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Full Length Article

Acute intake of *B. longum* probiotic does not reduce stress, anxiety, or depression in young adults: A pilot study

Michael P. Siegel [a,b], Sarah M. Conklin, Ph.D. [a,*]

[a] Neuroscience Program and Psychology Department, Allegheny College, USA
[b] Geisinger Commonwealth School of Medicine, USA

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ABSTRACT

Background: The gut microbiome communicates bidirectionally with the brain, linking the gut to psychological phenomena such as stress, depression, and anxiety. Probiotics, or ingestible supplements containing billions of mutualistic bacteria, have demonstrated the mechanistic potential to influence mood; however, few studies have experimentally examined the acute effects of these compounds on individuals not recruited for psychopathology or gut dysfunction. The present study hypothesized reductions in stress, anxiety and depression symptoms following an acute, one week dosing period of *B. longum* intake.

Methods: The efficacy of a one-week period of orally administered *B. longum* was tested utilizing a double-blind experimental design. Participants were randomly assigned to either placebo or probiotic capsules under double blinded conditions and completed the Perceived Stress Scale (PSS), the Center for Epidemiological Studies Depression scale (CES-D), and the State-Trait Anxiety Inventory (STAI Y2 form) to assess for differences before and after one-week intervention.

Results: No significant reduction in symptoms between groups over the one-week period was found.

Conclusions: These findings suggest that 7-days of B. longum does not reduce stress, depressive symptoms, or anxiety in generally healthy young adults.

1. Introduction

The gut communicates bidirectionally with the brain via the gut-brain axis (GBA) (Foster and Neufeld, 2013; Carabotti et al., 2015; Cussotto et al., 2018). The GBA is comprised of segments of the central and enteric nervous systems, connecting higher-function cerebral regions such as the hippocampus and amygdala with peripheral intestinal function (Foster and Neufeld, 2013; Carabotti et al., 2015). This axis is mediated by bidirectional communication between the brain and microbiota, which have been shown to modulate immune (Carabotti et al., 2015; Mayer et al., 2015), nervous (Cussotto et al., 2018), and endocrine functions (Carabotti et al., 2015; Cussotto et al., 2018).

One mechanism by which the GBA is thought to communicate is via the hypothalamic pituitary adrenal (HPA) axis. The HPA axis coordinates organismal responses to both exogenous and endogenous stressors (Carabotti et al., 2015). Elevated HPA-axis responses were elicited among mice maturing in a germ-free environment when responding to confinement stress, thereby indicating a stress-reducing, mutualistic effect of bacteria (Foster and Neufeld, 2013). Another study with rats showed that *B. longum* reduced serum levels of pro-inflammatory cytokines involved in the HPA-axis (Bisson et al., 2010). Another study observed changes in hippocampal neurogenesis and hypothalamic genes involved in synaptic plasticity with *B. longum* (Carabotti et al., 2015). These changes were thought to be induced by changes to the gut microbiome, linking the HPA axis to the GBA.

The GBA may also modulate this bidirectional communication via neurotransmitter (NT) production and secretion from various strains of mutualistic bacteria. *Lactobacilli* and *Bifidobacteria* spp. interact with NTs such as acetylcholine, gamma-aminobutyric acid (GABA), and serotonin (5-HT) (Desbonnet et al., 2008; Cussotto et al., 2018). In fact, *Lactobacilli* and *Bifidobacteria* spp. produce both GABA and 5-HT inducing anxiolytic and depressiolytic effects (Bravo et al., 2011; Cussotto et al., 2018). One strain, *B. infantis,* altered 5-HT and tryptophan kinetics, inducing depressiolytic effects (Desbonnet et al., 2008; Yano et al., 2015).

Probiotics are hypothesized to achieve such effects by introducing beneficial microbiota like *B. longum* and many others in order to strengthen the gut-mucosal barrier and modulate immune function via changes in membrane permeability (Holzapfel et al., 1998; Abdel-Haq

* Corresponding author. Neuroscience Program and Psychology Department, Allegheny College, 520 N. Main St, Box 39, Meadville, PA, 16335, USA.
E-mail address: sconklin@allegheny.edu (S.M. Conklin).

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et al., 2018). Furthermore, bacteriocin production from strains like *B. longum* can further affect the gut microbiome by inhibiting the growth of harmful strains (Fooks and Gibson, 2002). By changing gut ecology, probiotics can affect NT levels, HPA-axis reactivity, and complex psychological processes.

One study linked the oral consumption of a probiotic formulation, consisting of *L. helveticus* and *B. longum*, to significant decreases in perceived stress (Messaoudi et al., 2011). Another showed how *B. bifidum* reduced chronic stress in college students within 2-3 weeks (Langkamp-Henken et al., 2015). *B. longum* has been shown to limit increases in stress as determined by the socially evaluated cold pressor test and by cortisol levels in humans (Allen et al., 2016). In that study, perceived stress was significantly reduced in participants following four weeks of daily probiotics. However, one study assessing acute effects of a two-week multi-strain probiotic intervention found no difference in cardiovascular reactivity to cognitive stress (Möller et al., 2017). Studies assessing psychological changes associated with probiotic intake have also yielded mixed outcomes. Neither the dose nor the duration of the interventions seem to predict efficacy (Mayer et al., 2015; Wallace and Milev, 2017; Cussotto et al., 2018). Neuroimaging research has yielded similarly conflicted findings. While one neuroimaging study found beneficial cognitive improvements upon *B. longum* administration corroborated by electroencephalography (EEG) (Allen et al., 2016), a neuroimaging study testing the efficacy of a multi-strain probiotic containing both *Lactobacilli* and *Bifidobacteria* spp. found no such cognitive changes indicated by lack of between-group differences on functional magnetic resonance imaging (fMRI) (Papalini et al., 2019). This study, however, showed fMRI evidence of HPA-axis modulation as a result of using the probiotic mixture, demonstrating an effect on stress-related cognition (Papalini et al., 2019).

How the microbiome, and the addition of probiotics, impacts mood and cognition is not yet well-understood. Nevertheless, various strains of probiotics have elicited salutary effects on stress, depression, and anxiety over a range of doses, mixtures, and durations across human and animal studies. Given that *Bifidobacteria* has been found to mutually benefit human health via bidirectional GBA communication linked to HPA-axis (Messaoudi et al., 2011; Carabotti et al., 2015; Allen et al., 2016; Agustí et al., 2018; Cussotto et al., 2018), the current study aimed to test the hypotheses that a very acute, 7-day administration of *B. longum* would show salutary effects on (1) stress, (2) depression, and (3) anxiety in young adults.

2. Materials & methods

2.1. Participants

Participants (N = 84) consisted of undergraduate students and were recruited through Sona Experiment Management System, an online research recruitment system designed for recruiting research participants. Study methods were approved by the College Ethics Committee.

2.2. Questionnaires

The Perceived Stress Scale (PSS) was used to assess perceived stress. The PSS is a widely used, 10-item, questionnaire that measures the degree to which a participant's life is perceived as stressful (Cohen and Williamson, 1988). Participants were asked to report the degree to which they have felt a certain way, with responses ranging from *never* (0) to *very often* (4) (Cohen and Williamson, 1988; Deckro et al., 2002). Recent data showed that an average PSS score among an 18–29 year old cohort (N = 645) was 14.2(6.2) (Cohen and Williamson, 1988).

The Center for Epidemiological Studies Depression scale (CES-D) was used to assess depression in participants. The CES-D is a 20-item questionnaire that measures depressive symptoms among the general population (Radloff, 1977). Participants were asked to report how they felt over the past week, with responses ranging from *rarely* (0) to *mostly* (3) (Radloff, 1977). An average CES-D score was 9.25(8.58), (N = 2514) (Radloff, 1977). This questionnaire allowed for assessing weekly changes in depression, the duration of the intervention period.

Table 1
Sample demographic statistics.

Demographic Characteristics	Placebo (N = 39)	Probiotic (N = 40)	*t or* χ^2 *scores*	*p-value*
Age	19.9(1.1)	19.4(1.0)	t = 2.22	$p < 0.05$
Sex (% female)	76.9	70.0	$\chi^2 = 2.11$	$p = 0.35$
Ethnicity (% white)	71.8	75.0	$\chi^2 = 1.88$	$p = 0.60$
Previous use of probiotics			$\chi^2 = 3.46$	$p = 0.49$
never	56.4	45.0		
1-2 times/year	12.8	25.0		
1-2 times/month	12.8	12.5		
1-2 times/week	10.3	15.0		
3 + times/week	7.7	2.5		
Previous use of antibiotics[a]			$\chi^2 = 3.78$	$p = 0.44$
1-5 times	43.6	52.5		
5-20 times	38.5	35.0		
≥ 20 times	2.5	5.0		
Not sure	15.4	5.0		

Age was reported as average(standard deviation); all other *Demographic Characteristics* were reported as % frequency.

[a] *0 times* was not displayed but was still used upon analysis. One participant from the probiotic group chose this response.

The State-Trait Anxiety Inventory (STAI) is a two-part questionnaire that was used to assess state and trait anxiety in participants. It is divided into two, 20-item sections, namely the Y1 and Y2 forms assessing state and trait anxiety, respectively (Spielberger et al., 1970). Both its validity and reliability have been tested, demonstrating strength within both metrics (Spielberger et al., 1970). Participants were tasked with answering questions on a Likert scale from *not at all* (1) to *very much so* (4). The STAI has been used to assess anxiety level changes upon probiotic use (Allen et al., 2016). The distribution of Y1 and Y2 STAI forms are as follows: (Y1) women (N = 210) 36.17(10.96), men (N = 446) 36.54(10.22); (Y2) women 36.15(9.53), and men 35.55(9.76) (Spielberger, 1983).

2.3. Probiotic supplement

The probiotic supplement contained *B. longum* in powder form. The probiotic was presented in capsules, each containing ~4.0×10^{10} colony-forming units (CFUs) that were taken orally (Ait-Belgnaoui et al., 2012). The capsules were each filled with 400 mg (4.0×10^{10} CFUs) of *B. longum* powder; placebo capsules contained equivalent volumetric measures of corn starch (Kailaspathy and Chin, 2000). Gelatin capsules size-000 were used to create both supplement and placebo capsules. A capsule-filling machine (100 capsules/batch) was used to create batches of capsules with approximately equal quantities of either probiotic or cornstarch. The capsules were then filled into containers, which were coded A-J. The coding was unknown to the researchers who were administering the tests. Two capsules/day were placed into the containers, containing either the probiotic (400 mg/capsule *B. longum*) or placebo (400 mg/capsule cornstarch).

2.4. Protocol

Participants provided informed consent and completed study questionnaires online. They scheduled a time to pick up a 7-day pill container filled with either probiotics or placebo capsules as well as study instructions. Group assignment was double blind. Randomization was carried out via random number generation upon pre-test completion. The

Table 2
Mixed-model ANOVA analyses of questionnaire responses.

Questionnaires	Placebo		Probiotic		*F-within* *F-between* *F-interaction*	*p-value*
	T1	T2	T1	T2		
PSS	19.7(6.5)	18.0(6.9)	20.4(5.9)	18.0(5.7)	$F(1,77) = 24.20$	$p < 0.05$
					$F(1,77) = 0.060$	$p = 0.81$
					$F(1,77) = 0.810$	$p = 0.37$
CES-D	18.6(9.9)	16.3(10.9)	19.6(9.8)	16.2(9.4)	$F(1,77) = 15.31$	$p < 0.05$
					$F(1,77) = 0.550$	$p = 0.46$
					$F(1,77) = 0.053$	$p = 0.82$
STAI (Y1)	41.0(11.4)	40.1(11.4)	41.3(12.2)	39.5(11.0)	$F(1,77) = 2.016$	$p = 0.16$
					$F(1,77) = 0.002$	$p = 0.96$
					$F(1,77) = 0.234$	$p = 0.63$
STAI (Y2)	44.0(12.1)	43.2(12.3)	46.7(11.4)	44.3(11.0)	$F(1,77) = 4.728$	$p < 0.05$
					$F(1,77) = 0.562$	$p = 0.46$
					$F(1,77) = 1.137$	$p = 0.29$

T1, pre-experimental scores; T2, post-experimental scores; PSS, Perceived Stress Scale; CES-D, Center for Epidemiological Studies of Depression scale; STAI (Y1/2), Y-1/2 forms of the State-Trait Anxiety Inventory.

control group was given a one week-supply of placebo cornstarch in capsule form, while the experimental group was given the *B. longum* powder in capsule form. Participants were instructed to (1) ingest two capsules/day for a one-week period, (2) to not throw out missed doses, and (3) to not ingest more than the daily. Containers were returned and pills were counted as a compliance measure. Participants with <70% compliance were not included in analyses. After one week, participants were asked to complete the questionnaires again. Participants were provided a debriefing form upon completion of the study. Data were recorded, coded and saved for further statistical analyses.

2.5. Data analysis

Data were analyzed using the SPSS Statistical Package 19.0 (IBM Corp., Armonk, NY). A 2x2 mixed-model ANOVA ($\alpha = 0.05$) was performed to assess for change in perceived stress, depression, and anxiety between the two groups. Mean differences and standard error values were computed. Between-group and within-group main effects were assessed to account for potential concerns of internal validity. Significant Group x Time Interaction effects would demonstrate confirmation of study hypotheses.

3. Results

Out of 84 participants who provided consent, 79 met criteria for compliance and completion. The remaining participants (N = 79, M = 19.7 years old, 73.4% female, 73.4% white) were randomly assigned to a placebo (N = 39) group or a probiotic (N = 40) group. Sample demographics were assessed and presented by group as shown in Table 1. The placebo group was roughly 0.5 years older than the probiotic group (Table 1). The statistical significance found, $p < 0.05$, can be attributed to sample size and limited age range of participants (18–22 years of age); this difference is not clinically significant under the scope of this study (Mitsuoka, 1996; Yatsunenko et al., 2012). There were very similar previous probiotic and antibiotic exposures between the groups.

The present study aimed to assess if daily intake of *B. longum* probiotic for seven days would reduce symptoms of stress, depression, and anxiety. To determine such changes, a series of 2x2 mixed-model ANOVAs (Time x Group) were run. The results failed to support any of the proposed hypotheses, indicated by non-significant Time x Group interaction effects (Table 2). Significant within-subjects main effects, $p < 0.05$, were found, as may be expected, in responses to the PSS, CES-D, and STAI (Y2), indicating a significant reduction in symptoms from Time 1 to Time 2 for the entire sample regardless of condition (Table 2).

4. Discussion

The current study aimed to examine the efficacy of an acute administration of *B. longum* on perceived stress, anxiety and depression in young adults. The sample size utilized was comparable to other probiotic investigations that found significant results, and no group differences in probiotic or antibiotic history were present (Table 1). Nevertheless, contrary to predictions, the present study did not observe significant reductions in stress, depression, or anxiety following one week of *B. longum* intake.

A number of possible explanations could explain the null effect. Relative to typical scores for the mood questionnaires used in the current study, participant scores were higher on average for all groups regardless of time. This can account for the significant main-effect of time on responses for the PSS, CES-D, and STAI (Y2) (Table 2). Since the sample had elevated initial scores, it is likely that the scores regressed toward the expected mean scores over the study's duration. Additionally, there are cross-cultural differences in gut microbiota concentrations across the life span (Mitsuoka, 1996; Yatsunenko et al., 2012). The sample is comprised solely of young U.S. residents, whose microbiomes are less diverse and contain relatively higher concentrations of *B. longum* when compared to their adult counterparts (Yatsunenko et al., 2012). Thus, *B. longum* may have already been proportionately elevated by outcompeting other microbiota, dampening any observed effects from the *B. longum* powder (Mitsuoka, 1996; Yatsunenko et al., 2012).

Dosage could have also affected probiotic efficacy. Other studies have used a wide range of microbiota concentrations. Two studies found significant stress reductions using 2.4×10^9 CFUs and 3.2×10^8 CFUs/day–much less than the dose used in the present study (Rao et al., 2009; Sanchez et al., 2014). Even though there is no literature currently that analyzes dose-dependence of probiotics on psychological measures, one study on probiotic modulation of immune response found that lower doses of *Lactobacilli* increased T-cell response, alluding to a negative dose-effect relationship (Wen et al., 2011). These results could explain why a larger dose of *B. longum* could produce less pronounced reductions in relation to other human probiotic studies. Mechanistically, however, it remains unclear how a negative dose-effect relationship could be present. Future studies should continue to examine these poorly understood phenomena.

Duration of probiotic administration may also have an effect on bacterial proliferation in the gut. The present study intentionally utilized a very acute period in comparison to previous studies in order to assess for probiotic efficacy within a very acute period. Current literature reports a wide range of administration periods, ranging from days to months, yielding mixed results that do not correlate with duration (Messaoudi et al., 2011; Allen et al., 2016; Wallace and Milev, 2017). It is

very likely that the acute period utilized in the present study was not long enough to allow for the bacteria introduced to proliferate enough to observe a measurable effect. Future studies should continue to examine acute time frames in order to more broadly understand the time-effects of acute probiotic exposure on psychological symptomatology.

Another explanation for the lack of effect is that *B. longum* is less effective when ingested without additional mutualistic strains. Only one study to date has used *B. longum* alone, yielding mixed results (Allen et al., 2016). The majority of studies, however, utilized two or more strains of probiotic, typically from the genera *Lactobacillus* and *Bifidobacterium* (Wallace and Milev, 2017) and as such, the singular strain examined in this study could be considered a methodological weakness. Furthermore, multiple studies that used a single strain did not observe significant effects (Marcos et al., 2004; Benton et al., 2007; Chung et al., 2014). Based on these findings, it is plausible that a synergistic effect between *B. longum* and other strains of mutualistic bacteria could be responsible for observed changes in overall mental and physiological health. One study found that *both* counts of *Lactobacilli* sp. and *Bifidobacteria* sp. were elevated following administration of a strain of either genus, demonstrating the proposed synergistic effect (Rao et al., 2009). Bacterial strains could work synergistically to better improve overall gut health via a variety of proposed mechanisms mentioned in Section 1.

The mechanism of oral ingestion is not well-understood, with many of the studies listed in Section 1 yielding mixed results that vary widely across a range of participants, doses, durations, and strains. In the present study, *B. longum* was chosen for its relatively high survival rate in acidic environments like that found in the stomach (Kailaspathy and Chin, 2000). However, studies have shown that the presence of glucose or oligosaccharides can work to increase the survival rates of strains from the genera *Lactobacillus* and *Bifidobacterium* (Kailaspathy and Chin, 2000; Corcoran et al., 2005). Participants were not specifically instructed to take the capsules with food, which could have provided the necessary protection to increase survival and efficacy upon traveling through the stomach.

The current study is not without limitations. Sample size could have been a limitation in this study–a larger sample would have a reduced chance of Type II error. External validity could also have been a limitation, given the entire sample was of presumably generally healthy college students that were predominantly white and female. Additionally, participants were not instructed to take the capsules with food, which would have enhanced survival through the stomach. The study was designed to test only the very acute effect of a singular probiotic. Singular probiotics may be less effective than those consumed in combination. Again, and notably, the study was only designed to capture a very acute effect and so the results should be interpreted with caution since we did not assess whether or not measurable changes would occur with longer durations of supplementation. Future research should examine the potential effects of probiotics, in both singular and mixture form, preferably with longitudinal study designs. Since probiotics are available over the counter it is quite likely the general public consumes such products with hopes of rather acute action. Understanding the acute effect of common probiotics remains an important question for further study.

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Declaration of competing interest

None.

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