions. The assessments of these factors are particularly intended to capture the probiotic impact on different pathways along the brain-gut axis [7] including the endocrine (cortisol, ghrelin, leptin), immune (macrophage migration inhibitory factor, interleukin 1-beta), neural (EEG, structural and functional MRI, DTI, ASL) and microbial (metabolic profiling) system. Combining cutting-edge multidimensional analysis approaches with thorough machine learning algorithm [54, 55] are then needed to predict the individual clinical response to the probiotic augmentation. In other words, this kind of analysis allows to determining whether patients with a positive biomarker status will show an improved outcome (high responder), but the same treatment will have no (or reduced) benefit in patients with a negative marker status (low responder). In line with the concept of personalized medicine [56, 57], such predictive biomarkers would allow to targeting those patients who are actually going to benefit from probiotic supplements. Besides predicting personalized treatment responses, these prognostic models further help delivering biomarkers for early drug development. Extracting data features with significant contribution to the prognostic performance, potential targets can be discovered for the development of novel microbial therapeutics in depression treatment. This data-driven system medicine approach [58] allows developing more efficient treatments that are better tailored on comprehensive pathophysiological mechanisms.

Possible challenges and limitations

Study recruitment for such a comprehensive and demanding study can be challenging. It is therefore key to thoroughly explain patients the potential benefit of probiotic supplementation. In case of recruitment issues, additional sites can be considered or omission of predefined secondary outcomes can be proposed. Relaxing inclusion criteria by including patients with mild depressive episodes could be another option to facilitate recruitment. Medication adherence is often a challenge in affective disorders [59]. It is therefore suggested to including only inpatients to ensure optimal compliance and study supervision. Restricting the study sample to inpatients also entails the advantage that all participants take standard meals from the hospital over the course of the study. Successful study completion also depends on rigorous observance of the time constraints and sound execution of data collection. Adequate training of study personnel and regular monitoring is recommended.

Although intended to guarantee optimal compliance, four weeks probiotic augmentation therapy proposed as proposed in the present protocol is shorter compared with previous successful trials in patients with depression [50, 53]. However, a higher dosage is proposed and if successful, it offers a promising strategy to complement standard antidepressant treatment by inducing a faster treatment response. A possible further limitation is the choice of our probiotics, i.e. the mixture of certain probiotic strains. This point can be addressed by performing metagenomic analysis of faecal stool samples to evaluate engraftment of all strains in our recipients. Although our understanding how specific metabolites in the gut may influence mental health is increasing [60], more research is warranted to test which type of bacteria shows clinical effects while others do not and whether there are dose-response functions.

Conclusion

In summary, this protocol offers a template to assess the effectiveness of a short-term probiotic augmentation therapy in patients with severe depressive episodes. The proposed investigation fits with the nascent field of nutritional psychiatry [37, 61] and lays the ground for future investigations to establish predictive biomarkers for individualized probiotic augmentation therapies in depression and the development of more efficient microbiota-related treatments.

Trial status

The trial (NCT02957591: Probiotic Supplementation in Severe Depression, protocol version 3, 23rd November 2016) is currently in the recruitment phase at the University Basel, Department of Psychiatry (Universitäre Psychiatrische Kliniken (UPK) Basel). Recruitment started in March 2017 and completion is expected on December 2019.

Abbreviations

ASL, Arterial Spin Labeling;

CRF, case report form;

BDNF, brain-derived neurotrophic factor;

BMI, Body Mass Index;

DTI, Diffusion tensor imaging;

EEG, Electroencephalography;

HADRS, Hamilton Depression Scale;

HPA, hypothalamic-pituitary-adrenal;

IPAQ, International Physical Activity Questionnaire;

IEC, Independent Ethics Committee;

ISI, Insomnia Severity Index;

MADRS, Montgomery-Åsberg Depression Rating Scale;

MDD, major depressive disorder;

MRI, magnetic resonance imaging;

RCT, Randomised controlled trials;

STAI, State-Train Anxiety;

TMT; Trail making test;

UPK, Universitäre Psychiatrische Kliniken;

VAS, Visual analogue scales;

VLMT, verbal learning memory task; VAS, visual analogue scale

WMS-R, Wechsler Memory Scale Revised;

Declarations

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Dissemination policy and data availability

The findings of this trial will be published in a peer-reviewed scientific journals. The trial results might also be presented at scientific congresses. Data will be available for the study personnel and can be analysed with authorization from the PI (AS). No publication or communication involving the results of the study is authorized without prior written consent from the PI. No unpublished data may be transmitted to a third party without prior written approval by the PI. No use of professional writers is intended.

Consent for publication

Not applicable.

Authors' contribution

All authors confirmed that they have read and approved the final manuscript. NS: contribute to patient recruitment and data collection, manuscript preparation; CK: contribute to patient recruitment, data collection and management; LM: contributed to the study protocol; SB: performed randomisation; CB: contributed to study design; SB: contributed to study design; UEL:

Sponsor, contributed to study design; AS: Principal Investigator, contributed to study design and protocol, overall study supervision, will lead data analysis and dissemination activities.

Ethics approval and consent to participate

The study has been approved by the IEC (EKNZ: Ethikkommission Nordwest- und Zentralschweiz, project ID: 2016-01608, protocol version 3, 23rd November 2016) on 23rd January 2017. Informed consent will be obtained from all study participants.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Study aims and outcomes.

Objectives	Aims	Outcomes	Measures
Primary	Testing the effect of probiotic supplementation on depressive symptoms	Depressive symptoms	HADRS [62]
Secondary	Exploring the effect of probiotic supplementation on brain-gut interactions.	Immune signaling and inflammatory processes	Blood levels of macrophage migration inhibitory factor and interleukin 1 beta
		HPA axis responses	Salivary cortisol awakening responses.
		Neurogenesis	Blood levels of BDNF
		Release of appetite-regulating hormones	Blood levels of ghrelin and leptin
		Gut microbiota composition	Metabolic profiling of fecal samples
		Brain perfusion, structure and activation	ASL, structural and functional MRI, DTI
		Cognitive functioning	TMT, WMS-R, VLMT
		Subjective feelings of satiety, hunger, fullness and food craving	VAS
		State anxiety	STAI [45]
		Depressive symptoms	BDI [46], MADRS [63]
		Physical activity	IPAQ [47] and actigraph
		Sleep quality	EEG, ISI [48]
Safety	Assessing adverse events and side effects after probiotic supplementation	Adverse events and incidence/severity of gastrointestinal side effects	CRF, GSRS [49]

ASL, Arterial spin labeling; BDI, Beck Depression Inventory; BDNF, brain-derived neurotrophic factor; CRF, Case report form; DTI, Diffusion Tensor Imaging; EEG, Electroencephalography; GSRS, Gastrointestinal Symptom Rating Scale; HADRS, Hamilton Depression Scale; HPA, Hypothalamic-pituitary-adrenal; IPAQ, International Physical Activity Questionnaire; ISI, Insomnia Severity Index; MADRS, Montgomery-Åsberg Depression Rating Scale; STAI, State-Trait Anxiety Inventory; TMT; Trail making test; VAS, Visual analogue scales; VLMT, Verbal Learning and Memory Test; WMS-R, Wechsler Memory Scale-Revised.

Table 2. Assessment schedule.

	Study period							
	Enrolment/Allocation	Post-allocation						Follow-up
		Pre-intervention	Intervention				Post-intervention	
Timepoint	day 1	week 1	week 2	week 3	week 4	week 5	week 6	week 9
Enrolment:								
Eligibility screen	X							
Informed consent	X							
Allocation	X							
Interventions:								
Probiotic / Placebo			X	X	X	X		
Assessments:								
BMI		X	X	X	X	X	X	X
Clinical assessments (HADRS, BDI, MADRS, STAI)		X	X	X	X	X	X	X
Saliva sampling		X					X	
Blood collection		X					X	X
Neuroimaging (ASL, sMRI, DTI, fMRI)		X					X	
Physical activity (actigraph)			X	X	X	X		
Physical activity (IPAQ)		X					X	
Stool collection		X					X	X
Sleep quality (EEG, ISI)		X					X	
Cognitive assessments (TMT, WMS-R, VLMT)		X					X	
VAS (satiety, hunger, fullness, craving)		X	X	X	X	X	X	
Adverse events		X	X	X	X	X	X	X
Gastrointestinal side effects (GSRS)		X	X	X	X	X	X	X

ASL, Arterial Spin Labeling; HADRS, Hamilton Depression Scale; BDI, Beck Depression Inventory; MADRS, Montgomery-Åsberg Depression Rating Scale; STAI, State-Train Anxiety; sMRI, structural Magnetic Resonance Imaging; DTI, Diffusion Tensor Imaging; fMRI, functional Magnetic Resonance Imaging; IPAQ, International Physical Activity Questionnaire; EEG, Electroencephalography; ISI, Insomnia Severity Index; TMT, Trail Making Test; WMS-R, Wechsler Memory Scale Revised; VLMT, verbal learning memory task; VAS, visual analogue scale

Figures

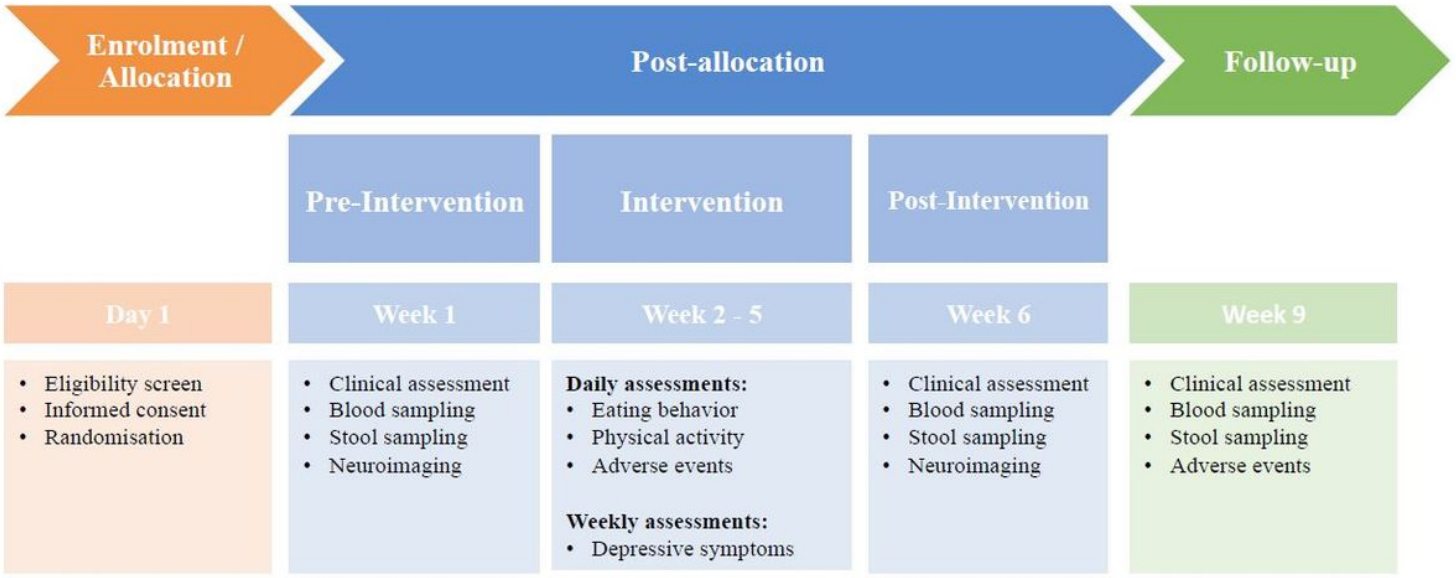

Figure 1

Course of the study.

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