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EFFECT OF LACTOBACILLUS ACIDOPHILUS L-92 FOR ADULT ATOPIC DERMATITIS

Efficacy of prolonged ingestion of *Lactobacillus Acidophilus* L-92 in adult atopic dermatitis patients

Kozo Yamamoto,* Kazuhito Yokoyama,* Takehisa Matsukawa,* Sayaka Kato,†

Shinji Kato,† Tatsuhiko Hirota,† and Kazuhisa Yamada†

* Department of Epidemiology and Environmental Health, Juntendo University Faculty of Medicine, Tokyo Japan

†Microbiology & Fermentation Laboratory, Calpis Co., Ltd., Kanagawa ,Japan

Corresponding aouther: Kozo Yamamoto

Address: Department of Epidemiology and Environmental Health, Juntendo University Faculty of Medicine, 2-1-1 Hongo, Bunkyoku,Tokyo 113-8421, Japan,

Phone: +81 +358021047

Fax: +81 +38121026

e-mail: kzyamamo@juntendo.ac.jp

Interpretive Summary

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Yamamoto

Atopic dermatitis is a chronic inflammatory skin disease with relapsing pruritic eczema. Atopic dermatitis often interferes with daily activities due to symptoms of itching and epithelial damage sustained. To evaluate the safety and efficacy of prolonged ingestion of *Lactobacillus acidophilus* L-92 on skin symptoms in adult atopic dermatitis patients, a placebo-controlled double-blinded parallel-group comparison study was conducted, which included administration of heat-killed L-92 or placebo for 24 weeks. The L-92 group showed significant decreases in skin symptom scores compared with the placebo group.

ABSTRACT

To evaluate the safety and efficacy of prolonged ingestion of *Lactobacillus acidophilus* L-92 (**L-92**) on skin symptoms in adult atopic dermatitis (**AD**) patients, a placebo-controlled double-blinded parallel-group comparison study was performed. This included daily administration of heat-killed and dried L-92 or placebo for 24 weeks in 50 AD patients who were 16 years old and older. The severity of skin symptoms was evaluated at baseline and at 4, 8, 12, 16, 20 and 24 weeks, during the intervention using the Investigator Global Assessment, Eczema area and severity index, and Scoring Atopic Dermatitis. Serum cytokine and blood marker levels were also measured at baseline and at 4, 8, 16 and 24 weeks during the intervention. No adverse events were reported during the study period. Compared with the placebo group, the L-92 group showed significant decreases in Investigator Global Assessment, Eczema area and severity index and Scoring Atopic Dermatitis scores. Subjective symptoms in adult AD patients were reduced by intake of L-92. Furthermore, it was suggested that sustained ingestion of L-92 resulted in suppression of scratching behavior and maintenance of remission status of skin symptoms. Sixteen weeks after the study commenced, a significant decrease in Lactate Dehydrogenase and a significant increase in Transforming Growth Factor-β were observed in the L-92 group compared with the

placebo group. In the L-92 group, significant elevation of IL-12 (p70) level before and after ingestion, was observed.

This study suggested that L-92 suppresses Th2-dominant inflammation by activating Treg and Th1 cells.

Key words

Lactobacillus acidophilus ; atopic dermatitis ; placebo-controlled double-blinded parallel-group comparison study ; Allergy

INTRODUCTION

Atopic dermatitis (**AD**) is a chronic inflammatory skin disease with relapsing pruritic eczema, involving allergic and immunological abnormalities. AD often interferes with daily activities due to symptoms of itching, scratching, and epithelial damage sustained through these activities. The cause of AD has not been fully elucidated but it has been reported that a variety of factors, such as genetic background and collapse of skin barrier function are very important in precipitating disease (Harris et al., 2001). One of the dominating hypotheses at this moment, is that AD is caused by penetration of allergens in the setting of Th2-predominant conditions, which arise from an imbalance of Type 1 helper T cells (**Th1**) and Type 2 helper T cells (**Th2**).

Probiotics are defined by live microorganisms which, confer health benefits on the host when administered in therapeutic quantities. A number of studies have reported the efficacy of probiotics over the last few years, and it is thought that probiotics provide various effects through improvement of the intestinal bacterial flora. Indeed, the number of reports related to the impact on allergic diseases is rapidly increasing in the last 20 years. With respect to allergic diseases, there are many reports on the effect of probiotics on atopic dermatitis. For example, *Lactobacillus rhamnosus* GG strain (Sistek et al., 2006) and various other strains including *L. acidophilus* (Torii et al., 2011),

and *L. plantarum* (Yoshida et al., 2013) have been shown to have significant effects on AD. In several studies, probiotics were shown to be effective in adult patients with refractory AD due to protracted inflammation (Kim et al., 2014). The most common mechanism underlying the efficacy of probiotics is considered to be the hygiene hypothesis (Yazdanbakhsh et al., 2002). The hygiene hypothesis is a theoretical concept that suggests that exposure to bacteria and viruses in the immediate environment in childhood has a strong influence on development of allergy. Although there are conflicting data through a number of studies, it has been definitively shown that environmental microbes have influences on the immune system of the host through fundamental studies (Matricardi et al., 2000). Th2-dominant responses result in mast cell degranulation and release of Immunoglobulin-E(**IgE**) in patients with allergy. In contrast, probiotic lactic acid bacteria suppress the Th2 reaction by enhancing Th1 response in such patients. Furthermore, some strains of lactic acid bacteria stimulate the release of cytokines involved in inflammatory suppression, such as Interleukin**(IL)**-10 and Transforming Growth Factor-β (**TGF-β**). These cytokines alleviate excessive inflammatory reaction by inducing regulatory T cells (**Treg**) (Doganci et al., 2005, Karimi et al., 2009, Konstantinov et al., 2008, Smits et al., 2005). Furthermore, Konstantinov and colleagues report that sufficient oral or topical administration of

non-viable microbial cells (intact or broken) and crude cell extracts (i.e. with complex chemical composition), confer a benefit to the human or animal consumer (paraprobiotics) (Konstantinov et al., 2008). However, in human, Boyle *et al*. concluded that intake of probiotics had no efficacy on AD based on data from 12 randomized controlled trials (**RCTs**), which assessed administration of probiotics to patients with atopic dermatitis (Boyle et al., 2008). The effects of probiotics on atopic dermatitis are dependent on appropriate administration, duration and dosage, as well as dosage of various strains. Therefore, further studies are necessary.

Lactobacillus acidophilus L-92 is a *Lactobacillus* strain selected from a number of strains and has been shown to have potential for anti-allergic properties (Ishida et al., 2003). Efficacy of heat-killed L-92 for treatment of hay fever (Ishida et al., 2005b), perennial allergic rhinitis (Ishida et al., 2005a), and atopic dermatitis (Torii et al., 2011) have been reported. Furthermore, Scoring Atopic Dermatitis (**SCORAD**) scores significantly improved in adult patients with atopic dermatitis when L-92 was administered over a period of 8 weeks in conjunction with their usual medication (Inoue et al., 2014). During clinical treatment, it would be useful to evaluate the efficacy of L-92 sustainably given that atopic dermatitis tends to follow a chronic course.

To date, there are few studies relating to the effects of long-term intake of lactic acid

bacteria in conjunctions with the prescribed medication in adult patients with atopic dermatitis. Therefore, we examined the safety and the additive protective effect of L-92 with prescribed medication in adult patients with chronic progression of atopic dermatitis. We did this by evaluating variations in skin symptoms and cytokines when L-92 was administered in addition to prescribed medication over a period of 24 weeks in adult patients with atopic dermatitis.

MATERIALS AND METHODS

Patients who satisfied all of the following registration criteria were included in this study: [1] patients with mild to moderate AD diagnosed according to the Guideline for Management of AD by the Japanese Dermatological Association; [2] patients who were prescribed standard medication in accordance with the Guidelines for the Management of Atopic Dermatitis of the Japanese Dermatological Association; [3] patients who did not receive administration of an antibiotic within a month of the observation period; [4] patients who refrained from positive ingestion of lactic acid bacteria containing foods as well as antibiotics and anti-flatulents, which may have had an impact on the intestinal flora during the study period, and [5] patients without complications of atopic dermatitis at the start of the study.

Exclusion criteria for the participation to this study were as follows: (a) patients with personal history or potential allergy against the test food; (b) patients with serious disease including diabetes, gastrointestinal disease, renal disease and heart disease; (c) patients who could not ingest tablets on a regular response and (d) other patients who were determined as excludable by a clinical investigator.

This study was implemented with consideration for the medical ethics in accordance with the spirit of the Declaration of Helsinki (adopted in 1964, amended in 2008). The ethics committee of Juntendo University approved this study on June 3, 2013.

In patients with atopic dermatitis who were over 16 years of age and received outpatient care at the International University of Health and Welfare, Shioya Hospital and the Kawasakinanbu Hospital, 57 patients were included in this study. Incident cases were not included, All subjects provided written consent. The subjects were assigned by an envelope method; 28 patients and 29 patients were assigned into L-92 group and placebo group, respectively.

All subjects received standard prescribed medication appropriate to disease severity of symptoms, including moisture retention using heparin analog and application of external steroid external, in accordance with the Guidelines for the Management of Atopic Dermatitis of the Japanese Dermatological Association.

Test Samples

Tablets containing 20.7 mg of heat-killed and dried L-92 were prepared (**L-92**). Placebo tablet containing no L-92 with the same shape, flavor and appearance as the L-92 tablet was also prepared. Maltose, starch and vegetable oil were used to shape these tablets. The L-92 tablets or placebo tablets were provided to the subjects in a double-blinded manner, and the subjects were asked to take the tablets every day for a period of 24 weeks.

Assessment of Clinical Conditions

Severity of atopic dermatitis was evaluated using the Investigator Global Assessment (**IGA**), Eczema area and Severity Index (**EASI**), and SCORAD scores in all subjects before the intervention and at 4, 8, 12, 16, 20 and 24 weeks . Furthermore, blood was collected before the intervention and at 4, 8, 16 and 24 weeks after the start of ingestion, while the eosinophil count, serum thymus and activation-regulated chemokine (**TARC**), Lactate Dehydrogenase (**LDH**) and total IgE were examined.

Measurement of Cytokines

Several cytokines were measured before the intervention and at 4, 8, 16 and 24 weeks after the start of ingestion. Venous blood samples (5-10 ml) were collected from subjects under aseptic conditions. The collected samples were immediately centrifuged to obtain serum samples. The obtained serum samples were frozen and stored at -20ºC until subsequent measurement.

The Bio-Plex Pro Human Cytokine Assay, 17-plex Panel (M5000031YV; Bio-Rad; Hercules, Calif., USA) was used for measurement of cytokine levels to evaluate the concentration of cytokines including IL-7, IL-8, Monocyte Chemotactic Protein-1 (**MCP-1**), Macrophage inflammatory protein-1β (**MIP-1β**), and Tumor Necrosis Factor-α (**TNF-α**). IL-12 (p70) and TGF-β were measured by Enzyme-Linked ImmunoSorbent Assay (**ELISA**) using human IL-12 (p70) ELISA kit (BD Biosciences) and LEGEND Free Active TGF-β1kit (Biolegend; San Diego, CA, USA), respectively.

Statistical Analysis

The Wilcoxon test was implemented for comparisons of skin symptoms (IGA, EASI, SCORAD), measured blood test values, and serum cytokine levels at each time point of observation from 4 to 24 weeks after the start of ingestion with values before the intervention. In addition, Mann-Whitney U test was used to test variations (Δ

measured values) at each time point for the values before the intervention with a significance level of $p < 0.05$. All statistical analyses were conducted using SPSS Version 20.0 J(SPSS Inc., Chicago, Ill.)

USA).

RESULTS

No significant differences were observed for age, sex, and IGA score between each group, before the start of the study. Initially, 57 patients were included in the study. 7 subjects were excluded (the remainder being 22 males and 28 females). The reasons for exclusion were antibiotic intake (1 patient from the active group), non-attendance at scheduled visits (1 patient from the placebo group and 1 patient from the active group), and a physician disqualified four individuals for reasons unrelated to the test samples (2 patient from the placebo group and 2 from the active group) but who had discontinued the medication. No adverse events were observed in groups given the L-92 tablet or placebo during the study period (Table 1).

Comparison of Changes in Skin Symptom Scores in L-92 and Placebo Groups

Improvements were observed in all IGA, EASI and SCORAD scores in the L-92

group compared with the placebo group. The IGA, EASI and SCORAD scores of the L-92 group were significantly lower at week 16, week 24, and week 8 after the start of ingestion compared with the placebo group, respectively (Table 2).

For changes in blood markers, serum total IgE and eosinophil count were significantly decreased in both the L-92 and placebo groups. LDH of the L-92 group was significantly decreased at week 16 compared with the placebo group (p = 0.049) (Table 3).

Comparison of Changes in Serum Cytokines

At each time point of measurement, the concentrations of IL-7, IL-8, MCP-1, MIP-1β, TNF-α, IL-12 (p70), and TGF-β were compared with the concentration at week 0. A decrease in TGF-β was observed in the placebo group at week 16 after ingestion. For variations from week 0 (**ΔTGF-β**) at each time point of measurement, the serum TGF-β level of the L-92 group at week 16 was significantly elevated compared with the placebo group (p =0.03) (Table 4, Figure 1).

Furthermore, a significant increase in IL-12 (p70) was observed in the L-92 group at week 24 (p = 0.049) (Figure 2).

DISCUSSION

The effects of L-92 ingestion over a period of 6 month in addition to prescribed medications for adult patients with atopic dermatitis were examined in this study.

Three scoring methods were used for assessment of skin symptoms (Schmitt et al., 2007, Schram et al., 2012) . A variety of scoring methods for skin symptoms have been used previously. Of all these methods, SCORAD is the most widely used method in Japan. This score calculates the condition of dermatitis as the sum of a severity score rated by an evaluator and a score of subjective symptoms rated by the diseased subject. In contrast, EASI is often used for the assessment of skin conditions in Europe and the United States. However, the condition of skin rash is rated by an independent evaluator and subjective symptoms of the patient are not taken into account. For IGA, an evaluator scores the condition of dermatitis in a subject based on a holistic impression of the body (Eichenfield et al., 2002). IGA is considered to be similar to a general practice evaluation when a physician determines the courses of treatment based on assessment of overall severity of the patient's dermatitis.

Of these three evaluation indicators, improvement was observed with SCORAD at the earliest time point in this study. Therefore, it is suggested that subjective symptoms related to itch and attendant lack of sleep were alleviated by the combined use of L-92

with prescribed medication. Significant differences in changes in IGA and EASI in the L-92 group compared with the placebo group were observed later than the change in SCORAD. Although the change in scores were small, subjective improvements were confirmed in this study of combined use of L-92 with prescribed medication in atopic dermatitis in adults.

In part, the effects of combined use of L-92 can be summarised by observing amelioration in symptoms of atopic dermatitis using several indicators in this study. A number of evaluation indicators for the skin symptoms of atopic dermatitis have been proposed in addition to the indicators used in this study, and the number of these indicators is estimated to be more than 20 (Schmitt et al., 2007). Each indicator is calculated by the different aspects and this may affect the result. Currently, dermatologists are engaged on the harmonizing outcome measures for eczema (HOME) initiative, which aims to define the core outcome domains to be used for clinical trials (Schmitt et al., 2015). Use of such indicators would be necessary in evaluating the additional effect of L-92, in the future.

A significant improvement was observed in the total IgE and eosinophil count in both L-92 and placebo groups in this study, while significant suppression of elevation of LDH was also observed at week 16 in the L-92 group. A significant correlation between

LDH and the condition of patient with atopic dermatitis has previously been reported, and LDH is a key measurement, which determines the progress of atopic dermatitis (Mukai et al., 1990). It is thought that LDH becomes elevated as a result of inflammatory conditions induced by a collapse of the skin tissue structure due to scratching behavior. Therefore, we believe that the collapse of skin tissue was abrogated by suppression of itch in the L-92 group compared with the placebo group. As a consequence, L-92 suppressed the scratching behavior in patients with atopic dermatitis, which appears to be consistent with the evaluation of the afore-mentioned skin symptom scores.

For further analysis regarding the mechanism of action during the combination use of L-92, measurement of cytokines in the peripheral blood was performed. From these data, a significant elevation of TGF-β was observed in the L-92 group compared with the placebo group. TGF-β is known as an immunosuppressive cytokine, and it has been reported that TGF-β induces Foxp3-positive regulatory T cells (Treg) in the periphery (Chen et al., 2003). Treg cells are reported as cells capable of acting on inflammatory suppression. A previous study confirmed that there was an increase in production of TGF-β in the Peyer's patch in mice sensitized to OVA and given L-92 and OVA (Torii et al., 2007). Furthermore, elevation of TGF-β in the L-92 group was also confirmed in a

study conducted in adult patients with atopic dermatitis, and reproducibility of this result was also confirmed in this study (Inoue et al., 2014).

In addition, a significant elevation of IL-12 (p70) level was observed in the L-92 group compared to before and after intake of L-92. In a previous study, the production of IL12 (p70) was increased when the L-92 strain was added to isolated and cultured spleen cells of micee sensitized to OVA at the time of antigen stimulation with OVA (Sagitani, 2010). Thus, it seems that allergic symptoms are controlled by improving the balance between Th1 and Th2 cells, through activation of Th1 cells and suppressing Th2 cells in Th2-dominant allergic diseases. There are a number of *in vitro* studies, which have showed that lactic acid bacteria can induce Th1 cytokines (Christensen et al., 2002, Zeuthen et al., 2006). However, a significant variation in human peripheral blood has been confirmed only in a few cases (Vliagoftis et al., 2008). Additionally, the activation of Th1 and induction of TGF-β have been confirmed. These data may appear to be conflicting. However, different cytokines are induced depending on the condition of the disease and subjects. Therefore, further analysis of the detailed immunological mechanism is required before the most suitable method of lactic acid bacteria use for treatment of atopic dermatitis is resolved.

In conclusion, in adult patients showing chronic progression of atopic dermatitis, we

show that atopic dermatitis symptoms could be improved when long-term intake of the probiotic L-92 strain is combined with prescribed medications.

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Table 1. Background of subjects

	L-92 group (n=24)	placebo group (n=26)	*P* value
age, year	25.5(16 to 46)	27 (16 to 49)	.82
IGA	3(2 to 4)	3 (1 to 4)	.43
EASI	10.40 (1.40 to 32.60)	9.20 (0.70 to 32.10)	.52
SCORAD	27.75 (10.20 to 66.70)	30.05 (12.30 to 50.30)	.94
TARC, pg/mL	195.50 (125.00 to 2,512.00)	230.50 (125.00 to 906.00)	.86
total IgE, IU/mL	250.00 (16.10 to 12,700.00)	483.50 (5.00 to 10,200.00)	.73
Eos, /μl	333.00 (90.00 to 1,086.00)	255.00 (114.00 to 1,083.00)	.79
LDH, IU/mL	184.50 (134.00 to 283.00)	179.50 (140.00 to 268.00)	.46
IL-7, pg/mL	5.71 (1.16 to 12.82)	4.61 (2.29 to 18.59)	.59
IL-8, pg/mL	5.38 (2.01 to 22.78)	5.13 (2.85 to 16.34)	.86
IL-12(p70), pg/mL	26.69 (1.62 to 111.14)	35.38 (2.15 to 214.87)	.73
MCP-1, pg/mL	28.25 (9.21 to 45.40)	28.86 (19.20 to 52.74)	.56
MIP-1β, pg/mL	96.89 (65.50 to 187.18)	115.19 (32.60 to 220.24)	.92
TNF-α, pg/mL	3.72 (0.38 to 14.60)	3.74 (1.04 to 23.32)	.12
TGF-β, pg/mL	15.01 (2.12 to 39.78)	13.54 (3.21 to 143.25)	.94

Values are presented as median (range).

Table 2. Changes in skin symptom score

	L-92	placebo	*P* value
4week			
IGA	0(-1 to 0)	0(-3 to 0)	.77
EASI	-1.85(-11.00 to 9.60)	-1.30(-10.60 to 11.60)	.95
SCORAD	-2.60(-19.5 to 25.90)	-3.60(-28.1 to 16.80)	.74
8week			
IGA	0(-1 to 0)	0(-1 to 1)	.22
EASI	-2.90(-13 to 9.00)	-0.40(-12.90 to 5.00)	.05†
SCORAD	-4.90(-20.8 to 17.80)	-0.30(-16.30 to 14.20)	.02*
12week			
IGA	0(-1 to 0)	0(-1 to 2)	.10
EASI	-3.65(-15.80 to 8.80)	-0.70(-12.60 to 6.70)	.09†
SCORAD	-7.25(-19.70 to 20.20)	-0.90(-18.20 to 19.50)	.01*
16week			
IGA	-1(-2 to 1)	0(-1 to 1)	.03*
EASI	-4.15(-18.60 to 7.80)	-1.50(-14.60 to 3.90)	.16
SCORAD	-8.50(-25.30 to 19.50)	-2.00(-16.40 to 14.40)	.02*
20week			
IGA	-1(-3 to 0)	0(-1 to 1)	.03*
EASI	-5.30（-19.00 to 7.70)	-1.50(-19.90 to 7.30)	.08†
SCORAD	-9.45(-24.70 to 16.60)	-1.60(-17.50 to 17.30）	.01*
24week			
IGA	-1(-2 to 0)	0(-2 to 1)	.0004***
EASI	-10.65(-23.50 to 6.90)	-0.15(-12.30 to 12.10)	.01*
SCORAD	-6.30(-19.20 to 4.30)	-1.10(-16.60 to 5.60)	.00003***

Scores represent changes in values from Week 0

Value are presented as median (min to max)

Mann-whitney *U* test † *P*<0.1, **P*<0.05, ***P*<0.01, ****P*<0.001.

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Table 3. Changes in blood test values

	L-92	placebo	*P* value
4week			
TARC, pg/mL	5.00(-2,006.00 to 190.00)	0.00(-508.00 to 355.00)	.90
total IgE, IU/mL	-0.60(-640.00 to 1,100.00)	-3.00(-660.00 to 2,400.00)	.30
Eos, /μl	3.50(-504.00 to 308.00)	-9.00(-422.00 to 381.00)	.73
LDH, IU/mL	-12.50(-58.00 to 25.00)	-4.00(-37.00 to 80.00)	.34
8week			
TARC, pg/mL	5.00(-1,342.00 to 532.00)	4.00(-479.00 to 423.00)	.83
total IgE, IU/mL	-5.80(-1,390.00 to 1,100.00)	-8.60(-1,710.00 to 800.00)	.94
Eos, /μl	-33.00(-670.00 to 267.00)	-34.00(-345.60 to 474.00)	.63
LDH, IU/mL	0.50(-85.00 to 31.00)	3.50(-37.00 to 39.00)	.26
16week			
TARC, pg/mL	-5.00(-670.00 to 3,650.00)	-8.50(-628.00 to 518.00)	.71
total IgE, IU/mL	-33.00(-3,020.00 to 2,000.00)	-65.00(-2,710.00 to 280.00)	.68
Eos, /μl	-29.00(-599.00 to 223.00)	-69.00(-614.00 to 133.00)	.27
LDH, IU/mL	-5.50(-80.00 to 77.00)	4.50(-24.00 to 36.00)	.049*
24week			
TARC, pg/mL	0.00(-547.00 to 2,150.00)	-4.00(-442.00 to 522.00)	.66
total IgE, IU/mL	-47.00(-2,400.00 to 620.00)	-108.50(-2090.00 to 1,330.00)	.63
Eos, /μl	-88.50(-784.00 to 833.00)	-67.50(-722.00 to 79.00)	.78
LDH, IU/mL	-11.50(-94.00 to 46.00)	-1.50(-34.00 to 53.00)	.13

Scores represent changes in values from Week 0

Value are presented as median (min to max)

Mann-whitney *U* test * $P<0.05$.

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Table 4. Changes in serum cytokine level before administration in L-92 and placebo groups (pg/ml)

		L-92	placebo	*P* value
IL-7, pg/mL		-1.23(-5.61 to 8.52)	-0.70(-8.75 to 38.37)	.53
IL-8, pg/mL		-1.63(-5.46 to 6.82)	-0.86(-8.35 to 6.30)	.88
IL-12(p70) , pg/mL		11.21(-79.00 to 69.92)	1.31(-129.81 to 53.82)	.29
MCP-1, pg/mL		16.14(-32.54 to 17.14)	-16.20(-39.16 to 5.39)	.43
MIP-1β, pg/mL		36.33(-61.04 to 124.73)	48.23(-96.17 to 153.25)	.21
TNF-α, pg/mL		-2.64(-10.31 to 3.89)	-2.87(-12.86 to 48.01)	.86
TGF-β, pg/mL				
	4 week	9.73(-38.29 to 91.21)	-5.70(-58.84 to 44.76)	.08†
	8 week	1.92(-35.37 to 105.07)	-1.42(-113.18 to 111.29)	.22
	16 week	2.52(-20.08 to 84.90)	-5.21(-73.07 to 22.61)	.03*
	24 week	-1.51(-29.63 to 85.85)	-0.56(-90.44 to 58.77)	.91

Scores represent changes in values from Week 0 to Week 24

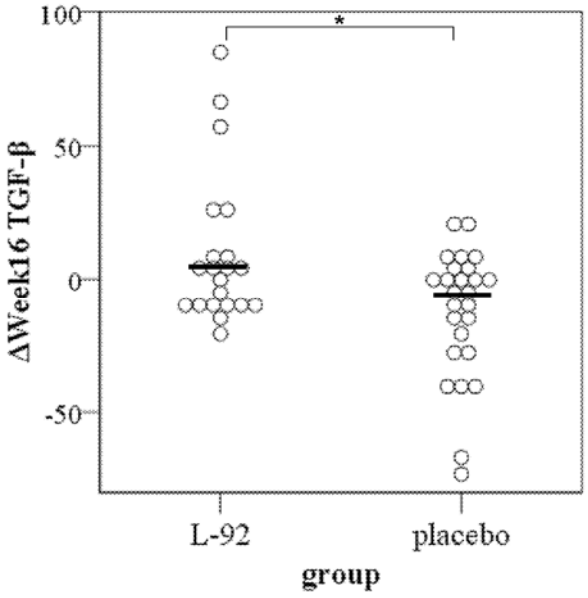
Value are presented as median (min to max)

Mann-whitney *U* test † $P<0.1$, * $P<0.05$.

Figure 1. Changes in TGF-β 16 weeks after intake. Mann-Whitney U test was used for the analysis. *****:A significance level was set at $P < 0.05$.

Figure 2. Changes in IL-12 (p70). Value are presented as average ± S.E. Wilcoxon signed rank test was used a test. *****:A significance level was set at $P < 0.05$.

Yamamoto Figure 1



Yamamoto Figure2