Association between the Distal Gut Microbiome and Anxiety in Highly Active Individuals

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Abstract

Background: Traditional thinking is that physical activity benefits mental and physical health, however, excessive physical activity can increase anxiety, depression, and affect the gut microbiome. Considering the strong connection between the gut and the brain, the purpose of the present study was to evaluate the association between gut microbiota composition and anxiety as well as depression in highly active individuals.

Methods: Participants included 55 young adults (ages 18-25, 51% males). All participants were highly physically active, as determined by 7 days of SenseWear monitoring. Anxiety and depression were measured with the Beck Anxiety and Depression Inventories. Alpha diversity, beta diversity, and microbial composition were evaluated via 16S rRNA gene sequencing using distal gut samples.

Results: Greater anxiety was associated with both lower distal gut alpha diversity ($P < 0.05$) and higher beta diversity (PERMANOVA test; R-squared: 0.17562, $P = 0.027$), which appeared stronger in males. Genus level taxonomic abundance analysis showed *Prevotella* relative abundance as higher in males with higher anxiety ($P = 0.03$, $q=0.06$). However, adjusted linear regression analysis, controlling for fiber intake and sex nullified the association between *Prevotella* and anxiety. Additional analysis demonstrated a strong association between lower dietary fiber intake and higher anxiety scores (Est.= -0.48, SE= 0.20 , $P = 0.021$).

Conclusion: In highly active individuals, specifically males, there is a strong relationship between the gut microbiome, fiber intake, and anxiety. These data suggest highly active males with anxiety may benefit from increased dietary fiber intake.

Introduction

The gastrointestinal tract and the brain possess a bidirectional communication known as the gut-brain axis [1]. Recent research has suggested the gut microbiome as an important communication conduit between these two organs [1]. The gut microbiome plays a role in the establishment of neurogenerative processes such as the formation of the blood-brain-barrier [2, 3], myelination [4, 5], neurogenesis [6, 7], and microglia maturation [8–10], as well as sleep [11, 12], appetite [13, 14], cognition [15, 16], and mood [15, 17]. One of the proposed mechanisms of action through which bacteria communicate with the brain is by regulating the release of peptide hormones. Peptide hormones produced by the gut and the microbiota are able to diffuse into the systemic circulation where they can either bind to the vagus nerve receptors or bypass the blood brain barrier, enabling gut-brain communication [3, 18–25]. Bacterial diversity and composition influence the release of gut peptides such as glucagon-like peptide [26], serotonin [27], peptide YY [28, 29], corticotropin-releasing factor [30], oxytocin [31, 32], and ghrelin [33, 34]. It is possible that these gut peptides could in turn be associated with anxiety and depression, although further research is needed [18]. Likewise, communication between the microbiota and the brain also takes place through the presence of microbial membrane molecules and the production of microbial-derived

metabolites. The main microbial molecules that demonstrate an external response are lipopolysaccharides, which are molecules located on the external membrane of gram-negative bacteria that induce a pro-inflammatory response [35, 36]. Some of the metabolites produced by gut microbiota are: gamma-aminobutyric acid, serotonin, dopamine, norepinephrine, and acetyl-choline [35, 37–39]. These microbial molecules and metabolites stimulate afferent neurons and enterochromaffin cells located on the intestinal wall therefore conveying information to the nervous system [37, 39]. Afferent neurons extend their axons into the gut mucosa where they can interact with microbial components and metabolites, whereas enterochromaffin cells work as intestinal chemosensors [37, 39]. The neural signals initiated by these microbial molecules may travel to the nucleus tractus solitarius in the brain by passing through the vagus nerve [40]. Therefore, gut microbiota may be able to regulate mood through production of hormones, metabolites, and membrane molecules.

There is emerging evidence for a connection between the gut microbiota and anxiety and depression [41–47]. Overall, it has been demonstrated that 1) gut microbial composition differs across patients with major depressive disorder and healthy controls [41, 42], 2) pro- and pre-biotic supplementation have shown potential to decrease anxiety and depression [44–49], and 3) fecal microbial transplants (FMT) from patients with depression to germ-free mice results in the appearance of depression-like symptoms [50]. It is crucial to highlight, however, that although individuals with mood disorders possess a different gut microbiota composition, the high interindividual variability has precluded researchers from characterizing a “depression/anxiety-like” gut microbiota profile.

Regular physical activity reduces anxiety and depression, whereas excessive physical activity significantly increases these factors [51–53]. However, it remains unknown if the gut microbiome in highly active individuals is related to anxiety and depression, which is important as this population has been shown to have a different gut microbial composition [54–56]. Changes in the gut microbiota occur as a response to athletes’ different diet composition as well as gastrointestinal modifications in response to acute intense exercise such as splanchnic hypoperfusion and increased intestinal permeability [57–61]. Therefore, the purpose of the present study was to evaluate the association between gut microbiota composition and psychological variables (anxiety and depression) in highly active collegiate adults.

Methods

Study Population and Design

By using a cross-sectional design, we evaluated whether anxiety and depression were associated with distal gut microbiota diversity and composition. A total of 55 male ($n = 28$) and female ($n = 27$) participants, with a BMI between 18.5 to 27.0 kg/m², within 18 to 25y were recruited from September 2017 to March 2018. All eligible subjects were provided informed consent approved by the Institutional Review Board for Human Subjects at Baylor University (#1109598). All experimental procedures involved in the study conformed to the ethical considerations of the Helsinki Code.

During the first visit, participants were admitted to the study if they did not have any of the following exclusionary criteria: presence of metabolic (diabetes, cardiovascular diseases, etc.), autoimmune, or sleep disorders; having consumed antibiotics, probiotics or metformin in the last month; having suffered from any diarrheal or gastrointestinal infections in the last month; currently taking medications that affect sleep, anxiety or depression, as well as, currently consuming laxatives or fiber supplements; following a weight loss intervention; being a smoker; being vegetarian, and being pregnant. Eligible participants were scheduled for a second visit.

During the second visit, participants completed a general medical history questionnaire (history of diagnoses, previous surgeries, and current medications) as well as the Beck Anxiety and Depression Inventories. The Beck anxiety inventory is a 21-item self-report assessment of anxiety symptoms experienced over the previous month. We classified anxiety level scores from 0–21 as “very low anxiety”, from 22–35 as “moderate anxiety”, and above 36 as “high anxiety” [62]. The Beck Anxiety inventory has shown to have excellent internal consistency (alpha: 0.93) and good construct validity when compared to other anxiety scales [62, 63].

The Beck Depression Inventory is a 21-item self-report assessment of depression severity. Items are rated from 0 to 3 and include sadness, feelings about the present and the future, satisfaction, guiltiness, feeling of being punished, self-dislike, self-criticalness, suicidal thoughts or wishes, crying frequency, agitation, loss of interest, indecisiveness, worthlessness, energy loss, sleeping pattern changes, irritability, changes in appetite, concentration difficulty, tiredness or fatigue and loss of interest in sex. We classified participants whose summed scores < 10 as “low depression, 10–18 as “mild depression” and > 18 as “moderate to severe depression” [62]. The Beck depression inventory has excellent internal consistency (alpha: 0.92), high content validity, validity in differentiating between depressed and non-depressed subjects, sensitivity to change, and international propagation [62, 64].

Objective Assessment of Physical Activity

SenseWear™ monitor (BodyMedia Inc., Pittsburgh, PA, USA) was utilized in the present study to objectively measure physical activity duration, energy expenditure, and number of steps per day. Biometric information including date of birth, sex, height, weight, and handedness of the participant were introduced in the equipment before providing the participants with the armband. SenseWear™ was worn on the non-dominant upper arm, over the triceps muscle. During visit 3, participants were instructed to wear the armband for 7 continuous days, removing it only to shower and to charge the monitor according to manufacturer guidelines. Compliance was directly measured, as skin contact is needed in order for the device to collect data. Adherence time is expressed as the average time per day at which each monitor was used out of 24 h. A day was considered valid if the monitor was worn at least 22 h; duration that was determined according to estimated non-wear time spent on personal grooming (e.g. bath, shower) and required charging time. Once the participants concluded the study, SenseWear™ data were downloaded into the computer using the SenseWear™ 7.0 Professional Software (2012; Pittsburg, PA). Physical activity variables included average time spent in physical activity, average time spent in physical activity including only bouts above 10 min, average energy expenditure, and average number of steps per day.

Days were divided from midnight to midnight for analysis involving physical activity. For the purpose of this research, any activity with energy expenditure between 0 and 3 METs was considered as sedentary, whereas activities above 3 METs were classified as physical activity.

Dietary Assessment

Participants filled out a 24 h food log evaluating the food consumption of the day prior to the stool sample collection. Food log included columns for food (including brand or restaurant), cooking method, and amount (in grams or household measure). Participants were instructed on how to properly fill out this form by specifying the food ingredients of each of their meals. Energy intake, macronutrient composition, fiber content, and fruits and vegetables consumption were assessed by a nutritionist using the ESHA Research Food Processor Nutrition Analysis Software (Salem, OR).

Stool Sample Processing and Analysis

During the second visit (2–3 days after visit 1), participants were provided with a stool sample kit (OMNIgene® Gut kit) and were asked to follow directions to collect a stool sample prior their third visit (3–5 days after visit 2). OMNIgene Gut kits keep samples stable at ambient temperature for up to 60 days [65, 66]. Sample tubes were frozen at -80 degrees immediately upon receipt, and later sent on dry ice to Baylor College of Medicine Alkek Center for Metagenomics and Microbiome Research for 16S rRNA gene sequencing and bioinformatic analysis.

Stool samples were analyzed using high throughput sequencing of the V4 region of the 16S rRNA gene in three batches, the first batch contained 30 samples, whereas the second and third batch contained 15 samples each, with the same control sample for each batch across sequencing runs. The 16S rRNA gene sequencing methods were adapted from the methods developed for the Earth Microbiome Project and NIH-Human Microbiome Project [67–69]. Briefly, bacterial genomic DNA was extracted using MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories). The 16S rDNA V4 region was amplified by PCR and sequenced in the MiSeq platform (Illumina) using the 2 × 250 bp paired-end protocol yielding pair-end reads that have near complete overlap. The primers used for amplification contained adapters for MiSeq sequencing and single-index barcodes to enable pooling of PCR, which were sequenced directly, targeting at least 10,000 reads per sample.

The read pairs were demultiplexed based on unique molecular barcodes added via PCR during library generation, then merged using USEARCH v7.0.1090 [70]. The subsequent analysis steps of the pipeline leveraged custom analytic packages (developed at the CMMR) to produce summary statistics and quality control measurements for each sequencing run, as well as multi-run reports and data-merging capabilities for validating built-in controls and characterizing microbial communities across large numbers of samples or sample groups as previously described [71]. 16S rRNA gene sequences were clustered into operational taxonomic units (OTUs) at a similarity cutoff value of 97% using the UPARSE algorithm [72]. OTUs were subsequently mapped to an optimized version of the SILVA Database [73] containing only sequences from the V4 region of the 16S rRNA gene to determine taxonomies. Abundances were recovered by mapping the demultiplexed reads to the UPARSE OTUs. Alpha diversity,

beta diversity, and relative taxonomic abundance were evaluated and were also calculated with the CMMR pipeline (Supplementary Table 1).

Statistical Analyses

An a priori sample of 26 participants per group was determined to be required to have 80% power to detect significant two-tailed associations of effect size ≥ 0.5 , with alpha of 0.05. According to these numbers, the sex-stratified population would allow us to detect moderate to strong correlations. Sex stratification was performed as previous research has established a sex-differential connection between gut microbial composition and health biomarkers [74–77]. After being divided by sex, all variables were checked for normality by using Shapiro-Wilk tests, histograms, and QQ plots.

Alpha diversity, which measures the taxonomic diversity within a sample, was measured through four diversity metrics: number of unique OTUs, Shannon index, Inverse Simpson, and Fisher indexes. Number of OTUs represents microbial richness, which is the number of different species in a habitat/sample without taking into consideration their abundance [78]. Shannon index takes into consideration microbial richness and evenness, although it is more sensitive to richness [79]. Inverse Simpson estimates the probability that two random reads from a particular sample come from different taxa, with a higher value representing greater diversity [80]. Fisher index describes the relationship between the number of species and the number of individuals of the corresponding species by using logarithmic distribution [81]. Fisher index is not influenced by the sample size and is less affected by the abundance of the most common species [80].

Relationships between non-taxonomic variables were analyzed through Pearson correlations. Two complimentary approaches were taken to identify taxa associated with anxiety, linear regression analysis and linear discriminant analysis effect size (LEfSe) [82]. LEfSe determines the OTUs that are most likely to explain differences between classes, using linear discriminate analysis to estimate the effect size of each differentially abundant feature [83].

To determine associations between taxonomic differential abundance and Beck Anxiety/Depression scores, microbiome data were plotted using Log10 normalized counts from taxonomic assignment at the genera level. Multiple hypothesis correction (Benjamini-Hochberg procedure) was applied to all p-values (< 0.05), and q-values < 0.2 were determined to be significant given the small sample size [84, 85]. In addition, linear regression analysis was performed on Log10 normalized counts from taxa found to be associated with anxiety in LDA testing, with multiple hypothesis correction applied to all p-values to generate q-values. LEfSe at the genera level was utilized to identify differences in bacterial abundance between groups. Further, *a priori* dietary fiber intake (calorie corrected) and sex were chosen as factors for adjustment in linear regression with *Prevotella* abundance as a predictor of anxiety scores. Separately, linear regression analysis between anxiety scores in males and fiber intake (calorie corrected) was performed.

Results

A total of 114 participants underwent screening with 60 satisfying inclusion criteria and completing all aspects of the study. Five participants were excluded from statistical analysis because they did not satisfy the minimum time (23 h per day) required for wearing the SenseWear monitor to guarantee their monitor data would accurately represent their normal physical activity patterns. In the remaining 55 subjects, the average wearing time of the monitors was 23 h 20 min $\pm$ 12 min/day. Among participants, 49% were females and 62% classified themselves as being White. Ethnicity stratified by sex is shown in Table 1. There were no differences in ethnicity between sex. All participants ranged in age from 18–25 years, with an average age of 20.8 years (SD = 1.7), with no significant age differences between male and females. Male participants showed significantly greater physical activity than females measured as total physical activity time ($P < 0.001$), physical activity longer than 10 min ($P < 0.001$), and energy expenditure ($P < 0.001$). The prevalence of depression (above normal) and anxiety (moderate levels) was 18.5% and 11% in females, and 16.8% and 7% in males (no significant differences between males and females; Figs. 1 and 2). No significant sex differences in gut alpha diversity were found with exception of the Inverse Simpson measure, which showed a significantly higher alpha diversity in female than males.

Table 1
Distribution of variables by gender.

Variable	Total (n = 55)		Female (n = 27)		Male (n = 28)		P
	Average	SD	Average	SD	Average	SD	
Ethnicity							
White	34		17		17		0.960
Hispanic	9		4		5		
Asian	7		3		4		
Others	5		3		1		
Age							
Years	20.84	1.72	20.6	1.8	21.1	1.6	0.238
Anthropometrics							
Height (m)	1.7	0.1	1.7	0.1	1.8	0.1	0.000***
Weight (kg)	158.0	28.2	138.2	17.3	177.0	23.3	0.000***
Waist Circumference (cm)	79.4	8.6	75.3	8.1	83.4	7.1	0.001***
Hip Circumference (cm)	98.5	6.9	97.5	6.9	99.5	6.8	0.297
BMI (kg/m2)	23.8	2.4	23.0	2.2	24.7	2.3	0.007**
Physical Activity							
Physical Activity (h:min)	2:32	1:23	1:48	0:47	3:15	1:28	0.000***
Physical Activity > 10 m (h:min)	1:47	1:22	1:04	0:43	2:28	1:28	0.000***
Number of Steps	8919.8	3491.3	8069.1	2898.4	9740.0	3855.8	0.076
Energy Expenditure (Kcal)	2703.9	720.8	2138.6	238.9	3249.0	597.2	0.000***
Sedentary with sleep (h:min)	17:10	1:54	17:47	1:38	16:32	1:58	0.017*

P values represent comparison between genders.

* Significance at the 0.05 level (2-tailed).

** Significance at the 0.01 level (2-tailed).

*** Significance at the 0.001 level (2-tailed).

Variable	Total (n = 55)		Female (n = 27)		Male (n = 28)		P
	Average	SD	Average	SD	Average	SD	
Sedentary no sleep (h:min)	10:17	1:47	10:29	1:34	10:04	1:59	0.396
Psychological variables							
Anxiety (Beck Inventory)	8.93	7.54	10.26	8.59	7.64	6.26	0.201
Depression (Beck Inventory)	6.44	5.31	6.96	5.63	5.93	5.03	0.475
Alpha Diversity							
Observed OTU	81.22	25.89	84.59	28.49	77.96	23.16	0.347
Shannon	2.54	0.68	2.68	0.74	2.40	0.60	0.132
Simpson	0.80	0.16	0.83	0.17	0.78	0.16	0.278
Inv Simpson	8.42	5.54	9.95	6.03	6.94	4.67	0.042*
Fisher	14.13	5.46	14.87	6.08	13.43	4.79	0.331
P values represent comparison between genders.							
* Significance at the 0.05 level (2-tailed).							
** Significance at the 0.01 level (2-tailed).							
*** Significance at the 0.001 level (2-tailed).							

To determine if depression or anxiety were correlated with community structure, we first conducted a Pearson correlation analysis between depression or anxiety with alpha diversity. Depression was not associated with alpha diversity in males or females. However, interestingly, anxiety showed a strong negative correlation with alpha diversity in male participants (Shannon index $r = -.552$, $P = .003$, and Inverse Simpson $r = -.422$, $P = .028$) (Supplemental Fig. 1A and 1B) such that low anxiety in males was associated with a higher number of bacterial species. A similar pattern emerged when examining β -diversity, a measure of the dissimilarity in community composition between two individuals or samples. β -diversity was significantly associated with anxiety in male participants (PERMANOVA, $P = 0.027$) (Fig. 3), but not in female participants.

Given this association between anxiety and community dissimilarity, we next examined the association between genus level taxonomic abundance and anxiety using two complimentary approaches. First, we ran a linear regression analysis correcting for multiple hypothesis testing. We calculated a false discovery rate q-value for each genus and applied it for the all taxa using a q-value cutoff < 0.2 [84, 85]. Using this approach, we identified *Prevotella* as significantly associated with anxiety (Fig. 4). Second, we conducted

linear discriminant analysis, which detected 25 bacterial taxa able to identify the different anxiety categories (Fig. 5). *Prevotella* and *Catenibacterium* showed significantly higher relative abundance in participants with “moderate anxiety”; *Paraprevotella*, *Lachnospiraceae-UCG-004*, *Lachnospiraceae-NK4A136*, *Ruminococcus*, *Fusicatenibacter*, *Blautia* and *Butyricimonas* were more abundant in low anxiety participants; whereas *Bacteroides*, *Eubacterium rectale*, *Lachnoclostridium*, *Lachnospiraceae-UCG* and *Barnesiella* were more abundant in very low anxiety participants (Fig. 5). A separate linear regression was performed between *Prevotella* and anxiety scores, which showed *Prevotella* to be positively associated with anxiety levels in males ($P = 0.03$, q-value = 0.06) (Fig. 6A). Together these data indicate a relationship between higher abundance of *Prevotella* and higher anxiety levels in highly active males.

Lastly, given the strong link between dietary fiber intake and gut microbiome composition, we analyzed the relationship between dietary fiber intake (calorie corrected), *Prevotella* relative abundance and anxiety scores. In adjusted linear regression analysis, controlling for fiber intake and sex, the association between *Prevotella* and anxiety scores was nullified; however, we did observe a strong association between lower dietary fiber intake and higher anxiety scores (Est. = -0.48, SE = 0.20, $P = 0.021$). A separate linear regression analysis between anxiety scores in males and dietary fiber intake also demonstrated a significant inverse relationship ($P = 0.014$) (Fig. 6B).

Discussion

Our results demonstrate that higher anxiety scores were associated with lower alpha diversity in highly active male participants [31, 86]. This is the first study showing that the majority of highly active males with low to very low anxiety had more similar microbial composition, while those with moderate anxiety were more dissimilar in comparison. Sex-specific connections between gut microbial composition and health biomarkers have been previously identified [74–77], indicating a need for sex-specific interpretation of gut microbial research.

While previous research in different populations has connected psychological symptoms (or disorders) to bacterial diversity, the current work advances the literature by identifying the specific genus driving this association. In this respect, we interrogated the individual taxonomic abundance and observed a strong association between anxiety and *Prevotella* in highly active males but not females. *Prevotella* has been found to be higher in males versus females [87, 88], and in patients with depression [41, 89], insulin resistance [90], non-alcoholic fatty liver disease [91], hypertension [92], and colon cancer [93]. However, paradoxically, *Prevotella* has also been shown to be related to fiber consumption (due to its capacity to digest and ferment complex carbohydrates) [94–97] and to be higher in vegetarians and rural communities [97]. Therefore, it is possible that the observed correlation between *Prevotella* and anxiety is not a causal relationship but rather an indirect one. This is, if higher anxiety levels are positively correlated with physical activity levels, it could be that the higher *Prevotella* is in fact directly related to higher physical activity and not anxiety *per se*. However, we did not observe a significant correlation between physical activity levels and anxiety scores in our male participants ($p = 0.32$).

Another potential explanation is the presence of several strains within the *Prevotella* genus that exert pathogenic actions, which could help to explain the bi-directional and almost opposing effects that *Prevotella* has shown to have in human health [97]. It is also possible that the effects of *Prevotella*'s on the host depend on the co-presence of other bacteria, as research has shown syntrophic relationships between different bacterial species [98–100]. This is, if the gut microbiota has insufficient amounts of SCFA-producing bacteria, and/or if there is an overpopulation of mucin degrading bacteria (i.e. *Prevotella*) likely due to insufficient fiber consumption, there is a risk for increased intestinal permeability and potential bacterial translocation from the intestinal lumen into the systemic circulation thus creating inflammation [101, 102]. Therefore, it is possible that the interpretation of *Prevotella* in the gut could depend on the concentration of other bacterial species that are co-habiting the intestine. Lastly, because of the bidirectional communication between the gut and the brain, we can't discard the possibility of anxiety regulating food consumption [103] and therefore gut microbiome composition through nutrient availability. In this case the relationship between *Prevotella* and anxiety would not be a causative.

Our results also showed a significant negative association between fiber intake and anxiety levels in active male participants. Consistent with our results, previous research has also found both sex and fiber intake to affect the relationship between mood and the gut microbiota [104]. In addition, a literature review found an improvement in anxiety and depression when fiber intake (from fructooligosaccharides and galactooligosaccharides) increased > 5 g/day [105]. Experimental animal research seems to point towards a similar direction, with fiber consumption decreasing anxiety-like behavior to a stressful event [106]. However, it is also possible that changes in fiber intake are reflective of other dietary changes that have shown to directly affect mood (omega 3, vitamin C, tryptophan, etc.) [107–110]. In this case, it could be that the other nutrients explain the relationship between diet and anxiety levels and not merely fiber intake.

One of the limitations of the present study is the possibility of other psychological variables affecting the relationship between anxiety with gut parameters; however, this study attempted to minimize the potential effect of extraneous variables by careful selection of participants. A second limitation of the study was the lack of control of female's menstrual cycle which could have created higher variations in gut diversity as it has been shown that food consumption (a main modulator of gut microbiota composition) is altered through the menstrual cycle [111]. Lastly, a third limitation of the study is the inability of 16S rRNA to identify gut microbiota composition down to the strain level as well as to provide information regarding gut microbial functionality.

Conclusion

The present study conducted in highly trained participants found 1) a negative relationship between anxiety and gut alpha diversity, 2) a higher beta diversity with higher anxiety levels, 3) a positive correlation between anxiety and *Prevotella*, and 4) a lower anxiety with higher fiber consumption. Further

research in highly active individuals will be needed in order to fully elucidate the sex-specific connection between gut bacterial strains and anxiety as well as depression.

Clinical Perspective

The study of psychobiotics has shown promising results in regard to the effect of probiotics on anxiety and depression, however, this area of research is still on its infancy [112, 113]. Probiotic supplementation of a single or few strains may augment the population of that specific microbe(s) and therefore change overall gut microbial function [114]. This effect may be sought in patients with depressive or anxiety disorders, however, whether healthy adults can be beneficated by the consumption of a single or few strains of probiotics is still not well understood [112]. FMT provide a wider array of microbes that have shown to reduce anxiety and depression; however, they also involve risks such as unintentional transplantation of multi-drug resistant organisms [114, 115]. Novel psychobiotics would create an FMT supplement-like probiotic to provide patients with the positive effects of FMT without the detrimental consequences. Currently, however, the most prudent recommendation for healthy individuals is to consume a variety of different sources of fiber-rich food to maintain a gut microbial composition that has been related to positive health outcomes.

Abbreviations

FMT

fecal microbial transplant

LEfSe

linear discriminant analysis effect size

OTU

operational taxonomic units

Declarations

Ethics approval and consent to participate: All eligible subjects were provided informed consent approved by the Institutional Review Board for Human Subjects at Baylor University (#1109598). All experimental procedures involved in the study conformed to the ethical considerations of the Helsinki Code.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: Authors have no conflicts of interests to declare in relation to this article.

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performance, and Recreation Department at Baylor University.

Authors' contributions: EMM conceived the initial research idea, obtained the funds, coordinated the research team, and wrote the initial draft of the article. EF, PH, CSK, and SS collaborated with data collection. KLG and NP assisted with statistical analysis and graphs design. KLG, PG, and MKS provided feedback throughout grant writing, study design, collection process, data analysis, and article writing. All authors read and approved the final manuscript.

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Supplemental Figures

Supplemental Fig. 1A and 1B. Linear regression analysis between Beck Anxiety scores and Alpha diversity. A) The Shannon diversity (left) and B) Simpson diversity (right) were regressed on Beck Anxiety scores in males ($n = 28$). Significance was estimated at $p < 0.05$ based simple linear regression analysis.

Figures

Figure 1

Anxiety levels by gender. Each dot represents a participant, dark lines denote the cutting point for the different anxiety classifications according to the Beck Inventory.

Figure 2

Depression levels by gender. Each dot represents a participant, dark lines denote the cutting point for the different depression classifications according to the Depression Inventory.

Figure 3

β -Diversity among males by level of anxiety. Ordination plot representing β -diversity stratified by anxiety score; very low (score <10), low (score <20), and moderate (≥ 20). Beta diversity analysis was calculated using weight UniFrac distance measure; Statistical significance was calculated using PERMANOVA testing; R-squared: 0.17562; p-value < 0.027 .

Figure 4

Taxonomic differential abundance in males by Beck Anxiety score. Log 10 normalized counts from taxonomic assignment at the genera level were used in a linear regression to calculate the co-efficient of variation with Beck Anxiety scores in males. Multiple hypothesis correction was applied to all p-values, and q-values <0.2 were determined to be significant (dashed line). The only taxa passing this threshold was *Prevotella* (dot below dashed line).

Figure 5

Linear Discriminant Analysis (LDA) of differentially abundant features. Anxiety scores were divided into Very low (score <10), Low (score <20), and Moderate (≥ 20). Log transformed counts from taxonomic assignment at the genera level were used to calculate the effect size based on Beck Anxiety scores in males. Features are considered to be significant based on their adjusted p-value. The adj. p-value was considered significant at $p < 0.05$.

Figure 6

Association between Prevotella abundance and Fiber intake with Beck anxiety scores in males. A) Log 10 normalized counts from reads assigned to Prevotella, or B) calorie corrected fiber intake were used in a linear regression to calculate the co-efficient of variation with Beck anxiety scores in males. Multiple hypothesis correction was applied to all p-values, and q-values were calculated for taxonomic abundance analysis.

Supplementary Files

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