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Effects of ingesting milk fermented by *Lactococcus lactis* H61 on skin health in young women: A randomized double-blind study

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ABSTRACT

We conducted a randomized double-blind trial to evaluate the effects of fermented milk produced using only *Lactococcus lactis* strain H61 as a starter bacterium (H61-fermented milk) on the general health and various skin properties of young women. Healthy female volunteers (n = 23; age = 19–21 yr) received H61-fermented milk (10^{10} cfu of strain H61/d) or conventional yogurt (10^{10} cfu of both *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* per day), as a reference food, daily for 4 wk. Before and at the end of 4 wk, blood samples were taken, and skin hydration (inner forearms and cheek) and melanin content, elasticity, and sebum content (cheek only) were measured. Skin hydration at the inner forearm was higher at wk 4 than at wk 0 in both groups. Sebum content in cheek rose significantly after intervention in the H61-fermented milk group, but not the conventional yogurt group. Other skin parameters did not differ in either group. Serum analysis showed that total protein concentration and platelet count were elevated and reactive oxygen species decreased in both groups after the intervention. Although H61-fermented milk and conventional yogurt had similar effects on skin status and some blood characteristics of participants, an increase of sebum content in cheek is preferable to H61-fermented milk. As skin lipids contribute to maintaining the skin barrier, H61-fermented milk would provide beneficial effects on skin for young women.

Key words: fermented milk, skin health, human study

INTRODUCTION

Probiotics have been defined by the Food and Agriculture Organization-World Health Organization as "live microorganisms which, when administered in adequate amounts, confer a health benefit to the host." The probiotic properties of lactic acid bacteria have garnered great interest recently. For example, probiotic lactic acid bacteria can reduce serum lipid content (Higashikawa et al., 2010), correct imbalances of intestinal microbiota (Lidbeck et al., 1991), and exert immunomodulatory activity (Kalliomäki et al., 2003) in humans. Although many reports have addressed the effect of lactic acid bacteria on skin properties in subjects with skin diseases, such as atopic dermatitis (Shimada et al., 2004; Moroi et al., 2011), few studies have involved healthy humans. Thus far, it has been reported that skin elasticity and the degree of dryness in the cheek of healthy Japanese women were reported to be improved after 4 wk of consuming yogurt made by using *Lactobacillus delbrueckii* ssp. *bulgaricus* 2038 and *Streptococcus thermophilus* 1131 (Isawa et al., 2008). Our previous study also showed that oral administration of heat-killed *Lactococcus lactis* ssp. *cremoris* strain H61 improves some skin properties, such as skin hydration and self-surveyed apparent hair follicles in middle-aged (30–60 yr old) healthy women (Kimoto-Nira et al., 2012). However, in that study, skin hydration of women was improved by intake of strain H61 at 50 to 60 yr old, but not at 30 or 40 yr old. In addition, only the 30-yr-old participants in H61 group noted marked improvements in self-surveyed skin elasticity. Thus, it was suggested that the effect of strain H61 on skin properties is altered by age. In the present study, we focused on young (around 20 yr old) healthy women and assessed the effect of ingesting fermented milk made by only strain H61 (H61-fermented milk). In addition, the effect of strain H61 on skin properties were compared with other lactic acid bacteria by using conventional yogurt fermented by *L. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus* as a yogurt starter. Establishing the beneficial properties of strain H61-fermented milk could lead to recognition of strain H61 as an effective probiotic dairy starter. We also evaluated serum oxidative status and various blood parameters of our subjects to clarify the factor of ingesting strain

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H61 affecting skin properties and whether any effects on general health could be detected.

MATERIALS AND METHODS

Subjects and Study Design

Twenty-three healthy women (age = 19–21 yr) from Juntendo University were enrolled in our study, which was performed between February and March 2012 as a randomized (2 groups), double-blind application test. Exclusion criteria were pregnancy, breast-feeding, and routine use of medicinal products to treat various metabolic, cardiovascular, or hepatic diseases. In the H61-fermented milk group (n = 11), the mean values (SD) for age, height, and weight were 20.0 (1.1) yr, 164.5 (4.8) cm, and 55.1 (6.6) kg, respectively. In the conventional yogurt group (n = 12), the mean values for age, height, and weight were 20.0 (1.1) yr, 162.4 (4.9) cm, and 55.2 (6.3) kg, respectively. Age and body characteristics were comparable between the conventional yogurt and H61-fermented milk populations.

The subjects were randomly assigned to 2 groups according to the skin properties observed at wk 0. The study lasted 4 wk, with assessment of skin parameters and collection of fasting blood samples at wk 0 and 4. The current study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the ethics committees of NARO Institute of Livestock and Grassland Science and Juntendo University. All subjects received detailed information about purposes, methods, expected results, and ethical considerations, including possible adverse effects relevant to the study, and submitted a written declaration of consent to participate in the study. According to a study concerning the effects of yogurt intake on skin properties (Isawa et al., 2008), subjects enrolled in the present study were instructed to use stable cosmetic application before and during the study. They were also instructed to have stable life patterns of outdoor activity and food intake. They were all students and belonged to athletic clubs; thus, their lifestyle was very similar across treatment groups.

Test Foods

Many clinical studies testing probiotics have used doses ranging from 4×10^8 to 1×10^{11} cfu daily (Larsen et al., 2006; Savard et al., 2011). The H61-fermented milk and conventional yogurt were produced by Japan Agricultural Cooperatives Ibaraki Mizuho (Hitachiohta, Ibaraki, Japan) every 2 wk and distributed to the participants. Both products contained glucose, fructose, and oligosaccharide, and were similar in the level of all nutrients except for the lactic acid bacteria used. The H61-fermented milk was made by using only *Lactococcus lactis* ssp. *cremoris* H61 (MAFF 400007; The Genebank of National Institute of Agrobiological Sciences, Tsukuba, Japan) at a level of 10^8 cfu/mL. Conventional yogurt was made by using both *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* at 10^8 cfu/mL each as a starter culture (Chr. Hansen, Hørsholm, Denmark). Both products were packed in identical containers. Participants consumed either H61-fermented milk (150 mL) or conventional yogurt (150 mL) twice daily, at their morning and evening meals.

Mechanical Analysis of Skin Properties

The hydration of the skin of the right and left inner forearms and the hydration and other parameters of the lateral angles of the right and left cheeks were assessed in study volunteers. Before measurements, each subject washed her cheeks once with identical soap in tap water and sat quietly for 20 min to equilibrate to the conditions in the testing room (temperature = 22~25°C; relative humidity = 33 ± 3% in February, 53 ± 3% in March). Two symmetrical regions of the cheeks were evaluated. Skin hydration was measured by using a corneometer (CM 825, Courage and Khazaka Electronics, Cologne, Germany; De Spirt et al., 2009; Nagata et al., 2010). Malar skin elasticity was measured by using an absorption method (Cutometer SEM575, Courage and Khazaka Electronics; Izumi et al., 2007; De Spirt et al., 2009; Nagata et al., 2010). In brief, a 2-mm region of skin was aspirated at 300 mbar for 2 s and then released. The ratio of immediate retraction to total distension was recorded. The melanin and sebum contents in the cheeks were measured by using a Mexameter (Courage and Khazaka Electronics; Huh et al., 2010) and a Sebumeter SM810 (Courage and Khazaka Electronics; Nagata et al., 2010), respectively. Skin hydration and melanin content were measured 3 times at each location and time point. Skin elasticity was measured 4 times at each site and time point. Sebum content was measured once per time point.

Blood Analysis

The general health of the volunteers and the safety of the intervention were confirmed by blood analyses at the beginning and the end of the 4-wk intervention period. Blood count and serum total protein, creatinine, triglyceride, aspartate transaminase, alanine transaminase, lactate dehydrogenase, creatine phosphokinase, and iron were analyzed by a certified clinical laboratory (SRL, Tokyo, Japan).

The serum oxidative stress level was evaluated by using reactive oxygen metabolite tests (Wismerll Co. Ltd., Tokyo, Japan; Saigo et al., 2011). Briefly, serum samples were diluted in an acidic buffer (pH 4.0), which resulted in the production of free ferrous and ferric ions. These ions produce alkoxy and peroxy radicals from hydrogen peroxide, in accordance with Fenton's reaction, after which the radicals oxidize an alkyl-substituted aromatic amine in a chromogenic mixture, which transforms them into pink-colored derivatives. The Free Radical Analytical System 4 (FRAS4, Diacron International, Grosseto, Italy) was used quantify the colorization, and the results were expressed in conventional arbitrary units (Carr U), with 1 Carr U equal to 0.08 mg/dL of hydrogen peroxide.

The level of biological antioxidant potential (**BAP**) was measured by using FRAS4 (Kaneko et al., 2012). Briefly, BAP measurement is based on ability of reduction by antioxidant substances, including albumin and transferrin in serum. Serum (10 μL) was mixed with a colored solution containing ferric ions bound to a chromogenic substrate (thiocyanate derivative) and incubated at 37°C for 5 min. When ferric ions were reduced to ferrous ions by antioxidant substances, the thiocyanate derivative was decolorized. Absorption at 505 nm was determined by using FRAS4. The BAP levels were expressed as micromole per liter.

Statistical Analysis

Data were expressed as means with standard deviations or standard errors. For all skin properties evaluated by using mechanical analysis at all time points (wk 0 and 4), descriptive statistics were calculated and were analyzed by using a general linear model procedure (SAS version 9.1, SAS Institute, Cary, NC). Least squares means were compared by applying the Tukey–Kramer test. A P-value of less than 0.05 was considered statistically significant, and P-values <0.1 indicated trends.

RESULTS AND DISCUSSION

Participants demonstrated good compliance with study requirements, and no adverse effects or symptoms were reported.

Mechanical Analysis of Skin Parameters

Table 1 indicates the various skin parameters in young women who consumed conventional yogurt or H61-fermented milk. Conventional yogurt or H61 treatment had no effect on any skin parameter (Table 1). Stage had a significant effect on the hydration of inner forearm and melanin and sebum contents in cheek (Table 1). The skin hydration of the inner forearm ($P < 0.0001$), melanin content ($P = 0.009$), and sebum content ($P = 0.043$) in the cheek were significantly increased at 4 wk compared with 0 wk in both groups.

Interaction between group and stage on sebum content showed a trend toward significance ($P = 0.099$; Table 1). Sebum content in cheek was significantly ($P < 0.05$) higher after treatment with H61-fermented milk but not conventional yogurt. Interaction between group and stage had no effect on hydration of the in-

Table 1. Effects of fermented milk intake on various skin parameters of young women[1]

	Inner forearm	Cheek			
Item	Hydration (AU)	Hydration (AU)	Elasticity	Melanin (MI)	Sebum ($\mu g/cm^2$)
Group					
Yogurt	34.9 (1.9)	47.1 (2.4)	0.335 (0.009)	158 (10)	3.98 (1.84)
H61	37.1 (1.9)	40.6 (2.5)	0.321 (0.009)	162 (16)	7.20 (1.92)
P-value	NS	NS	NS	NS	NS
Stage					
Before intake	33.6 (1.5)[b]	43.1 (1.9)	0.336 (0.008)	155 (7)[b]	3.95 (1.55)[b]
After intake	38.3 (1.5)[a]	44.7 (1.9)	0.320 (0.008)	165 (7)[a]	7.24 (1.55)[a]
P-value	<0.0001	NS	NS	0.009	0.043
Group × stage					
Yogurt before	32.6 (2.0)[b]	45.1 (2.6)	0.338 (0.011)	153 (10)	3.67 (2.14)
Yogurt after	37.2 (2.0)[a]	49.1 (2.6)	0.331 (0.011)	162 (10)	4.29 (2.14)
H61 before	34.7 (2.1)[b]	41.0 (2.8)	0.334 (0.012)	157 (10)	4.23 (2.24)[b]
H61 after	39.4 (2.1)[a]	40.2 (2.8)	0.308 (0.012)	168 (10)	10.2 (2.24)[b]
P-value	NS	NS	NS	NS	0.099

[a,b]Within each category (group, stage, group × stage), values within a row that have unlike superscript letters were significantly ($P < 0.05$) different.

[1]AU = arbitrary units; MI = melanin index; values are expressed as least squares means and figures in parentheses are standard errors.

ner forearm or cheek hydration, elasticity, or melanin content in either group.

Blood Parameters

Blood count and serum biochemical parameters are summarized in Table 2. Ingestion of neither conventional yogurt nor H61-fermented milk caused any abnormality in any of the measured parameters (Table 2). Total protein ($P < 0.001$) and platelet count ($P = 0.0001$) were significantly increased at 4 wk compared with 0 wk in both groups. Serum parameters associated with Fe, such as red blood cell count, hemoglobin concentration, and hematocrit, were increased (but remained within reference ranges) during the intervention period. This difference may reflect the prestudy use of Fe supplements by 2 participants in the H61-fermented milk group and by 1 participant in the conventional yogurt group. The white blood cell counts showed significant ($P = 0.012$) group × stage effects, but Tukey-Kramer analysis revealed that a significant difference was not observed in the results of subjects.

Table 3 indicates the effect of consuming conventional yogurt or H61-fermented milk on antioxidant status. Reactive oxygen metabolite tests showed a significant ($P < 0.0001$) effect of stage. The interaction between group and stage was not significant in regard to antioxidant levels. The ingestion of conventional yogurt or H61-fermented milk had no effect on BAP.

The consumption of various foods is thought to affect skin condition (Heinrich et al., 2006; Stahl et al., 2006). For example, increased intake of vitamin C and linoleic acid and decreased consumption of fats and carbohydrates are associated with improved appearance in aging skin (Cosgrove et al., 2007). In our previous study, oral intake of heat-killed cells of strain H61 improved skin hydration at inner forearms, but not for sebum content in cheek of middle-aged women from autumn to winter (Kimoto-Nira et al., 2012). In the present study, we show that young women who consumed H61-fermented milk for 4 wk showed increased skin hydration at the inner forearm and sebum content in the cheek from winter to spring. It indicates that intake of strain H61 is effective on skin properties of young healthy women and middle-aged women, although differences exist for season and skin index.

Season-associated effects are an important factor in skin condition. Skin disorders such as psoriasis and senile xerosis tend to exacerbate in winter. Melanin provides varying degrees of brown coloration at the skin surface, and melanin content is affected by internal and external factors, such as age, race, and sunlight exposure. The changes by this intervention in hydration of the inner forearm and in the melanin in both

Table 2. Effects of fermented milk intake on blood parameters of young women[1]

Item	TP (g/dL)	CRE (mg/dL)	TG (mg/dL)	AST (IU/L)	ALT (IU/L)	LD (IU/L)	CPK (IU/L)	Fe (μg/dL)	WBC (10^3/μL)	RBC (10^6/ μL)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (%)	MCHC (%)	PLT ($\times 10^4$/μL)
Group																
Yogurt	7.4 (0.1)	0.69 (0.02)	106 (11)	27 (2)	19 (2)	194 (10)	331 (87)	108 (8)	5.9 (0.3)	4.46 (0.1)	13.7 (0.2)	41.6 (0.6)	93.4 (1.3)	30.7 (0.4)	32.9 (0.2)	26.5 (0.8)
H61	7.6 (0.1)	0.69 (0.03)	75 (11)	24 (2)	16 (2)	200 (10)	240 (91)	116 (9)	5.7 (0.3)	4.50 (0.1)	13.4 (0.2)	41.1 (0.6)	91.5 (1.3)	29.9 (0.5)	32.7 (0.2)	24.9 (0.9)
P-value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Stage																
Before intake	7.3 (0.1)[b]	0.69 (0.02)	88 (10)	24 (2)	16 (1)	196 (8)	253 (72)	100 (7)[b]	5.8 (0.2)	4.40 (0.1)[b]	13.4 (0.2)[b]	40.6 (0.4)[b]	92.3 (0.9)	30.4 (0.3)	32.9 (0.2)	24.7 (0.6)[b]
After intake	7.7 (0.1)[a]	0.70 (0.02)	93 (10)	27 (2)	19 (1)	199 (8)	319 (72)	124 (7)[a]	5.8 (0.2)	4.56 (0.1)[a]	13.8 (0.2)[a]	42.1 (0.4)[a]	92.6 (0.9)	30.2 (0.3)	32.6 (0.2)	26.7 (0.6)[a]
P-value	<0.001	NS	NS	NS	NS	NS	NS	0.003	NS	0.0001	0.0010	0.0002	NS	NS	NS	0.0001
Group × stage																
Yogurt before	7.3 (0.1)[b]	0.69 (0.03)	110 (14)	25 (3)	17 (2)	195 (10)	273 (99)	104 (10)	6.3 (0.3)	4.40 (0.1)	13.5 (0.2)	41.0 (0.6)	93.2 (1.3)	30.7 (0.5)	33.0 (0.3)	25.4 (0.9)[b]
Yogurt after	7.6 (0.1)[a]	0.70 (0.03)	102 (14)	30 (3)	21 (2)	194 (10)	389 (99)	111 (10)	5.6 (0.3)	4.52 (0.1)	13.8 (0.2)	42.2 (0.6)	93.6 (1.3)	30.6 (0.5)	32.7 (0.3)	27.5 (0.9)[a]
H61 before	7.3 (0.1)[b]	0.69 (0.03)	66 (15)	23 (3)	16 (2)	197 (11)	232 (104)	96 (10)[b]	5.3 (0.3)	4.41 (0.1)[b]	13.2 (0.2)[b]	40.2 (0.6)[b]	91.4 (1.3)	30.1 (0.5)	32.8 (0.3)	24.0 (0.9)[b]
H61 after	7.8 (0.1)[a]	0.69 (0.03)	84 (15)	25 (3)	16 (2)	203 (11)	248 (104)	137 (10)[a]	6.0 (0.3)	4.60 (0.1)[a]	13.7 (0.2)[a]	42.1 (0.6)[a]	91.6 (1.3)	29.8 (0.5)	32.5 (0.3)	25.8 (0.9)[a]
P-value	NS	NS	NS	NS	NS	NS	NS	0.028	0.012	NS	NS	NS	NS	NS	NS	NS

[a,b]Within each category (group, stage, group × stage), values within a row that have unlike superscript letters were significantly ($P < 0.05$) different.

[1]Total protein (TP), 6.7–8.3; creatinine (CRE), 0.47–0.79; triglyceride (TG), 50–149; aspartate transaminase (AST), 10–40; alanine transaminase (ALT), 5–40; lactate dehydrogenase (LD), 115–245; creatine phosphokinase (CPK), 32–180; Fe, 48–154; white blood cells (WBC), 3.5–9.1; red blood cells (RBC), 3.76–5.00; hemoglobin (Hgb), 11.3–15.2; hematocrit (Hct), 33.4–44.9; mean corpuscular volume (MCV), 79–100; mean corpuscular hemoglobin (MCH), 26.3–34.3; mean corpuscular hemoglobin concentration (MCHC), 30.7–36.6; platelet count (PLT), 13.0–36.9. Values are expressed as least squares means; figures in parentheses are standard errors.

Table 3. Effects of fermented milk intake on antioxidant levels in young women[1]

Item	BAP (μmol/L)	dROM (Carr U)
Group		
Yogurt	2,050 (57)	320 (12)
H61	2,119 (58)	332 (12)
P-value	NS	NS
Stage		
Before intake	2,157 (56)	346 (9)[b]
After intake	2,011 (57)	306 (9)[a]
P-value	NS	<0.0001
Group × stage		
Yogurt before	2,068 (79)	340 (13)[a]
Yogurt after	2,031 (79)	300 (13)[b]
H61 before	2,247 (79)	353 (13)[a]
H61 after	1,991 (83)	312 (13)[b]
P-value	NS	NS

[a,b]Within each category (group, stage, group × stage), values within a row that have unlike superscript letters were significantly ($P < 0.05$) different.

[1]BAP = biological antioxidant potential; dROM = diacron-reactive oxygen metabolites; Carr U = conventional arbitrary units. Values are expressed as least squares means; figures in parentheses are standard errors.

groups are likely in response to environmental changes, such as temperature, humidity, and UV exposure, due to the transition from winter to spring (February to March in the current study). Conversely, sebum content in the cheek was higher after consumption in the H61 group but not the conventional yogurt group. This finding indicates that this positive effect due to strain H61 is not season dependent. Skin lipids originating from sebaceous glands contribute to maintaining the skin in a hydrated state. Nagata et al. (2010) reported a negative correlation between surface lipid content of the forehead and facial wrinkles and a positive correlation between surface lipid content and elasticity of the inner side of the right upper arm. Increases in sebum content induced by H61-fermented milk intake may be useful for maintaining skin health.

To clarify the mechanism by which H61-fermented milk affects skin properties, we investigated the serum oxidative status of our subjects. Solar UV radiation induces the production in the skin of reactive oxygen species, such as singlet oxygen and superoxide, which have long been suspected of contributing to the deleterious effects of cutaneous photodamage (Carbonare and Pathak, 1992). Oral administration of antioxidant substances, such as vitamin C (Darr et al., 1992; Heinrich et al., 2006) and carotenoids (Alaluf et al., 2002), has been shown to have photoprotective effects on the skin, and several studies address the antioxidative activity of lactic acid bacteria (Kaizu et al., 1993; Lin and Chang, 2000). In addition, *Lactococcus lactis* has superoxide dismutase, which catalyzes the dismutation of unvalently reduced oxygen (Hosono and Satake, 1993), and we have found that strain H61 has potential superoxide dismutase activity (unpublished data). Our current study showed that oxidative status (evaluated by reactive oxygen metabolite test) decreased in both groups, thus it does not account for the benefit of H61-fermented milk compared with conventional yogurt in terms of improvement of the sebum content in the cheek. The amount of skin surface lipids drastically increases from approximately 10 yr of age, reaching a maximum after 20 yr, and decreasing gradually with age. Sebum production is partly regulated by hormones such as estrogens, growth hormone, and insulin. The effect of strain H61 on those hormone levels in young women needs to be investigated.

The beneficial effects of milk on skin status in humans have been reported. For example, whole milk intake was associated with alleviation for atopic dermatitis (Woods et al., 2003; Keller et al., 2014). Because an increase of sebum content in the cheek was only observed in H61-fermented milk group after intervention, it would not be responsible for the milk component itself. Furthermore, we previously reported that administration of strain H61 as living cells suspended in milk or milk fermented from the strain to mice was found to reduce skin trouble with aging (i.e., incidence of hair loss and skin ulcers) compared with control milk, and no significant difference in skin status between living cells and fermented milk was observed (Kimoto-Nira et al., 2007). Considering that those results in mice can be extrapolated to those in humans, and heat-killed cells provide beneficial effect on skin status in human (Kimoto-Nira et al., 2012), milk metabolites produced by strain H61 are unlikely to be associated with improvement of skin status observed in the current study.

CONCLUSIONS

We show herein that fermented milk made by using *L. lactis* strain H61 improved sebum content in the cheek and serum oxidative status in healthy young women. Strain H61 has been widely used over the last 50 yr in Japan to produce fermented dairy products. Our study enhances the value of strain H61 as an effective probiotic dairy starter.

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REFERENCES

Alaluf, S., U. Heinrich, W. Stahl, H. Tronnier, and S. Wiseman. 2002. Dietary carotenoids contribute to normal human skin color and UV photosensitivity. J. Nutr. 132:399–403.

Cosgrove, M. C., O. H. Franco, S. P. Granger, P. G. Murray, and A. E. Mayes. 2007. Dietary nutrients intakes and skin-aging appearance among middle-aged American women. Am. J. Clin. Nutr. 86:1225–1231.

Dalle Carbonare, M., and M. A. Pathak. 1992. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. J. Photochem. Photobiol. B 14:105–124.

Darr, D., S. Comb, S. Dunston, T. Manning, and S. Pinnell. 1992. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. Br. J. Dermatol. 127:247–253.

De Spirt, S., W. Stahl, H. Tronnier, H. Sies, M. Bejot, J. M. Maurette, and U. Heinrich. 2009. Intervention with flaxseed and borage oil supplements modulates skin condition in women. Br. J. Nutr. 101:440–445.

Heinrich, U., K. Neukam, H. Tronnier, H. Sies, and W. Stahl. 2006. Long-term ingestion of high flavonol cocoa provides photoprotection against UV-induced erythema and improves skin condition in women. J. Nutr. 136:1565–1569.

Higashikawa, F., M. Noda, T. Awaya, K. Nomura, H. Oku, and M. Sugiyama. 2010. Improvement of constipation and liver function by plant-derived lactic acid bacteria: A double-blind, randomized trial. Nutrition 26:367–374.

Hosono, A., and N. Satake. 1993. Isolation and characterization of superoxide dismutase from *Lactococcus lactis* ssp. *cremoris* R-48. Anim. Sci. Technol. (Jpn) 64:599–604.

Huh, S. Y., J. W. Shin, C. H. Huh, S. W. Youn, and K. C. Park. 2010. The efficiency and safety of 4-n-butylresorcinol 0.1% cream for the treatment of melasma: A randomized controlled split-face trial. Ann. Dermatol. 22:21–25.

Isawa, K., T. Noma, M. Yamamoto, K. Kimura, H. Ito, N. Taketomo, K. Numano, and M. Kawashima. 2008. Verifying the ability of yogurt prepared with LB81 lactic acid bacteria to improve skin function. J. Int. Microbiol. 22:1–5. (In Japanese).

Izumi, T., M. Saito, A. Obata, M. Arii, H. Yamaguchi, and A. Matsuyama. 2007. Oral intake of soy isoflavone aglycone improves the aged skin of adult women. J. Nutr. Sci. Vitaminol. (Tokyo) 53:57–62.

Kaizu, H., M. Sasaki, H. Nakajima, and Y. Suzuki. 1993. Effect of antioxidative lactic acid bacteria on rats fed a diet different in vitamin E. J. Dairy Sci. 76:2493–2499.

Kalliomäki, M., S. Salminen, T. Poussa, H. Arvilommi, and E. Isolauri. 2003. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet 361:1869–1871.

Kaneko, K., T. Kimata, S. Tsuji, T. Shimo, M. Takahashi, and S. Tanaka. 2012. Serum albumin level accurately reflects antioxidant potentials in idiopathic nephritic syndrome. Clin. Exp. Nephrol. 16:411–414.

Keller, S. H., Y. Le, C. Rodiger, U. C. Hipler, R. Kertscher, A. Malarski, L. M. Huntstock, M. Kiehntopf, M. Kaatz, J. Norgauer, and G. Jahreis. 2014. Supplementation of a dairy drink enriched with milk phospholipids in patients with atopic dermatitis—A double-blind, placebo-controlled, randomized, cross-over study. Clin. Nutr. http://dx.doi.org/10.1016/j.clnu.2014.01.014.

Kimoto-Nira, H., R. Aoki, K. Sasaki, C. Suzuki, and K. Mizumachi. 2012. Oral intake of heat-killed cells of *Lactococcus lactis* strain H61 promotes skin health in women. J. Nutr. Sci. 1:e18 http://dx.doi.org/10.1017/jns.2012.22.

Kimoto-Nira, H., C. Suzuki, M. Kobayashi, K. Sasaki, J. Kurisaki, and K. Mizumachi. 2007. Anti-aging effect of a lactococcal strain: Analysis using senescence-accelerated mice. Br. J. Nutr. 98:1178–1186. PubMed.

Larsen, C. N., S. Nielsen, P. Kastel, E. Brockmann, M. Bennedsen, H. R. Christensen, D. C. Eskesen, B. L. Jacobsen, and K. F. Michaelsen. 2006. Dose–response study of probiotic bacteria *Bifidobacterium animalis* ssp. *lactis* BB-12 and *Lactobacillus paracasei* ssp. *paracasei* CRL-341 in healthy young adults. Eur. J. Clin. Nutr. 60:1284–1293.

Lidbeck, A., U. Geltner-Allinger, K. M. Orrhage, L. Ottova, B. Brismar, J.-A. Gustafsson, J. J. Rafter, and C. E. Nord. 1991. Impact of *Lactobacillus acidophilus* supplements on the fecal microflora and soluble faecal bile acids in colon cancer patients. Microb. Ecol. Health Dis. 4:81–88.

Lin, M.-Y., and F.-J. Chang. 2000. Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356. Dig. Dis. Sci. 45:1617–1622.

Moroi, M., S. Uchi, K. Nakamura, S. Sato, N. Shimizu, M. Fujii, T. Kumagai, M. Saito, K. Uchiyama, T. Watanabe, H. Yamaguchi, T. Yamamoto, S. Takeuchi, and M. Furue. 2011. Beneficial effect of a diet containing heat-killed *Lactobacillus paracasei* K71 on adult type atopic dermatitis. J. Dermatol. 38:131–139. .

Nagata, C., K. Nakamura, K. Wada, S. Oba, M. Hayashi, N. Takeda, and K. Yasuda. 2010. Association of dietary fat, vegetables, and antioxidant micronutrients with skin ageing in Japanese women. Br. J. Nutr. 103:1493–1498.

Saigo, K., M. Takenokuchi, Y. Hiramatsu, H. Tada, T. Hishita, M. Takata, M. Misawa, S. Imoto, and S. Imashuku. 2011. Oxidative stress levels in myelodysplastic syndrome patients: Their relationship to serum ferritin and haemoglobin values. J. Int. Med. Res. 39:1941–1945.

Savard, P., B. Lamarche, M. E. Paradis, H. Thiboutot, E. Laurin, and D. Roy. 2011. Impact of *Bifidobacterium animalis* ssp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5-containing yoghurt, on fecal bacterial counts of healthy adults. Int. J. Food Microbiol. 149:50–57.

Shimada, T., L. Cheng, T. Enomoto, X. Yang, A. Miyoshi, and T. Shirakawa. 2004. Lysed *Enterococcus faecalis* FK-23 oral administration reveals inverse association between tuberculin responses and clinical manifestations in perennial allergic rhinitis: A pilot study. J. Investig. Allergol. Clin. Immunol. 14:187–192.

Stahl, W., U. Heinrich, O. Aust, H. Tronnier, and H. Sies. 2006. Lycopene-rich products and dietary photoprotection. Photochem. Photobiol. Sci. 5:238–242.

Woods, R. K., E. H. Walters, J. M. Raven, R. Wolfe, P. D. Ireland, F. C. Thien, and M. J. Abramson. 2003. Food and nutrient intakes and asthma risk in young adults. Am. J. Clin. Nutr. 78:414–421.