

# COMMERCIALLY-AVAILABLE HYDROLYZED FISH COLLAGEN PEPTIDES IN PREVENTIVE CONTROL OF RHEUMATOID ARTHRITIS USING MOUSE MODEL

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## ABSTRACT

Oral administration of cartilage-derived native type II collagen was previously reported to ameliorate rheumatoid arthritis (RA) in animal models of joint inflammation where the therapeutic activity may be resulted from accumulation of hydrolyzed collagen around limb joint leading to induced collagen production by chondrocytes. We study the ingestion of collagen hydrolysates (CHF, hydrolyzed fish collagen peptides from RABJ Co. Ltd.) in reduction of limb swelling using a mouse model (SKG/Jcl) with spontaneous onset of RA. CHF could permeate through synthetic skin model using cultured keratinocytes while high molecular collagen protein could not. CHF stimulated collagen synthesis in cultured dermal fibroblast suggesting the functionality of the percutaneous absorbed CHF. The intake of CHF by fibroblasts was statistically greater than the intake of either the native collagen protein or hydrolyzed collagen peptides from other producers. In order to determine the activity of CHF in control of RA, we administer CHF into the diet for SKG/Jcl mice that naturally develops a chronic T cell-mediated autoimmune disease mimicking RA. Oral ingestion of CHF contained the spontaneous progression of arthritis in SKG/Jcl mice, as demonstrated by both limb volume and arthritis scores suggesting that CHF could suppress or delay the onset of RA in SKG/Jcl mouse model. Administration of a formulated recipe containing CHF and N-acetyl glucosamine showed greater suppression of RA in laminarin(adjuvant)-induced SKG/Jcl mouse suggesting a likely synergy between collagen and glucosamine. We demonstrate here that non-native hydrolyzed collagen peptides could be used in preventive control of joint inflammation caused by rheumatoid arthritis.

## INTRODUCTION

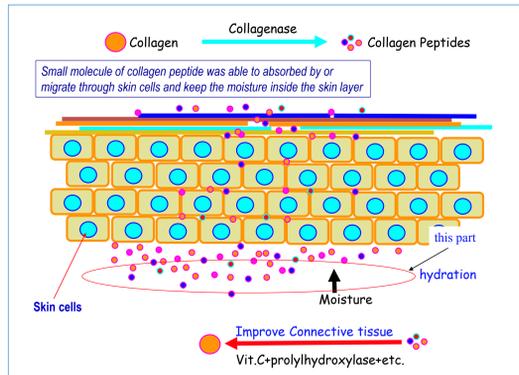
Rheumatoid arthritis is a chronic and progressive autoimmune disease that causes inflammation (swelling) and pain in the joints, the tissue around the joints, and other organs. The symptom of rheumatoid arthritis first occurs at the joints of the hands and feet, and progressively to other joints. The immune system of people with rheumatoid arthritis attacks the cartilage of joints, causing swelling (inflamed) and resulting to bone erosion and joint deformity, in the severe cases.

Destruction of collagen type II in the joint cartilage with people suffering arthritis may activate immune response and antibodies against collagen type II where cartilage degeneration results to rheumatoid arthritis (RA). Oral collagen type II was reported to prevent or delay an immune response through oral tolerance. Gut-activated lymphoid tissue (GALT) from intestine allows us to tolerate ingested protein or allergen. Therefore, repeated oral collagen intake will result to absent or minimal immune response to the collagen around the joints and lead to a reduction in pain and swelling associated with rheumatoid arthritis.

Clinical studies report that the oral ingestion of hydrolyzed collagen decreases joint pain where it is likely resulted from hydrolyzed collagen accumulation in the cartilage and stimulated production of collagen by the chondrocytes, the cells of cartilage. Oral administration of native type II collagen ameliorates two animal models of rheumatoid arthritis induced by type II collagen. Our study demonstrates the therapeutic possibility of oral administered commercially available collagen peptides and provides the foundation for the development of oral collagen as an easily administered nontoxic treatment for rheumatoid arthritis.

## CONCLUSIONS

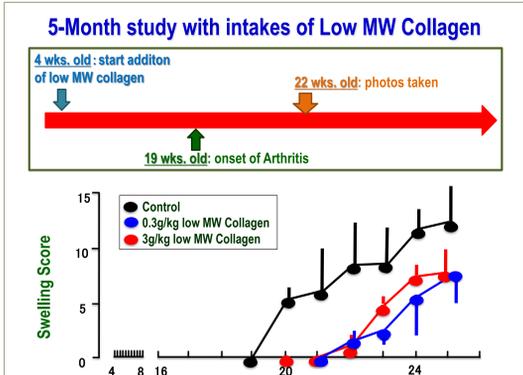
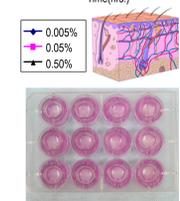
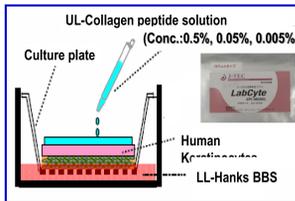
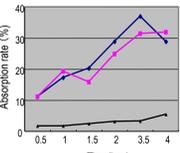
Our results show that commercially-available low molecular weight collagen peptides were able to permeate through cultured keratinocytes suggesting its permeability through skin cells. The inducible production of Pro Collagen Type I C-peptide synthesis in cultured dermal fibroblast model supports the biological efficacy of collagen peptides to synthesize inducible collagen in response to allergen stimulation. The preventive control of rheumatoid arthritis by collagen peptides was studied here using mouse model that develop symptoms of rheumatoid arthritis spontaneously or inducible for early occurrence. Collagen peptides were shown to significantly decrease the swelling of the limb joints suggesting that the intakes of collagen peptides are able to either develop oral tolerance or induce synthesis of collagen around the joints, nonetheless, both leading to alleviation of joint swelling associated with rheumatoid arthritis. The collagen peptide (a type I collagen) from RABJ Co. Ltd. is proven here for its biological efficacy and functionality in preventive control of rheumatoid arthritis.



## Collagen peptides could permeate through cultured skin keratinocyte.

### EXPERIMENT I

- Collagen peptide solution/Concentration : 0.5%, 0.05%, 0.005% collagen peptide in Hanks BSS
- Keratinocytes in vitro 3D model  
Upper layer (UL) : collagen peptide solution  
Lower layer (LL) : Hanks BSS  
Incubation time : 0.5hr., 1hr., 1.5hrs., 2hrs.
- HPLC analysis of lower solution



### EXPERIMENT III

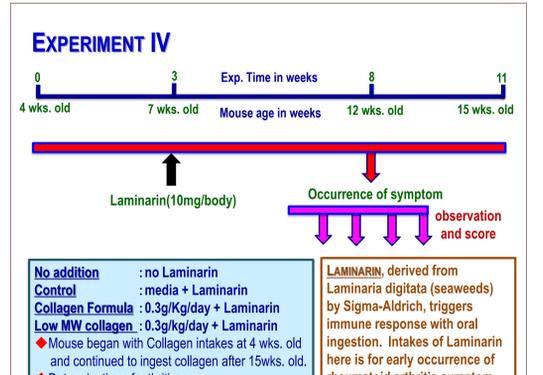
- SKG/Jcl mouse with spontaneous onset of rheumatoid arthritis (male, 4wks. old)
- Daily oral ingestion of low MW collagen at 0.3g/kg/day-3g/kg/day for 5 months
- Control with same amount of media without collagen

#### SCORES

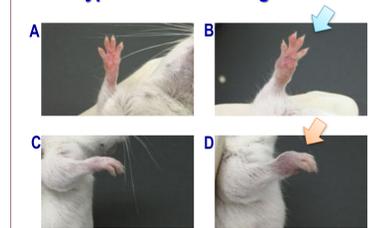
- Observation on mouse limbs for the 5-month time to determine the swelling of limbs & joints and fingers & knuckles according to the following methods.
  - According to the score to determine the activity of the Collagen intakes.
- 0 Point : No significant symptom.  
1 Point : Only 1 finger knuckle shows redness(rubor) and swelling.  
2 Point : Two or more finger knuckles or limb joints show redness and swelling.  
3 Point : One limb shows redness(rubor) and swelling.  
4 Point : Two or more limbs show redness(rubor) and swelling.

#### GROUPS

- Control with media intakes
- 0.3g/kg/day intakes of L MW Collagen
- 3g/kg/day intakes of L MW Collagen



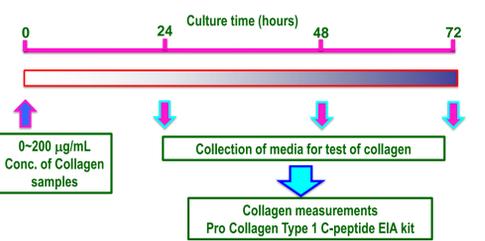
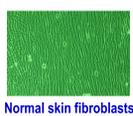
### Typical Joint Swelling Score



The contents of Collagen Formula: Hydrolyzed Collagen Peptide; Isomaltol oligosaccharide; Erythritol; Trehalose; N-acetyl Glucosamine; Hydrolyzed Silk Peptide; adlay (Coixseed) extracts; Haematococcus alga color containing astaxanthin; Pfaffia extracts; yeast extracts; Shark cartilage extracts; active lactic acid bacteria; Lactose; Vitamin C; Glutamine acid; Sodium Gluconate; Acesulfame K; Methyl parahydroxybenzoate; Nicotinamide; Hyaluronic acid pantothenate calcium; Vitamin B1, Vitamin B6; Vitamin B2, Vitamin B12

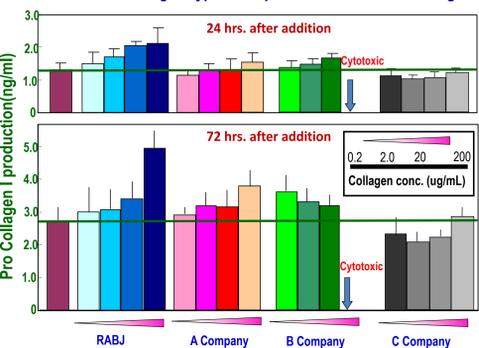
### EXPERIMENT II

Cells : human fibroblasts from normal skin  
Culture : Dulbecco's Modified Eagle media with Vitamin C

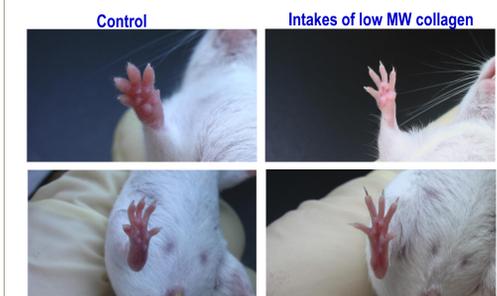


## Collagen peptides stimulates collagen synthesis in cultured dermal fibroblasts.

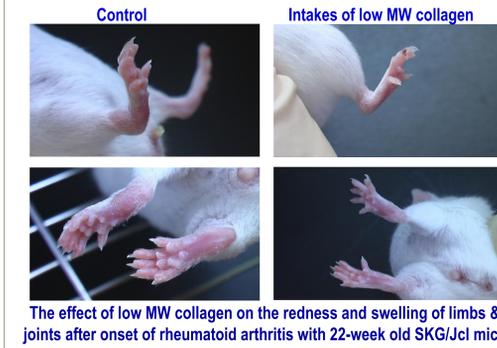
### Production of Pro Collagen Type I C-Peptide after addition of Collagens



## Collagen peptides could suppress or delay the spontaneous onset of rheumatoid arthritis in SKG/Jcl mouse model.



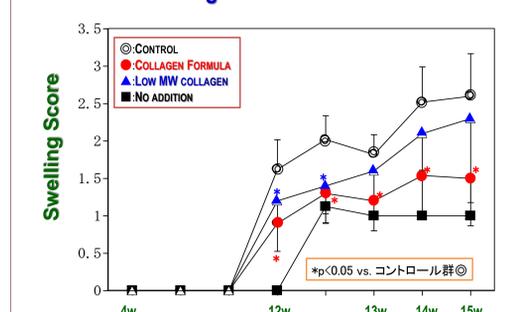
The effect of low MW collagen on the redness and swelling of limbs & joints after onset of rheumatoid arthritis with 22-week old SKG/Jcl mice



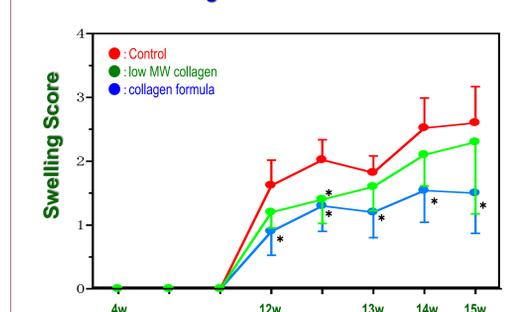
The effect of low MW collagen on the redness and swelling of limbs & joints after onset of rheumatoid arthritis with 22-week old SKG/Jcl mice

## Oral intakes of collagen peptides (type I) could decrease the swelling and redness on the limb joints of RA mouse model.

### Swelling Score on Limb Joints I



### Swelling Score on Limb Joints II



## Fish Source-Raw Material



## Production FlowCharts of Liquid Fish Collagen Peptides



## Certifications of Thai Nippon RABJ Factory

