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Effects of Acetic Acid Bacteria (*Gluconacetobacter hansenii* GK-1) on Fatigue Induced by Temporary Mental Stress: A Randomized, Double-Blind, Placebo-Controlled Study

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Abstract

Objective: This study assessed the effects of consuming acetic acid bacteria (*Gluconacetobacter hansenii* GK-1) for 12 weeks on fatigue induced by temporary mental stress. **Methods:** This randomized, double-blind, placebo-controlled, parallel-group study included 100 healthy male and female adults aged 20 - 64 years. Participants consumed either the *G. hansenii* GK-1 supplement (9×10^9 cells/day) or a placebo daily for 12 weeks. The impact of temporary mental stress on fatigue in *G. hansenii* GK-1 was assessed using a Visual Analog Scale (VAS) before the study began and after 12 weeks of supplementation. **Results:** Subjective fatigue measured by Visual Analog Scale (VAS) showed a significant decrease in fatigue induced by temporary mental stress after 12 weeks of consumption in the *G. hansenii* GK-1 group compared with the placebo group. No adverse events were attributed to *G. hansenii* GK-1. These findings confirm that continuous oral ingestion of *G. hansenii* GK-1 by healthy Japanese adults reduces feelings of fatigue caused by temporary mental stress.

Keywords

Acetic Acid Bacteria, *Gluconacetobacter hansenii* GK-1, Fatigue, Temporary Mental Stress, Double-Blind Study

1. Introduction

A 2002 survey on employee health by Japan's Ministry of Health, Labor, and Welfare revealed that 72.2% of workers experienced fatigue from their regular jobs, indicating that Japan was a fatigued country [1]. It is anticipated that fatigue and feelings of fatigue will become increasingly prevalent in society due to the increasing complexity and pace of social structures, as well as an aging population. Countermeasures are necessary to prevent overwork and stimulate economic activity. Despite the widespread nature of this issue, few foods or medicines have demonstrated effective results in combating fatigue.

Generally, Kitani *et al.* (from the Fatigue Research Group of the Ministry of Health, Labor, and Welfare) define fatigue in healthy individuals as an attenuation of mental and physical functions accompanied by unique pathological discomfort caused by excessive physical and mental activities, along with a desire to rest [2]. "Anti-fatigue" measures, therefore, aim to relieve the feeling of fatigue caused by discomfort (stress) during a fatigue challenge. Other substances known to alleviate discomfort (stress) during fatigue in healthy adults include L-theanine [3]-[6], which inhibits excitation and enhances the inhibition of neurotransmitters and neuroreceptors in the brain, and GABA [7] [8], which acts on the autonomic nervous system from the peripheral ganglia. The acetic acid bacteria (*Gluconacetobacter hansenii* GK-1) used in this study were reported to reduce nasal discomfort caused by pollen and house dust in humans [9]. It has also been shown to reduce the cumulative number of days of illness due to general fatigue and fatigue with cold-like symptoms by strengthening the immune system through pDC, IFN-α, and s-Ig pathways [10]-[12].

Previous studies have shown that temporary mental stress, experienced as discomfort (stress) during a fatigue challenge, reduces immune function. Thus, it is expected that *G. hansenii* GK-1 will help maintain immune function and reduce feelings of fatigue [13]. Functional food materials that alleviate discomfort during fatigue challenges have novel effects on immunity rather than the nervous system. However, previous research has only assessed fatigue by measuring the cumulative number of days of cold-like symptoms. The effects of these bacteria on fatigue caused by temporary mental stress have not yet been studied.

Based on previous studies, we conducted a 12-week randomized, double-blind, placebo-controlled, parallel-group study. Our aim was to investigate the effects of continuous oral ingestion of *G. hansenii* GK-1 on fatigue induced by temporary mental stress in healthy Japanese adults of both sexes [11]-[13].

2. Materials and Methods

2.1. Study Samples

The test foods consisted of 9×10^9 cells/day of *Gluconacetobacter hansenii* GK-1 (Kewpie Corporation, Tokyo, Japan) and dextrin (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan) as a placebo. Both were formulated as capsule supplements (Aliment Industry Co., Ltd., Yamanashi, Japan) with uniform taste, appearance,

and nutritional composition (Table 1).

Table 1. Nutrition facts of each test sample.

		Placebo	GK-1
Energy	(kcal)	1.1	1.1
Protein	(g)	<0.1	<0.1
Fat	(g)	<0.1	<0.1
Carbohydrates	(g)	0.2	0.2
Salt equivalent	(g)	0.0	0.0

2.2. Study Design

The study was conducted at Miura Medical Clinic, Japan, between March and June 2024 under the supervision of a physician. Participants were randomly divided into two groups—placebo or *G. hansenii* GK-1—for a 12-week, randomized, double-blind, placebo-controlled, parallel-group study. To avoid bias in subjective fatigue scores, allocation personnel not involved in laboratory tests used block randomization, considering sex, age, and Visual Analog Scale (VAS) scores from the pre-test. Allocation information remained sealed from allocation until study completion. Subjects, interventionists, and outcome assessors were all blinded. Both groups consumed one capsule daily for 12 weeks. Throughout the intake period, the participants maintained daily life diaries, recording test food consumption, body condition, drug intake, and alcohol and exercise habits.

Anthropometric measurements, physical examinations, and physician interviews were conducted before ingestion and after 12 weeks. Fatigue assessment involved temporary mental stress assessment using the Uchida Kraepelin test and subjective fatigue assessment using a Visual Analog Scale (VAS) before and after the stress load. This study adheres to the principles of the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (MEXT·MHLW·METI, Japan). The Ethics Committee of the Miura Clinic, Medical Corporation Kanonkai approved the study (approval number: S2308, approval date: January 25, 2024). The study protocol was pre-registered with the Clinical Trials Registry System (UMIN-CTR) (UMIN000053601).

2.3. Subjects

The subjects were 100 healthy Japanese males and females aged 20 to 64 years experiencing daily fatigue. (Table 2) presents the subject inclusion and exclusion criteria. Subjects should: 1) take test food as directed by the study director or investigator's staff, 2) avoid ingestion of test food by persons other than the study subjects, 3) avoid binge eating and drinking on the day before all tests, excessive exercise, and lack of sleep, and maintain daily activities, 4) not significantly change their diet and lifestyle before and during study participation, record any changes in eating habits and lifestyle in the diary, 5) during the study, record the name

Table 2. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
(1) Healthy males and females aged 20 - 64 years	(1) Serious diseases such as diabetes mellitus and metabolic diseases
(2) Individuals who were aware of daily fatigue	(2) Those treated for chronic fatigue syndrome (CFS)
(3) Those who volunteered and agreed to participate in the study by expressing a good understanding of the purpose and the content of the study after being provided the full explanation	(3) Those with food allergies related to research foods
	(4) Those with digestive diseases and a history of digestive surgery (excluding appendicitis)
	(5) Regular users of medicines (including Chinese herbal medicines) and quasi-drugs that may affect immunity, fatigue, and sleep
	(6) Those who habitually consume foods and healthcare products containing high amounts of acetic acid bacteria and bifidobacterial (including lactic acid bacteria, yogurt, and kimchi) at least three times a week
	(7) Those unable to discontinue the intake of specified health foods, foods with functional labels, and supplements
	(8) Those engaged in physical work such as day-night shift work or heavy lifting
	(9) Those engaging in excessive exercise during the study (including professional athletes) or on a diet
	(10) Pregnant and breastfeeding individuals
	(11) Subjects deemed inadequate during screening tests
	(12) Subjects who smoked an average of 21 or more cigarettes per day
	(13) Subjects with a history or current diagnosis of alcohol dependence
	(14) Subjects with an extremely irregular diet
	(15) Subjects participating in various tests during the study period
	(16) Individuals unable to perform the study as specified
	(17) Those who had participated in other clinical studies or were willing to participate
	(18) Those scheduled to travel abroad
	(19) Those considered inappropriate by the study director

and usage of drugs and supplements that may affect immunity and fatigue (including food for specified health and functional labels) or avoid their consumption, 6) during the study, record the name and usage of specified health foods, functional labeled foods, and health foods (including supplements), foods containing acetic acid bacteria, lactic acid bacteria, or bifidobacterial in the diary, 7) during the study, limit alcohol consumption (maximum daily dose: up to the equivalent of 20 g net alcohol), and if alcohol consumption exceeds this level, it should be recorded in the diary, 8) and during the study period, obtain permission from the study director before using any drugs, except in emergencies. If used, the

reason for use, drug name, dose, and duration should be recorded in the patient's diary. 9) if used without the study director's approval due to unavoidable circumstances, the same procedures apply. If unable to attend the study visit on the test day for any reason, the investigator should be contacted immediately, 10) any changes in physical condition during or after the study period should be reported immediately to the investigator or staff, 11) during the study period, subjects should not participate in studies involving ingestion of other foods or use of drugs, cosmetics, or medications, 12) influenza or coronavirus vaccinations received during the study period should be recorded in the diary. For sample size determination, the required sample size was calculated as 89 using the Cancer Research and Biostatistics Statistical Tools (https://stattools.crab.org/), with $\alpha = 0.05$ and power $(1 - \beta) = 90\%$. This calculation was based on the report (unpublished data) of a 12-week, placebo-controlled, double-blind, randomized, parallel-group pilot study involving 88 healthy adult males and females. Accounting for a 10% dropout rate, the total number of subjects in this study was 100, with 50 subjects per group.

The investigator thoroughly explained the study's purpose and content to the subjects. Before conducting the study, written informed consent was obtained from each participant, ensuring their voluntary participation.

2.4. Outcomes

A subjective fatigue survey using a Visual Analog Scale (VAS) was administered as the primary endpoint. Fatigue was assessed by measuring the mean fatigue sensation over the 3 days before the study and the fatigue sensation associated with a temporary mental stress load. Temporary mental stress load was induced using the Uchida Kraepelin test (Japan Institute of Mental Technology) for 30 min (two 15-minute sessions with a 5-minute rest between). The fatigue due to temporary mental stress was calculated by subtracting the pre-load fatigue score from the post-load fatigue score. The VAS used the "Fatigue VAS Test" developed by the Japanese Fatigue Society, to assess subjective fatigue [14]. Participants marked their current fatigue level on a 10-cm line, with the left end representing "not fatigued at all" and the right end representing "so fatigued as to be unable to do anything." The distance from the left end to the participant's mark was measured to quantify fatigue.

2.5. Statistical Analysis

The test results are presented as mean ± SEM, with a significance level set at $p < 0.05$. The statistical analyses were performed using IBM SPSS Statistics 29.0 (IBM Corp., NY, USA). The male-to-female ratio between groups was compared using a χ^2 test, whereas age was compared using the t-test. The feelings of fatigue 3 days before the test and fatigue due to temporary mental loading, as measured by VAS, were analyzed between groups using Welch's t-test after confirming normality with the Shapiro-Wilk test. Paired t-tests were used to compare before and after ingestion.

3. Results

3.1. Subject Characteristics

Table 3 presents the subject characteristics. No significant differences were observed between the placebo and *G. hansenii* GK-1 groups in terms of gender, age, or other demographic factors. During the study, four participants withdrew voluntarily—two from each group. Of the 100 *G. hansenii* patients in the intent-to-treat (ITT) group, 89 were included in the final analysis (Figure 1). This number excludes those who continued drug use or made lifestyle changes (four in the placebo group and three in the *G. hansenii* GK-1 group, as per the study protocol's exclusion criteria for the Per Protocol Set (PPS). The sample intake rate was 97.8%. The subjects included in the analysis maintained their pre-study dietary and lifestyle habits throughout the research period.

Table 3. Background characteristics of the patients.

	Placebo	GK-1	P-value
	Mean ± SE	Mean ± SE	
Number of participants	Male: 20 Female: 24	Male: 22 Female: 23	0.746
Age (y)	45.9± 1.39	45.8 ± 1.50	0.966

P-value for comparison was calculated by χ^2 test for the number of participants and unpaired t-test for age.

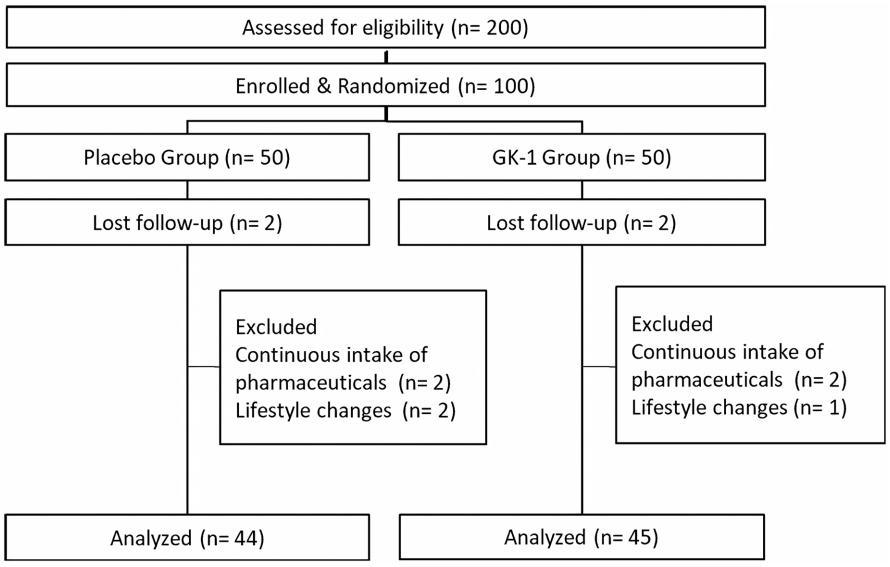

Figure 1. Flowchart of patient recruitment and assignment to the study groups.

3.2. VAS

Table 4 presents the Visual Analog Scale (VAS) results for subjective fatigue, showing the mean fatigue sensation 3 days before the test and the change in fatigue sensation from baseline due to temporary mental loading. At 12 weeks post-

ingestion, there was no significant difference in mean fatigue sensation between the *G. hansenii* GK-1 and placebo groups 3 days prior to the study. However, fatigue due to temporary mental challenges was significantly lower in the *G. hansenii* group than in the placebo group at 12 weeks (P = 0.0082). The placebo group showed a significant decrease in mean fatigue sensation during the 3 days prior to the test at 12 weeks compared with the pre-test levels (P = 0.003), but no significant difference in fatigue sensation due to temporary mental load. The *G. hansenii* group showed no significant differences in mean fatigue sensation 3 days before the study between the pre- and post-ingestion periods. However, this group showed a significant reduction in fatigue sensation due to temporary mental loading at 12 weeks compared with baseline (P = 0.0039).

Table 4. Changes from baseline on fatigue VAS score after 12-week ingestion of placebo or GK-1.

	Week 12 (Mean ± SE)				P-value	
	Chronic fatigue*[1]	Work Load		Temporary mental stress (Post-Pre)	Chronic fatigue	Temporary mental stress
		Pre	Post			
Placebo	−13.9 ± 3.53†††	−14.8 ± 3.48	−12.1 ± 2.94	2.7 ± 3.46	0.2563	0.0082**
GK-1	−7.8 ± 4.04	−4.3 ± 4.22	−14.9 ± 3.26	−10.6 ± 3.47††		

*[1]Average fatigue level three days before the test; **P-value (Welch's t-test), compared with GK-1 and placebo groups; **p < 0.01 vs. placebo groups; †††P-value (paired t-test) compared with baseline (P = 0.0003); ††P-value (paired t-test) compared with baseline (P = 0.0039).

3.3. Safety Evaluation

No adverse events related to the ingestion of *G. hansenii* GK-1 or placebo were reported during the study.

4. Discussion

This randomized, double-blind, placebo-controlled, parallel-group study examined the effects of daily *G. hansenii* GK-1 consumption in healthy Japanese adults aged 20 - 64 years. Participants ingested 9×10^9 cells/day for 12 weeks. Subjective fatigue was assessed using the Visual Analog Scale (VAS) at baseline, after 12 weeks, and following a temporary mental stress challenge. The primary endpoint of *G. hansenii*—change in VAS-measured fatigue due to temporary mental stress after 12 weeks—showed significant improvement in the *G. hansenii* GK-1 group compared with the placebo group.

Fatigue arises from multiple factors, including age, genetics, immune function, neuroendocrine processes, and psychological and behavioral influences [15] [16]. The mechanism by which *G. hansenii* GK-1 reduces temporary fatigue appears to be primarily related to its effects on immune function.

Fatigue results from a complex interplay of various factors with no single identifiable cause. The immune system plays a crucial role in this process. When temporary mental stress induces fatigue, overactive nerve cells generate oxygen radicals

that oxidize proteins and lipids, leading to cellular and intracellular damage. Immune cells detect this damage and signal the brain's nervous and endocrine systems via immune cytokines to repair and maintain the immune balance [17]. It is hypothesized that by adjusting the immune balance, the neuroinflammation generated by temporary mental stress can be suppressed, thereby reducing fatigue [18]. The mechanism appears to involve regulatory T cell activation, which controls excessive inflammation and suppresses mental fatigue. Studies have shown that orally ingested *G. hansenii* GK-1) can reach the small intestine with preserved functional activity. These bacteria bind to TLR2 and TLR4 receptors in intestinal epithelial cells, inhibiting them via regulatory T cells [19]-[24]. Further research on *G. hansenii* GK-1 and immunity by Tanaka T *et al.* (2022) [12] revealed significant enhancement of CD40 and CD80 expression, which play critical roles in maintaining immunity and reducing the cumulative number of days of exhaustion. CD40 promotes NK and T cell activation through IL-12, maintains IFN-α production, sustains immune cell activity [25] [26], and promotes IgA production while activating NK cells [12] [27]. Yamashita *et al.* (2022) [10] reported that *G. hansenii* GK-1 use was associated with increased immunization and sIgA *G. hansenii* levels [11]. Their study showed a significant reduction in the cumulative days of general fatigue onset in the *G. hansenii* GK-1 group compared with the control group. The interrelation between immune responses, stress, and sIgA suggests that increased sIgA levels may contribute to alleviating feelings of exhaustion during temporary mental stress [28] [29].

Fatigue is categorized into four stages: acute, subacute, diurnal, and chronic [30]. In this study, the improvement in fatigue sensation following the ingestion of *G. hansenii* GK-1 corresponds to acute fatigue induced by temporary mental stress. Although no significant group differences were observed in mean fatigue levels 3 days before testing, these measures assessed subacute fatigue without temporary loading and its potential progression to chronic fatigue. Participants were instructed to maintain their usual daily activities, including diet and exercise, and no major changes were reported or observed during the study. However, subjective fatigue assessment is susceptible to external factors and does not involve a standardized temporary load. Consequently, the effect on the mean fatigue sensation 3 days before the study could not be definitively determined. A limitation of this study is that various lifestyle elements and diets complexly influence fatigue induced by temporary mental stress [31] [32]. The extent to which these confounding factors affected the results was not fully explored. Future research should investigate the relationship between subject characteristics, lifestyle habits, and the impact of *G. hansenii* GK-1 ingestion on fatigue.

Oral ingestion of *G. hansenii* GK-1 for 12 weeks by healthy Japanese adults reduces feelings of fatigue caused by temporary mental stress and is expected to be used as a functional food that contributes to the maintenance of fatigue.

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Authors' Contributions

Conceptualization: K.S. and R.M.; Methodology, software, validation, formal analysis, and investigation: W.W., M.O., and M.W.Y.; Resources: M.R., Y.T., and K.K.; Data curation; W.W., M.O., and M.W.Y.; Writing—original draft preparation: W.W.; Writing—review and editing: M.R. and Y.T.; Visualization: W.W.; Supervision: K.S., N.M., and M.K.; Project administration and Funding acquisition: R.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The data underlying this article cannot be shared publicly due to ethical restrictions. The data will be shared on reasonable request to the corresponding author.

Institutional Review Board Approval

The study was conducted according to the Declaration of Helsinki Guidelines and approved by the Ethics Committee of Miura Clinic, Medical Corporation Kanonkai (protocol code R2308; date of approval January 25, 2024).

Informed Consent

Informed consent was obtained from all patients involved in the study.

Conflicts of Interest

W.W., M.O., M.W.Y., K.K., Y.T., M.K., and R.M. are employees of Kewpie Corporation. The remaining authors have no other conflicts of interest to declare.

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