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Full Paper

Effect of applying *Lactiplantibacillus plantarum* subsp. *plantarum* N793 to the scalps of men and women with thinning hair: a randomized, double-blind, placebo-controlled, parallel-group study

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***Lactiplantibacillus plantarum* subsp. *plantarum* N793 (N793) is a lactic acid bacterium (LAB) isolated from corn. We previously showed that N793 increases the level of keratinocyte growth factor, which is required for hair growth, in the culture supernatant of human follicle dermal papilla cells. Additionally, an open-label, single-arm study reported that applying a lotion containing N793 to the scalp for 24 weeks improved hair density in men and women with thinning hair. The present study was a double-blind, placebo-controlled, parallel-group study aimed at verifying the efficacy of N793 for thinning hair. A lotion containing N793, and a control lotion (placebo) were applied once daily for 24 weeks to 104 healthy Japanese men and women. Analysis of all participants revealed no difference in hair density between the N793 and placebo groups. However, an additional analysis limited to participants with relatively mild progression of thinning hair showed a significantly better hair density in the N793 group than in the placebo group. These findings suggest that topical application of N793 improves thinning hair in men and women when the condition's progression is relatively mild.**

Key words: *Lactiplantibacillus plantarum* subsp. *plantarum* N793, thinning hair, hair loss, lactic acid bacterium

INTRODUCTION

Scalp hair protects the scalp from sunlight, other external irritants, and temperature extremes and comprises both the hair and follicles that hold the hair [1]. Hair grows in a prescribed process called the hair cycle that repeats three phases: the anagen, catagen, and telogen phases. Thinning hair is a condition in which a range of factors (age, stress, lack of sleep, and genetics) lead to reduced activity of hair follicle dermal papilla cells (HFDPCs) and stem cells that disrupts the hair cycle, decreasing the amount and diameter of hair and making the scalp more visually prominent [1, 2]. Scalp hair normally grows for 2–6 years and then deteriorates over 2–3 weeks; however, it remains in the hair follicle for several months in its resting/telogen phase. Next, this telogen hair is shed from the follicle when new hair grows in the follicle [1]. The factors mentioned above that shorten the hair cycle reduce the growth/anagen phase from several years to several months, causing hair to shed from follicles before attaining full growth and resulting in thinning hair. The anagen phase is maintained by growth factors, such as keratinocyte growth factor (KGF), insulin-like growth factor 1, and vascular endothelial growth factor [2, 3]. Maintaining the anagen phase promotes hair growth and increased hair diameter. Thinning hair is obvious due to the reduced amount of hair, loss of hair, and change from terminal to vellus hair.

The growth factors mentioned above are produced by HFDPCs at the base of the hair follicle. Notably, KGF secreted by HFDPCs acts directly on and promotes the proliferation of hair matrix cells that form growing hair. Therefore, KGF is regarded as the growth factor with the most direct effect on hair growth. Pea extract has been reported to promote KGF gene expression in HFDPCs and increase hair density [4]. Therefore, we can assume that HFDPCs are necessary for hair growth; stimulating their activity and elevating KGF production will lead to hair growth.

Lactic acid bacteria (LAB) are reported to regulate intestinal function and to have other physiological roles [5, 6]. The effect

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of topical application of LAB to the skin has recently become a research focus [7, 8]. Topical application of *Lactiplantibacillus plantarum* USM8613 to wound sites is reported to potentially aid in wound healing [9]. Additionally, topical application of a *Latilactobacillus sakei* proBio-65 strain reportedly reduced inflammation in a mouse model of psoriasis [10]. Applying a cream containing heat-treated *L. plantarum* GMNL6 to the face was confirmed to alter the composition of the dermal bacterial flora, increase the moisture content in the stratum corneum, and reduce wrinkles [11]. Moreover, topical application of an ointment containing *Limosilactobacillus reuteri* DSM 17938 reportedly reduces symptoms in patients with atopic dermatitis [12]. However, few reports have examined the effects of applying LAB on the scalp or thinning hair.

We screened a bacterial strain that can stimulate HFDPC activity and promote KGF production from a microorganism library belonging to Nissin Foods Holdings Co., Ltd. (Nissin, Tokyo, Japan). Specifically, we selected the strain *L. plantarum* subsp. *plantarum* N793 (N793) because it produced the highest activity in experiments that assessed the ability of killed bacteria to stimulate cell activity in HFDPCs and elevate the level of KGF in the culture supernatant of HFDPCs [13, 14]. N793 is a unique LAB that was isolated from corn by Nissin. Based on the data collected, N793 promises to improve thinning hair by stimulating HFDPC activity and promoting growth factor production. Although an open-label, single-arm study in men and women with self-perceived thinning hair showed that hair density was improved by sustained topical application of a lotion containing N793 [15], the efficacy of N793 has yet to be evaluated thoroughly. Accordingly, in this study, we aimed to verify the efficacy of sustained topical application of N793 on thinning hair in a randomized, double-blind, placebo-controlled, parallel-group study.

MATERIALS AND METHODS

Participants

This study was conducted after review and approval by the Shiba Palace Clinic Ethics Committee (approved on June 24, 2021; approval number: 147015-30215). The study complied with the ethical principles established by the Declaration of Helsinki (adopted in 1964, revised in Fortaleza in October 2013), the Ethical Guidelines for Medical Research Involving Human Subjects (MEXT/MHLW Public Notice No. 3 of 2014), and the Act on the Protection of Personal Information (Act No. 57 of May 30, 2003). We recruited healthy Japanese men and women who had self-perceived thinning hair and were 20 to ≤59 years of age. Specifically, we selected 200 people who provided informed consent after receiving an explanation of the study details. A participant information and consent form approved by an ethics committee was used to thoroughly explain the details of the study, and consent to participate was obtained voluntarily and in writing from each of the 200 candidates willing to participate. After obtaining consent, the candidates underwent screening that involved photographing their heads and blood and urine tests. Based on the screening data, the study investigator and personnel selected 106 participants with thinning hair and no illnesses who met the inclusion criteria and had none of the exclusion criteria (53 men and 53 women; mean age: 40.00 ± 4.00 years). The number of participants in each group was established based on the results of a pilot study [15].

The inclusion criteria were as follows: (1) Japanese men or women who were 20 to 59 years of age at the time of consent, (2) self-perceived thinning hair, (3) capable of consenting to a partial haircut by a professional, and (4) able to agree to maintain normal haircut (hair length, hairstyle, and perm), hair-dyeing (hair dyeing agent), and hair-washing (hair washing agent) routines for the study duration.

The partial haircut was performed as follows: taking the intersection of the midline of the head and the line connecting the topmost part of the helix of the left and right ears (transverse line), clippers were used to trim a 1 × 1 cm area of hair to a length of approximately 0.5 mm at a position approximately 3 cm along the transverse line to the right of the intersection. The size and location of the haircut included a degree of error due to individual variability.

The exclusion criteria were as follows: (1) at risk of developing allergic symptoms to test product components, (2) use of oral or topical pharmaceuticals that may affect the test results (anti-hair loss agents), (3) habitual consumption of health foods that may affect the study results (supplements that claim to be effective for hair growth), (4) at risk of developing allergic dermatitis or persons with skin hypersensitivity, (5) current treatment by a dermatologist, (6) pregnant, nursing, or planning to become pregnant or nurse a baby during the study, (7) participation in another clinical study, (8) use of a shampoo or other hair cleaning product that claims to be effective for hair growth, (9) presence of an underlying disease (diabetes mellitus currently being treated with medication or diabetes mellitus with complications; chronic respiratory disease, chronic heart disease, including hypertension; chronic kidney disease; chronic liver disease, excluding fatty liver or chronic hepatitis; chronic blood disease, excluding iron-deficiency anemia; neurological or neuromuscular disease associated with an immune disorder; chromosomal abnormality; severe motor and intellectual disability, such as a combination of severe physical and intellectual disabilities]), and (10) current receipt of any type of drug treatment or outpatient treatment.

Test products

The N793 strain was grown in appropriate culture medium, washed with water, heat-killed, and frieze-dried (N793 powder) [15]. The test products used in this study were the investigational product and a control (placebo). The investigational product was a lotion comprising a mixture of heat-sterilized N793 powder, water, ethanol, dipropylene glycol, carbomer, potassium hydroxide, and PEG-40 hydrogenated castor oil. One measure of the investigational product (3 mL) contained 20 mg of N793 powder. The placebo was the same lotion without the N793 powder. All ingredients used in test samples were cosmetic grade. Both products were prepared to be indistinguishable. One measure (3 mL) of either product was applied to each participant's scalp daily for 24 weeks. The dose of the active component (20 mg) and application period (24 weeks) were set based on the results of a pilot trial [15]. Application was performed within 30 min after washing the hair in the morning. However, if the hair was not washed in the morning or the morning application was missed, application was performed within 30 min after washing the hair in the evening. Furthermore, if application was missed when the

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hair was washed in the evening, the product was applied before going to bed. Missed applications were not carried over to the following day.

Study schedule and control items

The participants were instructed to comply with control items for the study duration but to continue their daily routines. The control items during the study were as follows: (1) maintenance of normal haircutting (hair length, hairstyle, and perm) and hair dyeing (hair dyeing agents) habits, (2) prohibition of all procedures involving professional scalp massage and care, (3) continued use of usual hair washing agents and methods, and (4) abstinence from food on the day before an observation day that included a blood draw for screening but maintenance of adequate fluid (mineral water) intake from at least 12 hr prior to the visit.

Evaluation items and methods

An efficacy evaluation was undertaken as detailed in Table 1. For the study duration, participants were asked to record their daily activities in an online record (physical condition, health food and supplement consumption, pharmaceutical use, and haircare records). The condition of the head was recorded by taking digital photographs of the head from three directions: the top, back, and side. Moreover, the hair was assessed using a digital microscope to observe and record images of the area given a partial haircut. These images were used to determine hair density, mean hair diameter, and vellus and non-vellus hair densities and to calculate vellus (vellus hair density/hair density) and non-vellus hair percentages (non-vellus hair density/hair density). Next, 2 days after the partial haircut, the digital microscope was used to observe and record the partial haircut area and measure the anagen and telogen hair densities. Compared with day 0, hair that increased in length was considered anagen hair, and hair that did not increase in length was considered telogen hair. Subsequently, a hair growth coefficient was calculated as the anagen hair density/telogen hair density.

Hair loss was assessed by counting the number of hairs shed during washing. Specifically, the participant's hair was washed by a hairdresser for 3 min using only shampoo with no hair growth or treatment effects and rinsed thoroughly with warm water (approximately 40°C). Hairs shed during this washing process were recovered, laid out individually on a paper board, and counted manually by naked eye. Moreover, thinning hair was assessed subjectively by participants using a 10-question visual analog scale (VAS) questionnaire. For questions 1–9, the VAS was labeled with a score of 1 for "not concerned at all" on the leftmost side and 100 for "concerned" on the rightmost side. For question 10, the VAS was labeled with a score of 1 for "yes" on the leftmost side and a score of 100 for "no" on the rightmost side. Furthermore, the primary evaluation was based on hair assessment, hair loss count, and the VAS questionnaire, and a secondary evaluation was performed based on a lifestyle survey and digital images of the head.

The following data was collected during screening to exclude participants with illnesses. First, blood tests measuring the white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, total protein, albumin, urine nitrogen, creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, lactate dehydrogenase, creatine kinase, blood sugar, and hemoglobin A1c were conducted. Second, urine tests measuring the specific gravity, pH, protein, sugar, urobilinogen, bilirubin, and ketone bodies were conducted. Third, a urine occult blood test was conducted and scored as follows: 0, −; 1, +−; 2, +; 3, 2+; 4, 3+. These investigations were performed by BML, Inc., Tokyo, Japan.

The participants were assigned the investigational product or placebo in a manner that resulted in an even distribution of individuals between the groups based on thinning hair progression. Thinning hair progression in men and women was staged using the Hamilton–Norwood and Ludwig classification systems, respectively [16].

Table 1. Test schedule

Item		SCR	Baseline	Baseline +2 days	12 weeks	12 weeks +2 days	24 weeks	24 weeks +2 days
Subject visit		●	●	●	●	●	●	●
Obtaining consent		●						
Daily living condition				←	—	—	—	→
Test product application				←	—	—	—	→
Blood Test		●						
Urine Test		●						
Photographing subject heads		●	●		●		●	
Hair assessment	Hair density		●		●		●	
	Mean hair diameter		●		●		●	
	Percentage of vellus hairs		●		●		●	
	Percentage of non-vellus hairs		●		●		●	
	Anagen hair density			●		●		●
	Telogen hair density			●		●		●
	Anagen hair density / Telogen hair density			●		●		●
Number of hairs lost			●		●		●	
Visual Analogue Scale			●		●		●	

SCR: screening.

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Statistical analysis

Definition of the statistical analysis set

Statistical analysis sets and test methods were established in advance using the study protocol. Participants that met any of the following analysis exclusion criteria underwent a case review and were excluded from the analysis unless special circumstances were evident. The analysis exclusion criteria were as follows: (1) observation day delayed by ≥1 week, (2) participant violated the instructions for the participant control items during the study, (3) questionable data reliability due to problems with testing, (4) unused test product (participant did not use the amount prescribed for a single day) on over 15% of days scheduled for test product application, (5) discovery of the violation of an entry criterion or discovery of an applicable exclusion criterion, (6) collected data judged to include an abnormal value, and (7) any other evident reasons that indicated appropriate grounds for exclusion.

Efficacy assessment

Values are expressed as the mean ± standard deviation; tests used a 5% significance level (two-tailed). The results of hair assessments, hair loss counts, and VAS questionnaires performed after 12 and 24 weeks of application were compared against baseline data obtained before application. Additionally, the results of hair assessments, hair loss counts, and VAS questionnaires were compared between groups (Fig. 1). Comparisons of hair assessments, hair loss counts, and VAS questionnaire scores over time were performed using Dunnett's test. The statistical analysis software Dr. SPSS II for Windows (SPSS Japan, Tokyo, Japan) was used for analysis. A p-value of less than 0.05 was defined as statistically significant.

RESULTS

As one participant from the investigational product group (N793 group) and one participant from the placebo group dropped out of the ongoing study for personal reasons, the final efficacy evaluation was conducted with 52 participants in the N793 group and 52 participants in the placebo group. Among the remaining individuals, the partial haircut used in the hair assessment was performed in an incorrect position in one participant in the N793 group and in two participants in the placebo group. Therefore, these participants were deemed to meet the third analysis exclusion criterion and were excluded from the hair assessment part of the efficacy evaluation.

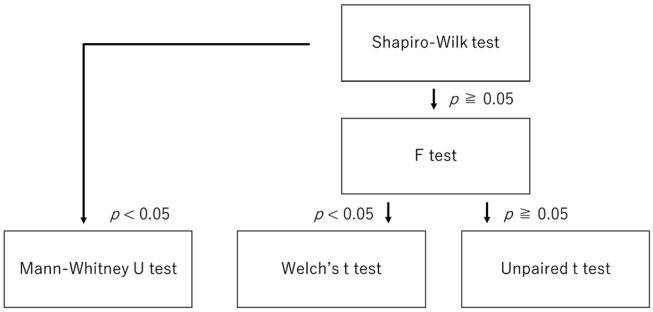

Fig. 1. Statistical analysis.
Confirmation of data normality, assessment of variance, and comparisons between the two groups were conducted by statistical analysis as shown here.

Unadjusted hair assessment data (raw data not adjusted for baseline) obtained with the digital microscope (on day 0 of partial haircut) were compared over time (Supplementary Table 1a). The hair densities in the N793 group after 12 (155.0 ± 32.7 hairs/cm^2) and 24 weeks of application (157.9 ± 32.3 hairs/cm^2) were significantly higher than the pre-application density (150.8 ± 31.8 hairs/cm^2). Similarly, the hair densities in the placebo group after 12 (152.6 ± 38.4 hairs/cm^2) and 24 weeks of application (154.7 ± 39.9 hairs/cm^2) were significantly higher than the pre-application density (149.3 ± 38.2 hairs/cm^2). Furthermore, the mean hair diameters in the N793 group after 12 (53.8 ± 16.8 μm) and 24 weeks of application (56.0 ± 17.2 μm) were significantly larger than the pre-application diameter (51.5 ± 16.4 μm). Similarly, the mean hair diameters in the placebo group after 12 (55.7 ± 15.0 μm) and 24 weeks of application (57.8 ± 16.3 μm) were significantly larger than the pre-application diameter (51.6 ± 15.0 μm). Defining vellus hair as hair with a diameter <40 μm, the vellus hair percentages in the N793 group after 12 (36.7 ± 27.4%) and 24 weeks (34.9 ± 26.9%) were significantly smaller than the pre-application percentage (39.7 ± 26.7%). Similarly, the vellus hair percentages in the placebo group after 12 (33.1 ± 21.7%) and 24 weeks of application (30.5 ± 21.6%) were significantly smaller than the pre-application percentage (37.9 ± 23.7%). Next, defining non-vellus hair as hair with a diameter ≥40 μm, the non-vellus hair percentages in the N793 group after 12 (63.3 ± 27.4%) and 24 weeks of application (65.1 ± 26.9%) were significantly higher than the pre-application percentage (60.3 ± 26.7%). Similarly, the non-vellus hair percentages in the placebo group after 12 (66.9 ± 21.7%) and 24 weeks of application (69.5 ± 21.6%) were significantly higher than the pre-application percentage (62.1 ± 23.7%).

Hair was also assessed using digital microscope images of the partial haircut area taken 2 days after the partial haircut (Supplementary Table 1b). The anagen hair density in the N793 group after 12 weeks of application (29.6 ± 25.6 hairs/cm^2) was significantly higher than the pre-application density (24.6 ± 21.5 hairs/cm^2). Similarly, the anagen hair density in the placebo group after 12 weeks of application (33.1 ± 28.1 hairs/cm^2) was significantly higher than the pre-application density (26.5 ± 21.6 hairs/cm^2). Additionally, the telogen hair density in the N793 group after 24 weeks of application (132.3 ± 35.0 hairs/cm^2) was significantly higher than the pre-application density (125.2 ± 38.0 hairs/cm^2). However, we observed no significant change in telogen hair density in the placebo group compared with the baseline. Moreover, the hair growth coefficient in the N793 group after 12 weeks of application (0.321 ± 0.381) was significantly larger than the pre-application coefficient (0.261 ± 0.343). Similarly, the hair growth coefficient in the placebo group after 12 weeks of application (0.370 ± 0.429) was significantly larger than the pre-application coefficient (0.245 ± 0.218).

Unadjusted and baseline-adjusted data (adjusted as a percentage of baseline) from the hair assessment were compared between the groups (Table 2 and Supplementary Table 1). There were no significant differences in the unadjusted data for any items between the groups (Supplementary Table 1a, 1b). In comparisons of the baseline-adjusted data, the telogen hair density after 24 weeks of application was significantly higher in

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the N793 group than in the placebo group; however, there were no significant differences for any other items between the groups (Tables 2, 3).

Furthermore, the hair loss count did not change significantly relative to the baseline in the N793 group. However, in the placebo group, the hair loss count after 24 weeks of application (42.1 ± 36.8 hairs) was significantly smaller than that before application (52.4 ± 35.4 hairs; Supplementary Table 2). A between-group comparison showed that the unadjusted hair loss count after 24 weeks of application was significantly smaller in the placebo group than in the N793 group. However, the baseline-adjusted hair loss count did not significantly differ between the groups (Table 4).

Table 2. Hair assessment (a) Baseline, 12 weeks, 24 weeks (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)
Hair density (hairs/cm^2)	Baseline	N793	51	100.0 ± 0.0	-
		Placebo	50	100.0 ± 0.0	
	12 weeks	N793	51	102.9 ± 5.6	5.2E-01
		Placebo	50	102.3 ± 4.4	
	24 weeks	N793	51	105.1 ± 6.9	3.1E-01
		Placebo	50	103.7 ± 6.9	
Mean hair diameter (μm)	Baseline	N793	51	100.0 ± 0.0	-
		Placebo	50	100.0 ± 0.0	
	12 weeks	N793	51	105.2 ± 10.3	8.0E-02
		Placebo	50	109.1 ± 9.7	
	24 weeks	N793	51	109.7 ± 12.9	1.8E-01
		Placebo	50	113.0 ± 12.7	
Percentage of vellus hairs (%)	Baseline	N793	51	100.0 ± 0.0	-
		Placebo	50	100.0 ± 0.0	
	12 weeks	N793	51	92.6 ± 27.7	2.4E-01
		Placebo	50	88.5 ± 18.9	
	24 weeks	N793	51	89.2 ± 31.5	1.2E-01
		Placebo	50	81.2 ± 32.0	
Percentage of non-vellus hairs (%)	Baseline	N793	51	100.0 ± 0.0	-
		Placebo	50	100.0 ± 0.0	
	12 weeks	N793	51	108.4 ± 36.4	2.4E-01
		Placebo	50	128.5 ± 117.6	
	24 weeks	N793	51	116.2 ± 51.9	1.2E-01
		Placebo	50	126.9 ± 67.9	

SD: standard deviation.

Table 3. Hair assessment (b) Baseline + 2 days, 12 weeks + 2 days, 24 weeks + 2 days (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)	
Anagen hair density (hairs/cm^2)	Baseline + 2 days	N793	51	100.0 ± 0.0	-	
		Placebo	50	100.0 ± 0.0		
	12 weeks + 2 days	N793	51	195.0 ± 287.2	6.8E-01	
		Placebo	50	192.5 ± 259.7		
	24 weeks + 2 days	N793	51	157.3 ± 192.3	2.8E-01	
		Placebo	50	151.0 ± 140.4		
Telogen hair density (hairs/cm^2)	Baseline + 2 days	N793	51	100.0 ± 0.0	-	
		Placebo	50	100.0 ± 0.0		
	12 weeks + 2 days	N793	51	100.5 ± 23.0	7.9E-01	
		Placebo	50	97.6 ± 18.7		
	24 weeks + 2 days	N793	51	109.0 ± 25.5	4.3E-02	*
		Placebo	50	100.5 ± 18.4		
Anagen hair density /Telogen hair density	Baseline + 2 days	N793	51	100.0 ± 0.0	-	
		Placebo	50	100.0 ± 0.0		
	12 weeks + 2 days	N793	51	226.2 ± 358.7	6.7E-01	
		Placebo	50	224.9 ± 316.0		
	24 weeks + 2 days	N793	51	164.2 ± 218.8	1.8E-01	
		Placebo	50	176.4 ± 215.2		

*p<0.05. SD: standard deviation.

Thinning hair was assessed subjectively using the VAS questionnaire (Table 5 and Supplementary Table 3). In this assessment, the lower the score, the better the improvement in thinning hair. The scores for all questions improved at every point surveyed throughout the study in the N793 and placebo groups, with significant differences observed after 12 and 24 weeks of application (Supplementary Table 3a, 3b). Moreover, a between-group comparison of unadjusted VAS data revealed no significant differences after 12 or 24 weeks of application. Analyzing the baseline-adjusted VAS data, the scores for questions 2 (hair loss during hair washing) and 10 (feel a reduction in hair loss) were significantly better in the placebo group after 24 weeks of application than in the N793 group. Additionally, the score for question 3 (hair loss during hair cutting and styling) in the N793 group after 12 weeks of application was significantly better than that in the placebo group (Tables 5, 6).

An additional analysis was performed after the progression of thinning hair was staged in men and women using the Hamilton–Norwood and Ludwig classification systems, respectively [16]. The results of staging were 5 to 7 (severe) for 18 participants, 4 for 6 participants, 3 for 17 participants, 2 for 10 participants, and 1 for 53 participants. Notably, participants with a staging ≥5 are considered to have severely thinning hair. Since hair growth is difficult in these cases, the hair assessments, hair loss counts, and VAS questionnaire data were reanalyzed for only participants with a thinning hair staging ≤4. This limited analysis set comprised 43

Table 4. Number of hairs lost-Baseline, 12 weeks, 24 weeks (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)
The number of hair loss (hairs)	Baseline	N793	52	100.0 ± 0.0	-
		Placebo	52	100.0 ± 0.0	
	12 weeks	N793	52	126.1 ± 117.6	3.3E-01
		Placebo	52	133.3 ± 146.2	
	24 weeks	N793	52	116.5 ± 119.5	2.0E-01
		Placebo	52	104.1 ± 103.5	

SD: standard deviation.

Table 5. Visual analogue scale (a) Baseline, 12 weeks, 24 weeks (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)	
Q1 Hair loss on waking	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	149.0 ± 296.8	7.4E-02	
		Placebo	52	145.5 ± 371.6		
	24 weeks	N793	52	246.7 ± 619.9	7.3E-02	
		Placebo	52	197.4 ± 773.5		
Q2 Hair loss during hair washing	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	95.5 ± 84.1	9.7E-02	
		Placebo	52	144.0 ± 478.1		
	24 weeks	N793	52	88.0 ± 55.4	1.3E-02	*
		Placebo	52	63.3 ± 32.5		
Q3 Hair loss during hairdressing	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	93.3 ± 48.3	4.8E-02	*
		Placebo	52	160.1 ± 497.5		
	24 weeks	N793	52	93.5 ± 87.9	2.3E-01	
		Placebo	52	86.6 ± 109.0		
Q4 Lack of firmness and elasticity of hair	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	97.5 ± 60.1	1.5E-01	
		Placebo	52	124.0 ± 215.6		
	24 weeks	N793	52	110.3 ± 207.8	3.0E-01	
		Placebo	52	93.6 ± 124.3		
Q5 Fine hair	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	238.8 ± 1,098.0	7.5E-01	
		Placebo	52	131.6 ± 215.0		
	24 weeks	N793	52	201.7 ± 842.8	2.6E-01	
		Placebo	52	93.1 ± 111.0		

*p<0.05. SD: standard deviation.

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Table 6. Visual analogue scale (b) Baseline, 12 weeks, 24 weeks (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)	
Q6 Lack of hair strength	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	91.2 ± 49.2	4.6E-01	
		Placebo	52	92.2 ± 82.0		
	24 weeks	N793	52	81.9 ± 27.5	4.3E-01	
		Placebo	52	78.2 ± 45.2		
Q7 Lack of hair volume	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	90.4 ± 33.1	9.7E-01	
		Placebo	52	98.0 ± 60.4		
	24 weeks	N793	52	81.0 ± 32.2	1.4E-01	
		Placebo	52	73.4 ± 38.6		
Q8 Scalp conspicuity	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	84.0 ± 28.5	9.0E-01	
		Placebo	52	92.4 ± 54.1		
	24 weeks	N793	52	79.6 ± 28.7	2.5E-01	
		Placebo	52	74.6 ± 34.5		
Q9 The conspicuity of the parietal, hairline and hair parting	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	88.9 ± 39.2	5.2E-01	
		Placebo	52	92.7 ± 38.7		
	24 weeks	N793	52	83.4 ± 41.0	3.3E-01	
		Placebo	52	80.4 ± 62.0		
Q10 Feeling of reduced hair loss	Baseline	N793	52	100.0 ± 0.0	-	
		Placebo	52	100.0 ± 0.0		
	12 weeks	N793	52	409.2 ± 1,511.5	1.8E-01	
		Placebo	52	311.0 ± 1,090.4		
	24 weeks	N793	52	483.0 ± 1,715.0	3.0E-02	*
		Placebo	52	101.9 ± 218.2		

*p<0.05. SD: standard deviation.

participants in the N793 group and 43 participants in the placebo group. However, the position of the partial haircut used in the digital microscope-based hair assessments was incorrect for one participant in the placebo group. This error was deemed to meet the third analysis exclusion criterion, and the individual was excluded from the hair assessment part of the efficacy evaluation.

Comparing the unadjusted data throughout the study in this limited analysis set, we observed a significant improvement in hair density, mean hair diameter, and non-vellus hair percentages after 12 and 24 weeks of application in the N793 and placebo groups compared with the pre-application data (Supplementary Table 4a). Additionally, the anagen hair density and hair growth coefficient after 12 weeks of application differed significantly from the pre-application data in the N793 and placebo groups. The telogen hair density after 24 weeks of application differed significantly from the pre-application level in the N793 group; however, this difference was not observed in the placebo group. Furthermore, the between-group comparison of unadjusted data showed no significant difference in any items (Supplementary Table 4a, 4b). However, the between-group comparison of baseline-adjusted data showed that hair density after 24 weeks of application was significantly better in the N793 group than in the placebo group (Table 7). Similarly, telogen hair density after 24 weeks of application was significantly higher in the N793 group than in the placebo group (Table 8). No significant difference was observed between the groups in the baseline-adjusted data for any other items. Analyzing the hair loss count over time, we observed no significant difference relative to the baseline in the N793 group. However, we observed a significant decrease in the placebo group after 24 weeks of application (42.3 ± 39.6 hairs) compared with the pre-application levels (51.0 ± 37.7 hairs; Supplementary Table 5). Additionally, a between-group comparison of the unadjusted data showed a significantly smaller hair loss count in the placebo group than in the N793 group after 24 weeks of application (Table 9). However, the baseline-adjusted hair loss count showed no significant difference between the groups.

Analyzing the VAS questionnaire data over time, we observed that the scores for questions 2 (hair loss during hair washing) to 4 (hair lacks firmness and elasticity) and questions 6 (lack of hair strength) to 10 (feel a reduction in hair loss) were significantly improved after 12 and 24 weeks of application compared with the pre-application scores in both groups (Supplementary Table 6a, 6b). However, the scores for question 1 (hair loss upon waking) were significantly improved after 12 and 24 weeks of application in the placebo group and after 24 weeks of application in the N793 group compared with the pre-application scores. Additionally, the scores for question 5 (fine hair) were significantly improved after 12 and 24 weeks of application in the N793 group and after 24

weeks in the placebo group compared with the pre-application scores. A between-group comparison of the unadjusted scores revealed a significant difference in the pre-application score for question 10 and no significant difference for any other items (Supplementary Table 6a, 6b). A between-group comparison of the baseline-adjusted data revealed that the score for question 10 after 24 weeks of application was significantly better in the placebo group than in the N793 group (Tables 10, 11); however, there were no significant between-group differences for any other items.

DISCUSSION

We verified the effect of N793 on thinning hair by applying it to the scalps of healthy Japanese men and women. Analyzing

Table 7. Hair assessment (stage 4 or less) (a) Baseline, 12 weeks, 24 weeks (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)	
Hair density (hairs/cm^2)	Baseline	N793	43	100.0 ± 0.0	-	
		Placebo	42	100.0 ± 0.0		
	12 weeks	N793	43	103.5 ± 4.3	2.3E-01	
		Placebo	42	102.4 ± 4.0		
	24 weeks	N793	43	106.3 ± 5.6	3.8E-02	*
		Placebo	42	103.9 ± 6.6		
Mean hair diameter (μm)	Baseline	N793	43	100.0 ± 0.0	-	
		Placebo	42	100.0 ± 0.0		
	12 weeks	N793	43	105.7 ± 9.7	1.3E-01	
		Placebo	42	108.5 ± 8.9		
	24 weeks	N793	43	110.3 ± 11.1	1.5E-01	
		Placebo	42	113.6 ± 13.0		
Percentage of vellus hairs (%)	Baseline	N793	43	100.0 ± 0.0	-	
		Placebo	42	100.0 ± 0.0		
	12 weeks	N793	43	91.2 ± 29.8	6.4E-01	
		Placebo	42	88.6 ± 19.4		
	24 weeks	N793	43	87.3 ± 33.8	1.4E-01	
		Placebo	42	79.6 ± 34.1		
Percentage of non-vellus hairs (%)	Baseline	N793	43	100.0 ± 0.0	-	
		Placebo	42	100.0 ± 0.0		
	12 weeks	N793	43	106.7 ± 17.5	5.0E-01	
		Placebo	42	110.2 ± 19.6		
	24 weeks	N793	43	111.3 ± 20.9	3.3E-01	
		Placebo	42	116.9 ± 29.0		

*p<0.05. SD: standard deviation.

Table 8. Hair assessment (stage 4 or less) (b) Baseline + 2 days, 12 weeks + 2 days, 24 weeks + 2 days (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)	
Anagen hair density (hairs/cm^2)	Baseline + 2 days	N793	43	100.0 ± 0.0	-	
		Placeb	42	100.0 ± 0.0		
	12 weeks + 2 days	N793	43	168.9 ± 233.8	4.4E-01	
		Placebo	42	183.3 ± 227.8		
	24 weeks + 2 days	N793	43	164.0 ± 198.3	3.3E-01	
		Placebo	42	156.9 ± 145.6		
Telogen hair density (hairs/cm^2)	Baseline + 2 days	N793	43	100.0 ± 0.0	-	
		Placebo	42	100.0 ± 0.0		
	12 weeks + 2 days	N793	43	101.0 ± 24.4	5.9E-01	
		Placebo	42	96.8 ± 120.2		
	24 weeks + 2 days	N793	43	110.7 ± 26.7	1.5E-02	*
		Placebo	42	100.0 ± 19.6		
Anagen hair density /Telogen hair density	Baseline + 2 days	N793	43	100.0 ± 0.0	-	
		Placebo	42	100.0 ± 0.0		
	12 weeks + 2 days	N793	43	192.8 ± 286.2	4.2E-01	
		Placebo	42	220.7 ± 299.5		
	24 weeks + 2 days	N793	43	170.6 ± 225.5	2.1E-01	
		Placebo	42	185.9 ± 227.5		

*p<0.05. SD: standard deviation.

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Table 9. Number of hairs lost (stage 4 or less) Baseline, 12 weeks, 24 weeks (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)
The number of hair loss (hairs)	Baseline	N793	43	100.0 ± 0.0	-
		Placebo	43	100.0 ± 0.0	
	12 weeks	N793	43	135.5 ± 125.6	4.2E-01
		Placebo	43	141.5 ± 158.1	
	24 weeks	N793	43	122.9 ± 125.0	1.4E-01
		Placebo	43	110.6 ± 112.2	

SD: standard deviation.

Table 10. Visual analogue scale (stage 4 or less) (a) Baseline, 12 weeks, 24 weeks (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)
Q1 Hair loss on waking	Baseline	N793	43	100.0 ± 0.0	-
		Placebo	43	100.0 ± 0.0	
	12 weeks	N793	43	144.0 ± 304.3	1.9E-01
		Placebo	43	139.6 ± 372.9	
	24 weeks	N793	43	216.6 ± 562.3	3.5E-01
		Placebo	43	227.4 ± 849.1	
Q2 Hair loss during hair washing	Baseline	N793	43	100.0 ± 0.0	-
		Placebo	43	100.0 ± 0.0	
	12 weeks	N793	43	96.3 ± 91.7	3.0E-01
		Placebo	43	84.1 ± 60.8	
	24 weeks	N793	43	80.4 ± 47.8	1.5E-01
		Placebo	43	65.6 ± 31.2	
Q3 Hair loss during hairdressing	Baseline	N793	43	100.0 ± 0.0	-
		Placebo	43	100.0 ± 0.0	
	12 weeks	N793	43	88.2 ± 47.2	2.0E-01
		Placebo	43	98.8 ± 113.3	
	24 weeks	N793	43	78.4 ± 35.4	6.5E-01
		Placebo	43	90.8 ± 118.5	
Q4 Lack of firmness and elasticity of hair	Baseline	N793	43	100.0 ± 0.0	-
		Placebo	43	100.0 ± 0.0	
	12 weeks	N793	43	95.2 ± 64.4	4.4E-01
		Placebo	43	102.6 ± 129.8	
	24 weeks	N793	43	112.8 ± 228.6	9.7E-01
		Placebo	43	97.4 ± 131.4	
Q5 Fine hair	Baseline	N793	43	100.0 ± 0.0	-
		Placebo	43	100.0 ± 0.0	
	12 weeks	N793	43	265.7 ± 1,208.1	3.6E-01
		Placebo	43	129.3 ± 220.9	
	24 weeks	N793	43	222.7 ± 929.3	8.8E-01
		Placebo	43	100.2 ± 120.0	

SD: standard deviation.

the data collected from all participants (full analysis) and those with a thinning hair staging ≤4 (limited analysis), we observed no significant difference between the N793 and placebo groups for any of the unadjusted data in the hair assessment. However, comparing the baseline-adjusted data between the groups, the full and limited analyses revealed a significantly higher telogen hair density after 24 weeks of application in the N793 group than in the placebo group. Moreover, analyzing the participants with a thinning hair staging ≤4 (limited analysis), we observed that the hair density was significantly improved after 24 weeks of application in the N793 group compared with the pre-application levels. Comparing the unadjusted hair loss count data between the groups, the full and limited analyses showed that the hair loss count was significantly higher after 24 weeks of application in the N793 group than in the placebo group; however, the baseline-adjusted data showed no significant difference between the groups. Comparing the unadjusted VAS questionnaire data between the groups, the full and limited analyses revealed no significant differences after 12 and 24 weeks of application; however, the baseline-adjusted VAS questionnaire data showed that the score for question 10 after 24 weeks of application was significantly higher in the N793 than in the placebo group.

The hair densities, mean hair diameters, vellus hair percentages, and non-vellus hair percentages determined in the

Table 11. Visual analogue scale (stage 4 or less) (b) Baseline, 12 weeks, 24 weeks (baseline-adjusted data)

Item	Week	Group	N	Mean ± SD	p-value (group)	
Q6 Lack of hair strength	Baseline	N793	43	100.0 ± 0.0	-	
		Placebo	43	100.0 ± 0.0		
	12 weeks	N793	43	91.3 ± 54.0	6.6E-01	
		Placebo	43	87.7 ± 55.9		
	24 weeks	N793	43	80.2 ± 27.3	8.8E-01	
		Placebo	43	81.8 ± 46.1		
Q7 Lack of hair volume	Baseline	N793	43	100.0 ± 0.0	-	
		Placebo	43	100.0 ± 0.0		
	12 weeks	N793	43	89.1 ± 35.5	7.2E-01	
		Placebo	43	93.0 ± 36.3		
	24 weeks	N793	43	78.4 ± 31.9	3.6E-01	
		Placebo	43	75.2 ± 38.6		
Q8 Scalp conspicuity	Baseline	N793	43	100.0 ± 0.0	-	
		Placebo	43	100.0 ± 0.0		
	12 weeks	N793	43	80.3 ± 29.0	3.6E-01	
		Placebo	43	89.0 ± 36.2		
	24 weeks	N793	43	76.1 ± 27.3	8.1E-01	
		Placebo	43	76.2 ± 35.2		
Q9 The conspicuity of the parietal, hairline and hair parting	Baseline	N793	43	100.0 ± 0.0	-	
		Placebo	43	100.0 ± 0.0		
	12 weeks	N793	43	86.8 ± 42.3	1.0E-01	
		Placebo	43	93.3 ± 32.6		
	24 weeks	N793	43	80.7 ± 43.3	8.2E-01	
		Placebo	43	75.0 ± 33.9		
Q10 Feeling of reduced hair loss	Baseline	N793	43	100.0 ± 0.0	-	
		Placebo	43	100.0 ± 0.0		
	12 weeks	N793	43	411.2 ± 1,625.7	3.9E-01	
		Placebo	43	300.1 ± 1,138.6		
	24 weeks	N793	43	415.4 ± 1,633.4	5.0E-02	*
		Placebo	43	73.6 ± 96.5		

*p<0.05. SD: standard deviation.

hair assessments improved over time in both groups. This is possibly due to scalp massage-induced improvement of blood flow in both groups. Massaging the scalp reportedly increases scalp blood flow, hair density, and mean hair diameter [17, 18]. In this study, the test products were rubbed onto the scalp; the resulting massage-like effect of this application method might have obscured differences between the groups.

We found no significant differences in any items when comparing unadjusted hair assessment data between the groups among all participants. However, some participants showed severe hair thinning, and the effects of N793 may have been obscured in them. Notably, as hair thinning progresses, the hair follicles in the scalp decrease in size, hair becomes finer, and hair shedding occurs [19, 20]. Among the study participants, the partial haircut area examined only overlapped the area of hair loss in participants with hair thinning stages of 5 to 7. Therefore, between-group differences may have been obscured in the analysis of all participants (full analysis) because the analysis included data for those with a hair thinning staging ≥5. People with this level of thinning hair progression have smaller follicles and a smaller number of, or even no, HFDPCs, which N793 targets, than those with a hair thinning stage ≤4. Hence, an additional analysis was performed among participants with a hair thinning staging ≤4, as they have more of the HFDPCs targeted by N793 than those with a hair thinning staging of 5 to 7. This additional analysis showed a significantly higher hair density in the N793 group than in the placebo group after 24 weeks of application. When applied to participants with a hair thinning hair stage ≤4, N793 likely acted on the HFDPCs in the hair follicles, stimulating cell activation and promoting KGF production, thereby maintaining the anagen phase and increasing the hair density. These findings suggest that the topical application of N793 stimulated hair growth and improved hair thinning.

Baseline-adjusted hair assessment data were also compared among all participants and those with a hair thinning stage ≤4. Both analyses showed that the telogen hair density after 24 weeks of application was significantly higher in the N793 group than in the placebo group. Telogen hair is shed from the hair follicle when pushed out by new hair in the early anagen phase of the hair cycle [21]. In this study, the high telogen hair density in the N793 group suggests a cessation of growth by exposed hair in the follicle. Although we did not identify a significant difference between the groups when we analyzed all the participants, we found that the hair density after 24 weeks of application was higher in the N793 group (105.1 ± 6.9) than in the placebo group (103.7 ± 6.9). Moreover, when we analyzed the participants with a hair thinning stage ≤4, the hair density after 24 weeks of application was significantly higher in the N793 group than in the placebo

group. Based on these results, the higher hair density in the N793 group may be due to cessation of the anagen phase, causing hair to enter the telogen phase and the promotion of new hair growth from their follicles. This possibly explains the temporary increase in telogen hair density in the N793 group.

The unadjusted VAS questionnaire data were assessed in all participants and those with a hair thinning stage of ≤4. We observed no significant difference between the groups; however, after 24 weeks of application, all items significantly improved in both groups compared with the baseline. The improvements noticed by participants were probably due to the improvements in hair density, mean hair diameter, vellus hair percentage, and non-vellus hair percentage observed over time in both groups. Furthermore, comparing the baseline-adjusted VAS questionnaire data between groups, the score for question 10 (feel a reduction in hair loss) after 24 weeks of application was significantly worse in the N793 group. Notably, one participant in the N793 group provided very different responses to question 10 at baseline and after 24 weeks of application (baseline score, 1; score after 24 weeks of application, 100), which skewed the baseline-adjusted data higher, possibly causing the significant difference between the N793 and placebo groups.

Digital photographs of the head (parietal, frontal, and occipital) were taken before and after application in this study, and many participants in the active group showed a decrease in scalp exposure due to improved hair density (data not shown). Because of the visual change in the head, it is believed that the participants themselves were able to perceive this as a beneficial difference. Because thinning hair is associated with physical, psychological, and social stress, the reduced scalp exposure and improvement in thinning hair due to increased hair density were considered improvements in the quality of life for the participants who were aware of their thinning hair prior to the study.

The pro-inflammatory cytokine interleukin-1β induces KGF production in fibroblasts, including dermal papilla cells [22]. Additionally, it has been reported that endogenous KGF enhances immune function by activating neutrophils and macrophages [23]. Therefore, there is a possibility of mutual influence between the immune response and KGF production. Considering these findings, it is conceivable that the immune response is involved in the mechanism by which N793 promotes KGF production by dermal papilla cells. Further research is needed to determine why heat-sterilized N793 has a beneficial effect, but previous studies have reported that the active substances in the immune induction of lactic acid bacteria are the cell wall and EPS (outer capsule polysaccharide) [24]. Therefore, it is assumed that dead N793 bacteria are effective.

The above results suggest that the topical application of N793 improves hair density in Japanese men and women with thinning hair that progresses at a relatively mild rate (thinning hair staging ≤4). We intend to undertake further studies to understand the mechanism of action of N793 on hair follicles and HFDPCs and determine whether N793 aids in improving hair thinning in humans.

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CONFLICT OF INTEREST

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