Viability of the Probiotic Strain

Effect of LcS on Upper Respiratory Tract Infection

Possible Role of LcS in Metabolic Disorders

Cross Talk Between Gut and Brain

Emerging Researches of LcS
VIABILITY

1. Recovery of *Lactobacillus casei* strain Shirota (LcS) from the intestine of healthy Vietnamese adults after intake of fermented milk.


   This study was done to demonstrate the gastrointestinal survival of *Lactobacillus casei* strain Shirota (LcS) in healthy Vietnamese adults.

   A fermented milk drink (65ml, $10^8$ CFU/mL *Lactobacillus casei* strain Shirota (LcS)) was administered daily for 14 days to twenty-six healthy Vietnamese adults in the age group of 18-35 years. Fecal samples were collected before, during and after consuming the fermented milk drink.

   LcS was confirmed by culture and Enzyme Linked Immunosorbent Assay (ELISA). After 7 and 14 days of ingesting the fermented milk drink, LcS was recovered from fecal samples at average of $5.0 \times 10^7$ CFU/g feces (n=26) and $5.4 \times 10^7$ CFU/g feces (n=26), respectively. LcS persisted in 8 volunteers until day 42 (after 14 days stopping fermented milk drink) at $0.0033 \times 10^7$ CFU/g feces (n=8). The study confirmed survival of LcS after passage through the gastrointestinal tract of Vietnamese adults.

IMMUNITY

2. Daily intake of fermented milk with *Lactobacillus casei* strain Shirota (LcS) reduces the incidence and duration of upper respiratory tract infections in healthy middle-aged office workers.


   The study was conducted to evaluate the effects of *Lactobacillus casei* strain Shirota-fermented milk (LcS-FM) on the incidence of Upper Respiratory Tract Infections (URTIs) in healthy middle-aged office workers.

   A total of 96 eligible male workers aged 30–49 years were enrolled in this randomized controlled trial. They were given LcS-FM containing $1.0 \times 10^{11}$ viable LcS cells or control milk (CM) once daily for 12 weeks during the
winter season. URTI episodes were evaluated by a physician via a questionnaire of URTI symptoms.

The incidence of URTIs during the intervention period was significantly lower in the LcS-FM group than in the CM group (22.4 vs. 53.2 %, \( P = 0.002 \)). The time to- event analysis showed that the LcS-FM group had a significantly higher URTI-free rate than the CM group over the test period (log-rank test: \( \chi^2 11.25, \ P = 0.0008 \)). The cumulative number of URTI episodes and cumulative days with URTI symptoms per person was lower in the LcS-FM group, and the duration per episode was shorter.

There was no reduction in Natural Killer (NK) cell activity in peripheral blood mononuclear cells and no increase in salivary cortisol levels in the LcS-FM group. These results suggest that the daily intake of fermented milk with LcS may reduce the risk of URTI’s in healthy middle-aged office workers, probably through modulation of the immune system.

**METABOLIC DISORDERS**

3. **Effect of *Lactobacillus casei* strain Shirota-fermented milk (LcS-FM) on metabolic abnormalities in obese prediabetic Japanese men: a randomised, double-blind, placebo-controlled trial.**


This study aimed to investigate the effects of *Lactobacillus casei* strain Shirota (LcS) on metabolic abnormalities in obese prediabetic subjects in a randomised, double-blind, placebo-controlled trial.

100 obese subjects (body mass index \( \geq 25 \)), who had moderate post-load hyperglycemia (1-hr post-load plasma glucose (PG) levels \( \geq 180 \) mg/dl during the oral glucose tolerance test), consumed LcS-fermented milk or placebo milk daily for 8 weeks. The post-load PG and fasting blood markers were evaluated. Although post-load PG levels were not significantly different between the groups, 1-hr post-load PG, glycoalbumin, and HbA1c levels decreased at 8 weeks compared with the baseline levels only in the LcS group (\( p=0.036, p=0.002, \) and \( p=0.006 \), respectively). The reduction in glycoalbumin levels was statistically significantly greater in the LcS group than in the placebo group (\( p=0.030 \)). Stratified analyses revealed significantly improved 1-hr post-load PG and glycoalbumin levels in the LcS group compared with the placebo group among subjects with severe glucose intolerance (2-hr post-load PG levels higher than the median at baseline; \( p=0.036 \) and \( p=0.034 \), respectively).
In terms of lipidic outcomes, total, low-density lipoprotein and non-high-density lipoprotein cholesterol levels were significantly lower in the LcS group than in the placebo group (p=0.023, p=0.022, and p=0.008, respectively).

These findings suggest that LcS may favorably affect metabolic abnormalities in obese prediabetic subjects, though the effects on glycemic control may be limited.

4. The effects of the Lactobacillus casei strain (LcS) on obesity in children: a pilot study.

The study was done to clarify the effects of Lactobacillus casei strain Shirota (LcS) containing beverages in obese children.

The intestinal microbiota and organic acid levels were compared between 12 obese (average age, 10.8 years; Body Mass Index (BMI) Z score, 2.7±1.7) and 22 control children (average age, 8.5 years; BMI Z score, 0.1±0.7). The obese group underwent diet and exercise therapy for 6 months and then were given an LcS beverage daily for another 6 months. Body weight and serological markers were monitored.

There was significant reduction in the faecal concentrations of Bifidobacterium (obese group, 7.9±1.5 vs non-obese group, 9.8±0.5 Log_{10} cells/g; P<0.01) along with a significant decline in the Bacteroides fragilis group, Atopobium cluster and Lactobacillus gasseri subgroup and acetic acid (obese group, 45.1±16.9 vs non-obese group, 57.9±17.6 μmol/g; P<0.05) in the obese group at baseline.

A significant decline in body weight (-2.9±4.6%; P<0.05) and an elevation in the high-density lipoprotein cholesterol level (+11.1±17.6%; P<0.05) was observed 6 months after ingestion of the LcS beverage compared to baseline. Furthermore, a significant increase in the faecal concentration of Bifidobacterium (7.0±1.2 before ingestion vs 9.1±1.2 Log_{10} cells/g after ingestion; P<0.01) and an apparent increase in acetic acid concentration (7.0±1.2 before ingestion vs 9.1±1.2 Log_{10} cells/g after ingestion; P<0.01) were observed 6 months after ingestion.

The study concluded that LcS contributed to weight loss and improved lipid metabolism in obese children via a significant increase in the faecal Bifidobacterium number and acetic acid concentration.
5. Probiotic reduces bacterial translocation in Type 2 Diabetes Mellitus: A Randomised Controlled Study.
Sato J et al. 2017. Scientific Reports. 7: 12115.DOI:10.1038/s41598-017-12535-9

This study was done to investigate whether probiotics cause changes in the gut microbiota and reduce bacterial translocation.

Seventy (70) Japanese patients with Type 2 Diabetes were randomised to receive probiotic drink Lactobacillus casei strain Shirota (LcS) or placebo for a period of 16 weeks. The gut microbiota composition in feces and blood, fecal organic acids, and other biochemical parameters were measured at baseline, 8 and 16 weeks.

The fecal counts of the Clostridium coccoides group and Clostridium leptum subgroup in the probiotic group were significantly higher than in the control group at the end of the study. As expected, the fecal counts of total Lactobacillus were significantly higher in the probiotic group. Intriguingly, the total count of blood bacteria was significantly lower in the probiotic group. However, fecal organic acids were comparable between the two groups.

The results showed that probiotic administration altered the gut microbiota and reduced bacterial translocation in Japanese patients with Type 2 Diabetes Mellitus.

GUT BRAIN AXIS

6. Beneficial effects of Lactobacillus casei strain Shirota (LcS) on academic stress-induced sleep disturbance in healthy adults: A Double-Blind, Randomised, Placebo-Controlled Trial.

The study aimed to examine whether Lactobacillus casei strain Shirota (LcS) improves sleep quality under psychological stress.

A double-blind, placebo-controlled trial was conducted in healthy fourth year medical students exposed to academic examination stress. The trial was repeated over two consecutive years in different groups of students, and the data were pooled. For 8 weeks prior to and 3 weeks after a National Standardized Examination, a total of 48 and 46 subjects received a daily dose of 100 ml of LcS-fermented milk or non-fermented placebo milk, respectively.
The study measured subjective anxiety, overnight single-channel electroencephalography (EEG) recordings, and the Oguri-Shirakawa-Azumi (OSA) sleep inventory scores of subjective sleep quality. Total OSA scores were significantly lower than baseline on the day before the exam and recovered after the exam, indicating a stress-induced decline in sleep quality. There was a significant positive effect of LcS treatment on OSA factors for sleepiness on rising and sleep length. Sleep latency measured by EEG lengthened as the exam approached in the placebo group but was significantly suppressed in the LcS group. The percentage of stage 3 non-REM (N3) sleep decreased in the placebo group as the exam approached, whereas it was maintained in the LcS group throughout the trial. Delta power during the first sleep cycle, measured as an index of sleep intensity, increased as the exam approached in the LcS group and was significantly higher than in the placebo group.

The study suggests that daily consumption of LcS may help to maintain sleep quality during a period of increasing stress. The observed retention of N3 sleep and increased delta power in the LcS group may have contributed to higher perceived sleep satisfaction.

7. **Fermented milk containing Lactobacillus casei Strain Shirota (LcS) preserves the diversity of the gut microbiota and relieves abdominal dysfunction in healthy medical students exposed to academic stress.** Kato-Kataoka A et al. 2016. *Appl Environ Microbiol.* 31;82 (12):3649-58

The study was conducted to investigate the effects of the probiotic *Lactobacillus casei strain* Shirota (LcS) on abdominal dysfunction, a double-blind, placebo-controlled trial with healthy medical students undertaking an authorized Nationwide examination for academic advancement.

23 and 24 subjects consumed an *L. casei* strain Shirota-fermented milk and a placebo milk daily, respectively for 8 weeks, until the day before the examination. In addition to assessments of abdominal symptoms, psychophysical state, and salivary stress markers, gene expression changes in peripheral blood leukocytes and composition of the gut microbiota were analyzed using DNA microarray analysis and 16S rRNA gene amplicon sequence analysis, respectively, before and after the intervention.

Stress-induced increases in a visual analog scale measuring feeling of stress, the total score of abdominal dysfunction, and the number of genes
with changes in expression of more than 2-fold in leukocytes were significantly suppressed in the *L. casei* strain Shirota group compared with those in the placebo group. A significant increase in salivary cortisol levels before the examination was observed only in the placebo group.

The administration of *L. casei* strain Shirota, but not placebo, significantly reduced gastrointestinal symptoms. Moreover, 16S rRNA gene amplicon sequencing demonstrated that the *L. casei* strain Shirota group had significantly higher numbers of species, a marker of the alpha-diversity index, in their gut microbiota and a significantly lower percentage of Bacteroidaceae than the placebo group.

The study demonstrated that the daily consumption of the probiotic strain *L. casei* strain Shirota, preserves the diversity of the gut microbiota and may relieve stress-associated responses of abdominal dysfunction in healthy subjects exposed to stressful situations.

**OTHERS**


The study investigated the effects of the probiotic strain *Lactobacillus casei* Shirota (LcS) on immune profiles and intestinal microbial translocation among children infected with human immunodeficiency virus (HIV). This prospective study included 60 HIV-infected children—31 without antiretroviral therapy (ART) (HIV (+)) and 29 who received ART for a median of 3.5 years (ART (+)) and 20 children without HIV infection (HIV (-)).

Participants were recruited in Vietnam. All children were given fermented milk containing LcS (6.5 × 10⁸ cfu) daily for 8 weeks. Before and after LcS ingestion, blood samples were collected for virological, immunological, and bacteriological analyses. After LcS ingestion, peripheral CD4⁺ T-cell and Th2 (CXCR3-CCR6-CD4⁺) counts significantly increased in both HIV-infected groups; Th17 (CXCR3-CCR6⁺CD4⁺) counts increased in all three groups; regulatory T-cell (CD25highCD4⁺) counts decreased in the ART(+) and HIV(-) groups; activated CD8⁺ cells (CD38⁺HLA-DR⁺CD8⁺) decreased from 27.5% to 13.2% (p < 0.001) in HIV(+) children; and plasma HIV load decreased slightly but significantly among HIV(+) children. No group
showed a significantly altered frequency of bacterial 16S/23S rRNA gene
detection in the plasma. No serious adverse events occurred.
These findings suggest that short-term LcS ingestion is a safe supportive
approach with immunological and virological benefits in HIV-infected
children.

9. The effect of probiotic Lactobacillus casei Shirota (LcS) on knee
osteoarthritis: A Randomised Double-Blind, Placebo-Controlled
Clinical Trial.

The study investigated the effect of probiotic Lactobacillus casei Shirota
(LcS) on patients with knee Osteoarthritis (OA).

537 patients with knee OA were enrolled in this double-blind, placebo-
controlled trial, who were randomised to receive skimmed milk
containing either LcS or placebo daily for 6 months.

Primary outcome was defined as changes in WOMAC (Western Ontario
and McMaster Universities Osteoarthritis Index) and VAS (visual analog
scale) scores. Secondary outcome was defined as changes in serum levels
of high sensitivity C-reactive protein (hs-CRP). After 6 months of
treatment, both WOMAC and VAS scores were significantly improved in
the LcS groups of patients compared to the placebo group. Serum levels of
hs-CRP were also significantly lower in patients receiving LcS than
placebo.

Strong linear correlations were observed between serum hs-CRP levels
and WOMAC and VAS scores. LcS consumption could serve as a novel
therapeutic option in the clinical management of knee OA, improving
treatment outcome likely through reducing serum hs-CRP levels.