



# KeepCalm

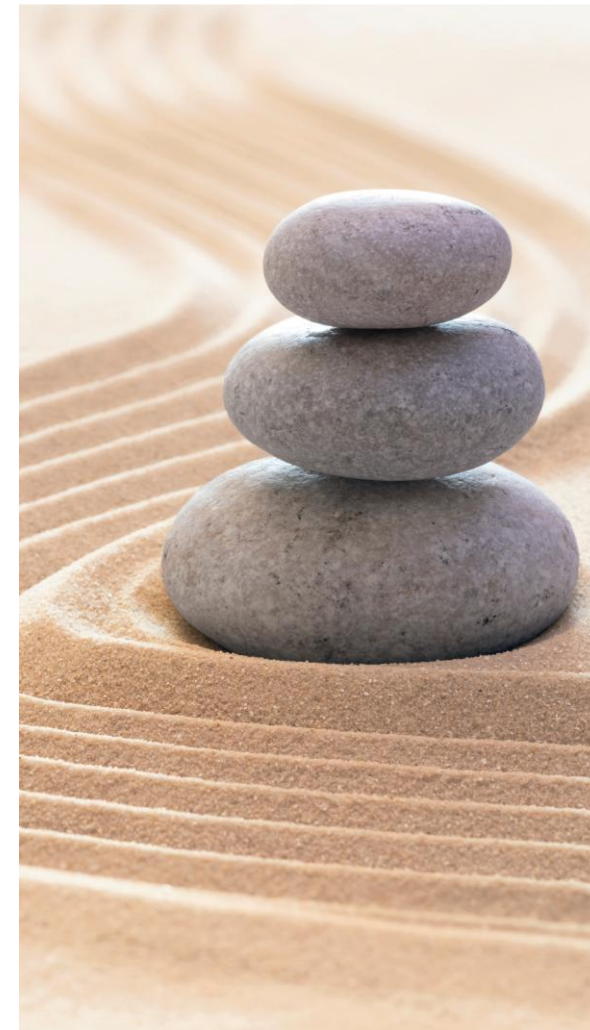
**Slow down your rhythm  
and enjoy your time**



## KeepCalm

Probiotic complex designed for mental health composed by  
*L. reuteri* - PBS072, *B. breve* - BB077

- ▶ Modulation of the epigenetic response to stress
- ▶ Stimulation of GABA production
- ▶ Improvement of Serotonin secretion
- ▶ Clinically tested on stress management, cognitive function and post-partum blues



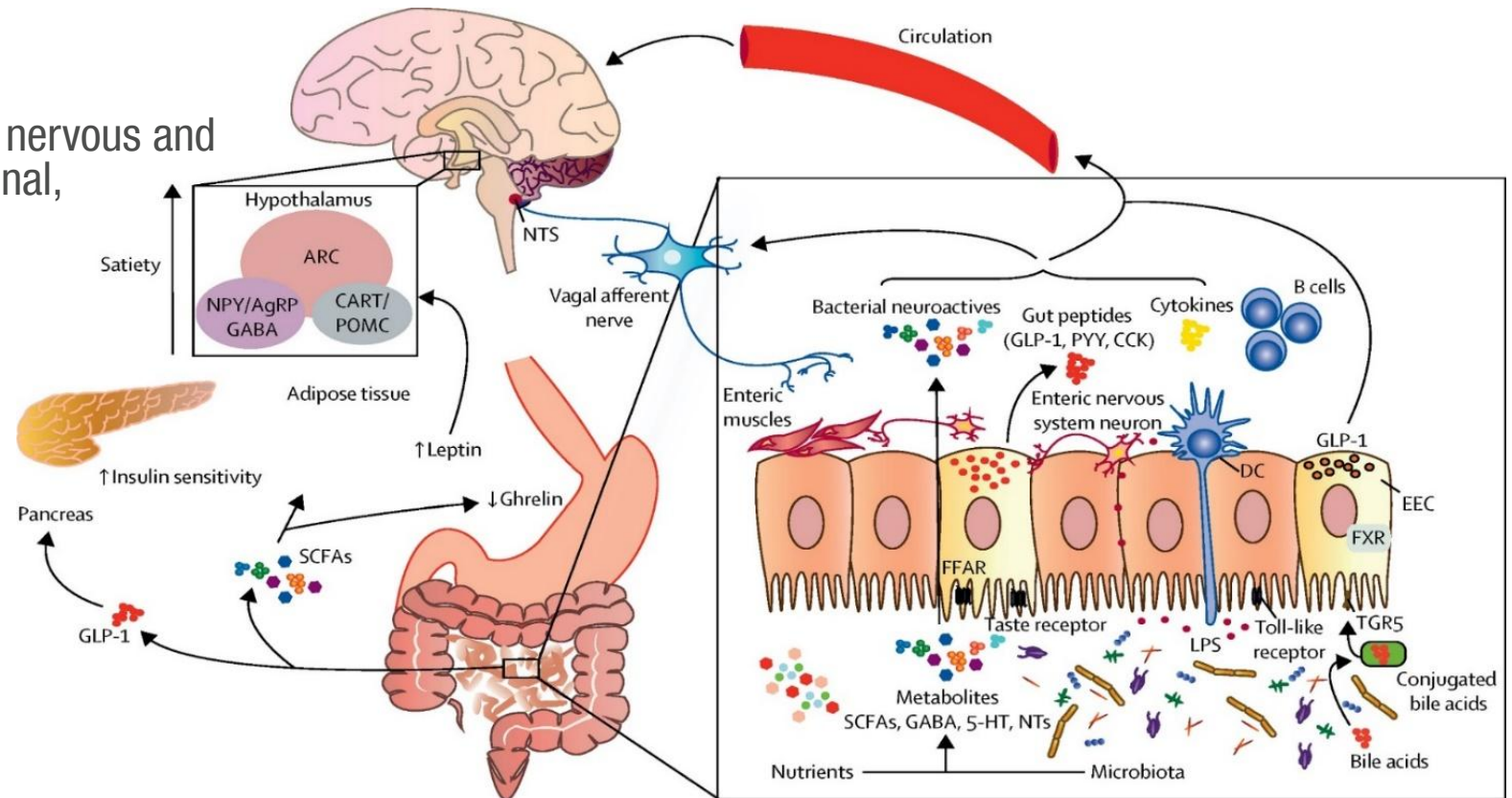


# The gut-brain axis & the microbiota

The bi-directional dialogue between our nervous and digestive systems is mediated by hormonal, immunological and neural signals.

Gut microbiota can play a role in serotonergic system modulation by regulating 5-HT production both at CNS and peripheral levels.

Moreover, it seems involved in other neuroactive biochemical production like dopamine,  $\gamma$ -aminobutyric acid (GABA), tryptophan and histamine.



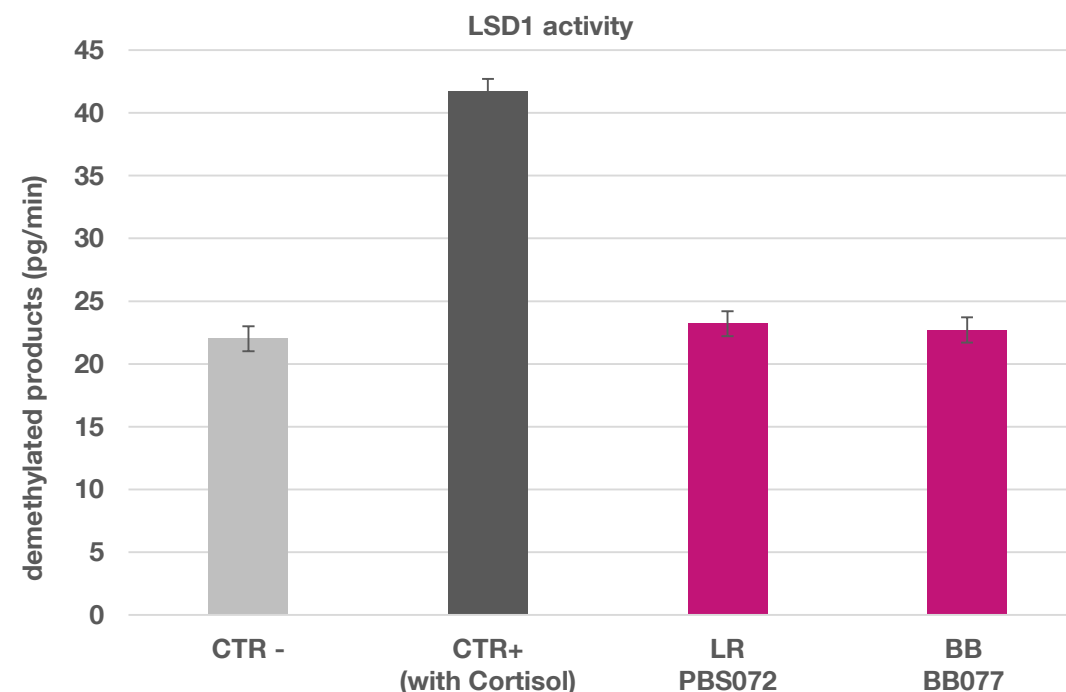
Torres et al. 2017 *The Lancet* ©



In-vitro

# Modulation of epigenetic response to environmental stress

- LSD1 modulation could help control the adaptive response to stressful stimuli and improve emotional behavior.
- Both *L. reuteri* PBS072 & *B. breve* BB077 probiotic strains **dramatically reduce** the induced **stressful effect of Cortisol**, bringing back the model into the basal conditions.



Nobile V, Giardina S, Puoci F: The Effect of a Probiotic Complex on the Gut-Brain Axis: A Translational Study. Neuropsychobiology 2021. doi: 10.1159/000518385

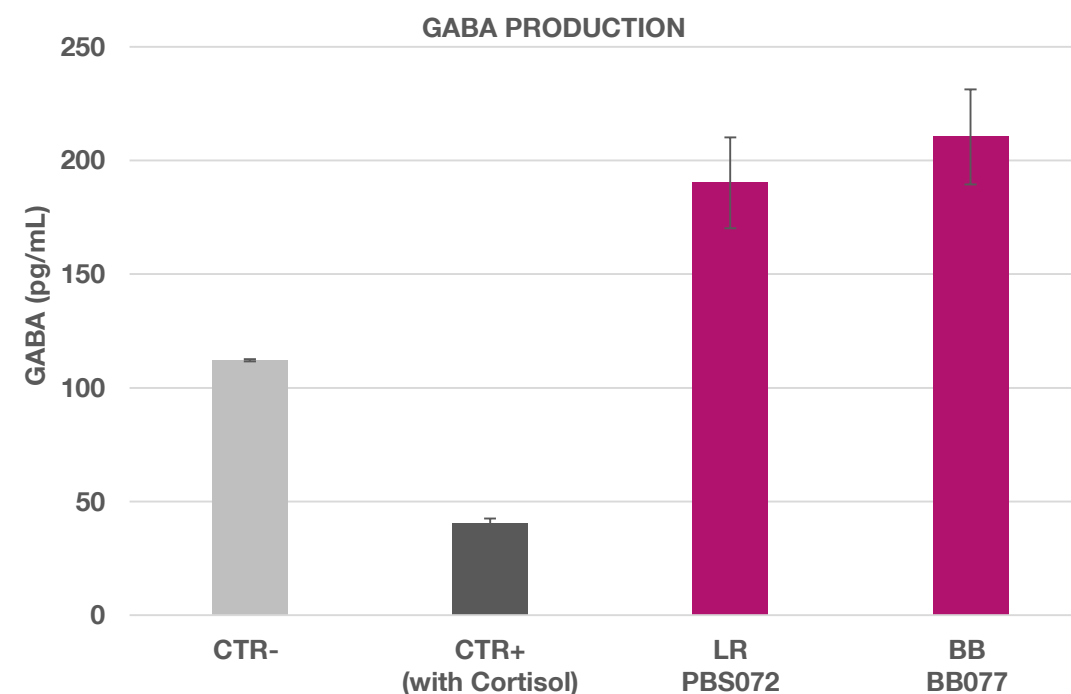
**Protocol:** evaluation of the demethylase activity of LSD1 enzyme expressed in neuroblasts SK-N-DZ after inducing stress with Cortisol and treating with the probiotic culture medium.



In-vitro

# Stimulation of GABA secretion

- GABA plays a major role in the modulation of cognitive and behavioral processes, as well as in the central modulation of anxiety and stress responses.
- Both *L. reuteri* PBS072 & *B. breve* BB077 probiotic strains **consistently increase GABA concentration** after stress induction, showing to further stimulate GABA production over the basal levels.



Nobile V, Giardina S, Puoci F: The Effect of a Probiotic Complex on the Gut-Brain Axis: A Translational Study. Neuropsychobiology 2021. doi: 10.1159/000518385

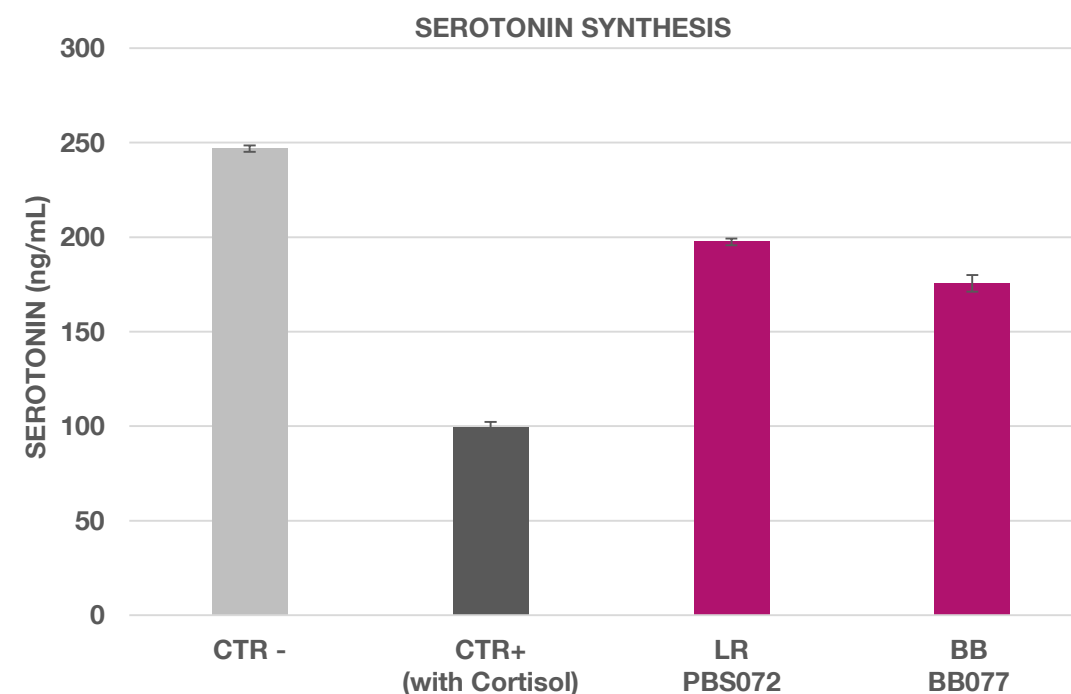
**Protocol:** detection of GABA levels expressed by neuroblasts SK-N-DZ after inducing stress with Cortisol and treating with the probiotic culture medium.



In-vitro

# Promotion of serotonin synthesis

- Serotonin is a neurotransmitter, precursor in the biosynthesis of melatonin, popularly known as contributor to feelings of well-being and happiness with several effects on mood, anxiety and sleep.
- *L. reuteri* PBS072 & *B. breve* BB077 probiotic strains demonstrated to **promptly enhanced Serotonin synthesis** in a stressed situation with respect to the CTR-.



Nobile V, Giardina S, Puoci F: The Effect of a Probiotic Complex on the Gut-Brain Axis: A Translational Study. Neuropsychobiology 2021. doi: 10.1159/000518385

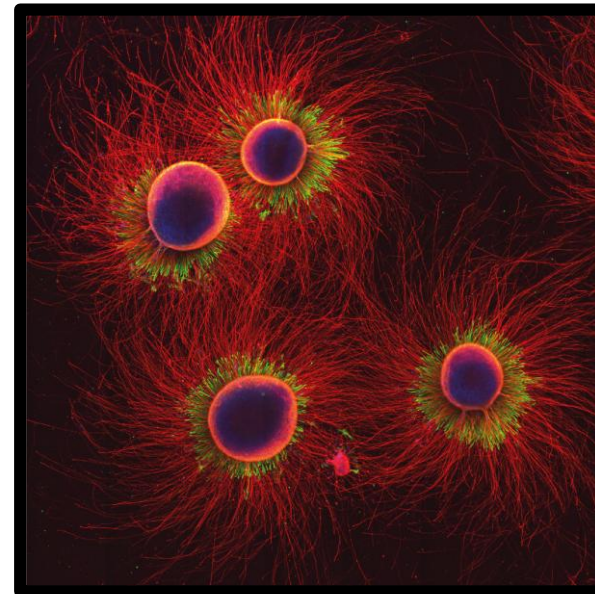
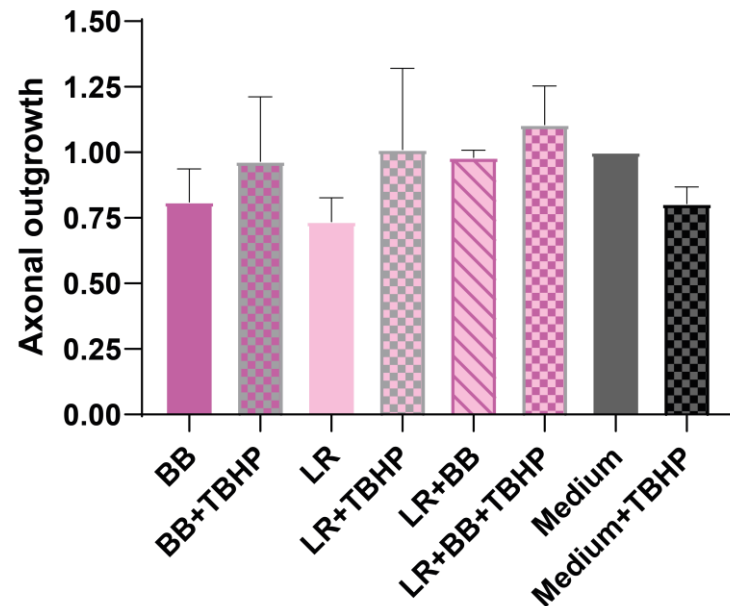
**Protocol:** valuation of the capability to modulate the serotonin synthesis in Caco2 cells. Experimental model for 48 hours with the medium in which strains were cultured with cortisol (stress agent).





# Brain Organoids

- The potential neuroprotective effects of probiotic metabolites were investigated using a human brain organoid model. Neurons and astrocytes were exposed to a stressor agent (TBHP), and analyzed through immunostaining.
- In this 3D brain model, treatment with metabolites derived from *L. reuteri* PBS072 and *B. breve* BB077 demonstrated a synergistic effect. This combination significantly enhanced axonal outgrowth and mitigated oxidative stress, thereby promoting neural homeostasis.

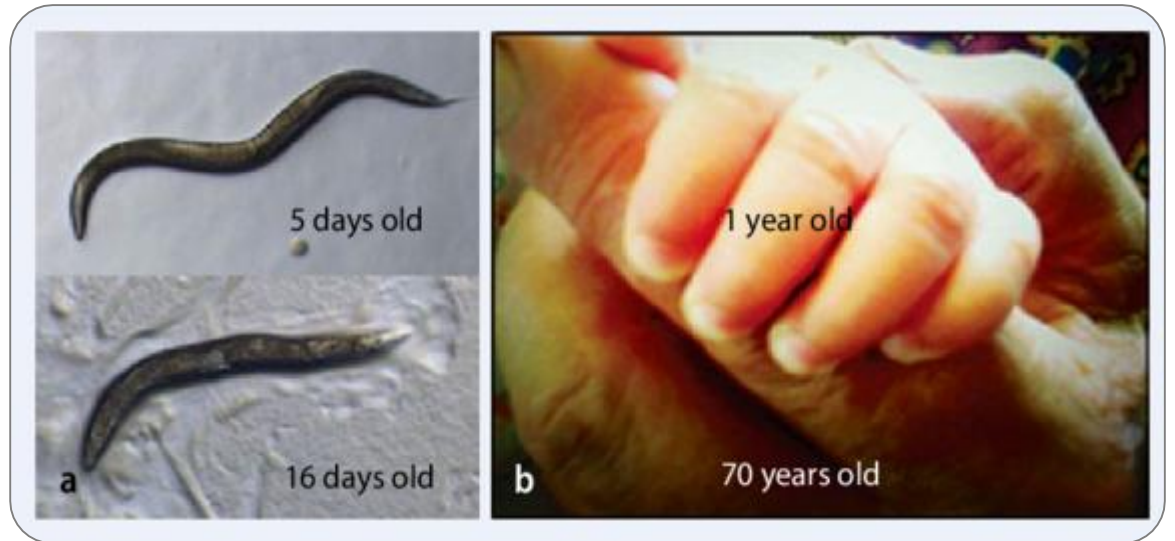


Nuclei are shown in blue  
Astrocytes (GFAP staining) in green  
Neurons (TUJ1 staining) in red  
Picture are taken with confocal microscope.



## Who is *C. elegans*?

- *C. elegans* shares about 40% of its genome with humans, making it a valuable model for aging research.
- Its lifespan is short, lasting only 2–3 weeks.
- *C. elegans* is hermaphroditic, producing approximately 300 offspring per adult.
- The nematode displays aging-related phenotypes, such as motility decline and oxidative stress.
- Bacteriophages influence the nematode's interactions with its microbial environment, adding complexity to its aging study.

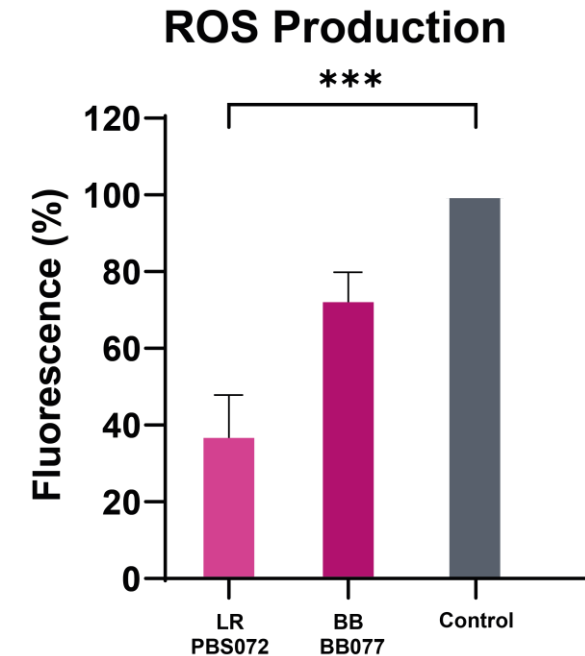
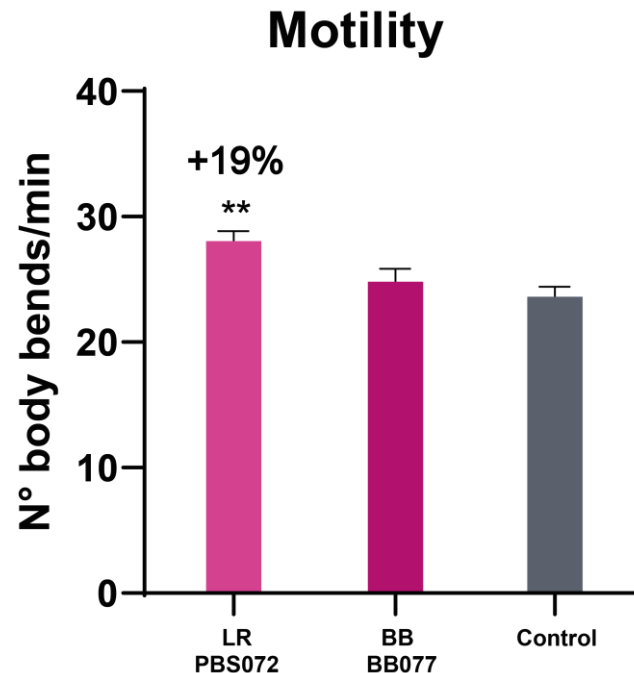
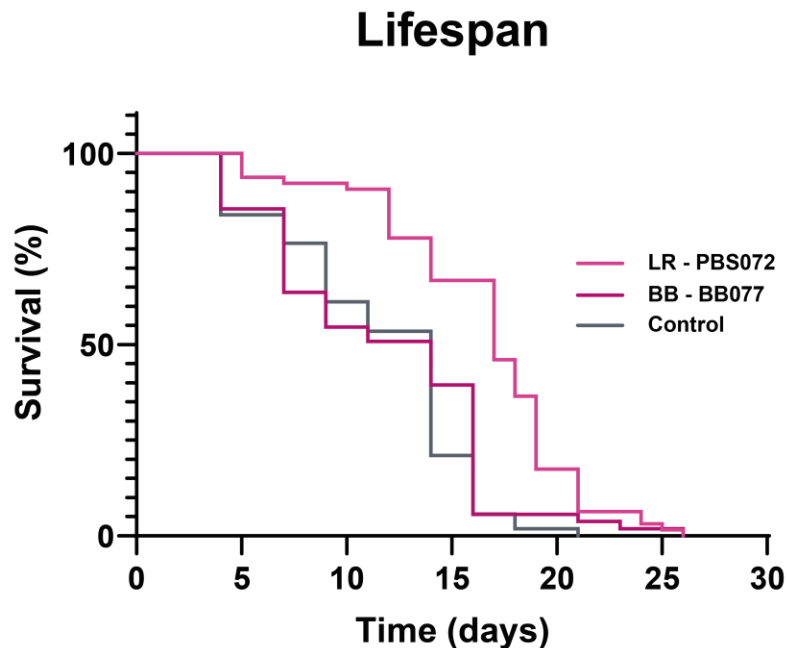






# Lifespan, Motility & Antioxidant activity

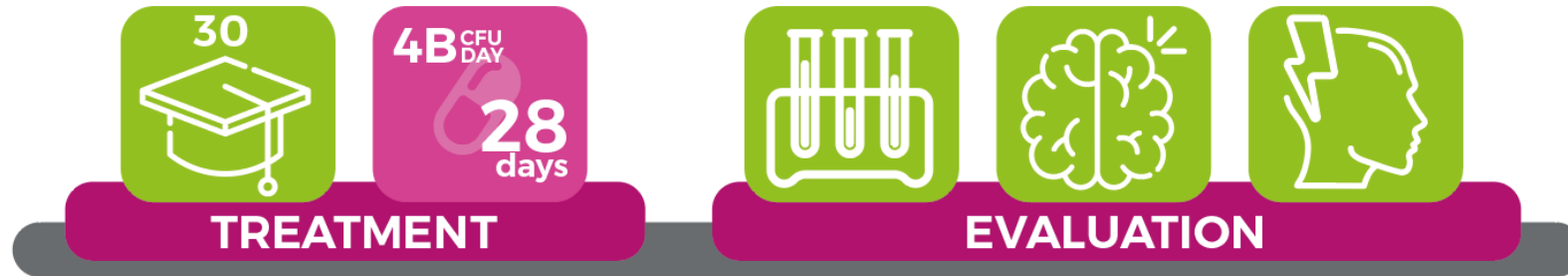
- *L. reuteri* PBS072 and *B. breve* BB077 extended lifespan by approximately 5 days and improved early survival. Both probiotics increased motility, indicating delayed aging as well as a significant ROS reduction 63% and 30% respectively for *L. reuteri* PBS072 and *B. breve* BB077.



**Protocol:** The effects of probiotics on healthy aging in *C. elegans* (N2 strain) were assessed by measuring lifespan, motility (body bend counts on day 11), and ROS levels. Probiotics were administered every two days, with *E. coli* OP50 as the control.



# Clinical study design - Cognitive function



## EVALUATED PARAMETERS:

- Short-term memory
- Attention
- Executive performance
- Physiological stress level
- Sleep quality
- Anxiety

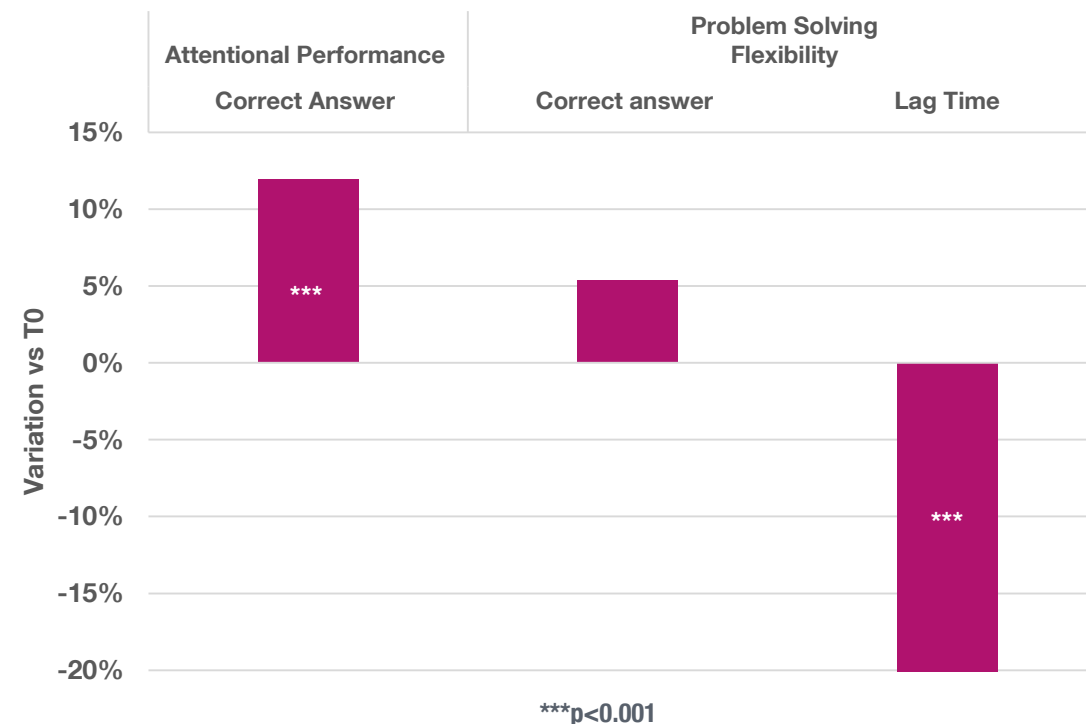
Nobile V, Giardina S, Puoci F: The Effect of a Probiotic Complex on the Gut-Brain Axis: A Translational Study. Neuropsychobiology 2021. doi: 10.1159/000518385



Human

## Improvement of executive performance

- Facing daily high-stress events negatively influence the ability to take decisions.
- KeepCalm promotes a **higher level of attention** and concentration under stress, together with an **improved flexibility to change strategy** during the test course.

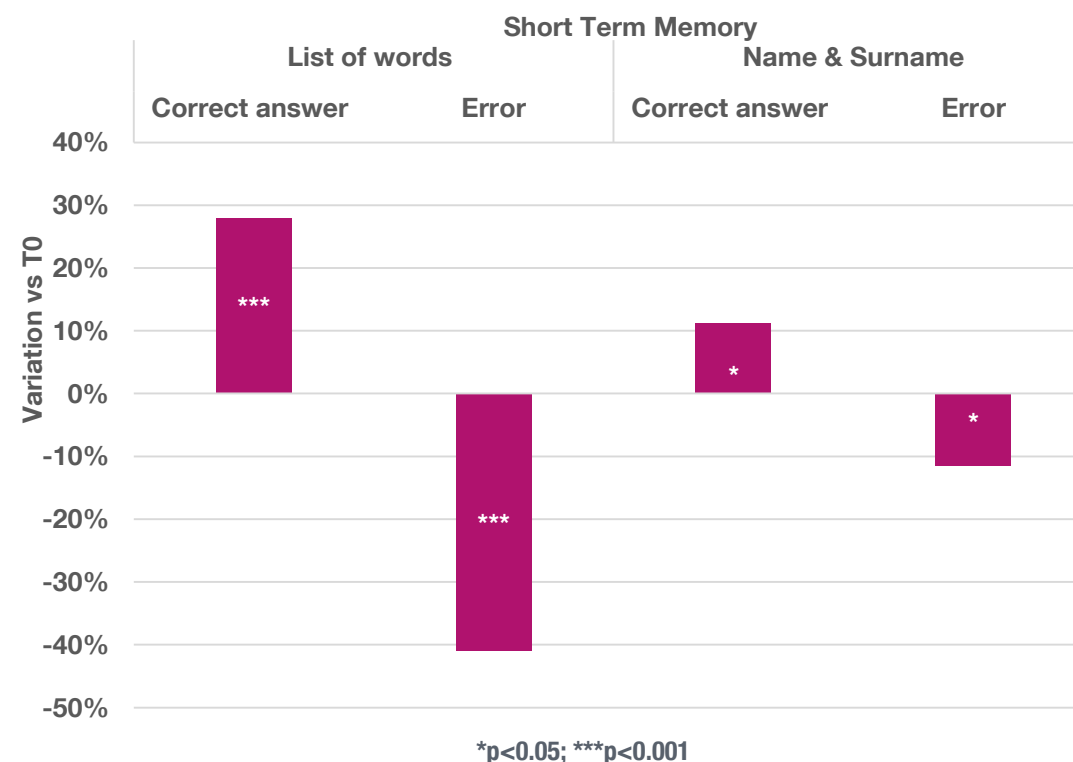


The (Divided) Attentional performance test measures the capacity to focus the attention on 2 different irregular stimuli (acoustic and visual). Problem solving flexibility indicates the ability to adapt to a sequence of input, by providing the expected feedback within the time (Wisconsin card sorting test).



# Enhancement of cognitive functions

- Performance anxiety also leads to a cognitive failure, with impact on short term memory and concentration.
- KeepCalm **promotes intellectual responsiveness under pressure** by improving short term memory with significant results in terms of correct answers ( $p < 0.001$ ).



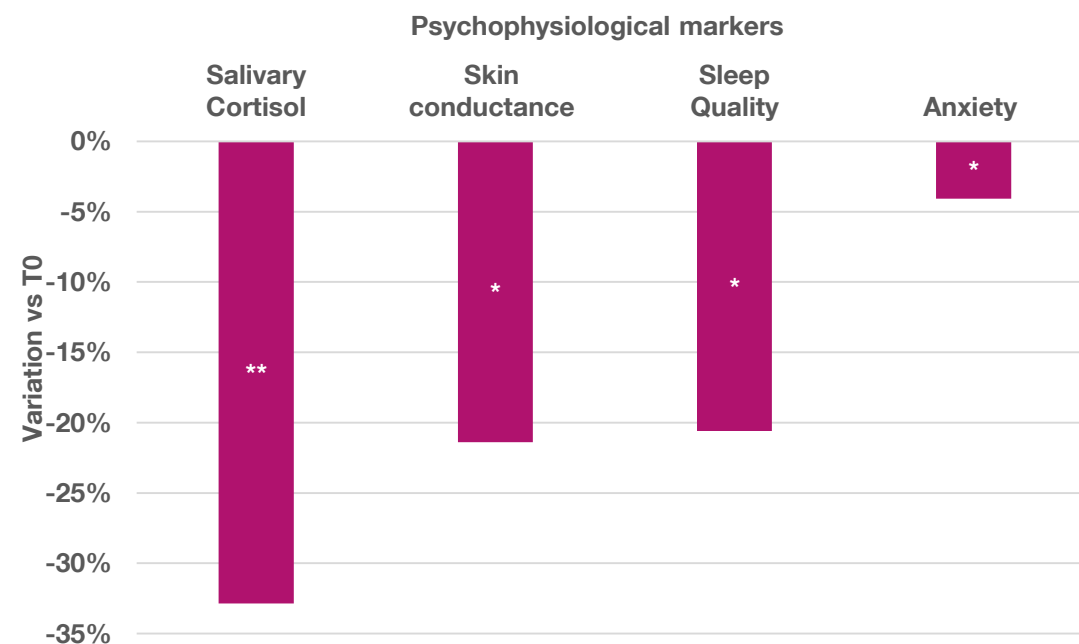
The short term memory test consists into the memorization of a first set of common words and a list of names and surnames, each followed by a retention time where patients are required to run an alternative task as "interference factor".



Human

## Improvement of psychophysiological markers

- Stress relief comes with the recovery of both physiologic and psychological markers.
- KeepCalm **improves the quality of life** in stressed subjects, evident by reduction of salivary cortisol and skin conductance (sweat), as well as **amelioration of sleep quality and anxiety perception**.



\*p<0.05

Athens insomnia scale assesses nocturnal sleep performance and daytime dysfunction on a 8 parameters base, rated on a 4 points Likert scale. The State-Trait Anxiety Inventory (STAI) is commonly used to measure trait (personal characteristic) and state anxiety (transitional, provoked by a certain event) by means of 40 items on a 4-point scale.





# Clinical study design - Work-related stress



## IMPROVEMENT FROM WORK-RELATED STRESS:

- Sleep quality
- Mood

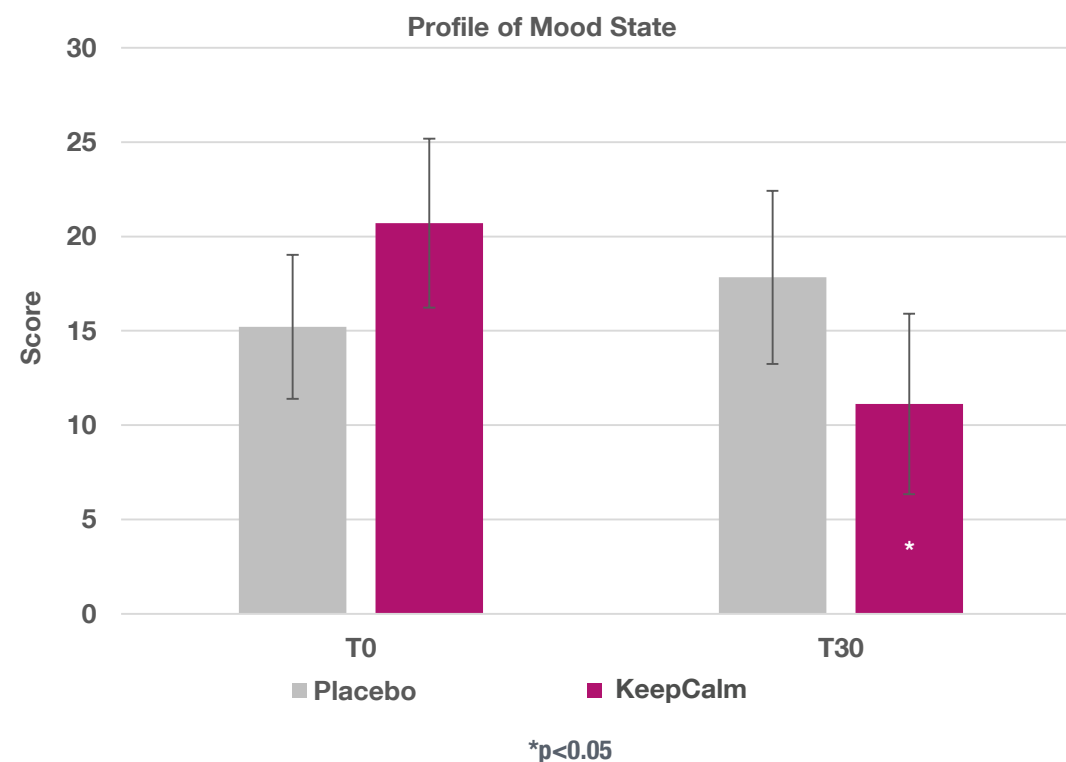
Nobile V, Puoci F: Effect of a multistrain probiotic supplementation to manage stress during the COVID-19 pandemic: a randomized double-blind placebo-controlled cross-over clinical trial Submitted at Neuropsychobiology



Human

## Mood & tone improvement

- Mood disorders cause the largest disease burden in general population and loss of work productivity in working population.
- KeepCalm has shown to positively modulate subjects mood states, with a **total score reduction of -44%** compared to T0.



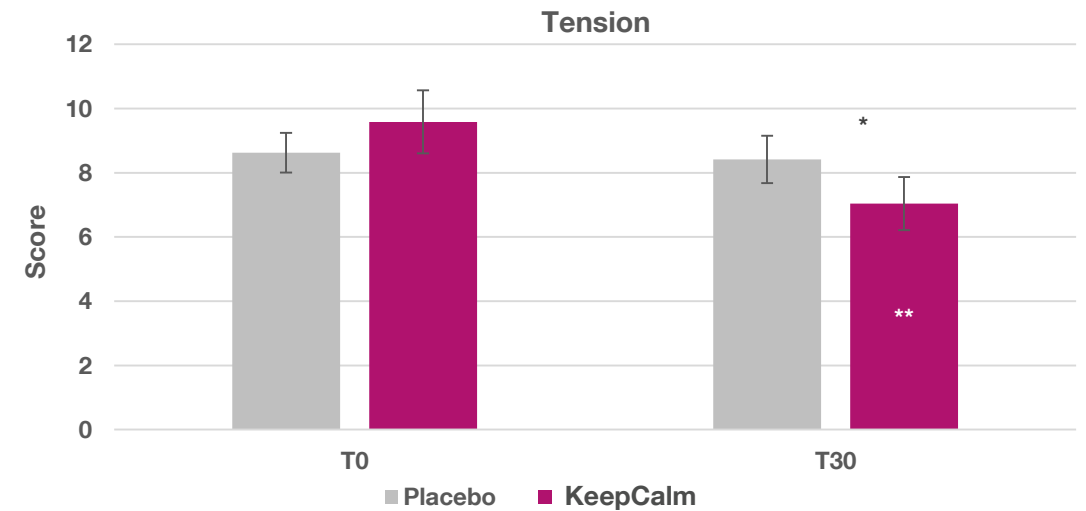
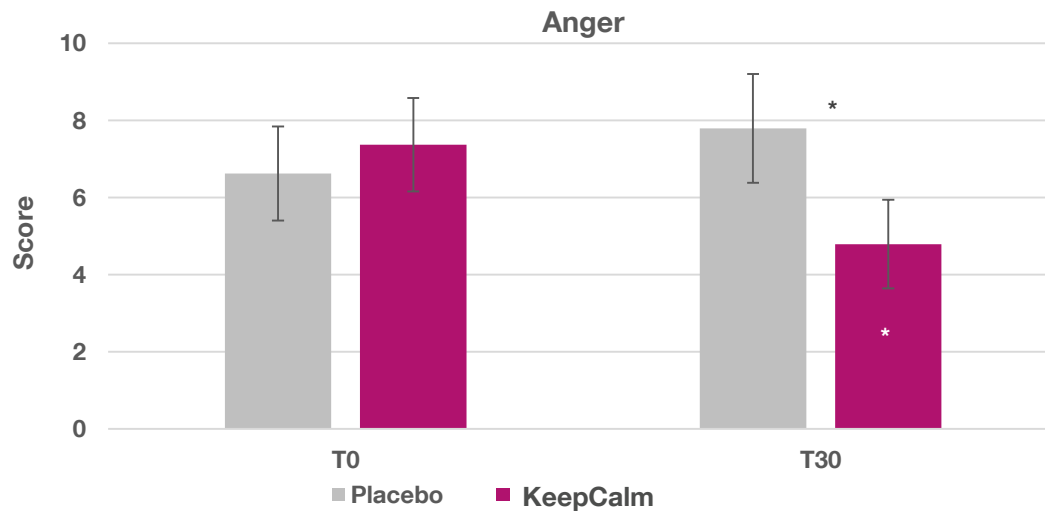
The Profile of Mood State, POMS, is a self-report questionnaire that assesses mood describing feelings that people usually have. The items are grouped in 6 subscales (Anger, Tension, Depression, Fatigue, Confusion & Vigor) the total score for each scale is obtained by adding the related item, wherein the higher the total score of the scale, the higher the level of the related mood.



Human

## Mood & tone improvement - subgroups analysys

- Workplace may leave employees feeling overwhelmed, increasing their tension and anger feelings.
- KeepCalm groups reported a significant reduction of both angry and tense mood state.



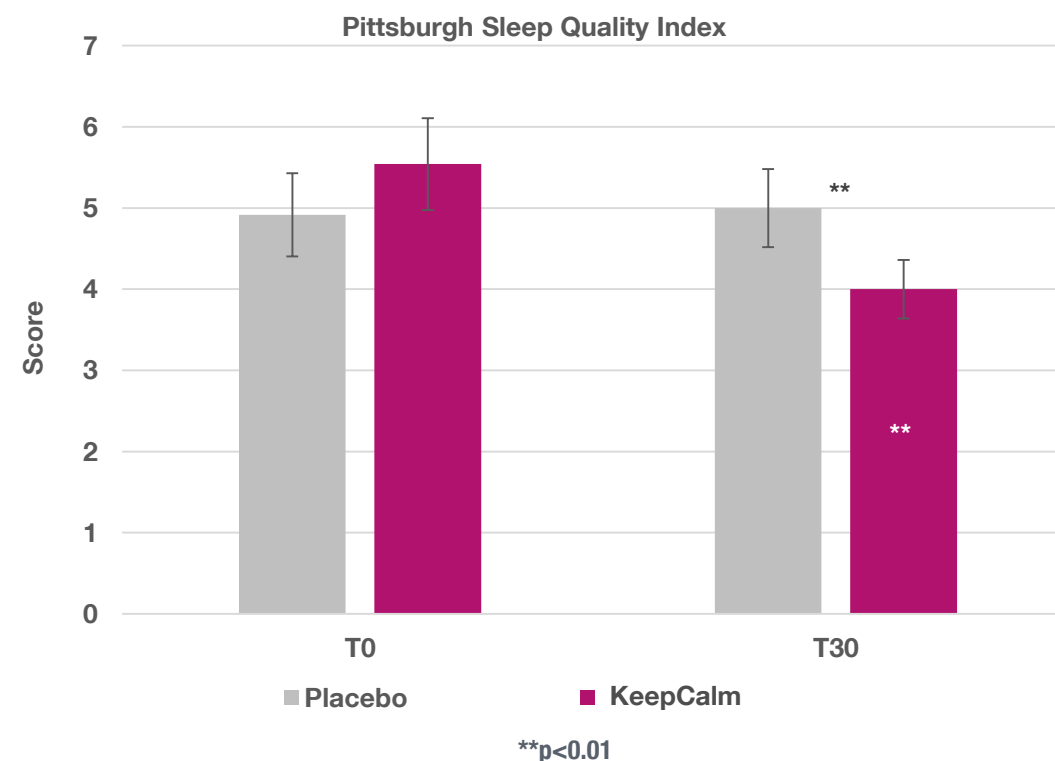
\*p<0.05; \*\*p<0.01

The Profile of Mood State, POMS, is a self-report questionnaire that assesses mood describing feelings that people usually have. The items are grouped in 6 subscales the total score for each scale is obtained by adding the related item, wherein the higher the total score of the scale, the higher the level of the related mood.



# Sleep quality evaluation

- Impaired sleep was defined as difficulties falling asleep and/or maintaining sleep, which is typical condition of subjects who are facing an intense period at work.
- KeepCalm has shown to significant improve sleep quality, with **-20% reduction of the global score** compared to T0.



The Pittsburgh Sleep Quality Index (PSQI) is a self-report questionnaire that assesses sleep quality for the majority of days and nights in the past months. It consists of 19 questions investigating usual bedtime and other aspects of sleep quality. The lower the PSQI global score (0-21), the better the sleep quality.



# Clinical study design - Post partum blues



## EVALUATED PARAMETERS:

- New-mothers mood & quality of life evaluation
- Breastfeeding quality and baby's crying assessment
- Mastitis incidence recording

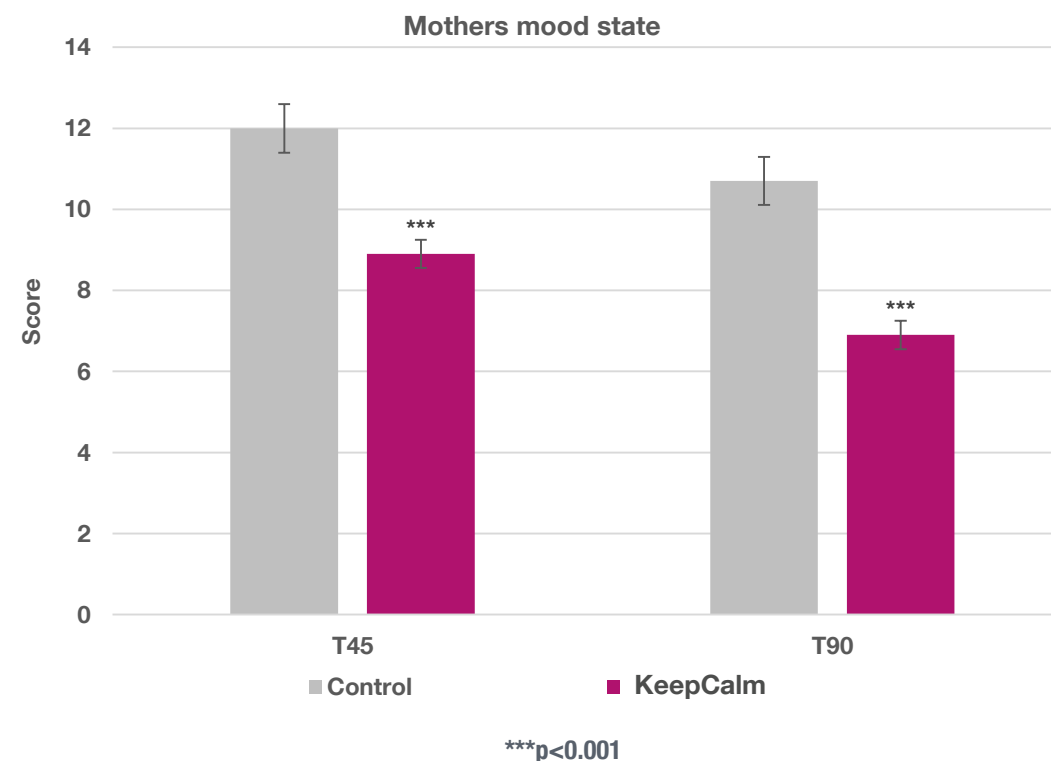
Vicariotto, Franco, et al. "Beneficial Effects of Limosilactobacillus reuteri PBS072 and Bifidobacterium breve BB077 on Mood Imbalance, Self-Confidence, and Breastfeeding in Women during the First Trimester Postpartum." Nutrients 15.16 (2023): 3513.





# Mothers mood state

- *Baby blues* and *post-partum depression* (PPD) may be consequently one to each other.  
*Baby blues* usually occurs a few days after giving birth and resolves around the first weeks after birth while PPD may arise after the first 4 weeks.
- KeepCalm has shown to significantly support mothers mood:
  - at T90 reduction of 22% compared to T45
  - compared to control, a reduction of 26% and 35% at T45 and T90 respectively



The Edinburgh Postnatal Depression Scale is a 10-item self-report questionnaire (range 0–30) designed to screen for possible depression in new mothers. Each question is scored 0 to 3, with a total score ranging from 0 to 30. The cut-off score linked to the pathological status of a woman is set at 10. The lower is the global score, the better is the mother's mood.



Human

## Self-confidence in breastfeeding

- One of the most important consequence and cause, at the same time, of women depressive mood after delivery is the possible difficultness in breastfeeding.
- KeepCalm has shown a significant improvement of lactation performance in the new-mothers, keeping a positive trend throughout the treatment, **+24%** compared to the control.

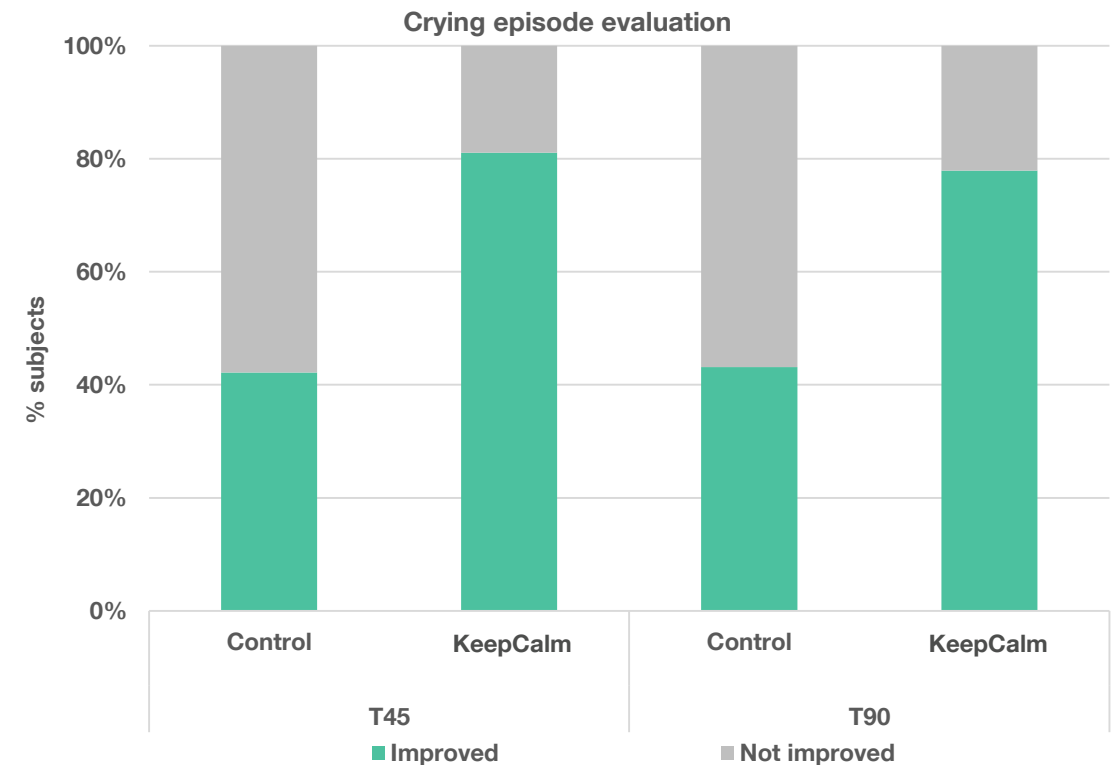


The Breastfeeding Self-Efficacy Scale Short Form (BSES-SF) is a 14-item questionnaire that was developed to measure a mother's confidence in her ability to breast-feed her new infant. Each question is scored 1 to 5, with a total score ranging from 14 to 70. Higher scores correspond to higher levels of breast-feeding self-efficacy and possibly to a higher likelihood of successful breast-feeding.



# Baby's crying reduction

- The first six months after birth is an important period for child's physical development. The modification of breast milk composition, mediated by gut and skin microbiota, could positively influence baby gut microbiota with a reduced gas production, thus a reduction of crying episodes.
- KeepCalm has shown to significantly improve the percentage of mothers reporting a **reduction of baby's crying episodes, up to 80%** compared to the control (only 40%).



The Breastfeeding Self-Efficacy Scale Short Form (BSES-SF) is a 14-item questionnaire that was developed to measure a mother's confidence in her ability to breast-feed her new infant. Each question is scored 1 to 5, with a total score ranging from 14 to 70. Higher scores correspond to higher levels of breast-feeding self-efficacy and possibly to a higher likelihood of successful breast-feeding.



# Clinical study in progress - PMS

**KEEP AN EYE OUT FOR  
THE LATEST UPDATE!**





## Summary

**Description:** Probiotic complex for stress relief composed by *L. reuteri* - PBS072, *B. breve* - BB077

**Dosage:** 4B CFU/day (2B CFU/ strain)

**Treatment:** minimum 28 days



Capsules



Tablets



Sachets  
or sticks



Triphase  
vials



Bulk / Full  
service

### RESULTS:

- ✓ Recovery from stress
- ✓ Modulation of GABA
- ✓ Improvement of Serotonin synthesis
- ✓ Clinical positive results on cognitive functions, work-related stress and post partum blues

### TYPICAL APPLICATIONS:

- ✓ Stress & anxiety conditions
- ✓ Psychological distress
- ✓ Memory lack
- ✓ Improved cognitive functions
- ✓ Sleep disorders
- ✓ Mood enhancer





Discover more:



[synbalance.care](https://synbalance.care)



**+39 02 9679 9831**



**[info@synbalance.care](mailto:info@synbalance.care)**