

Dampening exam anxiety with probiotics

Fermented Milk Containing Lactobacillus casei Strain Shirota Preserves the Diversity of the Gut Microbiota and Relieves Abdominal Dysfunction in Healthy Medical Students Exposed to Academic Stress 📎



Introduction

If you're down in the dumps, in some cases you may be able to use your microbiome as a scapegoat. The links between the bugs in your gut and mood are slowly and ploddingly [becoming better understood](#) in recent years. ERD #16, Volume 2, "Little Bugs For Big Depression," [discussed a study](#) that showed mood improvements can be achieved by people with major depressive disorder with the help of a mix of three probiotic strains: *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*.

The pathway through which microbes in your gut may influence mood is called the [microbiota-gut-brain axis](#) (or more colloquially as the "gut-brain axis"). The bi-directional communication that occurs between the [gut](#)

[and the brain](#) is mediated through various endocrine, neural, and immune pathways. Trying to figure out which strains of probiotics produce favorable outcomes can be an onerous task, with a lot of trial and error involved. [Many effects of probiotics are strain-specific](#) (as shown in Figure 1), so swapping out one for another may produce an altogether different effect.

Lactobacillus casei strain Shirota (LcS), the probiotic strain used in the study under review, has been previously researched for its effects on [cancer](#), [immune modulation](#), and [intestinal health](#). LcS is one of the more well-known probiotics (commonly sold under the brand name Yakult) and [has passed through the rigorous process](#) of being approved as Generally Recognized As Safe (GRAS) by the Food and Drug Administration of the United States. Preliminary evidence has indicated

Figure 1: Recommendations for probiotic use

Condition	Effectiveness	Probiotic Strains
Prevention of Antibiotic-Associated Diarrhea	A	<i>S. boulardii</i> , LGG, combination of <i>L. casei</i> DN114 G01, <i>L. bulgaricus</i> , <i>snf Streptococcus thermophilus</i>
Pouchitis – Preventing and maintaining remission	A	VSL#3
Ulcerative colitis – Maintenance	A	<i>E. coli</i> Nissle, VSL#3
Ulcerative colitis – Inducing Remission	B	<i>Escherichia coli</i> Nissle, VSL#3
Necrotizing enterocolitis	B	<i>L. acidophilus</i> NCDO1748, <i>B. bifidum</i> NCDO1453
Prevention of Clostridium difficile-Associated Disease (CDAD)	B/C	<i>S. boulardii</i> , LGG
Pouchitis – Inducing remission	C	VSL#3
Ulcerative colitis – Crohn's	C	<i>E. coli</i> Nissle, <i>S. boulardii</i> , LGG
Vaginosis and vaginitis	C	<i>L. acidophilus</i> , <i>L. rhamnosus</i> GR-1, <i>L. reuteri</i> RC14
Nonalcoholic fatty liver disease (NAFLD)	C	VSL#3, combinations of <i>L. plantarum</i> , <i>L. delbrueckii</i> , <i>L. bulgaricus</i> , <i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>B. bifidum</i> , <i>S. thermophilus</i> , <i>B. longum</i>
Alcoholic liver disease	C	VSL#3, LGG, <i>L. acidophilus</i> , <i>L. bulgaricus</i> , <i>B. bifidum</i> , <i>B. longum</i> with oligosaccharides

Source: Floch et al. J Clin Gastroenterol. 2015 Nov-Dec.

that LcS may be able to [ameliorate mood disturbances](#) in elderly people with initially poor mood and decrease anxiety in people [with chronic fatigue syndrome](#). In the paper under review, researchers looked to primarily investigate LcS's effect on stress-induced abdominal dysfunction. This can include symptoms like cramping, bloating, and general gastrointestinal discomfort. In addition, they examined secondary outcomes such as psychological state, salivary stress markers, and genetic expression changes related to stress response.

The microbiome has the ability to modulate certain aspects of mood through pathways in the gut-brain axis. *Lactobacillus casei* strain Shirota (LcS) has been shown to reduce symptoms of anxiety and mood disturbances in select populations. This study expands that line of research and investigates LcS's effects on stress-induced abdominal dysfunction, psychological state, salivary stress markers, and genetic expression patterns responsible for immune system activity.

Who and what was studied?

The researchers conducted a double-blind, placebo-controlled, parallel-group trial using 49 young Japanese fourth-year male and female medical students about to undertake a nationwide examination for academic advancement. Students are required to pass these examinations to continue on to their fifth year, when they will begin their clinical bedside training. This one-day exam covers all subjects in basic and clinical medicine and is one of the most stressful events for aspiring Japanese medical doctors.

Participants were divided into two groups and received either milk fermented with LcS (n=24) or non-fermented milk without LcS (n=25) that acted as the placebo. The LcS milk contained 1.0×10^{11} (100 billion) colony-forming units (CFU) of living LcS bacteria per 100-milliliter bottle.

What is a Colony-Forming Unit?

A CFU or colony-forming unit is a measure used to determine the amount of viable bacteria within a sample. It's essentially the number of bacteria in a given sample. This measure can be used in a few different applications, such as trying to determine the microbiological load of an infection in a blood sample.

In clinical trials, it is used to find out how many living probiotic bacteria are in the supplement being given to the participants. For most probiotic treatments to be effective, the bacteria need to be alive so that they can proliferate in the gut. That being said, heat-killed bacteria are sometimes also used in trials, as discussed in ERD issue #20, volume 1, due to bacterial components potentially having a physiological effect.

The placebo or LcS milk were consumed every day for eight weeks until the day before the exam. During this period, participants were prohibited from consuming other fermented milks, yogurt, lactic acid beverages, and pro- or prebiotic supplements that could possibly interfere or confound the study results. To assess the primary outcome of LcS's effect on stress-induced abdominal dysfunction, participants answered questionnaires every two weeks during the trial. The first looked at five common symptoms: abdominal discomfort and pain, feeling of incomplete bowel evacuation, abdominal distension, straining during bowel movement, and gastric pain. Gastrointestinal dysfunction was also examined every two weeks using the gastrointestinal symptom rating scale (GSRS), which evaluates acid reflux, abdominal pain, indigestion, diarrhea, and constipation. Both used rating scales with the lowest values indicating no issues and the highest indicating severe issues or discomfort.

Many secondary measures were also assessed. Psychological anxiety levels were monitored using two questionnaires (State-Trait Anxiety Inventory and NEO Five-Factor Inventory) and feelings of stress were measured using a visual scale from 0 (no stress) to 100 (severely stressed). Biomarkers for stress (cortisol and [alpha-amylase](#)) were acquired via saliva samples. Immune system function was gauged through blood measures of natural killer (NK) cells and by measuring changes in gene expression. Changes in the microbiome of the students were analyzed via fecal samples. Finally, a general questionnaire was administered about lifestyle and health practices.

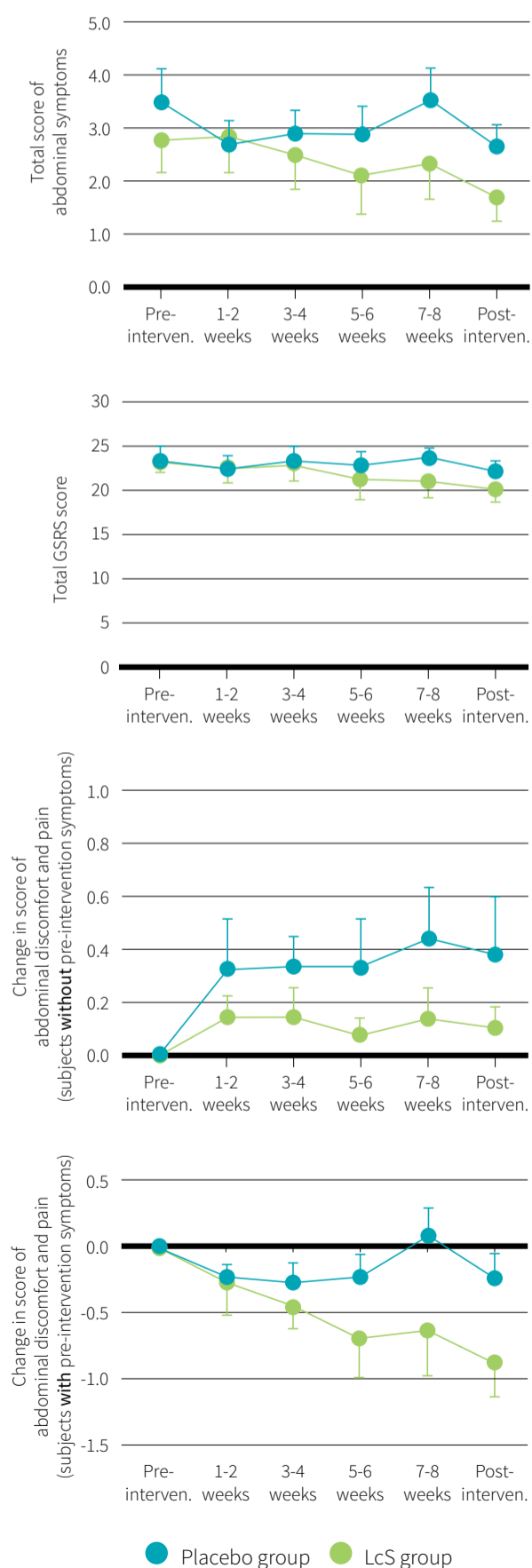
Researchers recruited 49 young Japanese medical students, who were randomized to consume either milk fermented with LcS (n=24) or non-fermented milk without LcS (n=25) during the eight weeks leading up to a stressful one-day examination critical to their career advancement. The primary objective was to assess LcS's effect on stress-induced abdominal dysfunction, which was measured via two questionnaires. The many secondary outcomes included monitoring changes in the microbiome, psychological anxiety levels, biomarkers of stress, and alterations in immune system activity level.

What were the findings?

There were no significant differences in any measures between groups at the start of the trial. Only two people dropped out, which means the study had an excellent retention rate. Both groups were 99% compliant with their treatments, according to daily diaries completed by the participants. Three to one days prior to the examination, live LcS was detected in all fecal samples for the intervention group and CFU content had more than doubled from baseline. In contrast, only two people tested positive for LcS in the placebo group and CFU had slightly decreased but remained largely unchanged.

Figure 2 shows the main study findings. There were significantly lower scores (that is, improvement) in abdominal symptoms and gastrointestinal symptoms with the use of LcS. These effects were still seen even two weeks after the exam, during which period all supplementation had ceased. The placebo group saw no significant changes in these measures.

Figure 2: Changes in gastrointestinal symptoms



For secondary outcomes, no significant differences were seen in the activity or number of NK cells between the groups during any point of the study. Psychological measures of anxiety were within normal ranges until the day before the exam, when both groups saw a significant spike. No significant differences in anxiety were observed. Feelings of stress peaked in the two weeks prior to the exam. Participants taking the LcS milk reported significantly lower feelings of stress during the eight-week trial. The LcS group also saw significantly lower salivary cortisol the day before the exam. However, no significant changes were seen in salivary alpha-amylase. LcS was able to significantly reduce the activity of stress-responsive genes one day before the test (86 genes in the LcS group compared to 179 genes in the placebo group). Lastly, the researchers noted the exam pass rates between the two groups was not significantly different.

The study had a low dropout rate and high compliance with taking the milk supplements. Those taking LcS experienced less abdominal and gastrointestinal issues as well as decreased feelings of stress and salivary cortisol levels. Fewer stress-responsive genes expressions were changed in the LcS group. There was no difference between groups in measures of anxiety or NK cell activity or number.

What does the study really tell us?

Study results indicate that daily consumption of LcS may decrease the stress response and help prevent related abdominal dysfunction in healthy participants exposed to brief periods of stress.

The results of the primary trial outcome should be given the most weight when looking at the results of this study. Secondary outcomes may be used to help evaluate or explain other effects of the intervention,

or produce results that can be explored in later trials. The primary results tell us that LcS can reliably but moderately reduce stress-induced abdominal and gastrointestinal dysfunction in young people living in Japan. There may be a difference in the magnitude of this effect from population to population as the microbiome [can vary over geographic location and age](#), so these results cannot be wholly applied to everyone.

The secondary results of the study help give legitimacy to the primary findings. In particular, the fact that the placebo group experienced double the change in stress-responsive genes compared to the LcS group. In this study, a gene had to change its expression by more than two-fold compared to baseline to be counted. Despite all the positive results seen in the students provided LcS, there was no significant difference in the examination pass rates between the two groups. So, even though the LcS students experienced lower levels of stress and anxiety, this did not necessarily translate into better testing performance.

It should be noted that 14 of the 17 authors on this study were financially supported by Yakult Honsha Co., Ltd., a company that sells LcS supplements and provided the supplements for this trial. This study was a collaboration between Yakult Central Institute and Tokushima University Graduate School. The acknowledgments state that Yakult “did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.”

LcS can reliably but moderately reduce stress-induced abdominal and gastrointestinal dysfunction in young people living in Japan. It’s unknown how well this effect would be in other populations. The fact that fewer stress-responsive genes were active in the LcS group gives credence to the findings that LcS reduced stress-related gastrointestinal events in the population studied.

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The big picture

The researchers acknowledged the moderate sample size of the study and called for further research to be conducted to verify these results. However, there have been three other studies looking at the effects of LcS on different components of mood.

An RCT of [132 healthy, elderly volunteers](#) found that three weeks of supplementation with LcS was able to improve mood in those with the poorest mood responses at baseline. A pilot RCT of [39 people with chronic fatigue syndrome](#) showed decreases in anxiety after two months of supplementation. Researchers conducting the [pilot study precursor](#) to the one reviewed here also observed a suppression of physical symptoms of stress in healthy students.

One important takeaway from this trial should be that the gut has an amazing ability to rapidly adapt. For instance, the concentrations of LcS in students taking the LcS milk supplement had fallen back to baseline levels only two weeks after supplementation had ceased. If you are hopeful about reaping the moderate stress reduction benefits of LcS, know that its effects have only been shown in the short term and that long-term sup-

plementation may not elicit the same benefits over time.

Three other trials of LcS have all seen improvements in mood, stress, and anxiety levels across various populations. The positive effects of LcS in the long term are not yet known.

Frequently asked questions

Are there any downsides to LcS supplementation?

Being one of the more popular probiotic strains, LcS has been tested on mice, hamsters, rats, rabbits and in healthy and health-compromised adults, children and infants. Previous reviews have noted that [LcS is well-tolerated](#) even in populations suffering from constipation.

The trickiest part of trying to supplement with LcS is ensuring that the product you pay for actually contains live cultures of the probiotic. ERD #15, Volume 2, “Your Probiotic May be Lying to You” reviewed a study that investigated the label claims of 17 probiotics. Only one product was a perfect match to its label. The others were either missing strains of bacteria claimed on the label or contained strains not listed. Buyer beware!

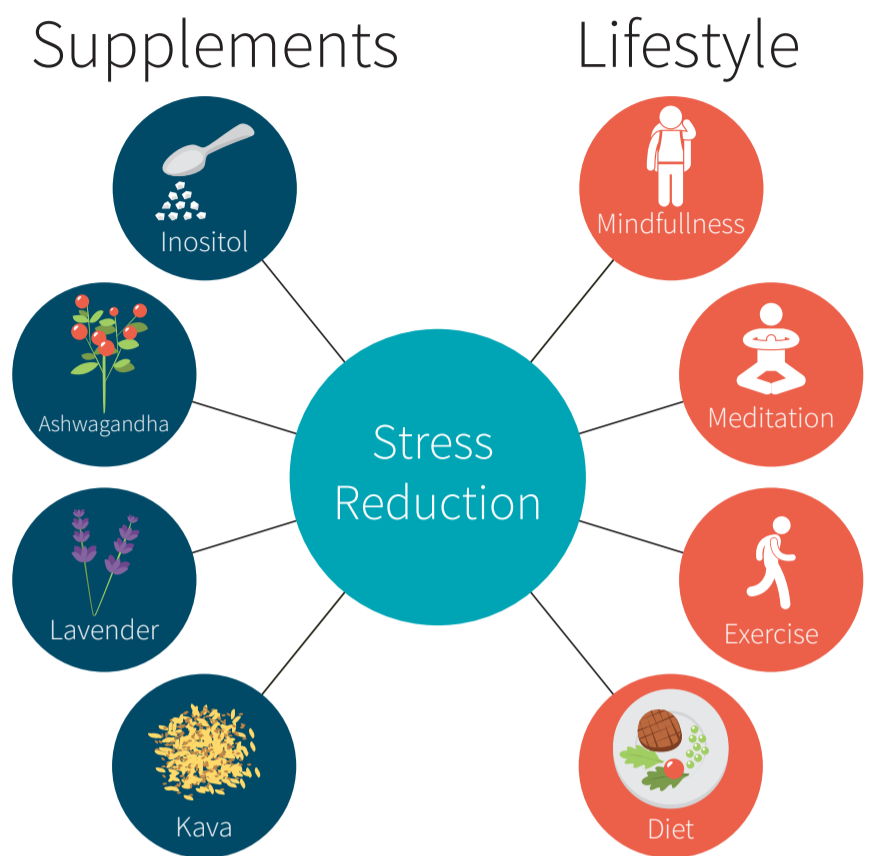
What else can be done to help alleviate stress/anxiety/depression?

Some of the most common strategies are shown in Figure 3. Non-supplement lifestyle-intervention routes to relieving stress can include [mindfulness-based therapy](#), [meditation](#), and [exercise](#). It never hurts to ensure your diet is primarily coming from whole-food sources either. On the supplement side of the equation, [lavender](#), [inositol](#), [Ashwagandha](#), and [Kava](#) have all been shown to reduce stress or anxiety to some degree.

What should I know?

Supplementation with LcS during periods of short-term stress may be able to moderately reduce physical symptoms of stress such as gastrointestinal upset. Psychological symptoms may also see a modest decrease as well. It is not yet known if these effects would continue over the long term (two or more months) or dissipate over time. ♦

Figure 3: Stress reduction strategies



You may not be a Japanese medical school student getting ready for your big exam (or maybe you are?), but this trial is noteworthy nonetheless. Discuss it at the [ERD private Facebook forum](#).