**Research Protocol**

***A feasibility study in the use of probiotics as an adjuvant in the treatment of Major Depressive Disorder***

**Study Investigators:**

1.Principal Investigator: Dr. Frederick Sundram

2.Co-Investigator: Dr. Venkat S Krishnamurthy Naga

1. Senior Lecturer and Consultant Liaison Psychiatrist, Dept of Psychological Medicine, University of Auckland and Waitemata DHB
2. Consultant Psychiatrist, Waitemata DHB and Dept of Psychological Medicine, University of Auckland

**Introduction:**

Major depressive disorder (MDD) is a psychiatric disorder characterised by persistent low mood or anhedonia, among other symptoms, for at least two weeks1. MDD is a particularly challenging disorder to treat, with approximately 30% of all patients responding ineffectively to available treatments2, and more than 50% of patients failing to reach remission after the initial treatment phase3.

Within New Zealand, there is a substantial burden of disease from neuropsychiatric disorders; according to the New Zealand Health Survey (2019), at least 15.7% of all adults are formally diagnosed with depression at some point during their lives4. Neuropsychiatric conditions also constitute the most significant individual health loss amongst New Zealand youth and young adults, accounting for 35% and 31% (respectively) of all health lost in Disability Adjusted life years42 (DALY) within this population5. Previous studies have additionally implicated MDD as the single most common mood disorder in New Zealand - at a rate of around 5.7%6. The relative prevalence of MDD in New Zealand, combined with the unique challenges (presented by this particularly difficult disorder3), indicates that innovation in addressing MDD is especially crucial for the New Zealand health care system.

Globally, MDD has increased significantly in the last two decades, yet our possible treatment options remain limited. Like most psychiatric disorders, MDD has multifactorial origins. Hitherto, our understanding of the role of the Gut in MDD was limited to healthy eating and bowel motion. Recent advances have led to a better understanding of our gut microbiota and their significant involvement in health and illness19.

Probiotics are defined as "living organisms which confer health benefits" as per the World Health Organisation (WHO)30. Numerous studies have looked at the effect of probiotics in anxiety and depressive states with fewer studies focused on MDD. Recent systematic reviews and meta-analyses 13,19,20,21,22 in this area of the study shows the possible beneficial effect of using probiotics in this patient group.

Based on the published literature and information available in the public domain, this will be the first research trial in New Zealand that will study the use of probiotics as a treatment option for depression in the adult population. Although probiotics are widely available on the market, they are not funded for the treatment of depression due to insufficient evidence-based research in this area. The current proposed study may help enhance the evidence-base.

Patients with MDD have reliably demonstrated raised inflammatory markers, e.g., cytokine levels compared to healthy controls35, indicating some type of inflammatory mechanism is at work in depressed patients. However, there is little evidence to suggest that inflammation aids in the clinical progression of MDD34.

Over recent decades, several studies have shown the association of inflammatory markers, especially interleukins such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β)  with MDD26. Recent studies, including the large NESDA study25 from the Netherlands, show that IL-6 and other inflammatory markers are associated with MDD severity/chronicity and treatment response. Although antidepressants (mainly SSRIs) decrease the level of cytokines, it is not consistent with other medications, and more chronic and resistant patients appear to have less of this response27,28.

Recent research in the study of the gut microbiota's role in MDD has shown correlations between dysbiosis (alteration in the gut microbiota that is associated with illness) and depression and anxiety disorders 7,8,11,12. Dysbiosis can be addressed by using multiple approaches, but the most plausible in humans has been by adding probiotics. Probiotics have been studied as a treatment option with acute gastroenteritis, inflammatory bowel diseases, allergic disease more than ten years ago32.But only in the last few years, there has been a renewed interest in understanding the role of probiotics in neuropsychiatric disorders, including depression14,15. Animal studies have shown the various pathways in which gut microbiota affects our brain, including immune, humoral, and neural pathways14. When there is dysbiosis in the Gut, it causes 'leaky gut' syndrome, which can lead to stimulation of various inflammatory pathways leading to brain dysfunction15,16. In animal studies29,  IL-6 blockage peripherally appears to have antidepressant effects. It does not show similar results when it is centrally mediated, explaining the role of the gut microbiome in inflammatory pathways.

**Aim:**

The primary aim of this work is to study the feasibility of probiotics as an adjuvant treatment for mild to moderate MDD in a community setting in New Zealand. The study will also explore whether serum levels of peripheral inflammatory markers mediate any possible beneficial effect arising from the above. The study will also plan to understand people's food and activity levels.

**Hypothesis**:

People with mild to moderate MDD would have a better response to adjuvant probiotics when treated for at least eight weeks. There will be a pattern of change in inflammatory marker levels as MDD improves ((Interleukin 1β and 6) will show a negative correlation with treatment response on probiotics).

**Secondary hypothesis:**

Food and activity levels will be explored for any possible correlation with treatment response.

**Study Design:**

Our study will be a feasibility study.However It will be double blinded randomised controlled study to reduce any possible bias during the study.

N=20

Referral from GP/other referral centre

Randomisation

Control group=10

Probiotics group =10

Initial Assessment

Psychiatric assessment for eligibility + MADRS score, AHEI for eating and baseline exercise activity

Blood test to check levels of Interleukins - 1β and 6

Weekly Visits for 8 weeks

Dispensing of Probiotics

Monitoring MADRS Score, and exercise activity

End of 8th week

Repeat scores of MADRS, AHEI and Physical activity

Repeat Blood Tests for Interleukins - 1β and 6

**Eligibility Criteria:**

**Inclusion criteria**

* Adults aged between 18 and 65 years
* Living in the community
* English speaking
* Able to give informed consent
* Currently experiencing an episode of MDD of mild to moderate\* severity based on the Diagnostic and statistical manual of mental disorders (5th ed.)43criteria.
* Attending an existing health care provider

\* *Mild: Few,If any, symptoms in excess of those required to make the diagnosis are present,the intensity of the symptoms is distressing but manageable,an the symptoms result in minor impairment in social or occupational functioning.*

*Moderate: The number of symptoms,intensity of symptoms,and/orfunctionalimpairment are between those specified for ‘mild’and “severe”.*

*Severe: The number of symptoms is substantially in excess of that required to make the diagnosis,the intensity of the symptoms is seriously distressing and unmanageable,and the symptoms markedly interfere with social and occupational functioning.*

**Exclusion criteria:**

* Illnesses that contribute to immunosuppression or inflammatory conditions
* People who take immunosuppressants
* Currently suffering from an infection and have been taking antibiotics
* On regular medications other than antidepressants especially those which affect the gut microbiota (e.g., Omeprazole, over the counter probiotics) or anti-inflammatory medications like NSAID’s.
* Acutely suicidal or at high risk of harming others or properties
* Currently diagnosed with Schizophrenia, Schizoaffective disorder or eating disorder
* Have been diagnosed with Bipolar Affective disorder in the past
* Any form of substance abuse or dependence in the last two years.
* Significant comorbid physical illnesses, e.g., autoimmune disorders, inflammatory bowel conditions, traumatic brain injury, neurological disorders

**Primary outcome:**

Change in the MADRS score of individuals taking probiotics with antidepressants in comparison to the control group. The control group will be receiving an antidepressant alongside an inert placebo—a relationship between peripheral inflammatory markers and probiotics as an adjuvant treatment.

**Secondary outcome:**

The study will help to understand any possible correlation between food and exercise activity and the improvement of MADRS scores.

**Study setting:**

At the University of Auckland, Clinical Research Centre, clinic rooms will be booked to see participants in a private environment. A clinical room in the same centre will be used to draw blood for the measurement of inflammatory markers**.**

**Intervention:**

A scientifically validated probiotic called Winclove EcologicR Probiotics will be used as the primary intervention. This probiotic is a multi-strain,human species probiotic (Lactobacillus casei,L.acidophilus,L.brevis, L.lactis, L.salivarius and Bifidobacterium bifidum,B.lactis). it contains 5billion colony forming units(CFU) . Winclove Probiotics have also been utilized as the probiotic intervention of choice in similar studies conducted overseas39.

This probiotic comes in powder form and remains stable at room temperature. Probiotic powders will be placed in identical sachets to the placebo. It will not be possible for participants to distinguish probiotics from placebo as they will be deidentified. Participants will be requested to mix it with water/fluid and ingest it once a day.

**Placebo:**

Maltodextrose powder will be used as a placebo. The powder will be placed in identical sachets to the probiotics, and they will be deidentified after buying it in bulk.

**Measurement tools used:**

1. MADRS will be used to measure the symptoms of MDD40. It has 10 rating scale questions.

The MADRS scale is a validated36 scale to measure the severity of depressive symptoms in patients who are taking antidepressants.

2. Australian Healthy Eating Index (AHEI) to measure the eating habits of participants16

Scoring criteria for the AHEI-2013 consist of 11 components based on different aspects of a healthy diet. These components include the five core food groups, that is, vegetables, fruits, grain (cereal) foods, milk and milk alternatives, and meat and protein food alternatives. The components also include discretionary foods high in saturated fat and/or added sugars, added salt, or alcohol that does not fit into the five core food groups, and should be limited because they are unnecessary for a healthy diet. Each component measures the degree to which a person's diet conforms to the serving recommendations for each food group.16

3. The participant's activity levels will be noted using a self-reported simple questionnaire and rated based on the below Ministry of Health’s guidelines for adults, as to whether they are physically active or at present have limited activities level.

Physically active people are defined as someone who will do at least 2 ½ hours of moderate or 1 ¼ hours of vigorous physical activity spread throughout the week.37

* Moderate intensity is defined as an activity that causes a slight but noticeable increase in breath and heart rate. You can still carry on a conversation.
* Vigorous-intensity is an activity that makes you out of breath – you can't do these activities and chat at the same time.37

**Cultural considerations/Maori consultation:**

We will obtain the ethnicity data using the Ministry of Health guidelines. There will be consultation with the Maori responsiveness team at the University of Auckland to ensure that our research will be beneficial to the Maori community. It is recognised that mental health disorders, including MDD, are approximately 1.5 – 2 times more prevalent in the adult Maori community. We will endeavour to keep our research responsive to the health inequities that exist for Maori.

We are planning to engage with Maori health providers in our area to recruit participants. We hope that this will help in appropriate sampling and reduce inequities in our recruitment strategies.

We will also explicitly mention and obtain consent from participants about our study involving the withdrawal of blood and our plan to store it safely in university of Auckland and use it for the measurement of interleukin 1β and 6. We will be discarding the blood material left over after it is used for measuring the above inflammatory markers.

**Participant recruitment:**

The recruitment strategy involves placing information leaflets in all the primary care centres that allow us to do so in the greater Auckland region as well as placing advertisements in social media, local universities notice boards, and local hospitals notice boards. In order to reduce inequity in the sample, we will put our information leaflets in our local Maori health providers who might be involved in seeing patients with depression-like Kereru psychotherapy and counseling services limited (after duly obtaining their consent). Once a participant contacts the study team, we will obtain their name and their contact details. We will then send them our Participant information sheet and request them to contact us, if they are still interested in participating in our study. When they contact us,their demographic information, including age, sex, address, email address, and their primary care provider details, will be obtained. We will collect ethnicity information using the standard ministry of health format. We will also organise a time to see them for an initial assessment. During the initial assessment session, the participant information sheet will be given again, and the research proposal will be summarised in layman’s terms before getting signed consent. We will specifically inform them about our plan to withdraw blood, store them, for interleukin assay. All study participants will be given a $50 supermarket voucher at the end of the study.

**Participant withdrawal:**

Participants who withdraw from the study are encouraged to advise the principal study investigator of the reason(s) for discontinuing. They will be notified that they have the right to withdraw from the study up to three months after participation without explaining. This is to allow adequate time before data is de-identified and added to the final sample for analysis. Any reasons for withdrawal from the study will be noted.

**Safeguarding data:**

All data related to this study will be stored securely in the Department of Psychological Medicine at the University of Auckland. Electronic data will be stored in password-protected folders within the university managed storage during collection and analysis with files identified by participant number with no personal identifying details. All data will be kept for a maximum of 10 years.

Unique participant numbers will be used to identify them, and their name and address will be de-identified unless they have opted for receiving the results of the overall study. However, individual results of participants will not be shared. All the blood samples drawn from participants will be de-identified using their unique identification number. These unique numbers will be used to share their information in any future publications or dissemination of the research data.

**Participants safety:**

Participants of the study have MDD and are considered a vulnerable population. If there is any deterioration in any participant’s mental state during the study, they will be redirected to their local Community Mental Health Centre's acute crisis team. If there is a critical emergency during their assessment visits to the Clinical Research Centre, Auckland District Health Board's Emergency mental health crisis line will be contacted. In the case of an unlikely emergency arising during venepuncture for drawing blood samples, an ambulance will be called, and the participant will be brought to the Emergency Department at Auckland City Hospital. A physical health assessment in the Emergency Department for the participant will be coordinated.

Probiotics are an over-the-counter health supplement and it does not cause major side effects to any health participants apart from a bloating sensation.

**Ethical considerations:**

Ethical approval will be obtained from the Health and Disability Ethics Committee. During the eight weeks of research, participants’ mental state will be monitored weekly. If during the study that participants' mental health deteriorates, relevant health care providers (based on their demographics) will be contacted, and their participation in our trial will be reviewed. As mentioned above, all the participants' data will be de-identified throughout the process. Throughout the process, participants can withdraw their consent anytime. Participants in the control arm of the study will receive the current evidence-based treatment for MDD from their primary care provider, and the research team will not be altering their currently prescribed treatment.

**Study procedure:**

1. There will be the recruitment of 20 participants with mild to moderate MDD who have presented to primary care, private psychologists, Maori health providers, and counsellors. Research study information leaflets will be placed on social media and in primary care liaison services of secondary care services. Research leaflets will be placed in various GP practices and GP education meetings, which happen throughout the Auckland Metro region to disseminate information about the study.
2. The study involves competent adult participants between ages 18-65 who have presented to their health care provider with MDD of mild to moderate severity, who have started antidepressant treatment. Participants will be given a participant information sheet and consent forms through email after they get back to us after reading our leaflets. A screening session will be booked with the participants.
3. We will inform them that our study involves withdrawing 5ml of blood. We will inform them that blood withdrawn will be stored safely in the University, and it will not be used for any other tests, including genetic analysis. We will only use the blood tissue for assessing inflammatory markers called interleukins. After obtaining the blood sample of the 20 participants, all the samples will be stored and used for assessing the interleukin levels in the school of pharmacy at University of Auckland.
4. The screening session will be performed by a mental health professional, e.g., a Psychiatrist or psychiatric nurse who will confirm the MDD diagnosis and its severity by a comprehensive psychiatric assessment including clarification of eligibility criteria and use the Montgomery-Asberg Depression Rating Scale (MADRS) to rate their depression severity.
5. After a comprehensive psychiatric assessment, if the participant is eligible for the study, they will be allocated to the Control group or the probiotic group based on a random number generator.
6. Participants will complete questionnaires to clarify their eating habits (Australian Healthy Eating Index (AHEI) for adults) and a rating scale for the level of activities they undertake in a week based on the Ministry of Health guidelines.
7. They will be giving their blood sample to study the baseline measure of Interleukin 6 (IL 6) and Interleukin 1β. Co-ordinating investigator will be collecting the blood sample from the patient.
8. Based on randomisation, the Principal Investigator will give the relevant sachets to the co-Investigator to be dispensed to the participants for the next eight weeks.
9. The Principal investigator will not be blinded to the interventions provided, but the co-investigator doing the above rating scales during screening and monitoring sessions will be blinded.
10. The co-investigator will give participants a powder sachet to be taken every day for a week, and the co-investigator will see them every week to collect the empty sachets and do the rating scales ( MADRS,AHEI for adults and level of activities).
11. At the end of eight weeks, another blood test will be taken, blood samples will be stored, and they will be analysed in a batch using CBA kits, for measuring inflammatory markers Interleukin 1β and Interleukin 6 with school of pharmacy at Universtiy of Auckland .
12. If there are any concerns about a participant's physical or mental health during the study, their GP and relevant crisis team numbers of their locality will be provided to the participant or the Emergency Department at Auckland City Hospital will be contacted by the research team and their participation in the study will be reviewed.

**Statistical Analysis:**

The MADRS, which will be done every week, has various levels for the severity of the depression. For a participant to reach remission of MDD, he/she should have scored less than or equal to ten38.

The proportion of participants who reach remission in both the control and probiotics groups will be examined along with the number of individuals whose pro-inflammatory marker levels decrease between pre and post-study for both the groups will be assessed. A summary of the various demographic factors, including gender, age, and the participants' healthy eating index scores, activity levels scores, and the interleukin levels of the participants, will be assessed.

Given the limited sample size, there will not be enough statistical power for the study; nevertheless, if this feasibility study does show improvement on probiotics, there are plans to do a larger trial.

**Outcome and Significance:**

This study will be the first in New Zealand to look at the effect of Probiotics on MDD. Most of the previous studies in this area have not explored the confounding factors of food and exercise, and only half of the previous studies could show any correlation between inflammatory markers and response to probiotics.

Factors including polypharmacy, food, activity levels influencing Gut microbiome's ecology are often not routinely assessed. Therefore, this study to be done in the community among relatively healthy individuals will concentrate on those factors and will help in understanding the role of these factors better and help with designing a larger study in the same area for the near future.

**Dissemination of findings:**

After completion of the study, the plan is to publish in a peer-reviewed journal and present at various professional mental health forums and conferences as well as use the findings to apply for funding from various funding bodies for a larger study that will adopt a randomised controlled trial design.

**Funding:**

Funding for the research project is from the Oakley Mental Health Research Foundation.

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| --- | --- |
| Items | Cost Estimate in NZD |
| Printing information sheets regarding research for dissemination | 100 |
| Patient information sheet for research | 100 |
| Getting Locality approval from relevant health practice's | Nil |
| Patient participation gift | 1000 |
| Probiotics | 1600 |
| Placebo Powder | 11 |
| Interleukin testing | 5000 |

**Timelines and milestones:**

Current COVID -19 related lockdown has affected our timelines for the study.

2019

Literature review and making funding application

Funding approved for a feasibility study in October.

Jan-Jun 2020

Summer student did systematic review of the literature

HDEC ethics application and Research protocol drafted

June-December 2020

Distributing the research leaflets in relevant centres

Start the study and store blood samples in the University

December- March 2020-21

Collate all the results and analysis of the results

March-June 2021

Research protocol and Funding applicated drafting for a larger study

**In 2019**,

After going through a preliminary round of literature review in the area of utilizing probiotics as an adjuvant treatment in MDD, a funding application was made to the Oakley mental health research foundation, and successful funding was obtained at the end of October.

After the funding approval came through, a systematic review of the literature in the area of probiotics, inflammation, and MDD was carried out to help confirm the research approach via a University of Auckland Summer research studentship project.

**January to June 2020:**

Work on the ethics approval, create a Participant information sheet, pamphlet, and research protocol prior to starting participant recruitment. Due to the COVID 19 situation, this part of the research has been delayed.

**August 2020-December, 2020:**

Participant recruitment, study procedure is anticipated to be conducted during this time.

**January 2021-June, 2021**:

We will be analysing the results and potentially presenting findings at local mental health conferences. This is a feasibility study, and there is a plan to do extensive investigation for a larger population if there are positive treatment findings or correlations found in this study. A larger study will help us understand whether there is any robust correlation between healthy eating and activity with a positive response to probiotics. Funding applications for the larger study will be done during this time period.

**Reference:**

1. National Institute of Mental Health. (2018). Depression. National Institute of Mental Health. <https://www.nimh.nih.gov/health/topics/depression/index.shtml>

2. Souery, D., Amsterdam, J., de Montigny C., Lecrubier, Y., Montgomery, S., Lipp,O., Racagni, G., Zohar, J., Mendlewicz, J. (1999). Treatment-resistant depression: methodological overview and operational criteria. European Neuropsychopharmacology, 9(1-2), 83-91.

3. Mrazek, D. A., Hornberger, J. C., Altar, A., Degtiar, I. (2014). A Review of the Clinical, Economic, and Societal Burden of Treatment-Resistant Depression:1996–2013. Psychiatric Services, 65(8), 977-987.

4. Ministry of Health. (2019). Annual Data Explorer 2018/19: New Zealand Health Survey [Data File]. URL: <https://minhealthnz.shinyapps.io/nz-health-survey-2018-19-annual-data-explorer/>

5. Ministry of Health. (2016). Health Loss in New Zealand 1990–2013: A report

from the New Zealand Burden of Diseases, Injuries and Risk Factors Study.Wellington: Ministry of Health.

<https://www.health.govt.nz/publication/healthloss-> new-zealand-1990-2013

6. Wells, J. E. (2006). Twelve-month prevalence. In Oakley Browne, M. A., Wells, J.

E., Scott, K. M. (eds). Te Rau Hinengaro: The New Zealand Mental Health Survey, 37-56. Wellington: Ministry of Health.

7. Bercik, P., Collins, S. M., & Verdu, E. F. (2012). Microbes and the gut‐brain axis. Neurogastroenterology & Motility, 24(5), 405-413

8. Mayer, E. A., Tillisch, K., & Gupta, A. (2015). Gut/brain axis and the microbiota. The Journal of clinical investigation, 125(3), 926-938.

9. Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., ... & Li, L. (2015). Altered fecal microbiota composition in patients with major depressive disorder. Brain, behavior, and immunity, 48, 186-194.

10. Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E. F., Wang, J., Tito, R. Y., ... & Claes, S. (2019). The neuroactive potential of the human gut microbiota in quality of life and depression. Nature microbiology, 1.

11. Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linløkken, A., Wilson, R., & Rudi, K. (2014). Correlation between the human fecal microbiota and depression. Neurogastroenterology & Motility, 26(8), 1155-1162.

12. Wallace, C. J., & Milev, R. (2017). The effects of probiotics on depressive symptoms in humans: a systematic review. Annals of general psychiatry, 16(1), 14.

13. Chrobak, A. A., Nowakowski, J., & Dudek, D. (2016). Interactions between the gut microbiome and the central nervous system and their role in schizophrenia, bipolar disorder and depression. Archives of Psychiatry and Psychotherapy, 2, 5-11.

14. Park, C., Brietzke, E., Rosenblat, J. D., Musial, N., Zuckerman, H., Ragguett, R. M., ... & McIntyre, R. S. (2018). Probiotics for the treatment of depressive symptoms: An anti-inflammatory mechanism?. Brain, behavior, and immunity.

15. O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behavioural brain research, 277, 32-48.

16. Roy, R., Hebden, L., Rangan, A., & Allman-Farinelli, M. (2016). The development, application, and validation of a healthy eating index for Australian adults (HEIFA—2013). Nutrition, 32(4), 432-440.

17. Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. Nature, 489(7415), 220.

18.Ng, Q. X., Peters, C., Ho, C. Y. X., Lim, D. Y., & Yeo, W. S. (2018). A meta-analysis of the use of probiotics to alleviate depressive symptoms. Journal of affective disorders, 228, 13-19

19.Huang, R., Wang, K., & Hu, J. (2016). Effect of probiotics on depression: a systematic review and meta-analysis of randomized controlled trials. Nutrients, 8(8), 483.

20.Liu, R. T., Walsh, R. F., & Sheehan, A. E. (2019). Prebiotics and probiotics for depression and anxiety: a systematic review and meta-analysis of controlled clinical trials. Neuroscience & Biobehavioral Reviews

21.Nikolova, V., Zaidi, S. Y., Young, A. H., Cleare, A. J., & Stone, J. M. (2019). Gut feeling: randomized controlled trials of probiotics for the treatment of clinical depression: Systematic review and meta-analysis. Therapeutic advances in psychopharmacology, 9, 2045125319859963.

22.Cheung, S., Goldenthal, A. R., Uhlemann, A. C., Mann, J. J., Miller, J. M., & Sublette, M. E. (2019). Systematic review of gut microbiota and major depression. Frontiers in psychiatry, 10, 34.

23.Chahwan, B., Kwan, S., Isik, A., van Hemert, S., Burke, C., & Roberts, L. (2019). Gut feelings: A randomised, triple-blind, placebo-controlled trial of probiotics for depressive symptoms. Journal of affective disorders, 253, 317-326.

24.Lamers, F., Milaneschi, Y., Smit, J. H., Schoevers, R. A., Wittenberg, G., & Penninx, B. W. (2019). Longitudinal association between depression and inflammatory markers: Results from the Netherlands Study of Depression and Anxiety. Biological psychiatry, 85(10), 829-837

25. Chu, A. L., Stochl, J., Lewis, G., Zammit, S., Jones, P. B., & Khandaker, G. M. (2019). Longitudinal association between inflammatory markers and specific symptoms of depression in a prospective birth cohort. Brain, behavior, and immunity, 76, 74-81.

26.Liu, J. J., Wei, Y. B., Strawbridge, R., Bao, Y., Chang, S., Shi, L., ... & Lu, L. (2019). Peripheral cytokine levels and response to antidepressant treatment in depression: a systematic review and meta-analysis. Molecular psychiatry.

27.Hannestad, J., DellaGioia, N., & Bloch, M. (2011). The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. Neuropsychopharmacology, 36(12), 2452

28.Zhang, J. C., Yao, W., Dong, C., Yang, C., Ren, Q., Ma, M., & Hashimoto, K. (2017). Blockade of interleukin-6 receptor in the periphery promotes rapid and sustained antidepressant actions: a possible role of Gut–microbiota–brain axis. Translational psychiatry, 7(5), e1138.

29.FAO/WHO. (2001). Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report.

30.Dianda, L., Hanby, A. M., Wright, N. A., Sebesteny, A., Hayday, A. C., & Owen, M. J. (1997). T cell receptor-alpha beta-deficient mice fail to develop colitis in the absence of a microbial environment. The American journal of pathology, 150(1), 91.

31.Isolauri, E., Kirjavainen, P. V., & Salminen, S. (2002). Probiotics: a role in the treatment of intestinal infection and inflammation?. Gut, 50(suppl 3), iii54-iii59.

32. Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J. A., & Colzato, L. S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, behavior, and immunity*, *48*, 258-264.

33.Wells, J. E. (2006). Twelve-month prevalence. In Oakley Browne, M. A., Wells, J.E., Scott, K. M. (eds). Te Rau Hinengaro: The New Zealand Mental Health Survey, 37-56. Wellington: Ministry of Health.

34. Verduijn, J., Milaneschi, Y., Schoevers, R. A., van Hemert, A. M., Beekman, A. T.F., Penninx, B. W. J. H. (2015). Pathophysiology of major depressive disorder: mechanisms involved in etiology are not associated with clinical progression. Translational Psychiatry, 5, 649-657. doi:10.1038/tp.2015.137.

35. Kohler, C. A., Freitas, T. H., Maes, M., de Andrade, N. Q., Liu, C. S., Fernandes,B. S., Stubbs, B., Solmi, M., Veronese, N., Herrmann, N., Raison, C. L., Miller, B.J., Lanctot, K. L., Carvalho, A. F. (2017). Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. Acta Psychiatrica Scandinavica, 135(5), 373-387.

36. Galinowski, A., & Lehert, P. (1995). Structural validity of MADRS during antidepressant treatment. *International Clinical Psychopharmacology, 10*(3), 157–161. [https://doi.org/10.1097/00004850-199510030-00004](https://psycnet.apa.org/doi/10.1097/00004850-199510030-00004)

37. <https://www.health.govt.nz/your-health/healthy-living/food-activity-and-sleep/physical-activity/how-much-activity-recommended>

38. Zimmerman, M., Posternak, M. A., & Chelminski, I. (2004). Derivation of a definition of remission on the Montgomery–Asberg depression rating scale corresponding to the definition of remission on the Hamilton rating scale for depression. *Journal of psychiatric research*, *38*(6), 577-582.

39. Chahwan, B., Kwan, S., Isik, A., van Hemert, S., Burke, C., &amp; Roberts, L. (2019). Gut feelings: A randomized, triple-blind, placebo-controlled trial of probiotics for depressive symptoms. Journal of affective disorders, 253, 317-326.

40. Montgomery, S. A., & Asberg, M. (1979). A new depression scale designed to be sensitive to change. *The British journal of psychiatry: the journal of mental science*, *134*, 382–389. <https://doi.org/10.1192/bjp.134.4.382>.

42. World Health Organisation. Health Statistics and information systems. Metrics: Disability- Adjusted Life Years. <https://www.who.int/healthinfo/global_burden_disease/metrics_daly/en/>

43. American Psychiatric Association. (2013). Depressive Disorders. In Diagnostic and statistical manual of mental disorders (5th ed.). <https://doi.org/10.1176/appi.books.9780890425596.dsm04>