Research Article

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# Ingestion of bioactive collagen hydrolysates enhance facial skin moisture and elasticity and reduce facial ageing signs in a randomised double-blind placebo-controlled clinical study

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## Abstract

**BACKGROUND: Several human studies have demonstrated occurrence of two major collagen peptides, prolyl-hydroxyproline (Pro-Hyp) and hydroxyprolyl-glycine (Hyp-Gly), in human peripheral blood. Some *in vitro* studies have demonstrated that Pro-Hyp and Hyp-Gly exert chemotaxis on dermal fibroblasts and enhance cell proliferation. Additionally, Pro-Hyp enhances the production of hyaluronic acid by dermal fibroblasts. These findings suggest that the amounts of Pro-Hyp and Hyp-Gly in blood are important factors to show the efficacy of collagen hydrolysates on skin health.**

**RESULTS: We conducted a randomised double-blind placebo-controlled clinical trial of ingestion of two types of collagen hydrolysates, which are composed of different amounts of the bioactive dipeptides Pro-Hyp and Hyp-Gly, to investigate their effects on the improvement of skin conditions. Improvement in skin conditions, such as skin moisture, elasticity, wrinkles, and roughness, were compared with a placebo group at baseline, and 4 and 8 weeks after the start of the trial. In addition, the safety of dietary supplementation with these peptides was evaluated by blood test. Collagen hydrolysate with a higher content of bioactive collagen peptides (H-CP) showed significant and more improvement than the collagen hydrolysate with a lower content of bioactive collagen peptides (L-CP) and the placebo, in facial skin moisture, elasticity (R2), wrinkles and roughness, compared with the placebo group. In addition, there were no adverse events during the trial.**

**CONCLUSION: This study demonstrated that the use of the collagen hydrolysate with a higher content of Pro-Hyp and Hyp-Gly led to more improvement in facial skin conditions, including facial skin moisture, elasticity, wrinkles and roughness.**


**Keywords:** clinical study; collagen hydrolysate; collagen peptide; skin health; bioactive peptides; skin roughness

## INTRODUCTION

There are many products that have beneficial effects on skin health available in the current health food market. Collagen hydrolysates have been developed over the past two decades as supplements or cosmeceutical products for use worldwide. Although a number of studies have demonstrated the efficacy of collagen hydrolysates on skin conditions, little is known regarding what peptides derived from collagen hydrolysates function as bioactive peptides and have physiological effects, which is fundamental information for the maintenance of healthy facial skin.

Denatured collagen forms a substance called gelatin, which when treated by enzymatic hydrolysis results in what are called collagen hydrolysates. Collagen hydrolysates are soluble in water at ambient temperature due to low molecular weight, and possess no gelation ability. This high solubility of collagen hydrolysates allow for the development of products in drink- and jelly-stick-form.

Pharmacological bioavailability trials revealed that two types of collagen dipeptides, prolyl-hydroxyproline (Pro-Hyp) and hydroxyprolyl-glycine (Hyp-Gly), were available at high concentrations for several hours in the human blood stream after oral administration.[1–5] It has been demonstrated that $^{14}$C-labelled Pro-Hyp reaches the skin and bone tissues rapidly after ingestion by mice.[6] Moreover, in a clinical study, Pro-Hyp was identified in urine after collagen hydrolysate intake.[7] These findings suggest that Pro-Hyp and Hyp-Gly are stable and relatively resistant to peptidases in the blood,[4,8] and are able to reach the skin tissues.

In addition, some *in vitro* studies demonstrated the physiological function of Pro-Hyp and Hyp-Gly in skin dermal fibroblasts. Pro-Hyp stimulated chemotaxis of dermal fibroblasts[9] and both Pro-Hyp and Hyp-Gly enhanced cell proliferation activity.[10,11] Additionally, it was observed that Pro-Hyp enhanced the production of hyaluronic acid in dermal fibroblasts.[11]

Pro-Hyp and Hyp-Gly involvement in such physiological roles may be important to improve the efficacy of collagen hydrolysates on the maintenance of skin health. The current study, a randomised double-blind placebo-controlled clinical trial, was carried out to evaluate the efficacy of two types of collagen hydrolysates with differing contents of the bioactive dipeptides Pro-Hyp and Hyp-Gly.

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## MATERIALS AND METHODS

### Investigational products

The placebo, maltodextrin TK-16, was purchased from Matsutani Chemical Industry Co., Ltd. (Itami, Japan). Two forms of collagen hydrolysates derived from fish gelatin, which were composed of different ratios of free-formed Pro-Hyp and Hyp-Gly, were used in this study. One form of collagen hydrolysate (L-CP) had a low ratio of dipeptide-to-product content, with about 0.1 g $kg^{-1}$ of product. The other form of collagen hydrolysate (H-CP) had a high ratio of dipeptide-to-product content, with more than 2 g $kg^{-1}$ of product. These products were provided by Nitta Gelatin Inc. (Osaka, Japan), and are commercially available under the Wellnex brand. Each 5 g test sample was packed in an aluminium sachet and could not be distinguished by the subjects or investigators.

### Study design

This clinical study was conducted in the Shanghai Skin Disease Hospital (Shanghai, China), under the supervision of Dr Xuemin Wang, MD. Randomised administration of the products was carried out in 85 Chinese female subjects who were shown to have no medical issues by blood test performed prior to the study.

The randomised double-blind placebo-controlled study consisted of three groups: Placebo, L-CP and H-CP. Participants were randomly assigned to one of the three groups in a 1:1:1 ratio using a computer generated randomisation schedule. This study was conducted from February to April in 2012. At the start of the trial, each group contained 28 or 29 subjects. Five-gram samples were ingested orally in hot milk, coffee, or any other beverages, once a day after dinner for 8 weeks. Efficacy was assessed at baseline, week 4 and week 8. The amount of daily protein except for collagen peptides was not confined in the study but sustainable intake amount was continued through this trial.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study protocol was approved by the ethics committee of Shanghai Skin Disease Hospital, and written informed consent was obtained from all subjects participating in the study. Selection criteria were: age between 35 to 55 years, subject conscious of their dry and rough skin, body mass index less than 30, not regularly using other supplements or health foods, no treatment with sex hormones over the prior 3 months, and not pregnant. The subjects were advised to avoid excessive eating, drinking, exercise, strong sunburn, change in lifestyle, and change cosmetics.

### Physiological measurements of the skin

Instrumental measurements of skin condition were evaluated at three points: at baseline prior to regular ingestion (baseline), and after 4 weeks and 8 weeks of ingestion. The subjects washed off their make up by conventional methods, and were acclimatised for 30 min in the waiting lounge at a constant temperature of 20 ± 2 °C and humidity of 50 ± 5% before facial skin evaluation.

*Skin moisture*

The change of the dielectric constant measured by an electrical capacitance method was used as an estimate of the amount of skin moisture at the cheek and canthus using a Corneometer CM820 (Courage and Khazaka, Cologne, Germany). Three measurements were taken and averaged.

*Skin elasticity*

Skin elasticity was measured by the suction method using a Cutomater SEM575 (Courage and Khazaka). Decompression suction was carried out for 5 s with a pressure of 300 mbar and a mouth diameter of 2 mm. The return rate, R2 (skin elasticity: Ua1/Uf1), after expansion was assessed at the cheek and canthus.

*Skin wrinkles and roughness*

Analysis of the cutaneous surface of the area from the cheek to the canthus was conducted using a VisioFace SSA (Skin Surface Analysis; Courage and Khazaka) on the following items: number of wrinkles, wrinkle area, wrinkle depth, and roughness.

**Table 1.** Panel demographics

| Group | Number of subjects: Baseline | Dropout | Week 8 | Mean age at week 8* |
|---|---|---|---|---|
| Placebo | 28 | 2 | 26 | 42.31 ± 4.80 |
| L-CP | 29 | 1 | 28 | 43.25 ± 4.06 |
| H-CP | 28 | 2 | 26 | 42.31 ± 4.92 |

*Data are expressed as mean ± SD.
L-CP, lower content of bioactive collagen peptides; H-CP, higher content of bioactive collagen peptides.

### Statistical analysis

Comparison of skin moisture, elasticity, and VisioFace SSA data at different time points within a group were carried out with paired Student's *t*-test. Comparison between the two experimental (H-CP and L-CP) and placebo groups was performed using one-way analysis of variance (ANOVA) with Tukey's post hoc test for evaluation of significance. Comparison of skin moisture and elasticity between the groups was performed using the difference of these variables before ingestion and after 4 weeks (changing rate week 4) or 8 weeks (changing rate week 8) of ingestion.

Significance was defined as $P < 0.05$ using the data analysis software SPSS Ver. 13.0 (IBM Inc., Armonk, NY, USA). Each value was expressed as the mean ± standard deviation (SD).

## RESULTS

### Panel demographics

Five subjects dropped out over the course of the study, due mainly to difficulty in visiting the hospital. There was no significant difference in age between the groups (Table 1). None of the subjects involved in the study demonstrated any dietary problems.

### Skin moisture

Skin moisture results are summarised in Table 2. Skin moisture at the cheek and canthus in both the L-CP and H-CP groups showed a significant increase between baseline and weeks 4 and 8 ($P < 0.05$), while the placebo group did not show such an increase. In the L-CP group, skin moisture at the canthus was significantly higher than the placebo group by week 8. On the other hand, both cheek and canthus skin moisture in the H-CP group was significantly higher by week 8 ($P < 0.05$) when compared to the placebo group.

The change of skin moisture from baseline, namely changing rate (%) week 4 and 8, at the cheek and canthus in both the L-CP and H-CP groups showed a significant increase compared to the placebo group ($P < 0.05$).

Moreover, the change in skin moisture from baseline in the H-CP group was significantly greater ($P < 0.05$) at the cheek by week 8 and at the canthus by weeks 4 and 8, when compared to the L-CP



  

**Table 2.** Facial skin moisture throughout treatment

| Group | Baseline | Week 4 | Changing rate (%), week 4 | Week 8 | Changing rate (%), week 8 |
|---|---|---|---|---|---|
| Cheek | | | | | |
| Placebo | 25.53 ± 14.31 | 26.68 ± 15.96 | 4.5 ± 14.92 | 25.42 ± 14.88 | −0.43 ± 14.54 |
| L-CP | 23.25 ± 12.51 | 27.73 ± 12.88* | 19.27 ± 15.14† | 28.63 ± 12.57* | 18.79 ± 12.85† |
| H-CP | 23.14 ± 12.40 | 29.08 ± 12.05* | 25.67 ± 23.55† | 33.53 ± 12.52*† | 30.99 ± 16.78†‡ |
| Canthus | | | | | |
| Placebo | 72.49 ± 11.58 | 71.76 ± 10.73 | −1.01 ± 7.27 | 70.85 ± 10.20 | −2.31 ± 6.87 |
| L-CP | 70.11 ± 11.25 | 75.56 ± 9.57* | 7.77 ± 8.91† | 78.42 ± 8.21*† | 10.60 ± 11.55† |
| H-CP | 65.50 ± 11.75† | 75.87 ± 10.82* | 13.67 ± 22.78†‡ | 82.78 ± 7.47*† | 20.87 ± 10.75†‡ |

Data are expressed as mean ± SD, in arbitrary units.
*Intragroup comparison ($P < 0.05$, vs. baseline).
†Intergroup comparison ($P < 0.05$, vs. placebo group).
‡Intergroup comparison ($P < 0.05$, L-CP group vs. H-CP group).
'Changing rate (%)' shows the changing rate in % figures between baseline and the time after ingestion, baseline, which was calculated by the equation: (score after ingestion − score at baseline) × 100/score at baseline.

**Table 3.** Facial skin elasticity (R2) throughout treatment

| Group | Baseline | Week 4 | Changing rate (%), week 4 | Week 8 | Changing rate (%), week 8 |
|---|---|---|---|---|---|
| Cheek | | | | | |
| Placebo | 0.736 ± 0.060 | 0.750 ± 0.041 | 1.90 ± 6.59 | 0.738 ± 0.045 | 0.27 ± 7.34 |
| L-CP | 0.739 ± 0.058 | 0.745 ± 0.044 | 0.81 ± 7.71 | 0.749 ± 0.039 | 1.35 ± 7.44 |
| H-CP | 0.725 ± 0.058 | 0.751 ± 0.059* | 3.59 ± 5.74 | 0.767 ± 0.058*† | 5.79 ± 7.59†‡ |
| Canthus | | | | | |
| Placebo | 0.735 ± 0.121 | 0.681 ± 0.088* | −7.35 ± 13.61 | 0.697 ± 0.087* | −5.17 ± 10.48 |
| L-CP | 0.689 ± 0.138 | 0.673 ± 0.099 | −2.32 ± 13.93 | 0.677 ± 0.105 | −1.74 ± 17.13 |
| H-CP | 0.721 ± 0.124 | 0.737 ± 0.106 | 2.22 ± 10.26† | 0.785 ± 0.097*† | 8.88 ± 13.18†‡ |

Data are expressed as mean ± SD, in arbitrary units.
*Intragroup comparison ($P < 0.05$, vs. baseline).
†Intergroup comparison ($P < 0.05$, vs. placebo group).
‡Intergroup comparison ($P < 0.05$, L-CP group vs. H-CP group).
'Changing rate (%)' shows the changing rate in % figures between baseline and the time after ingestion, baseline, which was calculated by the equation: (score after ingestion − score at baseline) × 100/score at baseline.

group. Additionally, the changing rate of H-CP showed a two-fold increase in the L-CP group by week 8 in cheek moisture and by weeks 4 and 8 in canthus.

### Skin elasticity (R2)

Skin elasticity (R2) results are summarised in Table 3. The placebo group showed elasticity of the canthus decreased significantly between baseline and weeks 4 and 8. The L-CP group showed no significant improvement in facial skin elasticity between baseline and weeks 4 and 8, and no significant differences between the placebo group at weeks 4 and 8. On the other hand, in the H-CP group, elasticity of the cheek increased significantly between baseline and weeks 4 and 8, as well as the elasticity of the canthus by week 8. Skin elasticity of both the cheek and canthus in the H-CP group was significantly higher ($P < 0.05$) than in the placebo group by week 8. Moreover, improvement of elasticity from baseline in the H-CP group was significantly higher ($P < 0.05$) than the placebo group by week 4 at the canthus and by week 8 at both the cheek and canthus. Furthermore, there was a significant difference in change rate of elasticity improvement at both the cheek and canthus between the L-CP and H-CP groups by week 8 ($P < 0.05$).

### Skin surface analysis by VisioFace SSA

Skin surface analysis results by VisioFace SSA are summarised in Table 4. In the L-CP group, wrinkle area by weeks 4 and 8 was reduced significantly ($P < 0.05$), and roughness also improved significantly ($P < 0.05$) by week 8, when compared to baseline.

On the other hand, the H-CP group showed significant improvement compared to baseline in many categories, including the number of wrinkles by week 8, and wrinkle area, wrinkle depth, and roughness by weeks 4 and 8. Moreover, comparison between the H-CP and placebo groups showed significant differences ($P < 0.05$) in the number of wrinkles by week 8, and both wrinkle depth and roughness by weeks 4 and 8.

Additionally, there were significant differences ($P < 0.05$) between the H-CP and L-CP groups, including the number of wrinkles and wrinkle depth by week 8, and roughness by weeks 4 and 8.

### Blood test

Blood test analysis results are shown in Table 5. Each value at baseline and after 8 weeks of ingestion was within the limits of standard values. Furthermore, no adverse effects were observed during the clinical trial.

**Table 4.** Facial skin wrinkles and roughness throughout treatment

| Group | Baseline | Week 4 | Week 8 |
|---|---|---|---|
| Number of wrinkles | | | |
| Placebo | 0.021 ± 0.004 | 0.020 ± 0.003 | 0.021 ± 0.004 |
| L-CP | 0.021 ± 0.005 | 0.021 ± 0.005 | 0.020 ± 0.006 |
| H-CP | 0.021 ± 0.004 | 0.021 ± 0.004 | 0.017 ± 0.005*†‡ |
| Wrinkle area | | | |
| Placebo | 0.73 ± 0.24 | 0.73 ± 0.21 | 0.73 ± 0.22 |
| L-CP | 0.69 ± 0.15 | 0.68 ± 0.14* | 0.67 ± 0.15* |
| H-CP | 0.71 ± 0.15 | 0.68 ± 0.12* | 0.65 ± 0.11* |
| Wrinkle depth | | | |
| Placebo | 56.79 ± 3.60 | 56.91 ± 3.06 | 56.52 ± 2.29 |
| L-CP | 56.60 ± 4.48 | 56.21 ± 4.70 | 55.93 ± 5.03 |
| H-CP | 55.86 ± 2.49 | 54.08 ± 3.02*† | 51.78 ± 3.26*†‡ |
| Roughness | | | |
| Placebo | 23.69 ± 1.74 | 23.58 ± 1.60 | 23.42 ± 1.60 |
| L-CP | 23.32 ± 1.42 | 22.93 ± 1.49 | 22.32 ± 1.63*† |
| H-CP | 23.15 ± 2.26 | 21.65 ± 2.23*†‡ | 20.27 ± 2.18*†‡ |

Data are expressed as mean ± SD, in arbitrary units.
*Intragroup comparison ($P < 0.05$, vs. baseline).
†Intergroup comparison ($P < 0.05$, vs. placebo group).
‡Intergroup comparison ($P < 0.05$, L-CP group vs. H-CP group).

## DISCUSSION

The present study demonstrated that ingestion of H-CP, which contains a higher content of the free-formed bioactive peptides Pro-Hyp and Hyp-Gly, resulted in significantly better improvements in facial skin conditions compared to ingestion of L-CP, which has a lower content of these bioactive peptides. These results suggest that, despite using the same raw material, it may be possible to control the effects of collagen hydrolysates on facial skin conditions by modifying the manufacturing process and thus the dipeptide content. Previous reports have demonstrated the effects of Pro-Hyp and Hyp-Gly on skin dermal fibroblasts as signal transducers, which can stimulate metabolism, migration, proliferation, and production of hyaluronic acid.[9–11] In addition, these dipeptides are absorbed into the blood by peptide transporters of the small intestinal epithelial cells in the human digestive and absorption process.[12] Taking into account the bioavailability of these oligopeptides, we hypothesise that it may be possible to enhance uptake of bioactive peptides like Pro-Hyp and Hyp-Gly by increasing the concentration of free-formed bioactive peptides in collagen hydrolysate products. Another type of collagen hydrolysate product, which we have previously reported on, contains more than 3 g kg$^{-1}$ of Pro-Hyp and Hyp-Gly and may have similar or improved effectiveness in enhancing facial skin moisture, elasticity (R2) and roughness, with as little as half the ingested dose (2.5 g) utilised in the present study.[13] On the other hand, we need to consider an effect of beverage co-ingested with collagen hydrolysate for better absorption of collagen bioactive peptides. In the presence study, we reflected the actual use of powder type of collagen hydrolysate by ingestion with tea, coffee, juice, milk, a kind of hot soup like miso soup, etc. Further studies are needed to better understand the optimum combinations with drink type and general food to enhance the functional effects of collagen hydrolysate.

Skin moisture and elasticity depends on the condition of the extracellular matrix, which consists of primarily collagen, hyaluronic acid, and elastin. In an *in vitro* study using human dermal fibroblast cells, Ohara *et al.*[11] reported that Pro-Hyp enhanced cell proliferation and hyaluronic acid synthesis with up-regulated hyaluronic synthase 2 (HAS2) mRNA levels. In addition, they demonstrated that Pro-Hyp stimulates phosphorylation of signal transducer and activator of transcription 3 (STAT3), which is a fundamental intracellular signaling factor.[11] Recently, we have reported the daily oral administration of Pro-Hyp + Hyp-Gly improved skin barrier dysfunction and moisture in HR-1 hairless mice.[14] These reports suggest that Pro-Hyp and Hyp-Gly have a crucial effect in improving the barrier function to enhance skin moisture. We hypothesise that Pro-Hyp and Hyp-Gly stimulated production of hyaluronic acid in the dermis. Hyaluronic acid has been shown to play crucial roles in skin moisture and elasticity.[15] Additionally, several animal studies demonstrated that oral intake of collagen hydrolysates stimulated the synthesis of type I collagen and other extracellular matrix molecules.[16–18]

Regarding the degree of the efficacy between the cheek and the canthus, moisture and elasticity were slightly better in the canthus. In general, the elasticity and thickness of human skin depends on age and measurement site.[19]

In the present study, the H-CP group showed improvement in the number of wrinkles and depth of wrinkles by VisioFace SSA.

Proksch *et al.*[20] have shown that the synthesis of procollagen Type I and elastin, components of the dermal extracellular matrix, led to a pronounced, statistically significant reduction in eye wrinkle volume in a double blind clinical trial. Their data support the idea that a decline in the number of eye wrinkles and wrinkle depth around the eye area effectively improves eye wrinkles, which was similar to the results of the present study (any data not shown)[21].

Regarding the effect of collagen hydrolysate on facial spots, we have previously reported in a clinical study that collagen hydrolysates help reduce ultra-violet spots after 4 weeks of ingestion.[22] Gu *et al.*[23] reported that hyaluronan plays a beneficial role by interacting with fibroblasts to enhance epidermal morphogenesis in a co-culture system. Okawa *et al.*[24] suggested that induced hyaluronic acid in dermal fibroblasts followed by oral administration of collagen hydrolysate may provide beneficial effects on maintaining epidermal and dermal homeostasis in mice. Additionally, Le Vu *et al.*[25] demonstrated that Pro-Hyp induced an increase in expression of Krtap and Krt genes in keratinocytes in co-culture with fibroblasts. These findings suggest that Pro-Hyp may affect signalling to change the phenotype of keratinocytes through the regulation of dermal cells.

Further studies are needed to better understand the mechanisms of the bioactive peptides, Pro-Hyp and Hyp-Gly, which may be associated with their bioavailability. The findings would contribute not only to a better understanding of collagen hydrolysate but also to further the understanding of fundamental mechanisms in anti-ageing.

## CONCLUSIONS

The present study demonstrates that both L-CP and H-CP are effective supplements for the improvement in skin moisture and roughness in women who were conscious of their dry and rough skin. Fortified collagen hydrolysate, H-CP, demonstrated a greater improvement in skin elasticity and reducing wrinkles on facial skin.

The present study is the first of its kind to demonstrate that there is a significant difference between conventional collagen hydrolysate and new types of collagen hydrolysate with higher



  

**Table 5.** Blood test of subjects in the clinical study

| Item | Unit | Placebo group ($n = 26$) | | L-CP group ($n = 28$) | | H-CP group ($n = 26$) | |
|---|---|---|---|---|---|---|---|
| | | Baseline | Week 8 | Baseline | Week 8 | Baseline | Week 8 |
| Total protein | g $L^{-1}$ | 75 ± 4 | 72 ± 4 | 76 ± 4 | 74 ± 3 | 77 ± 5 | 74 ± 4 |
| Albumin | g $L^{-1}$ | 43 ± 2 | 42 ± 2 | 45 ± 2 | 42 ± 2 | 44 ± 2 | 42 ± 2 |
| Albumin/globulin | Ratio | 2 ± 3 | 3 ± 9 | 1.4 ± 0.2 | 1.3 ± 0.2 | 1.4 ± 0.2 | 1.4 ± 9 |
| GPT | IU $L^{-1}$ | 20 ± 13 | 14 ± 6 | 17 ± 10 | 18 ± 8 | 17 ± 10 | 18 ± 6 |
| ALP | IU $L^{-1}$ | 109 ± 36 | 92 ± 27 | 106 ± 28 | 101 ± 27 | 102 ± 29 | 100 ± 27 |
| $\gamma$-GTP | IU $L^{-1}$ | 19 ± 11 | 18 ± 8 | 23 ± 11 | 23 ± 13 | 20 ± 8 | 21 ± 8 |
| GOT | IU $L^{-1}$ | 22 ± 8 | 18 ± 4 | 22 ± 7 | 19 ± 5 | 21 ± 7 | 19 ± 4 |
| LDH | IU $L^{-1}$ | 190 ± 24 | 189 ± 22 | 195 ± 27 | 188 ± 26 | 186 ± 25 | 202 ± 22 |
| Total bilirubin | μmol $L^{-1}$ | 8 ± 4 | 9 ± 3 | 7 ± 3 | 9 ± 4 | 8 ± 4 | 10 ± 3 |
| BUN | mmol $L^{-1}$ | 5 ± 1 | 5 ± 1 | 5 ± 1 | 5 ± 1 | 5 ± 2 | 5 ± 1 |
| Creatinine | μmol $L^{-1}$ | 56 ± 8 | 60 ± 7 | 54 ± 8 | 59 ± 8 | 56 ± 9 | 57 ± 7 |
| UA | μmol $L^{-1}$ | 256 ± 74 | 253 ± 34 | 236 ± 47 | 251 ± 56 | 252 ± 60 | 260 ± 34 |
| CPK | U $L^{-1}$ | 88 ± 26 | 82 ± 24 | 83 ± 32 | 77 ± 27 | 82 ± 31 | 90 ± 24 |

Data are expressed as mean ± SD.
GPT, glutamic pyruvate transaminase; ALP, alkaline phosphatase; $\gamma$-GTP, $\gamma$-glutamyltransferase, GOT, glutamic oxaloacetic transaminase; LDH, *lactate dehydrogenase*; BUN, blood urea nitrogen, UA, urinary acid; CPK, creatinine phosphokinase.

contents of specific bioactive dipeptides such as Pro-Hyp and Hyp-Gly for improvement of human skin conditions.

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