# Supplemental Ingestion of Collagen Peptide Improves T-cell-related Human Immune Status

## —Placebo-controlled Double-blind Study—

■

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**ABSTRACT**

***Objective*** The effects on human immunity of oral supplementation with fish-derived collagen peptide were investigated with a placebo-controlled double-blind trial.

***Methods*** Healthy Japanese men and women（30-59 years of age）with daily tiredness and fatigue and relatively low Scoring of Immunological Vigor（SIV）scores, a comprehensive score reflecting immune status, were randomly assigned to two groups（$n$＝25）. Each participant ingested 10 g of placebo or collagen peptide every day for 8 weeks. Their immunological functions were measured with the SIV score and other parameters.

***Results*** The ingestion of 10 g of collagen peptide for 8 weeks significantly improved the SIV score relative to that of the placebo group（$P$＝0.030）. A within-group analysis showed that the SIV scores（$P$＝0.002）and numbers of T cells（$P$＝0.017）, memory T cells（$P$＝0.008）, $CD8^+$ $CD28^+$ T cells（$P$＝0.039）, and NK cells（$P$＝0.038）increased significantly, and CD4／CD8 T cell ratio（$P$＝0.001）and the T-lymphocyte age（$P$＝0.047）, an index calculated from the $CD8^+CD28^+$ T-cell number, decreased significantly in the collagen peptide group, but not in the placebo group. Significant improvement in the subjective symptoms of diarrhea（$P$＝0.041）and appetite（$P$＝0.043）were only observed in the collagen peptide group. No adverse effects attributable to collagen peptide ingestion were observed.

***Conclusions*** These results suggest that the ingestion of 10 g of collagen peptide for 8 weeks improves the immunological status of humans, especially the number of T lymphocytes and their subsets.

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**KEY WORDS**　Collagen, Immunity, Lymphocyte, Double-blind, Humans

## INTRODUCTION

The immune system defends the body against infections by pathogenic organisms, the invasion of foreign toxic antigens, and the growth of malignant cells. The immune functions in our bodies can be ascribed to two systems：innate immunity and adaptive immunity. Innate immunity provides a quick response but lacks specificity and memory function. In contrast, adaptive immunity is slow but specific, and retains a memory of its target. Innate

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immunity is assumed to involve natural killer（NK）cells and phagocytic cells, such as neutrophils and macrophages. In contrast, adaptive immunity involves immune cells such as T cells, B cells, and antigen-presenting cells. Immune competence decreases with age and stress, and malnutrition also causes the immune functions to deteriorate. Many food components, including epigallocatechin-3-gallate in green tea,[1] probiotics,[2] indigestible oligosaccharides,[3] and vitamins,[4] modulate the immune functions.

To measure the comprehensive immunological strength of an individual as a simple numeral, the scoring of immunological vigor（SIV）score was developed,[5] which combines several immunological parameters. The SIV score has been used to evaluate the immune status of cancer patients[6] and to investigate the effects of foods in improving immune strength.[7]

Collagen is the most abundant protein in the human body, and is extracted from animal bone or hide or from fish scales as gelatin by heating them in water. Collagen peptide is prepared by partially digesting gelatin with enzymes, and is widely used as a safe ingredient. The ingestion of collagen peptide has beneficial effects on the human skin,[8] joints,[9] and circulatory system.[10] However, its effects on human immunity have not yet been elucidated. In this study, a placebo-controlled double-blind trial was conducted to investigate the effects of ingested fish-derived collagen peptide（FCP）on the human immunological status by measuring the SIV score and other immunological, physical, biochemical, and subjective symptom parameters.

## MATERIALS AND METHODS

### 1　Subjects

This study was conducted by Orthomedico Inc.（Tokyo, Japan）in compliance with the Helsinki Declaration and was approved by the Ethics Committee of the Seishin-kai Medical Association Inc., Takara Medical Clinic（Tokyo, Japan；approval date, February 6, 2014；protocol no. 1312-NP01-01）. The study participants were recruited by Orthomedico Inc. and selected according to the following criteria：1）healthy Japanese men and women aged 30-59 years with daily tiredness and fatigue；2）with a relatively low SIV, but＞9（see an explanation of SIV below, section 3）；3）were informed with written documentation on the content, methods, and possible adverse effects of the study；and 4）gave their signed informed consent before the study.

The following participants were excluded：1）those who had previously suffered malignant tumors, heart failure, or cardiac infarction；2）those who were under the care of a doctor for the treatment of chronic diseases such as atrial fibrillation, uneven heartbeat, rheumatism, diabetes, high blood pressure, and diseases of the liver, kidney, cerebral system, circulatory system, and lipid metabolism；3）those who were taking medicines, including herbal medicines；4）those who were allergic to collagen peptide, gelatin, or any medicine；5）those with pollen allergy；6）those who smoked；7）those who habitually took supplements（vitamin, mineral, collagen, hyaluronic acid, placenta, glucosamine, chondroitin）, functional water（oxygenated water, hydrogen water）, or fortified food；8）those who ate collagen-rich foods（fish skin, eel, sea eel, angler fish, beef tendon, internal organs, liver, pig trotters, chicken thighs, chicken wings, chicken cartilage, gelatin, gelatinous juice, gelatin candy, or collagen stew）twice or more a week；9）those who were pregnant, nursing, or were likely to become pregnant during the trial；10）those who had participated in another clinical trial within three months of submitting their written informed consent；11）those who were judged to be unsuitable to participate in the test by the doctor responsible for the present study（Tsuyoshi Takara, MD）.

The participants were instructed as follows：to take the assigned items as indicated；to maintain their usual lifestyles and habits, avoiding too much food, drink, or alcohol, for 1 week before the trial period and during the trial；to avoid excessive exercise；to desist from eating or drinking（except water）for 6 h before all measurements；to keep a daily record that included the intake of the assigned item（or not）and lifestyle factors during the test period, and to send the diary by mail to the study coordinator every 7 days；and to contact Orthomedico Inc. if they felt unwell.

### 2　Test materials

FCP granules（weight-average molecular weight：4000-6000）were prepared from fish scales by Nippi Inc.（Tokyo, Japan）. Dextrin powder（Nippon Starch Chemical Co. Ltd, Osaka, Japan）was used as the placebo. All participants in each group ingested 5 g of FCP or placebo with 0.2% flavor（Peach no. 2039 F；San-Ei Gen F. F. I., Osaka, Japan）twice a day, after breakfast and dinner, with water.

### 3　Experimental design

The purpose of this study was to investigate the effects of FCP ingestion on human immunity. The study was performed as a placebo-controlled double-blind trial. Biochemical analyses of blood and urine, the evaluation of

**Table 1　Immunological parameters between groups**

| Item | Unit | Placebo | FCP | *P* value |
|---|---|---|---|---|
| Score of immunological vigor | — | 15.6±1.8 | 16.2±1.6 | 0.030 |
| T lymphocyte age | year | 50.4±5.8 | 48.6±10.0 | 0.121 |
| Neutrophil | /μL | 3254.8±815.4 | 3528.7±1605.6 | 0.315 |
| Lymphocyte | /μL | 1521.8±289.9 | 1624.4±391.4 | 0.110 |
| T cell | /μL | 1047.6±216.8 | 1143.5±295.0 | 0.079 |
| $CD4^+$ T cell | /μL | 681.8±188.9 | 746.7±234.3 | 0.161 |
| $CD8^+$ T cell | /μL | 389.4±131.1 | 439.7±164.5 | 0.148 |
| CD4/CD8 T cell ratio | — | 2.0±1.1 | 1.8±0.7 | 0.514 |
| Naive T cell | /μL | 255.0±128.4 | 278.4±118.2 | 0.277 |
| Memory T cell | /μL | 426.7±118.9 | 468.4±152.0 | 0.197 |
| Naive/memory T cell ratio | — | 0.6±0.3 | 0.6±0.3 | 0.911 |
| $CD8^+CD28^+$ T cell | /μL | 230.4±67.8 | 271.8±138.5 | 0.113 |
| B cell | /μL | 225.5±102.1 | 241.5±93.0 | 0.519 |
| NK cell | /μL | 195.4±103.5 | 187.4±112.8 | 0.765 |

subjective symptoms, and physical examinations were conducted before and after ingestion of FCP or the placebo for 8 weeks. The analysis of the immune cells in the peripheral blood included the quantification of neutrophils, lymphocytes, T cells, $CD4^+$ T cells, $CD8^+$ T cells, naive T cells, memory T cells, $CD8^+CD28^+$ T cells, B cells, and NK cells, the CD4/CD8 T-cell ratio, and the naive/memory T-cell ratio. Subjective symptoms were measured with Likert scales in the range of 1-6：1, does not describe me at all；2, barely describes me；3, really does not describe me；4, describes me slightly；5, somewhat describes me；and 6, describes me almost completely. The subjective symptoms measured were canker sore prone, shoulder stiffness or pain in the lower back, slight constipation, slight diarrhea, feeling cold more easily than others, loss of sleep, laughing less than usual, poor appetite, persistent fatigue even after 2 days of rest, unexplained tiredness, and feverishness.

The comprehensive immunological status of each individual was measured with the SIV method.[5-7] The score is calculated as follows. First, the numbers of T cells, $CD8^+CD28^+$ T cells, naive T cells, B cells, and NK cells, the CD4/CD8 T cell ratio, and the naive/memory T-cell ratio are each ranked on three levels：1, needs improvement；2, needs observation；and 3, safe. The seven scores are then summed to obtain the SIV score, in a range of 7-21, which represents the vigor of the immune system from low to high. The T-lymphocyte age was also calculated using a regression equation between the patient's age and the number of $CD8^+$ $CD28^+$ T cells, which describes human immunity in a way analogous to age.

**4　Statistical analyses**

SIV was set as the primary outcome and the other immune parameters as the secondary outcomes. The primary and secondary outcomes were analyzed with an analysis of covariance（ANCOVA）model that was adjusted for the values at baseline, to assess the differences between the placebo group and the FCP group. A paired *t*-test was used to assess the differences in within-group changes and in the biochemical blood analysis data. The statistical analyses were performed with Microsoft Excel 2007 and IBM SPSS version 18.0. $P<0.05$ was considered significant.

To evaluate the effect of FCP on SIV, the sample size was determined with a power analysis[11] using the EZR package ver. 1.25[12] in R 3.0.2,[13] with the following assumptions：the statistical power was 80%, the significance level was $P<0.05$, the effect size was 0.8, and the allocation ratio was 1：1. The analysis indicated that 25 participants per group were required.

## RESULTS

When 75 candidates with daily tiredness and fatigue were screened, 50 participants（aged 46.1±6.6 years）with relatively low SIV scores were selected and randomly assigned to each group（$n=25$）, so that the mean SIV scores, sex ratios, and mean ages did not differ significantly between the two groups. All the participants completed the test and were included in the analysis.

The results of the statistical analysis of the SIV scores, T-lymphocyte ages, and other immunological parameters are shown in **Tables 1-3**. The SIV score improved significantly after the ingestion of FCP for 8 weeks compared with that of the placebo group（$P=$

**Table 2　Immunological parameters within the placebo group**

| Item | Unit | Before ingestion | After ingestion | *P* value |
|---|---|---|---|---|
| Score of immunological vigor | — | 15.5±1.3 | 15.6±1.8 | 0.877 |
| T lymphocyte age | year | 50.4±6.8 | 50.4±5.8 | 1.000 |
| Neutrophil | /μL | 3466.4±1200.6 | 3254.8±815.4 | 0.339 |
| Lymphocyte | /μL | 1410.5±288.5 | 1521.8±289.9 | 0.034 |
| T cell | /μL | 1037.8±236.2 | 1047.6±216.8 | 0.791 |
| $CD4^+$ T cell | /μL | 677.7±190.9 | 681.8±188.9 | 0.870 |
| $CD8^+$ T cell | /μL | 332.2±108.2 | 389.4±131.1 | 0.003 |
| CD4/CD8 T cell ratio | — | 2.2±0.9 | 2.0±1.1 | 0.075 |
| Naive T cell | /μL | 276.7±131.2 | 255.0±128.4 | 0.068 |
| Memory T cell | /μL | 401.0±118.4 | 426.7±118.9 | 0.167 |
| Naive/memory T cell ratio | — | 0.7±0.4 | 0.6±0.3 | 0.005 |
| $CD8^+CD28^+$ T cell | /μL | 226.4±76.1 | 230.4±67.8 | 0.718 |
| B cell | /μL | 109.3±70.8 | 225.5±102.1 | ＜0.001 |
| NK cell | /μL | 167.2±86.0 | 195.4±103.5 | 0.081 |

**Table 3　Immunological parameters within the FCP group**

| Item | Unit | Before ingestion | After ingestion | *P* value |
|---|---|---|---|---|
| Score of immunological vigor | — | 15.4±1.4 | 16.2±1.6 | 0.002 |
| T lymphocyte age | year | 50.4±9.0 | 48.6±10.0 | 0.047 |
| Neutrophil | /μL | 3191.5±992.7 | 3528.7±1605.6 | 0.339 |
| Lymphocyte | /μL | 1396.0±350.4 | 1624.4±391.4 | ＜0.001 |
| T cell | /μL | 1050.8±287.8 | 1143.5±295.0 | 0.017 |
| $CD4^+$ T cell | /μL | 697.2±229.3 | 746.7±234.3 | 0.060 |
| $CD8^+$ T cell | /μL | 341.1±120.7 | 439.7±164.5 | ＜0.001 |
| CD4/CD8 T cell ratio | — | 2.2±0.9 | 1.8±0.7 | 0.001 |
| Naive T cell | /μL | 284.8±122.8 | 278.4±118.2 | 0.527 |
| Memory T cell | /μL | 412.4±151.8 | 468.4±152.0 | 0.008 |
| Naive/memory T cell ratio | — | 0.7±0.3 | 0.6±0.3 | 0.001 |
| $CD8^+CD28^+$ T cell | /μL | 235.7±95.3 | 271.8±138.5 | 0.039 |
| B cell | /μL | 109.4±54.7 | 241.5±93.0 | ＜0.001 |
| NK cell | /μL | 147.5±101.2 | 187.4±112.8 | 0.038 |

0.030)．(**Table 1**)．

The within-group analysis showed that the SIV score ($P$=0.002) and the numbers of T cells ($P$=0.017), memory T cells ($P$=0.008), $CD8^+CD28^+$ T cells ($P$=0.039), and NK cells ($P$=0.038) increased, and CD4/CD8 T cell ratio ($P$=0.001) decreased significantly after supplementation with FCP. Furthermore, the T-lymphocyte age decreased significantly ($P$=0.047) by 1.8 years in the FCP group (from 50.4 to 48.6 years) (**Table 3**), but not in the placebo group (consistently 50.4 years) (**Table 2**). Some other parameters showed significant changes in both groups.

In the analysis of subjective symptoms, a number of significant differences were observed in both groups, but significant improvements in diarrhea ($P$=0.041) and appetite ($P$=0.043) were detected in the FCP group only. No severe changes were detected on physical examination, although some significant changes were observed in both groups. The biochemical analysis of the blood showed significant increases in the lymphocyte ratio, basophil ratio, basophil number, creatinine, and creatine kinase in the placebo group only, whereas significant reductions were observed in the erythrocyte number, hemoglobin, and mean corpuscular hemoglobin concentration. In the FCP group, but not in the placebo group, significant increases were detected in the mean corpuscular hemoglobin, γ-glutamyl transpeptidase, lactate dehydrogenase, cholinesterase, total protein, blood urea nitrogen, sodium, total cholesterol, HDL cholesterol, and LDL cholesterol. However, all these values were within normal limits. In the urine analysis, no severe changes attributable to FCP ingestion were detected. No adverse effects attributable to FCP ingestion were observed.

## DISCUSSION

In clinical studies of the effects of nutrients, the basal nutrient status must be considered to determine the amount of test material that should be ingested.[14] In this study, the ingestion of 10 g of collagen peptide was used because the ingestion of 5 g or 10 g of collagen peptide showed significant changes in the parameters assessed in clinical trials of Japanese men and women[15-17] who ingested about 1.9 g of collagen from their diets in everyday life.[18,19]

In this study, a placebo-controlled double-blind trial revealed that the ingestion of 10 g of FCP for 8 weeks significantly improved the comprehensive immunological status of humans, particularly their T-cell-related parameters, such as the numbers of T cells, memory T cells, and $CD8^+CD28^+$ T cells. This suggests the possibility that adaptive immunity was improved by supplemental ingestion of FCP. It has been reported that the number of $CD8^+CD28^+$ T cells correlates strongly with age because the number of these cells decreases with age.[20] The number of $CD8^+CD28^+$ T cells increased significantly in the FCP group but not in the placebo group, so the T-lymphocyte age, which represents the immunological age, decreased by 1.8 years in the FCP group after the ingestion of FCP.

The within-group analysis of subjective symptoms revealed significant improvements in diarrhea and appetite only in the FCP group. These results suggest that the improvements caused by FCP ingestion are subjectively recognized by humans with daily tiredness and fatigue. Because the T-cell-related parameters were improved by ingesting FCP, the improvements in diarrhea and appetite may be associated with changes in the T-cell activities induced by the ingestion of FCP.

It has been reported that ingested collagen is partly digested and absorbed as oligopeptides, of which the dipeptide prolylhydroxyproline（Pro-Hyp）is a major constituent.[21,22] Prolylhydroxyproline has displayed several biological activities, including the stimulation of mouse skin fibroblast growth on collagen,[22] the modulation of lipid metabolism in adipocytes,[23] and the suppression of mineralization in chondrocytes.[24] Another collagen-derived dipeptide, hydroxyprolylglycine（Hyp-Gly）, is also reported to stimulate skin fibroblast growth on collagen.[25] This suggests that the beneficial effects of collagen peptide ingestion depend, at least in part, on the biological activities of collagen-derived dipeptides. It is possible that collagen-derived dipeptides modulate T-cell functions *in vivo* because our study has shown that the ingestion of FCP improved a number of T-cell-related parameters. Because immune competence decreases with age and stress, the supplemental ingestion of FCP should benefit elderly people or those suffering from stress by improving their immune status.

## CONCLUSIONS

Innate immunity involves natural killer（NK）cells and phagocytic cells, such as neutrophils and macrophages. In contrast, adaptive immunity involves immune cells such as T cells, B cells, and antigen-presenting cells. Immune competence decreases with age and stress, and malnutrition also causes the immune functions to deteriorate. This placebo-controlled double-blind trial suggests that the ingestion of 10 g of collagen peptide per day for 8 weeks improves the immunological status of humans, especially the numbers of T lymphocytes and their subsets, and improves subjective symptoms.

**【Conflict of interest】** The authors have no conflict of interest.

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