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Original article

Effects of yogurt containing *Lactobacillus plantarum* HOKKAIDO on immune function and stress markers

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ABSTRACT

Lactobacillus plantarum HOKKAIDO (HOKKAIDO strain) was isolated from well-pickled vegetables in Hokkaido, Japan. We report a randomized, double-blind, placebo-controlled study evaluating the effects of *L. plantarum* HOKKAIDO on immune function and stress markers in 171 adult subjects. Subjects were divided into three groups: the *L. plantarum* HOKKAIDO yogurt group, the placebo-1 group who ingested yogurt without the HOKKAIDO strain, and the placebo-2 group who ingested a yogurt-like dessert without the HOKKAIDO strain. Hematological tests and body composition measurements were performed before and after 4 and 8 weeks of blinded ingestion. Although no significant differences in natural killer cell activity were observed, it was found that neutrophil ratio significantly decreased and lymphocytes tended to increase in the HOKKAIDO strain yogurt group compared with the yogurt-like dessert group. In addition, the neutrophil-to-lymphocyte ratio, a stress marker, tended to improve in the HOKKAIDO strain yogurt group compared with the yogurt-like dessert group. These results suggest that the ingestion of HOKKAIDO strain yogurt tends to improve immune activity and decrease stress markers.

1. Introduction

Several types of microorganisms reside in the intestine and comprise an individual's "microbial flora."[1] Probiotic microorganisms serve various purposes, such as increasing beneficial bacteria-growth, promoting digestion and absorption, and suppressing infectious diseases.[2]

The human immune system performs activities of "innate immunity," otherwise known as the nonspecific immune system that functions as the body's first line of defense, and "adaptive immunity," referred to as the acquired immune system or the specific immune system. Natural killer (NK) cells are innate lymphoid cells that play a role in natural immunity against tumors and infected cells.[3] NK cell numbers decrease because of various reasons, such as aging,[4] stress,[5] and smoking cigarettes.[6,7] Thus, it is worthwhile to investigate whether functional foods or the bioactive components of certain foods can improve NK cell activity. Recently, probiotic organisms, such as *Lactobacillus*, have been shown to have several functional properties, including stimulation of the immune system.[8] The mechanism of NK cell activation by *Lactobacillus* is thought to involve increased production of several cytokines following macrophage phagocytosis of *Lactobacillus*.[9]

Abbreviations: BMI, body mass index; BW, body weight; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HPA, hypothalamic-pituitary-adrenal; LDL-C, low-density lipoprotein cholesterol; NK, natural killer; NLR, neutrophil-to-lymphocyte ratio; TC, total cholesterol; TG, triglyceride.

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The gut–brain-microbiota axis is increasingly recognized as an important regulator of intestinal physiology.[10] Exposure to psychological stress causes activation of the hypothalamic–pituitary–adrenal (HPA) axis that influences intestinal barrier function. Microbial flora affect postnatal development of the HPA stress response in mice,[11] and germ-free mice exhibit more anxiety than their conventional counterparts. These findings suggest that the modulation of intestinal microbiota, through probiotics or symbiotics, could alter human behavior and reduce stress. However, there are limited number of clinical trials that have investigated effects of probiotics on both the immune system and stress-induced disorders.

There are currently three *Lactobacillus* biotope classifications: vegetable *Lactobacillus*, animal *Lactobacillus*, and intestinal *Lactobacillus*. Vegetable *Lactobacillus* exists in fermented foods that are often eaten in Japanese cuisine. It was recently reported that vegetable *Lactobacillus* isolated from vegetarians' intestines[12] activates immune functions and prevents infection and cancer.[13] In addition, vegetable *Lactobacillus* contains plant-derived antibacterial agents, including tannins, and can persist in high-salt and low-pH environments.

Lactobacillus plantarum HOKKAIDO (HOKKAIDO strain) was isolated from well-pickled vegetables in Hokkaido, Japan, by the Hokkaido Food Processing Research Center. This strain is resistant to salt, alcohol, and pH, protects individuals who consume it from bacterial infection, regulates intestinal functions, and improves isoflavone absorption.[14] Regarding immune regulation, it was reported that coincubation of the HOKKAIDO strain with human dendritic cell lines increased Interleukin (IL)-12 mRNA expression.[15] In addition, the HOKKAIDO strain induced the production of IL-8, a neutrophil chemotactic factor, in human monocytes (HTP-1) and OSC70 epithelial cells. These reports demonstrate that the HOKKAIDO strain activates NK cells and likely improves immune function.

These findings warranted a clinical trial to determine whether yogurt containing the HOKKAIDO strain improves immune function and decreases stress markers. However, very few clinical studies have been conducted to specifically assess responses to the HOKKAIDO strain. Here we evaluated whether ingestion of HOKKAIDO strain yogurt improves immune activity and decreases stress markers in Japanese adults.

2. Methods

2.1. Test meal preparation and ingestion method

The composition of the HOKKAIDO strain yogurt investigated in this study is presented in Table 1. The production and packing of test meals were performed by the Hokkaido Milk Product Co. Ltd.

Table 1
Composition of HOKKAIDO strain yogurt compared with placebo yogurt and yogurt-like dessert per 90 g.

Component	HOKKAIDO strain yogurt	Placebo yogurt	Yogurt-like dessert
Calories (kcal)	77	77	82
Water (g)	73.0	73.0	71.8
Proteins (g)	3.1	3.1	2.5
Lipids (g)	2.6	2.6	2.4
Carbohydrates (g)	10.3	10.3	12.7
Ash (g)	0.8	0.8	0.6
Sodium (mg)	45	45	40
HOKKAIDO strain	$\geq 5.0 \times 10^9$ CFU	–	–

Subjects were instructed to ingest daily 90 g of yogurt containing *L. plantarum* HOKKAIDO ($\geq 5.0 \times 10^9$ CFU, Hokkaido strain yogurt) or yogurt fermented with *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus* (Chr. Hansen, Hoersholm, Denmark) without Hokkaido strain (placebo-1) or a yogurt-like dessert without the Hokkaido strain (placebo-2). The HOKKAIDO strain was originally isolated from well-pickled vegetables in Hokkaido, Japan, by the Hokkaido Research Organization.[14]

2.2. Subjects

In this study, 171 subjects (28 males and 143 females, age range 31–69 years) with NK cell activity below 50% were enrolled. Subjects with a recent history of gastrointestinal disorders, pregnancy, significant disease, surgery, severe allergic reaction to food, or current use of any medication were excluded. Mean subject age, body weight (BW), height, body mass index (BMI), body fat percentage, and NK cell activity for each group are reported in Table 2.

The clinical intervention was conducted as a double-blind, placebo-controlled trial. At randomization, the 171 eligible subjects were randomly assigned to one of the three groups (Hokkaido strain yogurt group, placebo-1 group, and placebo-2 group) with adjustment for age, sex, and NK cell activity. The time schedule for this clinical study is shown in Fig. 1.

We performed hematological examinations and body composition (BW, BMI, and body fat percentage) measurements at baseline (week 0) and post-intervention (weeks 4 and 8) for the three groups. The hematological examinations were performed by Sapporo Clinical Laboratory, Inc. (Sapporo, Japan). Each subject's body compositionwas measured with an In-Body device (Biospace Co., Tokyo, Japan).

All subjects provided written informed consent before undergoing any study-related tests, and the study protocol was approved by the Ethics Committee of Hokkaido Information University. The study protocol conformed to the Helsinki Declaration and was registered at the UMIN Clinical Trial Registration System (certificate number UMIN000014138).

2.3. Statistical analysis

The mean and standard deviation of subject characteristics were calculated for each group. Changes in subject values were analyzed using Student's *t*-tests comparing means between the test group and placebo-1 group or between the test group and placebo-2 group. Statistical analyses were performed using SPSS Statistic 19 (IBM, Armonk, NY, USA). *P*-values <0.05 were considered significant.

Table 2
Characteristics of the subjects in the placebo and HOKKAIDO strain yogurt intake groups.

Characteristic	HOKKAIDO strain yogurt	Placebo yogurt	Yogurt-like dessert	*P* value
Subjects, *n*	57	55	59	–
Males, *n* (%)	9 (15.79%)	8 (14.55%)	11 (18.64%)	0.831
Age, years	49.58 ± 8.62	51.15 ± 9.90	51.34 ± 10.94	0.275
Height, cm	159.89 ± 5.96	158.39 ± 6.77	158.11 ± 6.35	0.698
Body weight, kg	54.82 ± 8.96	54.04 ± 7.99	53.48 ± 8.62	0.954
BMI, kg/m^2	21.38 ± 2.83	21.51 ± 2.66	21.36 ± 2.97	0.667
Body fat percentage, %	27.04 ± 6.47	27.40 ± 7.02	26.30 ± 6.55	0.580
NK cell activity, %	32.46 ± 13.39	28.85 ± 11.91	28.85 ± 13.50	0.580

Values shown are mean ± standard deviation. Analysis by analysis of variance was performed for age, height, body weight, BMI, body fat percentage, and natural killer cell activity and by chi-square test for gender. BMI, body mass index; *n* = number of subjects.