A gut feeling: how your gut and brain changes your risk and time preferences

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Article

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Abstract

Recent research has shown that the gut microbiota can influence the interaction between the central and the enteric nervous systems via the gut-brain axis (GBA). Animal models and human neuroimaging studies have revealed that changes in the gut microbiota affect neural activity in brain regions linked to basic emotional and cognitive processes. Whether the gut microbiota also affect human decision-making and, more specifically, risk and time preferences, however, remains largely unknown. Here, we examine the role of the gut-brain axis in decision-making in the face of risk and intertemporal choices.

In a placebo-controlled double-blinded design, with two sessions separated by 28 days, during which participants received daily doses of probiotics (or a placebo), we investigate whether the prolonged and controlled intake of probiotics affects risk-taking behavior and intertemporal choices using incentivised economic tasks.

We found a significant decrease in risk-taking behavior and an increase in future-oriented choices in the probiotics group as compared to the placebo group. These findings provided the first direct experimental evidence suggesting a potential functional role on the part of the microbiota-gut-brain axis in decision-making, creating a path for potential clinical applications and allowing for a better understanding of the underlying neural mechanisms of risk-taking behavior and intertemporal choices.

Introduction

Each person carries a complex ecosystem of bacteria in their body [1]. The intricate relationship between bacteria and host plays an important role in human homeostasis, our internal balance [2], maintaining health, nutrition and immune defenses [3]. The gut hosts most of these bacteria [1]. Variations in the gut microbiota can be detected by the gut epithelium, which transmits signals via the enteric nervous system to the central nervous system via hormone and neurotransmitter production, immunological responses and neuronal signaling [4,5]. This complex system formed by the central and enteric nervous systems and the gut microbiota forms an important bidirectional network between the gut and the brain and is known as the gut-brain-axis [6]. This network allows for the central nervous system to affect the gut, but there is also direct communication from the gut and the gut microbiota to the central nervous system, allowing the gut to influence brain activity and behavior [7]. The proper ecological balance of the gut microbiota is known to affect brain development, cognitive performance [8], mood, reactivity to stress and socialisation [9–11], and it even plays a role in certain psychopathologies, such as autism, depression and Parkinson's disease [2,8,12,13].

Studies using animal models have already shown that antibiotics-induced changes in the gut microbiota lead to increases in exploratory behavior and the hippocampal expression of brain-derived neurotropic factor (BDNF) [14]. They also demonstrated that germ-free mice exhibit anxiety-like behavior and increased risk-taking behavior in the Elevated Plus Maze task, which revert to normal levels with bacterial colonisation[15]. Furthermore, it was revealed that the *Bifidobacteria infantis*, commonly present in healthy

intestines, plays an important role in tryptophan metabolism, influencing serotonin production, with subsequent antidepressant properties [16]. In a similar vein, research with germ-free mice revealed increased concentrations of cortical dopamine and the influence of the gut microbiota on the myelination of frontal brain areas [17]. In fact, BDNF, serotonin and, especially, dopamine play important roles in high-order cognitive processes, including executive control and decision-making [18–21]. The results obtained in animal models have been successfully replicated in humans, expanding our knowledge about the relevance of the gut microbiota for brain development, important cognitive processes and behavior [10,22]. For example, it is known that early life bacteria colonization affects brain development of areas such as the hypothalamic-pituitary-adrenal axis, which is fundamental for stress-related responses [23]. Furthermore, factors such as delivery method and antibiotic treatments can lead to gut microbiota dysbiosis, meaning lower microbiota variability and a higher presence of pathogens, which plays a fundamental role in psychopathologies such as autism spectrum disorder (ASD) [24] and attention-deficit/hyperactivity disorder (ADHD) [25]. Studying the influence of the gut-brain axis on human cognition requires either microbiome genetic exploration [26] or microbiota manipulations [23]. The manipulation of microbiome composition can be achieved by changes in diet, fecal transplantation or the use of probiotics to increase gut microbiota variability [27].

Studies with patients have demonstrated that probiotics reduce anxiety and depressive symptoms, indicating the clinical potential of the so-called psychobiotics [23]. Fewer studies have explored the effects of GBA on healthy participants [8]. Nevertheless, the results thus far presented confirm the correlation between the variability of the complex gut environment and high-level brain activity [9,10,23,28,29]. A recent study explored the multivariate associative patterns between gut microbiota composition and large-scale brain network connectivity [29]. It identified that the *Bifidobacterium* concentration is positively correlated with the increasing connectivity of the frontal nodes of the default mode network, while the prevalence of *Prevotella_9* and *Bacteroides* is negatively correlated with connectivity [29]. Other relevant findings appear in studies using probiotics to investigate the effects of changes in the gut microbiota on brain activity with functional magnetic resonance imaging (fMRI) [27].

These neuroimaging studies showed that, when compared to controls, participants who received probiotics had decreased functional connectivity between the frontal pole and frontal medial-cortex during resting-state fMRI [12], as well as a significant reduction in brain activity in sensory and affective regions, such as the insula, and increased activity in cortical regulatory regions, such as the dorsolateral prefrontal cortex (DLPFC) and medial prefrontal cortex (MPFC), during a standardised emotional face recognition task [28]. The DLPFC and ventromedial prefrontal cortex (VMPFC) play a fundamental role in decision-making [30] and are especially relevant to risk-taking behavior [31–35] and intertemporal choices [30,36,37], the focus of this study.

The goal of this study was to examine how the gut-brain axis affects risk-taking and intertemporal decision-making by changing the gut microbiome composition. To that end, we used a double-blind

protocol of 4 weeks of ingestion of either probiotics or placebo (two groups, between subjects) and incentivised tasks to measure risk-taking behavior and intertemporal choices. Specifically, we used the Maastricht Gambling Task (MGT) [33] to measure risk-taking behavior and the Maastricht Choice Game (MCG), which includes estimates of present bias and time discounting to assess intertemporal choices. We hypothesised that changes in the gut microbiota affect risk-taking decision and intertemporal choices via the gut-brain axis. Hence, we hypothesised that the processing of risk and intertemporal decisions goes beyond the central nervous system's limits.

Results

In this section, we present the main behavioral results of our experiment. The detailed research design and statistical methodology are reported in Section 4.

2.1 Risk-taking behavior

A linear mixed model was used to analyse the effects of the probiotics/placebo protocols on risk-taking behavior. The model included the main effects for group (placebo vs. Probiotic) and time (Session 1 versus Session 2), as well as the interaction effect of group and time.

The interaction effect of group*time, which tests our hypothesis by comparing the effects on both groups after the probiotics/placebo intervention, is negative and can be considered small and significant (beta = -0.42, SE = 0.16, t(14118) = -2.67, p = .008). This indicates that, despite the overall increase in risk-taking behavior over time, there was a significant reduction in risk-taking behavior in the probiotics group as compared to the placebo group in Session 2. More details can be observed in Figure 1.

As expected, the effect of group was not significant (p = 0.922), indicating no difference between groups in the first session. There was a small positive and significant effect on the part of time (beta = 0.42, SE = 0.11, t(14118) = 3.69, $p < .001$), indicating an increase in risk-taking behavior from Session 1 to Session 2 for both groups. To examine the observed increase in risk-taking behavior over time more closely, we ran an additional analysis. More specifically, we investigated whether the variation in the payout of the participant fee from the MCG task created a house money effect, or a payoff-based belief distortion [38,39], and consequently increased risk-taking behavior [40]. We therefore added the payments received by participant between Sessions 1 and 2 to the model. These payments included the participation fee for all participants and the immediate payment of the MCG for some of the participants (others received it 35 days later, in line with the incentivized MCG task). The inclusion of two regressors for the amount of the immediate payment from the MCG (payment) and the participation fee (participation) significantly improved the model's fit. The results yielded significant yet small effects on the part of the immediate payment (beta = -0.04, SE = 0.02, p < .05) and the participation fee (beta = 0.03, SE = 0.12, p < .01). Our main result is robust to the addition of the two payment factors into the model, still indicating a

significant negative effect on the part of the probiotics intervention, which can now be classified as a medium effect (beta = -0.50, SE = 0.16, p < .01).

2.2 Intertemporal choices

To estimate participants' time preferences, we included two main dependent variables: their present bias () and time discount (). Please refer to the Methodology section for further details on the model used to estimate these parameters.

The analyses of effects of the probiotics manipulation on present bias were performed using linear mixed models. Regarding the probiotics interaction (group*time), we observed a significant large positive effect on (t(13216) =12.028, p < .001). This effect means that the probiotics intervention leads to a significant increase in , to a value above 1, which, according to Andreoni and Sprenger (2012), is characterized as a future bias, meaning that these participants were more likely to make future-oriented choices. It is important to highlight that participants already presented values above 1, independent of the probiotics manipulation, indicating future bias, which is expected when using the convex time budget method [41]. Our results demonstrated a small, albeit significant, positive effect of session (t(13261) = 1.99, p = .046), meaning that there was a small significant increase in from Session 1 to Session 2 in both groups. As expected, no significant effect of group was observed (p = .506). Details can be seen in Figure 2.

Furthermore, we analyzed participants' time discounting. The effect of the probiotics protocol, analyzed via the interaction group*session, was negative and can be considered medium and significant (beta = -0.01, SE = 0.01, t(13261) = -4.911, p < .001). We did not find a significant main effect on the part of group, as expected (p = .24). There was a large and significant effect on the part of session (beta = 0.03, SE = 0.01, t(13261) = 20.785, p < .001).

2.3 Control variables

We controlled for a series of variables, such as mood, self-control, arousal and diet. No significant effects on the part of the probiotics protocol (time*group) were observed on the mood scores (p = .17), self-control (p = .49), arousal (p = .72) or diet (p = .48). There were also no significant changes in diet when comparing the two time points estimated (p = .92) (please find more details on the analyses of the control variables in the supplementary material, S1.4).

Discussion

Considering the large number of recent studies showing the fundamental relevance of the gut-brain axis as a bidirectional network in cognitive processes, here, we investigated the influence of the gut brain axis on decision-making in the face of risk and in the context of intertemporal choices [27]. To this end, we conducted a placebo-controlled double-blinded design with two sessions separated by 28 days, during

which participants received daily doses of probiotics (or placebo). We investigated whether the prolonged and controlled intake of probiotics affected risk-taking behavior and intertemporal choices using incentivised tasks.

When we examined the results of the risk-taking behavior task more closely, interestingly, we observed a significant increase in risk-taking from Session 1 to Session 2 in the group that underwent the placebo protocol. This means that, even though participants did not receive any probiotics, their risk-taking behavior increased after 30 days. Such an increase was not observed in the group that received probiotics.

Our results showed a significant reduction in risk-taking behavior after prolonged probiotic intake. Considering that there were no significant dietary or mood differences from Session 1 to Session 2 and the experimental conditions were identical, we can attribute the observed effects to the probiotic intake. Thus, participants who underwent the probiotics protocol were significantly less likely to choose risky options as compared to participants in the placebo group in Session 2, indicating a significant decrease in risk-taking behavior.

Regarding intertemporal choices, we used the MCG to estimate present bias and time discounting. Our results show that participants who went through the probiotics protocol for 30 days exhibited a significantly higher future-bias and a significant reduction in time discounting as compared to the placebo group in Session 2. These results indicate that, after the prolonged use of probiotics, participants were significantly more likely to make future-oriented choices, investing more in delayed options than participants who received a placebo for the same period.

To further explore the robustness of our findings on risk-taking behavior, we control for certain additional factors. One may think that the significant effect of probiotics on risk taking is due to the fact that the placebo group exhibited a significant increase in risk taking in Session 2. We explore the potential reasons for this and find that the effect of probiotics is still significant after controlling for the increase in risk-taking across sessions observed in the placebo group. Increases in risk-taking behavior could be caused by a house money effect, in which participants take greater risks in the presence of prior gains [38], or due to payoff-based belief distortion, in which participants become more optimistic and, therefore, risk-prone after a gain [39]. Participants were compensated for their participation and also incentivised to complete the MGT and MCT. This means that participants who received the money from Session 1 during Session 2 could be subjected to a payoff-based belief distortion or house-money effect, leading to an increase in risk-taking behavior [38,39].

This hypothesis was tested by adding the participation fee and immediate payments received between Session 1 and Session 2 as factors in our analysis. In this way, we were able to show that the increase in risk-taking behavior in the placebo group was indeed an effect of the payments received by the participants between sessions; when we controlled for these payments in our model, the effect of time was no longer significant for either group. The effect of the interaction group*time, meaning the effects

of the probiotics protocol on risk-taking after controlling for payments between sessions, not only remains significant but shows a larger effect size. Hence, we can affirm that the probiotics protocol led to a significant negative effect on risk-taking behavior. This means that, in the group that received probiotics, the significant increase in risk-taking behavior due to the payments between sessions seems to have been neutralised. This result is due to a significant reduction in risk-taking behavior after the probiotics protocol, considering that all other conditions were stable across groups and sessions. Although the mechanisms are, at this point, unclear, it can be speculated that the probiotics intervention involved a house money effect or payoff belief distortion affecting risk-taking behavior, leading to an overall stabilisation of the participants' risky choices from Session 1 to Session 2.

In terms of intertemporal choices, we also observed an increase in future bias and time discounting from Session 1 to Session 2. These increases in both the placebo and probiotics groups are not unexpected and can be attributed to increased familiarity with the task and more confidence in the researchers, establishing a different reference point for their choices [15]. The probiotics intervention seems to have attenuated the effect on time discounting, which can be seen as a significant reduction in time discounting when comparing the probiotics and placebo groups in Session 2. Moreover, the group that underwent the probiotics protocol showed a larger significant increase in future bias than the placebo group, confirming the significant effect of probiotics on intertemporal choices.

Another interesting finding with respect to intertemporal choices is that participants were inherently future biased in both groups in Session 1, with an average β of 1.01, indicating future bias [41]. This contradicts the expectation based on the economics literature [47], which holds that most people are present- rather than future-biased. However, deviations from present-bias are not uncommon in empirical studies [48]. Moreover, our results are in line with Andreoni and Sprenger (2012), who also use a convex time budget, as we do in our task. It is important to stress that our task already presents significant delay intervals and a wide variety of discount rates, which should lead to realistic representations of participants' time preferences. One potential explanation for the future bias is that the payoffs offered to participant were not large enough, making it "easier" to wait for the payoffs[48,49].

Interestingly, the parametric estimation of risk obtained from the MCG yields results is consistent with our findings from the MGT; both show a reduction in risk-taking (α) behavior for the probiotics group. These results can be seen in our supplementary material (S2.1). Given that the intertemporal choices also include a risk factor, it is not possible to rule out that the effects on intertemporal choices were more likely mainly driven by the effects of the probiotics manipulation on risk-taking behavior [41]. Further studies are needed to disentangle the effects the probiotics manipulation on risk and time preferences in an intertemporal-choices context.

More research is also needed to determine whether the VMPFC mediates the effect of changes in the gut microbiota on the brain. The VMPFC is a potential candidate for this role, considering that it is believed to be the hub for somatic information, integrating this type of information during decision-making processing [32]. Moreover, according to studies using neuroimaging, the prolonged use of probiotics leads

to differential activation in this same area [12,29,42]. Moreover, the communication between the gut and the brain during decision-making is still unclear [8]. Two main pathways are potentially involved, namely the vagus nerve and neurotransmitter production [8,27]. The role of the vagus nerve as the main nervous pathway from the ENS to the CNS [11,14,20] and the effects of the gut microbiota on the production of neurotransmitters are now well-established [14,20,25,43,44]. Nevertheless, we can only speculate, at this point, that the changes in gut microbiota affect decision making through these pathways, and the relative importance of each pathway in this neuronal network is still unclear [22].

Another interesting question for further exploration is the protocol length needed to obtain significant results. Our design, with an interval of 30 days, is based on previous studies [12,42,45], but it would be interesting to investigate whether similar effects can also be achieved within a shorter period of probiotic ingestion and how long these effects hold for.

Overall, our findings about the effect on probiotics on risk-taking corroborate the results obtained in previous studies. According to research using animal models, germ-free rodents exhibit increased risk-taking behavior, which is reversed to a normal levels after their gut microbiota are normalised via fecal transplantation or probiotic administration [15,46]. The administration of the same probiotic composition, Ecologic®Barrier (Ecologic®Barrier, Winclove probiotics, The Netherlands), for six weeks in rats led to a significant reduction in risk-taking behavior in the Elevated Plus Maze [15]. Regarding studies with humans, Bagga et al. (2018) investigated the effects of a similar probiotics protocol on emotional brain signatures. Although they used a different probiotics composition (Ecologic®825, Winclove probiotics, The Netherlands) and a non-incentivized, self-reported measure of risk, they also observed a significant reduction in risk-aversion after four weeks' administration of probiotics, in line with our findings [46].

To our knowledge, no study to date has explored how the GBA affects intertemporal choices. Roman et al. (2018) conducted a comparable study but used a two-choice task, which is considered a measurement of impulsivity rather than intertemporal preferences because the delay time is only 5 seconds [47]. Nevertheless, their results point in a similar direction as our findings because the prolonged consumption of probiotics (3 weeks with daily ingestion of a milk yogurt containing *Lactobacillus casei Shirota*) led to a significant reduction in impulsive choices [47]. We suggest here that changes in the microbiota of the gut due to the intake of probiotics affects intertemporal choices in humans.

Finally, it is important to highlight the potential practical impact of our current findings. Our results open doors for studies on the therapeutical use of probiotics in populations that present abnormal patterns of risk-taking behavior, such as patients with attention deficit and hyperactivity disorder (ADHD), addictions or depression [31,48,49]. Evidently, more studies in this direction are needed. In addition to potential clinical applications, the results that we observe for healthy participants calls for more research on the relationship between nutrition and decision-making. Various factors affect the gut microbiota, including genetics, health status, mode of birth, use of antibiotics, and stress levels [23]. However, diet is certainly one

of the main factors to guarantee a balanced gut microbiota [50]. In our study, we used probiotics as a method to interfere with the microbiota-gut-brain axis by increasing microbiota diversity. Similar effects could potentially be achieved with a rich and healthy diet, healthier habits, and the lower use of unadvised antibiotics [2,51,52]. This is interesting in light of the fact that people with economic constraints often struggle to have access to nutritious diets [53], which would facilitate higher risk taking and more present-bias. For example, participants with poorer diets could be more likely to prefer immediate consumption over investing in a pension plan, potentially compromising their future financial wellbeing. Alternatively, a poorer diet could lead to more risk-taking behavior, with significant financial, social and economic impacts.

Overall, our findings suggest that the gut-brain axis may be a fundamental player in the neuronal mechanisms underlying decision-making. This means that our current neuroeconomical models used to predict risk-taking behavior and intertemporal choices, among potentially other types of complex decision-making, should not be limited to the CNS.

Methods

We conducted a double-blind between-subjects study, with probiotics or placebo administration used as a between-subjects factor. The study included two experimental sessions with a 28-day interval, during which participants took daily doses of either active or placebo probiotics. The study included a questionnaire to control for diet, arousal, self-control, and mood effects, as well as to estimate time and risk preferences.

4.1 Sample

We recruited 72 participants using posters on campus and social media targeting Maastricht University's academic community. Due to the COVID-19 lockdown in March of 2019, twelve participants discontinued the experiment, and three participants were excluded from the sample because they did not follow the probiotic intake protocol. Therefore, 57 adult healthy, right-handed participants (29 women) with an average age of 23.4 years (SD=4) finished the experiment (29 in the probiotics group). All participants had normal or corrected-to-normal vision and gave written informed consent after being introduced to the experiment and screened for safety. The safety screening followed the procedures recommended by the manufacturer (Winclove probiotics, The Netherlands) [15,54], excluding participants who had any sort of gastrointestinal disease or were using any medication during the experiment, with the exception of contraceptive pills. The study was approved by the local ethical committee (OZL_208_15_05_2019) and carried out in accordance with the standards set by the Declaration of Helsinki (Fortaleza Amendments).

Participants were asked to not consume more than two units of alcohol or any drugs one day before, as well as during, the experiment. Additionally, they were required to not take any antibiotics, medication or

other probiotics throughout the entire experimental period. Participants were compensated for participation and rewarded according to task outcome.

4.2 Procedure

Each participant underwent one of the assigned conditions of microbiota manipulation (probiotics or placebo). The conditions were assigned randomly, and the experiment was conducted in a double-blind fashion. Participation included two experimental sessions separated by 28 days (+/- 1), during which participants took daily doses of probiotics or placebo. Participants were reminded daily about probiotics ingestion via email to improve compliance, and a follow-up questionnaire was used in Session 2 to check for proper probiotic/placebo intake. The experimental design is illustrated in Figure 1.

In each session, participants were invited to our laboratory, where they signed the written consent form and filled out a questionnaire via Qualtrics. They were instructed to not change their dietary patterns, not take any probiotics other than those provided to them as part of the experiment and not to take antibiotics during the 30 days of this experiment. Any deviations had to be reported, and participants who did not sufficiently comply with these requirements were excluded from the sample, with three participants being excluded from the sample for these reasons.

The first scale measuring risk and time preferences is a part of the Global Preference Survey [55] (GPS). This scale was adapted using the text from the English version, with values pertinent to the Dutch population (based on the Dutch version), considering that our international participant base is fluent in English and resides in the Netherlands. The next scales the participants were asked to fill out were the Brief Self-Control Scale [56] (BSCS), the Self-Assessment Manikin [57] (SAM) and a short diet assessment. This was done to control for self-control, mood and potential dietary changes, respectively. The full questionnaire is available in the supplementary material. The constructs of the questionnaire were used as control variables in our analyses.

After filling out the questionnaire, participants were instructed about the Maastricht Gambling Task (MGT) and the Maastricht Choice Game (MCG), which are computer tasks used to elicit and estimate risk-taking behavior and intertemporal choices, respectively. The task order was randomised to avoid any potential order effect. The explanation of each task was followed by ten practice trials before task execution. The order of the two tasks was counterbalanced across sessions. The tasks are described in more detail below.

After finishing the two tasks, we used an online random number generator, with which the participants could select a random trial that would be used for payment in each task. Following the completion of the first session of the experiment, participants either received the first dose of probiotics or placebo and a box with the remaining 29 doses in individual sachets. They were then instructed to take one dose daily for the next 28 days and reminded daily via mail to take their doses. The last dose was taken in Experimental Session 2.

For the MCG, the participants received payments for a randomly selected trial. The payment timing depended on their choice in that trial, in which they invested points in earlier or later options. To guarantee that there were no effects on the part of transaction costs, immediate or later rewards were paid via bank transfer. The remaining compensation (participation and MGT) was paid using vouchers with monetary value after Session 2. Considering that the MGT did not have a time factor (the reward was always paid by the end of the experiment regardless of their choices), we compensated participants for completing this task with the same method of payment used for the participation fee.

Session 2 took place on the 30th (+/- 1) day of the experimental period. Before starting the procedures, participants ingested the last dose of either probiotics or placebo at the lab. The participant also completed a pre-experimental check, in which they stated whether they missed any doses during the interval. Participants who missed three or more doses were excluded from the sample. The second session followed the same procedure as Session 1, with the exception that the questionnaire excluded the GPS, leaving the BSCS, SAM and dietary inquiry. At the end of Session 2, the compensation for participation and completing the MGT was paid, and the participants were debriefed. The remaining MCG payments were received via bank transfer according to the participants' allocation.

In each session, participants completed a series of questionnaires (please refer to the methods section for more details) and played two economic games to estimate risk-taking behavior (Maastricht Gambling Task, MGT) and intertemporal choices (Maastricht Choice Game, MCG).

4.3 Maastricht Gambling Task (MGT)

The MGT is a computerised gambling task developed by Dantas et al. (2021), in which participants are presented with six colored boxes (see Figure 2A for an example screen) that can be either pink or blue. The number of pink boxes was randomized and could range from one to five (the remaining boxes are blue). Participants are informed that one of the boxes will hide a token represented by a yellow X. They are asked to guess the color of the box that hides the token. Please see Dantas et al. (2021) for a complete description of the task.

4.4 Maastricht Choice Game (MCG)

We developed the MCG to elicit and estimate intertemporal choices based on the Convex Time Budget (CTB) method developed by Andreoni and Sprenger (2009) [41]. In contrast to the MGT, each participant is initially endowed with 100 tokens and must spend this endowment entirely on two options. For each trial, the task randomly assigns two different allocation dates. The earlier options (t) could either be immediate (zero days) or in 35 days (after the end of the experiment). The later options offered either 35, 72 or 90 days (k) after the earlier options (therefore, the last option is equal to t+k). The payback for the earlier option (t) was always multiplied by one of twenty potential discount factors (0.50, 0.525, 0.55, 0.575, 0.60, 0.625, 0.65, 0.675, 0.70, 0.725, 0.75, 0.775, 0.80, 0.825, 0.85, 0.875, 0.90, 0.925, 0.95 and 0.99). The

future option (t+k) was not discounted. Therefore, 120 combinations of discount rates and intervals were used. Each combination was displayed twice in a total of 240 trials in a random order, which were divided into five blocks of 48 trials.

Participants could freely allocate the endowment by choosing how to distribute ten boxes among the two colors that represented the assigned interval. Pink boxes represented the earlier reward, and blue boxes represented the later reward. Each box represents 10% of the total endowment (10 points). The number of "moves" per trial was restricted to 15 to limit the time spent on each trial. The number of tokens allocated to each date and the score for each date were displayed on the screen. An example screen is presented in Figure 2B.

The MCG controlled for memory effects using independent trials, in which the results of previous trials did not affect the following one. The compensation for this task was provided via bank transfer according to the trial randomly selected by the participant for payment.

To evaluate time preferences, we used the following model:

$$\underset{x_t}{Max}\, U(x_t) = x_t^{\alpha} + \beta^t \delta^k (x_{t+k})^{\alpha} \qquad (3)$$

Such a model states that the choice in trial X is the result of a maximisation of the utility of such a trial. In the model, *t* represents the earlier time (0 or 35 days), and *k* represents the delay from this first time point (35, 70 or 95). Therefore, is the payoff at time *t,* and is the payoff at the time *t+k*. This maximisation takes into account the participant's risk attitude *α*. In this model, *α* is the curvature parameter, and therefore, . indicates risk neutrality. Our estimation of *α* here provides an additional check for the results in our MGT regarding risk attitude. In order to deal with corner solutions, in which participants allocate all points to either the earlier or the later option, without distribution, our estimation strategy adopts the two-limit Tobit maximum likelihood regression [41].

The parameters of major interest in this model for our research are each participant's present-bias () and time discount (). According to Andreoni and Sprenger (2012), indicates present-bias, while indicates future-bias. This parameter indicates how sharply it the participant's discount between now and the immediate future. Finally, indicates a participant's time discounting, or how much each dollar of future reward would be worth in present terms.

4.5 Probiotics

The probiotic Ecologic®Barrier (Ecologic®Barrier, Winclove probiotics, The Netherlands) is composed of Bifidobacterium bifidum W23, Bifidobacterium lactis W52, Lactobacillus acidophilus W37, Lactobacillus brevis W63, L. casei W56, Lactobacillus salivarius W24, and Lactococcus lactis (W19 and W58), distributed as sachets containing 2 g of freeze-dried powder of the PF and indicated for oral intake.

Participants received the composition for 30 days [15,54]. For the same timeframe of 30 days, the control group received a bacteria-free placebo created by the same laboratory, which was based on corn starch and identical to the probiotic composition both visually and in flavor.

4.6 Statistical analyses

To facilitate transparency and reproducibility, our datasets and codes are available at http://dx.doi.org/10.17632/nbz385mhny.1. We analysed the data from the MGT to estimate risk-taking behavior and the data from the MCG to estimate present-bias and time discounting. The remaining variables included in both models were also analysed and presented in the supplementary material (S1 and S2).

All data were preprocessed using a custom Matlab (The Mathworks Inc., Massachusetts, US). Our design included a between-subjects factor (group = placebo or probiotics) and a within-subject factor (time = session 1 and session 2). All trials (250 for the MGT and 240 for the MCG) were analysed per session and per participant, totalising 500 observations for the risk analyses and 480 for the intertemporal choice analyses per participant.

The control measures were analysed with regressions using custom R scripts [58 63]. The intertemporal choice analyses included an extra step in preprocessing, in which the parameters (risk attitude), (present-bias) and (time discounting) were estimated by running a two-limit Tobit maximum likelihood regression [41]. These parameters were estimated for each session.

The statistical analyses began with an analysis of outliers using custom R scripts, where observations outside 1.5 times the interquartile range above the upper quartile and below the lower quartile were excluded [59]. No observations were excluded from the risk-taking behavior analyses. For the intertemporal choice analyses, 14 analyses were excluded as outliers from the analyses, leaving 100 observations. Seventeen observations were excluded as outliers for the analyses of , leaving 97 observations.

Afterward, we ran a series of linear mixed model analyses, which are robust considering the missing data and appropriate for our mixed design. We again used custom R scripts [58] to estimate the effects of the each factor and, more importantly, the interaction of time*group, which indicates the effects of the probiotics protocol versus the placebo protocol in Session 2. Our final models were fixed-effects models, with participant-specific and trial effects as the random effects. All the analyses presented normally distributed residuals and showed no heteroscedasticity.

Risk-taking behavior was analysed by fitting a linear mixed model (formula = risk ~ group + time + group * time) estimated using REML. The follow-up analyses, including the payments received by the participants between sessions, were again estimated using REML, including the payments received as part of the MCG compensation (payment) and the participation fee from Session 1 (participation) (formula = risk ~ group + time + payment + participation + group * time).

The results of the MCG were analyzed again using linear mixed models. The effects of the probiotics protocol versus placebo on present-bias was estimated using REML, with group and session as the main factors. More importantly, we focus on the group*time interaction to evaluate the effects of the probiotics intervention (formula = present bias ~ group + time + group*time). The analyses of time discount were estimated with the same method and using REML (formula = time discount ~ group + time + group*time).

Declarations

Competing Interests statement

The authors declare no competing interests.

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Figures

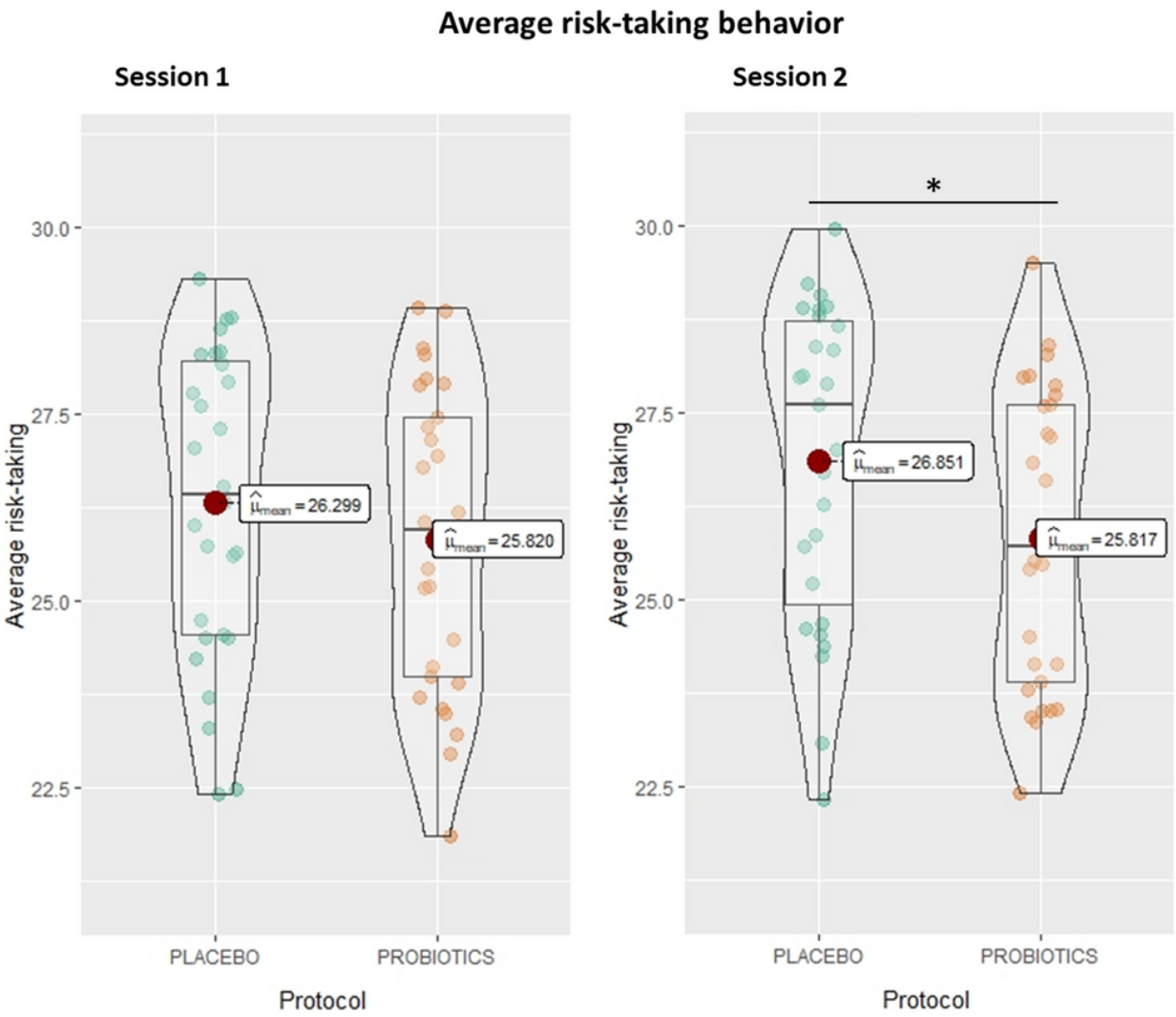

Figure 1

Average Risk-taking Behavior (n = 57) Average risk-taking estimated by the average standard deviation of each participant's choice across sessions and protocols (placebo in green and probiotics in orange). The MGT allows risk scores from 1.8 to 50. The present analyses show participants' average risk-taking scores. Average risk-taking scores vary between 21.84 and 29.31.

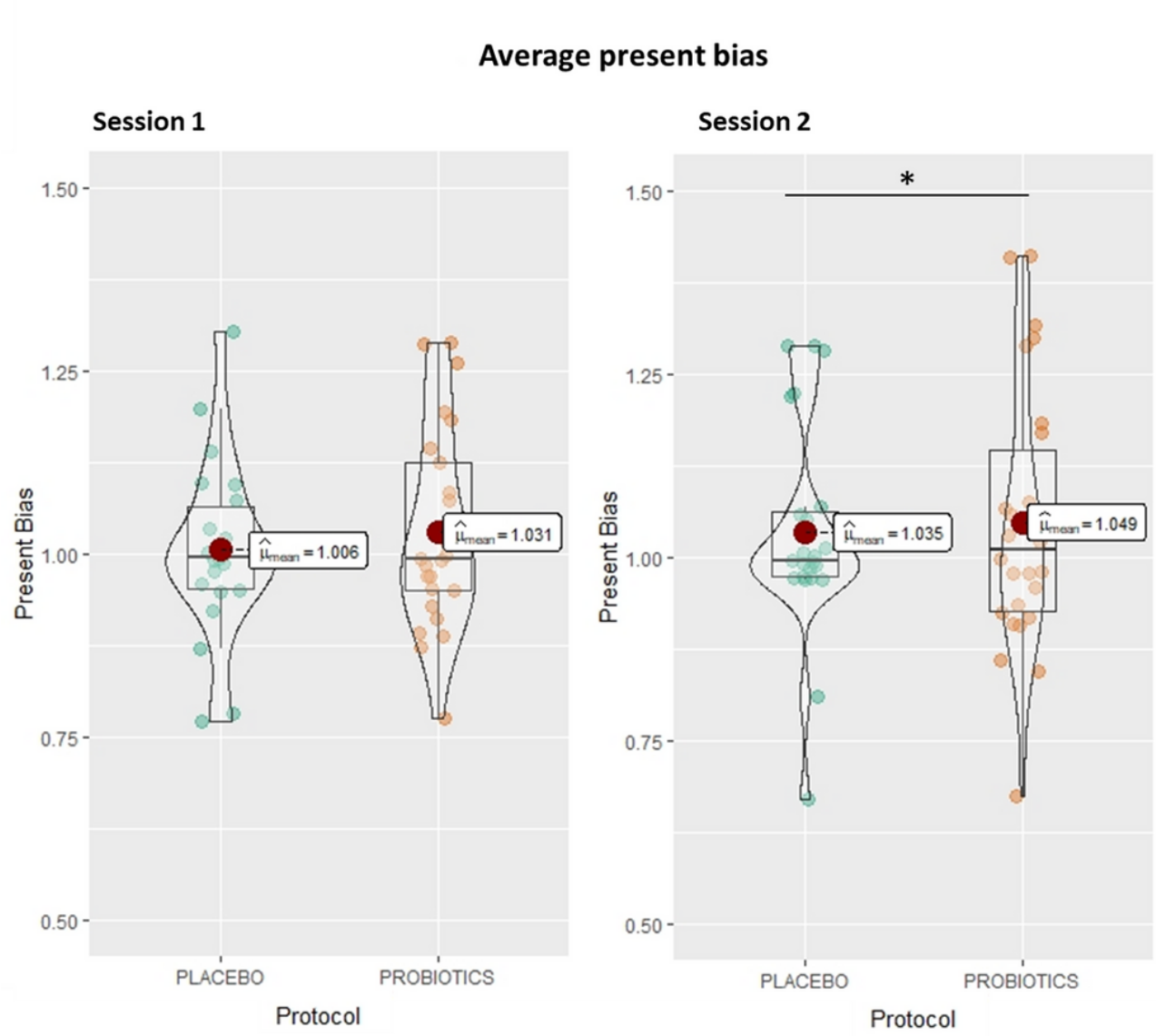

Figure 2

Average Present Bias (n = 57) Average present bias estimated by the model of convex budgets by Andreoni and Sprenger (2012). Participants' present bias (Beta) was estimated based on their responses during the MCG, with are averaged for each session. Blue circles represent participants' beta values for each session. The protocols are presented in different graphs (Placebo left and Probiotics right). Black bars indicate the 95% confidence interval of the linear model employed for data analyses. Values above 1 are interpreted as indicating future bias.

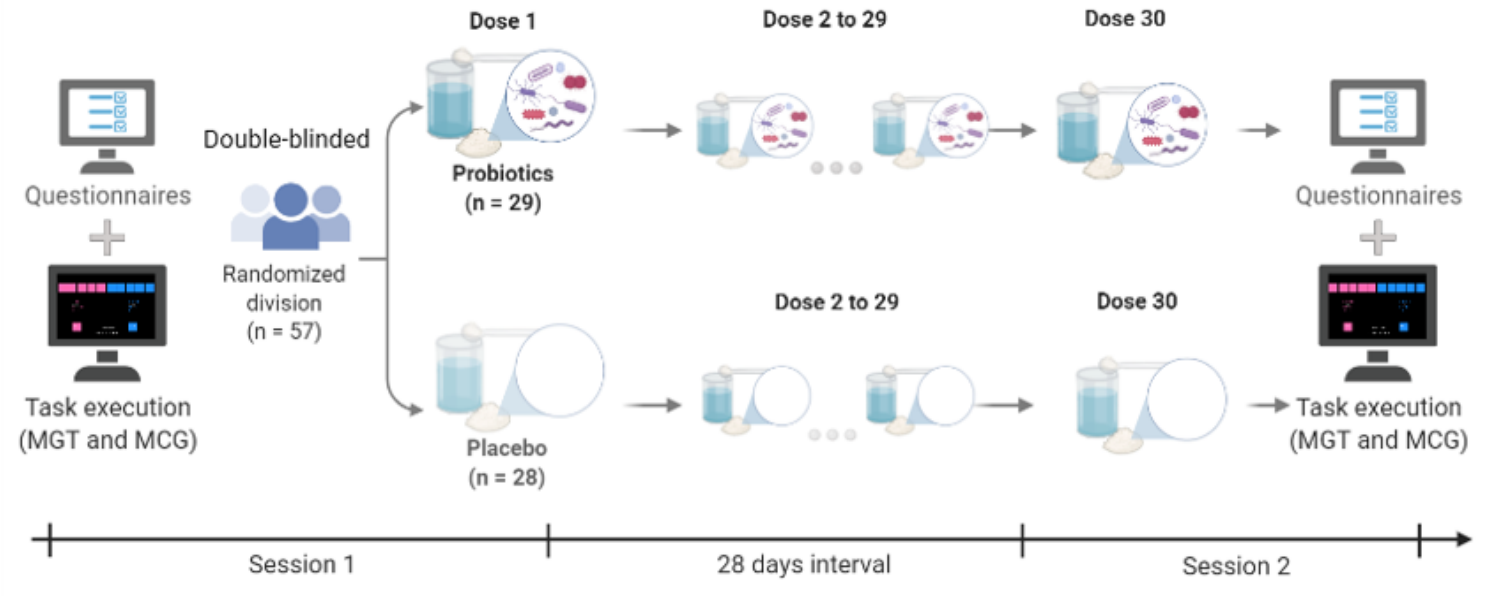

Figure 3

Experimental design Figure depicts the double-blinded placebo-controlled experimental design, along with the main procedures for both experimental sessions and the probiotics/placebo protocol followed by the participants during the 30 days of the experiment.

Figure 4

Example screens of the tasks used. Image A presents an example screen from the Maastricht Gambling Task (MGT), and image B shows an example screen from the Maastricht Choice Game (MCG).

Supplementary Files

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