Gut Feelings: Is there a Correlation between Microdiversity and Emotion Regulation, and is Sex a moderating variable?

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Abstract

Background: This study on gut feelings investigates the correlation between microdiversity in the gut and cognitive reactivity in the brain with sex as a potential moderator. Research on potential correlations and moderators is relevant to gain further insight into the networks involved in the gut-brain axis. The findings can influence treatment approaches in the field of clinical psychology to a greater focus on the gut-brain axis. Two hypotheses were examined: 1) a higher microdiversity in the gut is correlated with less emotion regulation difficulties, and 2) there are sex differences in the correlation between microdiversity and emotion regulation.

Methods: This research was an observational between-subjects design with a total number of 75 participants. The primary outcome was the alpha score, assessed with the Shannon Index, which gives insight into individual microdiversity. The Difficulties in Emotion Regulation Scale was used to assess self-reported difficulties in emotion regulation to have insight into individual cognitive reactivity. Sex was conceptualised as the moderator variable. A correlational analysis for alpha scores and DERS scores was carried out, followed by a multiple regression analysis, which tested for a moderation with the variable sex.

Results: There was no evidence for a negative correlation between high microdiversity and few emotion regulation difficulties. Sex did not have a significant moderation on this correlation either.

Implications: Further research is needed on the gut-brain axis and sex differences in gut microdiversity and emotion regulation to adapt the treatment of stress-related disorders such as post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), or other anxiety-related disorders from a cognitive- to a more holistic approach.

Keywords

Gut-brain axis (GBA), microdiversity, emotion regulation (ER), sex

Gut Feelings

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1. Introduction

Gut feelings is a term not only well known in the English language; there is even ancient evidence reaching back to Hippocrates of Kos (as cited in Valero-Cases *et al.*, 2020) mentioning that all disease starts in the gut. Phrases like "tell me what you eat, I tell you who you are" (Brillat-Savarin, 1842, p. 9 as cited in Sipe, 2009) or "having butterflies in the stomach" (Converse, 1917) imply that there is a universal understanding - established in common phrases - of some sort of connection between cognition and physiology. The assumption that emotions originate from limbic structures in the brain, where emotional processing occurs (LaBar & Cabeza, 2006), and that these emotions can be felt in the gut through biochemical and electrical reactions, has led to the ever-growing interest in researching this so-called microbiota-gut-brain axis (GBA) scientifically. So far it is known that the axis enables bidirectional communication between gut bacteria and the brain via neural, endocrine and immune pathways (Grossman, 1979; Grenham *et al.*, 2011; Mayer, 2011; Mayer *et al.*, 2014). This communication has a regulating role in maintaining homeostasis of the gastrointestinal, central nervous and microbial systems - the gut microbiota (Zarrinpar *et al.*, 2018; Hutter *et al.*, 2015) go so far as to write "being human is a gut feeling", reasoned by the fact that most human cells are bacterial cells. So how can gut feelings - a network of connections involving multiple biological systems - be researched in the field of clinical psychology?

Microdiversity - the amount of microorganisms - in the gut is insightful to study in regards to the GBA because it covers one part of the axis. For instance, a rich gut microbiota has been empirically proven to positively influence psychological functioning, neurodevelopment and neurotransmission, learning, memory, social behaviour and the regulation of emotion (Cryan & Dinan, 2012; Sherwin *et al.*, 2017). Additionally, Rinniella *et al.* (2019) state that richer and more diverse gut microbiota is related to a better ability to withstand external threats. Disturbances like an unbalanced diet, stress, antibiotic use or

disease affect microdiversity (Rinninella *et al.*, 2019; Osadchiy, Martin, & Mayer, 2019) and can interrupt the optimal performance of the metabolism and immune functioning, which can then result in various intestinal and neurological disorders (Rinninella *et al.*, 2019).

Researching *emotion regulation (ER)* is another method to approach one part of the GBA. This is the ability to monitor, evaluate, and modify emotional experience in accordance with one's desired goals according to Gratz and Roemer (2004). Researching ER is insightful because a dysregulated ER was found to mediate the onset of depression and stress (Velotti *et al.*, 2020).

Previous research has found some moderation of sex in the GBA, specifically on the aforementioned constructs 1) ER and 2) gut microbiota. Firstly, there is evidence that males and females differ in their self-reported experience of ER: females use more differentiated emotional language but also report more frequent use of problematic ER strategies in comparison to males (Anderson *et al.*, 2016). In addition to sex differences in ER, Gardener *et al.* (2013) found a greater sensitivity towards emotionally salient stimuli in females during emotional reactivity and in the following ER process in comparison to the male group. Even though Nolen-Hoeksema (2012) concludes that vital information is missing in the literature as to how men regulate their emotions, she does state that females engage more in rumination, leading to greater depression and anxiety levels, whereas males cope more by using alcohol to regulate their emotions. Laitinen, Ek and Sovio (2002), who state that predictors of engaging in stress-related consumption vary between the sexes, have reported additional findings of sex differences in ER. The best predictor for females was concluded to be a lack of emotional support, whereas greater predictors for males were concluded to be single, divorced, from a long history of unemployment, an academic degree, and a low level of occupational education. Secondly, some animal and human studies found significant sex-related differences in the gut microbiota, too. These were found to be caused by hormones (Yurkovetskiy *et al.*, 2013), bowel function and bile acid excretion (Lampe *et al.*, 1993), bacteria (Mueller *et al.*, 2006), and microbial structure (Takagi *et al.*, 2018). Though findings regarding the moderation of sex on gut microbiota remain inconsistent, possible explanations are that most evidence stems from animal studies without replications on humans (Kim *et al.*, 2019; Yoon & Kim, 2021), and that sex differences are generally difficult to measure in humans due to various confounding variables such as diet and medication (Yoon & Kim,

2021). In conclusion, researching a potential correlation between microdiversity, ER and a potential moderation of sex gives further insight into the networks of connections involved in the GBA, also affecting the field of clinical psychology.

The more scientific evidence there is for a bidirectional influence of microdiversity and ER, the more attention can be paid to the GBA in the prevention and treatment of psychopathology. Consequently, stress-related disorders such as post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD) or other anxiety-related disorders like depression could be approached in integrative treatment focusing specifically on microdiversity and ER. In that way, this study called "gut feelings" can also be seen as research contributing to the holistic optimisation of the human organism. Regarding the scientific background, research is growing on the GBA and the potential influence of sex on this bidirectional communication between the central and the enteric nervous system.

It is the objective of this study to contribute to the field by investigating if there is a correlation between microdiversity and emotion regulation and if this is moderated by sex. To do so, stool samples were used to quantitatively investigate the *microdiversity* in the gut, the amount of microbes in the gut. In addition, the responses to the Difficulties in Emotion Regulation Scale DERS (Gratz & Roemer, 2004, see Appendix A) will be analysed for insights into an individual's ER. Based on previous findings that high microdiversity increases stress resilience (Sherwin *et al.*, 2017) the first hypothesis is that 1) there is a negative correlation between a high alpha-score in microdiversity of the stool sample and a low total DERS score. Due to findings of significant sex differences in a) gut microbiota (Yurkovetskiy *et al.*, 2013; Lampe *et al.*, 1993; Mueller *et al.*, 2006; Takagi *et al.*, 2018) and b) sex differences in ER (Nolen-Hoeksema, 2012; Bardeen *et al.*, 2013; Anderson *et al.*, 2016; Gardener *et al.*, 2013) the second hypothesis is that 2) females will show a significantly higher DERS score with the same microdiversity in comparison to males.

2. Methods

2.1. Participants

According to the power analysis prior to the research's beginning, the aim was to use data from 80 participants in total. According to the G*Power 3.1.7 programme (Faul *et al.*, 2007),

74 participants guaranteed the necessary power of .9; allowance was made for a degree of attrition by testing six more participants. Furthermore, the researchers followed the recommended alpha of .005 (Benjamin *et al.*, 2017) and expected medium effect size (r≈ .20) and a within-factor correlation of 0.3.

Exclusion criteria were the intake of supplements such as pre-and/ or probiotics and/ or antipsychotics one month before participation. The same accounts for the intake of medication targeting the immune system or gastrointestinal functioning. A history of drug abuse and/ or addiction also led to exclusion: participants were not eligible if their alcohol consumption preceded 21 units per week and/ or when there had been consumption of (a) hard drug(s) in six weeks prior to participation. In addition, changes in diet in the last three months; past therapeutic sessions (except relationship therapy), and the history or diagnoses of stress-related or (auto-) immune-related disorders lead to exclusion, too. Altogether, the study includes data of 75 participants: 53 females and 22 males. Data was excluded from five participants in total: two subjects recently (less than 12 months ago) had used antibiotics; another three participants had a delay in data transfer; one other person sent a stool sample with an insufficient amount of genetic material.

Inclusion criteria were that the self-reported healthy males and females had to be between 18 and 40 years old, whereby the average age of the sample is 24 years, as seen in table 1. Additionally, all males and females had to be proficient in the Dutch language, needed sufficient internet connection for the computerised task, and accompanied questionnaires. Participants had to be willing to collect their saliva and stool samples and to spend around two hours on the entire study. Females had to use hormone preparations for at least one month or be post-menopausal. This precondition assured comparable hormonal levels between participants. All individuals had to give their legal consent (see Appendix D) to the study procedure.

The study subjects were recruited from 1) SONA[1] - the platform of Leiden University, which manages participants; 2) personal contacts; 3) hand-out flyers in the area of Leiden and The Hague, The Netherlands; 4) the sign-up form on the study's website and 5) the social media platforms Instagram, Facebook and LinkedIn. All information stated that

[1]https://ul.sona-systems.com/default.aspx?p_return_experiment_id=3111

participation was voluntary and that withdrawal was possible at any stage of the procedure without providing a reason. As compensation for effort and time for the entire study procedure, the participants were rewarded monetarily with either 20 euros or four SONA credits. The Financial Shared Service Centre (FSSC) of Leiden University carried out the payment via bank transfer. In case of a participant's withdrawal, they received a monetary reward for the time spent on the study. Participants who completed the entire study received access to a tailor-made report[2] of their gut microbiota worth 130 euros.

A description of the individuals in the sample follows and is based on self-reports. The average height of the participants in the sample is 173.5 centimetres. The lowest reported weight is 50 kilograms and ranges up to 100 kilograms. Most participants are non-smokers (N =58) or have stopped (N =10); seven females smoke actively. 9.33% of participants were born with a caesarean section; the other 90.67% (68 participants) had vaginal childbirth. The entire sample (N =75) scores a mean value of 83 on the DERS questionnaire with a standard deviation of 19.7 as seen in table 2. The scores of the total sample and the female sample ranged from 47 to 136. Males have a smaller range of 50 to 109 in their responses and have a smaller standard deviation (*SD* = 14.01) in their responses in comparison to females (*SD* = 21.12). The alpha diversity, calculated with the Shannon Index (Appendix B), results in a mean score of 3.86 with a standard deviation of .54 in the entire sample. The alpha diversity ranges from a minimum of 2.38 to a maximum score of 4.7. The average alpha diversity score in the female group (*M* = 3.94) is slightly higher than the average male score (*M* = 3.67) and does not range as low (2.41) in comparison to the male group (2.38). Specific information about means (*M*) standard deviation (*SD*) and ranges can also be found in table 1.

[2] www.mymicrozoo.com

Table 1

Self-reported characteristics of participants, analysis of self-reported responses on the Difficulties in Emotion Regulation Scale (DERS) and the alpha score

	male (N=22)	female (N=53)	total sample (N=75)
age	*M* = 24.04, *SD* = 2.76, Range: 18-28	*M* = 24.03, *SD* = 3.7, Range: 19-40	*M* = 24.04, *SD* = 3,43, Range: 18-40
weight	*M* = 81.56, *SD* = 7.11, Range: 67.5-95	*M* = 66.98, *SD* = 12.45, Range: 50-100	*M* = 71.26, *SD* = 12.96, Range: 50-100
length	*M* = 185.0, *SD* = 6.56, Range: 175.0-198	*M* = 168.8, *SD* = 6.34, Range: 153.0-182	*M* = 173.54, *SD* = 9.78, Range: 153-198
specific device	Desktop: 20, Samsung: 2, Apple iP: 0	Desktop: 48, Samsung: 4, Apple iP: 1	Desktop: 68, Samsung: 6, Apple iP: 1
smoking	never: 17, stopped: 5, active: 0	never: 41, stopped: 5, active: 7	never: 58, stopped: 10, active: 7
childbirth	vaginal: 20, caesarean section: 2	vaginal: 48, caesarean section: 5	vaginal: 68, caesarean section: 7
appendix	removed: 0, present: 22	removed: 1, present: 52	removed: 1, present: 74
DERS score	*M* = 76.5, *SD* = 14.01, Range: 50-109	*M* = 85.84, *SD* = 21.12, Range: 47-136	*M* = 83.1, *SD* = 19.7, Range: 47-136
alpha score	*M* = 3.67, *SD* = 0.639, Range: 2.38-4.67	*M* = 3.94, *SD* = 0.49, Range: 2.41-4.7	*M* = 3.8629, *SD* = .5442, Range: 2.38-4.7

2.2. Procedure

After potential participants showed their interest, they received a digital information form of eight pages (Appendix C) and the request of preferences in date and time for the intake interview. In the following phone or video call, the researcher screened the eligibility of the interested person by asking several questions from the brief and structured Mini International Neuropsychiatric Interview (M.I.N.I.). That intake interview screens for several psychiatric disorders, substance abuse and the individual's medical history (Sheehan *et al.*, 1998). Thereby, the participant has the choice of whether to turn on the camera or leave it off entirely, to ask the remaining questions, or withdraw without providing a reason. The answers needed to be in the range of inclusion criteria. The standardised interview includes 25 closed questions, which can be answered with yes or no; usually, it does not last longer than 15 minutes. As soon as the researcher decided on eligibility, the participant and researcher planned the test day.

The test day was explained in the screening: it could take place at the Faculty of Social and Behavioural Sciences (FSW) at Leiden University in Leiden, the Netherlands or any other location convenient for the participant. The test day started with the participant signing the statement of consent for the study and continued with collecting a saliva sample of 0.1 millilitres, which was put on dry ice instantly. In addition, the laboratory MyMicroZoo provided a microbiota test kit, which was handed over during the meeting. This kit included instructions on the collection of the stool sample, a sterile cotton stab, a tube with liquid to store the stool sample safely, and a free-return envelope to send back the sample to the MyMicroZoo in Leiden. It analysed the gut microbiota based on stool samples, whereby microdiversity was calculated with the Shannon Index and resulted in the so-called alpha diversity score. It is a metric, which is used to calculate diversity when a system contains too many individuals for each to be identified and examined: $H' = -\sum_{i-1}^{R} p_i ln p_i$ (Allaby, 2018).

The participants created an account on the laboratory's website and permitted access to their data to the researchers to gain insights into individual microbiota reports. These results were available within five weeks.

After the meeting of the researcher and participant, the test day continued with the online computerised task - the emotional N-back task, more specifically called the Facial

Emotion Expression Recognition task (FERT; adapted from Harmer *et al.*, 2001). This task was used to study responses to facial expressions of anger, fear, sadness, surprise, happiness, and disgust as a function of intensity (i.e. low, moderate or high) of an emotion expressed. The website and intelligible software *Gorilla*[3] collects behavioural data with validated reaction times. It guided and monitored the participants through the online assessment of the experiment. Participants completed the online part after having responded to five questionnaires: the Depression, Anxiety and Stress Scale (DASS; Lovibond & Lovibond, 1995), Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004), LEIDS-R (Van der Does & Williams, 2003), Multidimensional Assessment of Interoceptive Awareness (MAIA; Mehling *et al.*, 2012) and the Dysfunctional Attitudes Scale-form A (DAS-A; Beck, Steer, Brown, & Weissman, 1991). It is worth mentioning that the present study only includes data relevant to the specific research question (alpha diversity score, DERS scores, and the individual declaration of sex). The active participation in the study itself never exceeded three hours.

2.3. Measures

To measure microdiversity, the stool samples are analysed for their microbiota composition. 9687 different sorts of microbes have been observed and included in the analysis. Further conclusions on high or low microdiversity are based on the alpha diversity - calculated by MyMicroZoo based on the Shannon Index (Appendix B) - because this is an estimate of species diversity. According to MyMicroZoo an alpha diversity score below three is “low”, a score higher than 6 is “high”, and a score between 4.5 and 6 is labelled “normal”. Thus, the higher the alpha score, the higher the individual microdiversity (see Appendix B).

ER was measured using the Difficulties in Emotion Regulation Scale DERS (Gratz & Roemer, 2004), based on self-reports. This questionnaire has 36 items; these are rated on a 5-point ordinal scale from 1 (“almost never [0%–10%]”) to 5 (“almost always [91%–100%]”). It measures six facets of ER: nonacceptance of negative emotions (Nonacceptance), difficulties in engaging in goal-directed behaviours when distressed (Goals), difficulties controlling impulsive behaviours when distressed (Impulse), limited access to ER strategies

[3] https://gorilla.sc/

perceived as effective (Strategies), lack of emotional awareness (Awareness), and lack of emotional clarity (Clarity). The DERS gives a total score, which ranges from a minimum of 36 to a maximum of 180. Higher scores indicate greater ER difficulties (Gratz & Roemer, 2004). The DERS has been verified and applied in numerous studies due to its high levels of internal consistency and good test-retest reliability in a range of clinical and nonclinical samples (e.g., Gratz & Tull, 2010 and Hallion *et al.*, 2018). 3) The variable sex is based on the participant's response to a question from the intake interview whether they are male or female.

2.4. Statistical Analysis

The statistical analysis was conducted using the latest version of IBM SPSS Statistics 28.0.1.1 (2022). Figure 1 illustrates the conceptual model for our analysis. The analysis consisted of a bivariate Pearson correlation and a regression analysis in SPSS. 1) A bivariate Pearson correlation analysed the hypothesised negative correlation between the alpha score and the scores of the DERS ($p=.05$). 2) A regression analysis with PROCESS was performed to measure the moderation of sex on microdiversity and ER. The alpha diversity was the independent variable X, the scores on the DERS were the dependent variable Y and the variable sex was set as the moderator M to calculate the interaction. Means, standard deviation, and bivariate correlations were calculated independently for males and females to clarify sex differences. A significance level of $p < 0.05$ was adopted for all statistical tests. The between-subjects design was analysed by comparing the data of the test subjects with each other.

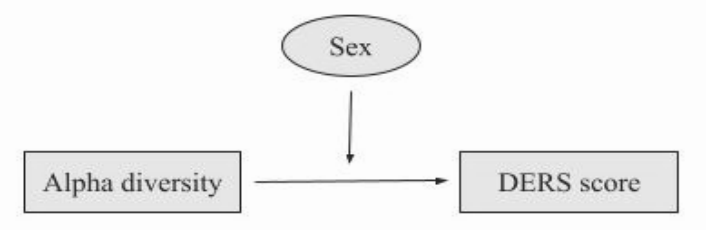

Fig. 1: The statistical analysis researches for (in-)significance in form of a negative correlation between alpha diversity (independent variable) and DERS scores (dependent variable) with sex as a potential moderator.

3. Results

3.1. Correlation Analysis of Alpha Diversity and DERS total Scores

A Pearson correlation coefficient was computed to assess the linear relationship between microdiversity and ER. The relationship between the two variables was positive, moderate in strength and statistically insignificant, r(73) = .047, p = .688.

3.2. Moderation Analysis of Sex on Alpha Diversity and DERS total Score

The moderator analysis was performed using PROCESS. It examined whether sex had a moderating effect on the correlation between Alpha Diversity (independent variable) and DERS scores (dependent variable). The overall regression model was statistically nonsignificant, R^2 = .049, F(3, 71) = 1.221, p = .309. Alpha diversity explains around 0.49 % of the variation in the DERS total score. The predictor alpha diversity on the DERS total score is insignificant, B = -.404, SE = 4.424, t(71) = -.091, p = .928, 95 % CI [-9.225, 8.417]. The predictor sex on the DERS total score is also insignificant, B = -9,097, SE = 5.152, t(71) = -1.766, p = .082, 95 % CI [-19.37, 1.17]. The interaction term is also nonsignificant, B = .831, SE = 2.181, t(71) = .381, p = .704, 95 % CI [-3.518, 5.181].

4. Discussion

This study aimed to give further insights into the GBA by investigating the relationship between microdiversity, ER and the influence of sex. Firstly, it was a-priori hypothesised that there is a negative correlation between alpha diversity and DERS scores. In other words, the higher the alpha diversity score, the lower the total score on the DERS, tested with a Pearson correlation. Secondly, it was hypothesised that sex does have a moderating effect on the previously mentioned correlation, specifically that females have a higher total score on the DERS than the male group, which was tested using linear regression analysis. Firstly, the results of the statistical analysis did not show a significant correlation between alpha diversity and the DERS total score, so the first hypothesis could not be confirmed. Secondly, there was no moderation of sex found in the relationship between microdiversity and ER, so the second hypothesis cannot be confirmed either.

4.1. Explanation of Findings

In explanation of the results concerning the total DERS score, the entire sample ranges from a minimum of 36 to a maximum of 180, whereby no one scored extremely high (<136) or extremely low (<80). The mean score in the entire sample is 83.1, which means that the total score is average: no greater difficulties in ER. The DERS scores are normally distributed. The absence of outliers can be explained by the precondition for participation: mental and physical health. In other words, a dysfunctional ER that interferes with daily life would have been an exclusion criterion. The mental health of the participants is represented by the normal distribution on the DERS. Nonetheless, even though the test subjects were tested for mental and physical health, there is still a surprising finding that females do have a greater range of reported ER difficulties in the DERS. The scores of the total sample and the female sample ranged from 47 to 136. This finding is in line with Anderson's *et al.*, (2016) research that females report problematic ER strategies more frequently compared to males. Additionally, the greater range in the female group is also in line with previous research that females reported a greater sensitivity towards emotional stimuli and a greater emotional reactivity (Gardener *et al.*, 2013). The smaller range of DERS scores in the male group can be explained by the finding of Gross and John (2003) that males are significantly more likely to engage in expressive suppression to regulate their emotions. They (Gross & John, 2003) mention this suppression can stem from "cultural indoctrination", as exemplified in phrases like "boys don't cry", as crying could be seen as a sign of weakness or unmanliness, resulting in males being less aware or expressive in their emotions.

In explanation of the results concerning the sample's mean alpha score (3.86), it is worth mentioning that MyMicroZoo defines a normal score ranging from 3,5 to 4,5 and a low alpha score is categorised as being below 3,5. This average score can be explained by the prerequisite for participation: mental and physical health. This average score represents the optimal performance of the metabolism and immune functioning without major disturbances to the microdiversity in the gut (e.g. unbalanced diet, stress and antibiotic use [Rinninella *et al.*, 2019; Osadchiy, Martin, & Mayer, 2019]). Otherwise, it is worth mentioning that the average alpha diversity of the entire sample is lower than expected. The overall alpha diversity score was expected to be higher with the

explanation that the sample mostly included acquaintances from the researchers who have high educational attainment (e.g. university). This is correlated with increased nutritional diversity and a lower caloric intake compared to subjects without qualifications, and thus a lower educational level (Azizi Fard *et al.*, 2021). One possible variable accounting for the lower alpha diversity score can be the COVID-19 pandemic during data collection, which has been shown to alter lifestyle choices like engagement in stress relief (Janssen *et al.*, 2021). There are individual differences in the tendency to increase or decrease habitual substance consumption in response to varying levels of stress (Conway *et al.*, 1981), for example by eating more and/ or drinking more alcohol (Laitinen, Ek, & Sovio, 2002). Altogether, these emotional reactivity strategies to external stimuli influence the mental and physical health of the participants, possibly explaining the overall low alpha diversity score in this study.

Even though the variable sex was identified as a non-moderator, the female group scored - unexpectedly - on average slightly higher in the alpha diversity score. This might be explained by findings like the one that females pay greater attention to their health than males do (Ek, 2015) and/ or that females reported more diet and health-related anxiety; and/ or that women were more likely than males to avoid certain foods like gluten, red meat, white flour and food additives due to perceived unhealthiness (Bärebring *et al.*, 2020). The findings of non-significance also contradict previous findings of De la Cuesta-Zuluaga (2019) that there are significant sex differences in alpha diversity.

The unexpected finding of a positive and insignificant correlation of microdiversity and ER - meaning that when the alpha diversity score increases, the DERS score increases too – needs to be explained. The correlation between microdiversity and ER difficulties has not been thoroughly researched, therefore there are no evidence-based results to compare the unrelatedness of microdiversity and ER difficulties.

One possible explanation of the overall insignificance in this study on gut feelings is chance. There might have also been a lurking variable in the study: the COVID-19 pandemic, which has proven to contribute negatively to physical and mental well-being. According to Kivelä *et al.* (2022), students reported more depressive

symptoms, academic stress and loneliness during the pandemic. Additionally, Ren *et al.* (2021) found that having been infected with Corona does change the gut microbiota in its composition. These two articles shed light on the negative consequences of the pandemic the sample might have been exposed to. Otherwise, the sample mostly had an academic background and consisted of contacts from the researchers, therefore most participants had a high SES and European background. It is to be discussed whether the results can be generalised to the general population. It is also possible that the sample was, despite the power analysis, too small.

Future research on the GBA including microdiversity and emotion regulation is recommended to increase the statistical power by increasing the sample size and/ or considering a longitudinal, between-subjects design. In that way, individual fluctuations in emotional states and microdiversity can be respected in the interpretation of data. Additionally, a between-group comparison could also shed light on the functioning of the GBA-axis. This could take the form of two experimental groups and one control group: the experimental group is being manipulated in the variable emotion regulation (e.g. by thinking of one positive memory a day) and the other one is manipulated in their gut microdiversity (e.g. adding vegetables, fruit and probiotics to the diet). The control group is only being observed in order to compare the two experimental groups for significant differences after some time (e.g. three months). Another recommendation is to continue exploring the moderation of sex on microdiversity and ER. It is worth considering whether males and females with insignificant differences in gut microdiversity might benefit from different treatment approaches for their ER (Matud, 2004). Previous research has shown that females cope more emotion-focused, and men engage more in problem-focused strategies (Matud, 2004). Sex differences can be found by between-group study designs. Lastly, it is recommended to add the variable childbirth into the analysis of future date since it has already been proven to influence microdiversity (Zhang *et al.*, 2021; Penders *et al.*, 2006). The sample of this study included seven participants, who were analysed like the other participants. Their data might needs to be reviewed differently when there are more findings on the GBA.

4.2. Limitations

This study includes several limitations such as a high level of reliance on self-reports of mental and physical health, which might include some uncontrollable subjective bias. Additionally, the data collection was conducted before, during and after the COVID-19 pandemic (WHO, 2020). To continue with the limitations, the analyses of microdiversity in stool samples might have been exposed to different conditions and analysis strategies in different laboratories, so there might be varying results in replication studies. Finally yet importantly, participants used different devices to finish the online part of the experiment. There might have been differences regarding the displayed design, which accounts for a bias the researchers cannot control.

4.3. Application

Even though findings were insignificant, future research is recommended to continue the exploration of the GBA in humans. Findings favouring bidirectional communication between brain and gut can affect the prevention and treatment of physiological and/ or psychological complaints. One application of such findings can be intensified coordination between physiological and psychological professionals to ensure an understanding of the underlying cause of an individual's complaint. Another application could have the form of an integrative treatment approach, which focuses on cognitive reactivity (e.g. ER difficulties) and microdiversity when approaching ER difficulties or gastrointestinal disorders (GI). Concretely this looks like a combination of cognitive behavioural therapy [CBT], self-relaxation techniques, psychoanalysis and talk therapy with microbiota analyses and possible probiotic intake (Mayer, 2018). In that way, (potentially traumatic) experiences from the past are reflected upon, which might influence the present.

5. Conclusion

High microdiversity did not predict fewer difficulties in emotion regulation in the present sample. There is no interacting effect of sex on emotion regulation difficulties and microdiversity. Further research on the gut-brain axis and sex differences in gut

microdiversity and emotion regulation is needed to have an impact on the treatment of psychopathology in clinical cases with stress-related disorders such as post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), or other anxiety-related disorders.

6. Acknowledgements

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7. Conflict of Interest

The authors declare that they had no conflicts of interest concerning their authorship or the publication of this article.

8. Ethics

The entire protocol and procedure of this study is conducted in line with several ethical codes: The "Declaration of Helsinki 2013" (World Medical Association, 2013) and its subsequent amendments, the applicable laws and regulatory requirements governing the conduct of biomedical research projects involving human subjects, the laboratory's site Standard Operating Procedures, and the Code of Conduct for Responsible Use of Human Tissue. The reasons are that 1) participants are neither exposed to invasive procedures, 2) nor are they required following rules of behaviour 3) nor does this study concern medical scientific research. Consequently, this committee needs no further review. The ethical approval of the research on *the microbiota-gut-brain axis in preventing stress-related disorders: antibiotics, cytokine activity and cognitive reactivity to emotion* was obtained on March 11, 2021, by the Psychology Research Ethics Committee, Leiden, the Netherlands. The reference number is 2021-03-09-L. Steenbergen-V3-3017. The Medical Research Ethics

Committee (MREC) Leiden Delft Den Haag; (in Dutch: Medisch-Ethische Toetsingscommissie [METC]) gave this study the reference number N21.016. The study was adapted to experienced issues with the study design (antibiotic vs. control group). Due to the deviations, the study got another CEP code: 2022-05-31-L. Steenbergen-V1-4048. The informed consent form (see Appendix C) was obtained from all participants included in this study.

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Appendices

Appendix A: DERS

Difficulties in Emotion Regulation Scal (DERS)

Geef aan hoe vaak de onderstaande uitspraken op jou van toepassing zijn door het betreffende cijfer uit de onderstaande tabel voor iedere uitspraak te plaatsen.

1	2	3	4	5
Bijna nooit (0-10%)	Soms (11-35%)	Geregeld (36-65%)	Heel vaak (66-90%)	Bijna altijd (91-100%)

1 ____ Ik ben helder over mijn gevoelens.
2 ____ Ik besteed aandacht aan hoe ik me voel.
3 ____ Ik ervaar mijn emoties als overweldigend en onbeheersbaar.
4 ____ Ik heb geen idee hoe ik me voel.
5 ____ Ik heb er moeite mee mijn gevoelens te begrijpen.
6 ____ Ik let op mijn gevoelens.
7 ____ Ik weet precies hoe ik me voel.
8 ____ Ik vind het belangrijk hoe ik me voel.
9 ____ Ik weet niet zeker hoe ik me voel.
10 ____ Als ik van streek ben, erken ik mijn emoties.
11 ____ Als ik van streek ben, word ik boos op mezelf.
12 ____ Als ik van streek ben, geneer ik me daarvoor.
13 ____ Als ik van streek ben, vind ik het moeilijk om werk uit handen te krijgen.
14 ____ Als ik van streek ben, raak ik buiten zinnen.
15 ____ Als ik van streek ben, denk ik dat dat lange tijd gaat duren.
16 ____ Als ik van streek ben, denk ik dat ik uiteindelijk heel depressief word.
17 ____ Als ik van streek ben, denk ik dat mijn gevoelens er mogen zijn en belangrijk zijn.
18 ____ Als ik van streek ben, heb ik er moeite mee me op andere dingen te concentreren.
19 ____ Als ik van streek ben, heb ik het gevoel dat ik mezelf niet meer in de hand heb.
20 ____ Als ik van streek ben, kan ik nog steeds dingen uit handen krijgen.
21 ____ Als ik van streek ben, schaam ik me dat ik me zo voel.

22 Als ik van streek ben, weet ik dat ik een manier kan vinden om me uiteindelijk beter te voelen.
23 Als ik van streek ben, heb ik het gevoel dat ik zwak ben.
24 Als ik van streek ben, kan ik mijn gedrag onder controle houden.
25 Als ik van streek ben, voel ik me schuldig.
26 Als ik van streek ben, vind ik het moeilijk om me te concentreren.
27 Als ik van streek ben, heb ik er moeite mee mijn gedrag te beheersen.
28 Als ik van streek ben, denk ik dat er niets is wat ik kan doen om mezelf beter te laten voelen.
29 Als ik van streek ben, raak ik geïrriteerd over mezelf.
30 Als ik van streek ben, begin ik me heel rot te voelen over mezelf.
31 Als ik van streek ben, denk ik dat ik er alleen maar in kan zwelgen.
32 Als ik van streek ben, verlies ik de controle over mijn gedrag.
33 Als ik van streek ben, kan ik aan bijna niets anders denken.
34 Als ik van streek ben, neem ik de tijd om uit te vinden wat ik werkelijk voel.
35 Als ik van streek ben, duurt het heel lang voor ik me weer beter voel.
36 Als ik van streek ben, word ik overspoeld door emoties.

Appendix B: Information Letter and Informed Consent Form

Proefpersonen-informatieformulier

Antibiotica en cognitie

Officiële titel: "De microbiota-darm-brein as in het voorkomen van stress gerelateerde stoornissen: antibiotica, cytokine activiteit en cognitieve reactiviteit naar emoties."

Inleiding

Beste Meneer/Mevrouw,

U bent gevraagd om deel te nemen aan een wetenschappelijk onderzoek. Deelname is geheel vrijwillig en u kunt op elk moment terugtrekken uit het onderzoek zonder daarvoor een reden te geven. Om deel te kunnen nemen aan dit onderzoek, hebben wij uw online schriftelijke toestemming nodig. De medisch-ethische toetsingscommissie Leiden Delft Den-Haag (METC LDD) heeft beoordeeld dat dit onderzoek niet onder de Wet medisch-wetenschappelijk onderzoek met mensen (WMO) valt. Voordat u een beslissing maakt over uw deelname zal er eerst informatie worden verstrekt over de aard van dit onderzoek. Neem uw tijd om dit door te lezen en mocht u nadien vragen hebben kunt u de onderzoeker benaderen voor verdere uitleg. Het is natuurlijk ook mogelijk om te overleggen met uw partner, vrienden of familie over u deelname.

1. Algemene informatie

Deze studie wordt uitgevoerd namens Universiteit Leiden. De studie is voltooid wanneer 80 participanten hebben deelgenomen. Toestemming voor de uitvoering van dit onderzoek is gegeven door de Commissie Ethiek Psychologie van de Faculteit van Sociale Wetenschappen, Universiteit Leiden. Dit is een observationele studie in gezonde proefpersonen en valt daarom niet onder medisch onderzoek.

2. Doel van het onderzoek

Het doel van deze studie is om te onderzoeken of het gebruik van antibiotica een effect heeft op cognitieve- en emotionele reactiviteit. Hiervoor zullen we individuen vergelijken die recentelijk **preventieve antibiotica (amoxicilline)** hebben gebruikt met individuen die recentelijk dit niet hebben gebruikt.

In de darmen bevinden zich veel bacteriën die niet alleen invloed hebben op het verteren van voedsel, maar ook invloed kunnen hebben op stemming, cognitie en gedrag. Het gebruik van antibiotica verstoort de balans in het darm microbioom door het reduceren van het aantal onschuldige bacteriën. Het microbioom van de darmen verwijst naar alle bacteriën die in de darmen leven samen met hun genetisch materiaal.

Het is echter nog onbekend hoe de verstoring van het darm microbioom kan zorgen voor veranderingen in stemming, cognitie en gedrag. In deze studie zullen we daarom onderzoeken wat het effect is van antibiotica op emotionele reactiviteit (de manier waarop

mensen reageren op emotionele opwekkende gebeurtenis) en welke mechanismen onderliggend zijn hieraan.

3. Achtergrondinformatie over dit onderzoek

Het is al enige tijd bekend dat de darmen en het brein zijn verbonden met elkaar. Eerdere studies hebben aangetoond dat de samenstelling van de darmmicrobiota (de populatie van bacteriën) een invloed kan hebben op stemming, cognitie en gedrag. Ondanks dat er nog veel onbekend is over de onderliggende mechanismen, is er bewijs dat sommigen darmbacteriën ontstekingsremmend zijn terwijl andere darmbacteriën juist voor ontstekingen kunnen zorgen. Verhoogde ontstekingsniveaus zijn op hun beurt weer geassocieerd met verminderd cognitief functioneren. Het begrijpen van deze mechanismen kan helpen in de ontwikkeling van nieuwe interventies die het verminderd cognitief functioneren tegengaan.

4. Hoe verloopt het onderzoek?

Wat deelname met zich meebrengt

Deelname aan het onderzoek kan vanuit huis worden gedaan en zal ongeveer 90 minuten van uw tijd nemen.

Stap 1: Screening

We willen eerst weten of u geschikt bent om mee te doen. Daarom zal er telefonisch een korte screening plaatsvinden voordat uw deelname begint. Gedurende deze screening zal de onderzoeker vragen naar bepaalde details van uw medische geschiedenis en levensgewoonten, om ervoor te zorgen dat u voldoet aan de inclusiecriteria van deze studie. Het is bijvoorbeeld niet mogelijk om deel te kunnen nemen wanneer u medicatie slikt naast het nemen van antibiotica.

Stap 2: De metingen

Meedoen aan deze studie omvat het invullen van enkele online vragenlijsten en het uitvoeren van een online computertaak. Daarnaast zullen we u vragen een monster van uw speeksel en uw ontlasting in te leveren. De online vragenlijsten zijn bedoeld om gegevens te verzamelen over uw stemming, gezondheid, manier van denken, en welzijn. Tijdens de online computertaak wordt uw reactietijd en correctheid gemeten.
Als laatst zullen er fysiologische metingen worden gedaan, waarbij speeksel en ontlasting door u zelf wordt verzameld. Voor de speeksel- en ontlastingsverzameling zult u kits thuisgeleverd krijgen die dit faciliteren. Voor de speeksel verzameling zult u gevraagd worden om te spugen in een buisje. Dit speeksel zal gebruikt worden om het effect van antibiotica op het immuunsysteem te onderzoeken. De verzameling van ontlasting kan in een speciale container, dit materiaal zal geanalyseerd worden op bacteriële diversiteit. Meer informatie over de ontlastingsverzameling kunt u lezen in bijlage B.

Stap 3: Debriefing

Nadat u alle vragenlijsten heeft kunnen invullen en de computertaak is gemaakt, zullen we u contacteren om te vragen hoe het is verlopen en hoe u het onderzoek heeft ervaren.

5. Verwachtingen

Om de kwaliteit van het onderzoek te beschermen is het belangrijk dat u **geen** supplementen slikt, overmatig alcohol gebruikt of (recreationele) drugs heeft ingenomen minder dan 7 dagen voor de start van de studie. Daarnaast is het ook belangrijk dat u **geen** radicale veranderingen in uw dieet aanbrengt tussen de screening en het einde van het onderzoek. Het is cruciaal dat u de onderzoeker op de hoogte brengt als u in deze periode (tussen de screening en het einde van het onderzoek) plotseling gezondheidsproblemen ervaart, opgenomen wordt in een ziekenhuis of wilt terugtrekken uit het onderzoek.

6. Mogelijke ongemakken

Alle procedures die onderdeel zijn van deze studie zijn veilig verklaard. Sommige procedures kunnen echter enig ongemak veroorzaken, zoals hieronder wordt beschreven.

Vragenlijsten en computertaken

Sommige vragen in de vragenlijsten kunnen confronterend zijn of voor ongemak zorgen. Deze vragen kunnen betrekking hebben op uw mentale welzijn, op eventuele negatieve gedachten van u en die van u kunnen vragen een sombere bui in te beelden tijdens het beantwoorden van vragen. Voor het belang van de studie is het belangrijk dat de vragen zo eerlijk mogelijk worden ingevuld. Daarnaast kan de computertaak als vermoeiend worden ervaren. Tijdens het maken van de computertaak is er de mogelijkheid om pauzes te nemen. Wanneer u zich vermoeid voelt tijdens het onderzoek, geef dit dan alstublieft aan bij de onderzoeker zodat dit kan worden meegenomen in de resultaten van het onderzoek.

Fysiologische procedures

De speeksel en ontlasting monsters worden door u zelf verzameld. Om het voor u zo makkelijk mogelijk te maken, zullen er speciale kits worden geleverd die het verzamelen van de monsters faciliteren. U kunt er ook voor kiezen om deze op te halen bij de Universiteit Leiden als u dit prefereert.

7. Mogelijke voor- en nadelen van deelname

Het is belangrijk om na te denken over de mogelijke voor- en nadelen van deelname voordat u de beslissing maakt om deel te nemen in deze studie.

Voordelen van participatie:

- U krijgt toegang tot de resultaten m.b.t. de bacteriestammen aanwezig in uw ontlasting. Let op: aan deze informatie kunnen géén medische conclusies ontleend worden. Mocht u uw darmmicrobioom medisch willen laten onderzoeken dan adviseren wij u contact op te

nemen met uw eigen huisarts. Meer informatie over de ontlastingsverzameling kunt u vinden in bijlage B.

● Uw participatie zal verder bijdragen aan het begrijpen wat de functie van het darmmicrobioom is in relatie tot cognitie, emotie en gedrag.

Potentiële nadelen van participatie:

● Blootstelling tot eventuele confronterende vragen in de vragenlijsten. Dit zijn vragen die onder andere reflecteren op uw mentale welzijn, op eventuele negatieve gedachten die u heeft en die van u vragen een sombere bui voor te stellen tijdens het beantwoordenvan vragen.

● Het ondergaan van eventuele ongemakkelijke procedures, zoals de verzameling van uw eigen speeksel en ontlasting.

● U moet zich houden aan de afspraken die horen bij het onderzoek.

Terugtrekken uit het onderzoek

Het besluit om deel te nemen aan het onderzoek is geheel vrijwillig.

Als u besloten heeft om deel te nemen, bent u altijd vrij om uw deelname op elk moment terug te trekken zonder hiervoor een uitleg te geven of verdere consequenties te ondergaan. Wanneer u besluit om uw deelname terug te trekken, is het belangrijk dat u meteen de onderzoeker op de hoogte stelt. De data die tot dan is verzameld zal meegenomen worden in de studie tenzij u daar bezwaar tegen hebt, dan wordt al uw data (waaronder de verzamelde speeksel- en ontlastingsmonsters) vernietigd.

8. Einde van de studie

U wordt direct op de hoogte gesteld wanneer er nieuwe informatie aan het licht komt die mogelijk uw beslissing tot deelname beïnvloedt. Daarbij wordt ook gevraagd of u nog verder wilt gaan met het onderzoek.

In deze situaties stopt voor u het onderzoek:

● alle procedures zijn volbracht en alle monsters zijn verzameld

● u beslist om u deelname te stoppen

● de onderzoeker beslist om de deelname te stoppen omdat dat het beste is voor u ● de wetenschapscommissie beslist om de studie te beëindigen

9. Wat gebeurt er na het onderzoek?

De volledige studie zal eindigen wanneer alle data van iedere participant is verzameld. Wanneer alle data verwerkt is, zal u geïnformeerd worden over de hoofdconclusies van de studie. U kunt ook ervoor kiezen om niet geïnformeerd te worden over de hoofdconclusies door dit aan te geven bij de onderzoeker. De onderzoeker zal deze informatie dan niet aan u verstrekken.

10. Gebruik en opslag van uw data en monsters

Wanneer u het toestemmingsformulier ondertekend heeft, geeft u toestemming tot het verzamelen, opslaan en verwerken van uw persoonlijke en medische informatie. Gedurende de studie zullen we persoonlijke en medische gegevens van u verzamelen. Daarnaast zullen ook speeksel- en ontlastingsmonsters worden verzameld. We verzamelen, gebruiken en bewaren uw gegevens om de vragen van dit onderzoek te kunnen beantwoorden en om de resultaten te kunnen publiceren. Al uw gegevens worden vertrouwelijk door ons behandeld.

Persoonlijke gegevens

De persoonlijke gegevens die tijdens het onderzoek worden bewaard zijn uw naam en adres. Deze worden bewaard om de materialen, die u nodig heeft voor het verzamelen van het lichaamsmateriaal (zie alinea hieronder), bij u te kunnen leveren. Mocht u dit liever niet willen, dan kunt u deze materialen afhalen aan de Universiteit Leiden. Uw naam en adres worden gewist zodra u het onderzoek heeft afgerond, of als u aangeeft te willen stoppen. Om uw privacy te waarborgen zullen andere verzamelde gegevens tijdens dit onderzoek niet gelabeld worden met uw naam of persoonlijke gegevens, maar met een code. Deze code, die te herleiden is tot uw persoonlijke gegevens, wordt bewaard in een beveiligd document. Uw gegevens worden **gecodeerd maar niet volledig anoniem** opgeslagen. Echter is de koppeling tussen uw code en uw persoonsgegevens alleen voor de volgende individuen beschikbaar: de hoofdonderzoeker (zie bijlage A), gekwalificeerde onderzoeksassistenten, vertegenwoordigers van de opdrachtgever en eventueel de gezondheidsinspectie. De zojuist genoemde individuen mogen uw persoonlijke gegevens niet delen met derde partijen. De onderzoeker zal uw gegevens voor 5 jaar bewaren.

Lichaamsmateriaal

Het speeksel dat we hebben verzameld wordt gebruikt om uw immunologisch functioneren te bepalen. De ontlasting zal gebruikt worden om de diversiteit van uw darmmicrobioom te bepalen. Al deze monsters worden gecodeerd voor de laboratoria die de analyses van de monsters uitvoeren. Deze laboratoria kunnen uw persoonlijke gegevens niet inzien; zij kunnen de koppeling tussen de code en uw persoonsgegevens niet maken. Alleen de eerder genoemde individuen krijgen toegang tot het document waarin de codes zijn gekoppeld met uw persoonlijke informatie.
De speekselmonsters zullen bewaard worden in het beveiligde laboratorium van de Universiteit Leiden totdat ze geanalyseerd worden in het Leiden Universitair Medisch Centrum (LUMC). Uw ontlastingsmonsters stuurt u zelf direct naar het analyselaboratorium. Nadat de speeksel- en ontlastingsmonsters zijn geanalyseerd, zullen de monsters vernietigd worden. Uw naam en adres worden gewist zodra u het onderzoek heeft afgerond, of als u aangeeft te willen stoppen, dus de gecodeerde lijn tussen uw persoonlijke gegevens en de gegevens verkregen uit uw ontlasting en speeksel zal dan ook verbroken worden.

Wilt u meer weten over uw rechten bij de verwerking van persoonsgegevens? Kijk dan op www.autoriteitpersoonsgegevens.nl Mocht u vragen hebben over uw privacy, dan kunt u ook contact opnemen met de privacy officer van de Universiteit Leiden via privacy@bb.leidenuniv.nl

11. Financiële compensatie

Om u te compenseren voor het deelnemen, zult u een geldbedrag van €20 (zegge twintig euro) ontvangen. Deze compensatie wordt als inkomen beschouwd door de Nederlandse belastingwet. Mocht u tijdens het onderzoek uw deelname terugtrekken, zal uw compensatie daarop worden aangepast.

12. Huisarts

Wij zullen uw huisarts **niet** informeren over het deelnemen in deze studie.

13. Vragen

Mocht u vragen hebben wat betreft dit onderzoek, kunt u contact opnemen met de hoofdonderzoeker van deze studie, Dr. Laura Steenbergen. Haar contactgegevens kunt u vinden in bijlage A. Heeft u een klacht? Bespreek dit dan met de onderzoeker van de studie. Wilt u dit liever niet? Mail dan naar Klachtenfunctionaris-Psychologie@fsw.leidenuniv.nl

14. Toestemmingsformulier

Nadat u voldoende tijd heeft gekregen om een beslissing te maken over uw deelname, wordt u gevraagd om het besluit te melden aan de onderzoeker. Wanneer u besluit om deel te nemen, zal u gevraagd worden om het bijgevoegde toestemmingsformulier online in te vullen. Bij het ondertekenen van het toestemmingsformulier stemt u ermee in dat u alle informatie in dit document hebt begrepen en akkoord gaat met participatie. U zult een digitale kopie ontvangen van het toestemmingsformulier.

Bedankt voor uw aandacht.

Bijlages

A. Contact gegevens

B. Informatie over de verzameling en analyse van uw ontlasting

C. Toestemmingsformulier

Bijlage A: Contactgegevens

Dr. Laura Steenbergen

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Bijlage B: Informatie over de verzameling en analyse van uw ontlasting

Uw ontlasting zal worden geanalyseerd door MyMicroZoo B.V.. Dit is een organisatie die de samenstelling van uw microbiota vaststelt en zo kan zien welke bacteriën en in welke mate deze bacteriën aanwezig zijn in uw dikke darm. Na ongeveer 3 tot 4 weken kunt u uw eigen resultaten inzien. In de resultaten kunt u zien welke bacteriën er aanwezig zijn in uw dikke darm en, indien wenselijk, krijgt u de mogelijkheid om dit verder te bespreken met een deskundige.

Wanneer u ervoor kiest om deel te nemen aan dit onderzoek, zullen er een aantal stappen worden ondernomen tot het analyseren van het ontlastingsmonster.

Stap 1: Account aanmaken

Wanneer u beslist te participeren in dit onderzoek, zult u gevraagd worden een individueel account aan te maken via www.mymicrozoo.com/nl.

Stap 2: Ontvangst van de kit

De hoofdonderzoeker zal de ontlasting kit (samen met de speeksel kit) bij u afleveren. Deze kit bevat een code en een instructieboekje voor het verzamelen van uw ontlasting. De code registreert u zelf op uw account van My MicroZoo. Uw identiteit blijft te allen tijde anoniem voor MyMicroZoo; alleen de onderzoekers zoals in de informatiebrief genoemd, kunnen de koppeling tussen uw code en uw persoonsgegevens maken. My MicroZoo zal u verzoeken enkele vragen te beantwoorden m.b.t. demografische kenmerken (bijv. uw geslacht, in welk land u geboren bent, en of u een bepaald dieet volgt). De antwoorden op deze vragen worden wederom anoniem opgeslagen. De kit bevat instructies en het nodige materiaal om de ontlasting te verzamelen. Wanneer er problemen optreden kunt u altijd contact opnemen met de onderzoekers. Na de verzameling van de ontlasting stuurt u dit naar MyMicroZoo middels de bijgeleverde enveloppe met antwoordnummer (frankering dus niet nodig!), die het vervolgens voor u gaan analyseren.

Stap 3: Resultaten

Na ongeveer 3 à 4 weken kunt u zelf de resultaten inzien via uw account. Dit kan door in te loggen bij MyMicroZoo met uw verkregen code. In de resultaten kunt u zien welke bacteriestammen er in uw ontlasting gevonden zijn en in welke mate elke bacterie voorkomt. Daarnaast kunt u ook uw bacteriepopulatie vergelijken met andere (anonieme) MyMicroZoo gebruikers. Ten slotte wordt u ook informatie aangereikt die uitleg geeft over de belangrijke darmbacteriën en een verklarende woordenlijst. Let op: aan deze informatie kunnen géén medische conclusies ontleend worden. Mocht u uw darmmicrobioom medisch willen laten onderzoeken dan adviseren wij u contact op te nemen met uw eigen huisarts.

Wilt u meer informatie hebben over hoe MyMicroZoo te werk gaat? Kijk dan op hun website www.mymicrozoo.com/nl

Bijlage C: Toestemmingsformulier

CEP code ''De microbiota-darm-brein as in het voorkomen van stress gerelateerde stoornissen: antibiotica, cytokine activiteit en cognitieve reactiviteit naar emotie''

Ik heb de informatiebrief gelezen en had de mogelijkheid om vragen te stellen. De vragen zijn naar mijn genoegen beantwoord en ik heb genoeg tijd gehad om een besluit te nemen wat betreft mijn participatie.		Ja/Nee
Ik ben ervan op de hoogte dat mijn participatie vrijwillig van aard is. Daarnaast ben ik ervan op de hoogte dat ik op elk moment mijn participatie kan terugtrekken zonder daar een reden voor te geven.		Ja/Nee
Ik ben ervan bewust dat sommige personen toegang hebben tot de gegevens die zijn verkregen gedurende het onderzoek. De personen die hier toegang tot hebben zijn vernoemd in de informatiebrief.		Ja/Nee
Ik ga akkoord met de verzameling van speeksel- en ontlastingsmonsters en het gebruik van deze monsters voor onderzoeksdoeleinden die staan beschreven in de informatiebrief.		Ja/Nee
Ik wil participeren in deze studie.		Ja/Nee
Ik geef akkoord voor het bewaren van mijn gegevens tot 5 jaar na het einde van het onderzoek beschreven in de informatiebrief.	Ja/Nee	
Ik ga akkoord dat mijn speeksel- en ontlastingsmonsters vernietigd zullen worden na de data-analyse hiervan.	Ja/Nee	

Naam participant:
Datum: __ / __ / __
Hoofdonderzoeker:
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