

Anti-aging effect of oral very high proline complex collagen (DERMOFIX®) on skin properties: a randomized, double-blind, placebo-controlled clinical study

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Abstract

Taking collagen supplement to rejuvenate skin is now finding public favor due to anti-aging trend. Synthesizing collagen, the body needs a specific amino acid group –Proline, Hydroxyproline and Glycine called “Proline complex” to make a core structure of every type of collagen fiber in human body. DERMOFIX®, which is a new very high proline complex containing-collagen supplement, helps promoting collagen synthesis naturally leading to anti-aging effects on skin properties as well as other collagen-containing organs.

The objective is to study the anti-aging effects of the oral very high proline complex collagen (VHPCC) primarily on skin properties compared to placebo and commercially available collagen (CAV) in Thailand, and secondarily on knee joint. In this randomized, double blind, placebo-controlled clinical trial, 50 women aged 30-45 years old were randomized to receive the VHPCC 10 g, CAV 10 g or placebo 10 g once daily for 8 weeks. Six aging related skin properties, which are skin elasticity, hydration, melanin index, transepidermal water loss, smoothness and wrinkle were objectively measured at 0, 1, 2, 4, 8 weeks. Knee joint assessments, photo-shooting, blood tests for CBC, creatinine and *sirt1* gene expression level were evaluated before and after the study.

Results: The VHPCC showed statistically significant improvement and gave faster effects than the CAV and placebo, in skin elasticity, hydration, melanin index, transepidermal water loss, smoothness and wrinkles. Most effects by VHPCC showed significant improvement since the first week while CAV showed improvement mostly at fourth or eighth week. Safety blood tests are normal in all groups. However, the Sirt1 gene expression did not increase in any groups. No adverse effect was reported throughout the study.

Conclusion: The study demonstrated that the VHPCC (DERMOFIX®) supplement was proved safe, gave much faster and more effective effects than CAV in anti-aging of skin properties, knee joints and collagen-containing organs.

Keywords: clinical trial, proline complex, collagen, collagen peptide, anti-aging, skin, elasticity, hydration, melanin index, transepidermal water loss, smoothness, wrinkle

Introduction

Nowadays, world population are turning to ‘grey population’ which means the number of elderly people is increasing, so is Thailand. The anti-aging health trend is also finding public

favor especially ‘beauty from within’ in order to keep beauty and healthy to one’s optimum. Taking collagen supplement to rejuvenate the skin is one of a kind.

Skin aging is caused by intrinsic and extrinsic factors such as UV radiation, oxidative stress, smoking, etc. After age of 20, the extracellular matrix especially collagen fiber starts decreasing gradually making unfavorable skin aging signs such as wrinkles, skin laxity, elasticity reduction, skin dryness, unevenness, aging spot and thinner skin thickness leading to loss of skin integrity and normal physiologic function. Moreover, collagen fiber in other collagen-containing organs are also depleting such as bone and joint. Knee joint degeneration problem by aging and weight bearing activities greatly becomes a point of concern due to increase in number of elderly.

The key difference between aging and young skin is pathologically physiological decline of extracellular matrix such as number, size and activity of fibroblast, number and size of collagen fiber, elastin and hyaluronic acid. To reverse (or anti-aging) those aging characteristics, the skin needs to have more aforementioned components in extracellular matrix especially collagen fiber. To have more, the body needs to synthesize more. Synthesizing new collagen fiber, the fibroblast needs a group of amino acids which are proline, hydroxyproline and glycine, called ‘proline complex’ to make an essentially core structure of every type of collagen fiber in human body. Not only skin will be rejuvenate, but also the other collagen-containing organs especially knee joint.

DERMOFIX® which is the new collagen peptide dietary supplement, contains very high content of selectively manufacturing ‘proline complex’ which is specific for collagen synthesis in human body. Compared to leading conventionally commercially available collagen peptide sold in modern drug store in Thailand, could DERMOFIX® give more anti-aging effectiveness and/or faster effects on skin properties? Moreover, this study measures the most number of aging-related skin properties so far, which are 6 parameters, blood test for safety-proven and longevity gene expression along with effect on knee joint in the same time.

Literature review

Watanabe-Kamiyama et al. studied the absorption and effectiveness of orally administered low-molecular-weight collagen hydrolysate in rats and found that these collagen peptides and free amino acids were simultaneously distributed in many parts of body, particularly to the dermis, cartilage, bone, brain, muscle, etc. So, not only skin, but every collagen-containing organ will be beneficially affected by orally administered collagen peptide.

Naoki et al. conducted a randomised double-blind placebo-controlled clinical trial and found that collagen hydrolysate with a higher content of bioactive collagen peptides (H-CP) showed significant 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. H-CP was considered as containing high ratio of free-formed Pro-Hyp or Hyp-Gly to product content with more than 2 gkg⁻¹, while DERMOFIX® has free-formed of Pro, Hyp, Gly more than 400 gkg⁻¹ so this is considered as ‘very high’ of Pro, Hyp and Gly content.

Ohara et al. performed an in vitro study to support that ingestion of collagen peptides helped stimulating dermal fibroblasts proliferation and synthesis of hyaluronic acid. The study showed Pro-Hyp maximally induced stimulation of fibroblast cell proliferation of 1.5 fold and induced a 3.8 fold increase in hyaluronic acid synthesis.

Tanaka et al. examined the effect of daily ingestion of collagen peptides on skin after damage induced by repeated UV-B irradiation. The 6-week study was done on hairless mice and showed that collagen peptides supplement were beneficial to suppress UV-B induced skin damage and photo-aging.

Nakatani et al. investigated the direct effect of prolyl-hydroxyproline (Pro-Hyp) on chondrocytes under in vivo and in vitro conditions and found that collagen hydrolysate and Pro-Hyp inhibited the loss of chondrocytes and thinning of the articular cartilage layer and increased staining area of glycosaminoglycan in the extracellular matrix.

Accordingly, we designed the randomized, double-blind, placebo-controlled clinical trial to study anti-aging effects of the orally administered very high proline complex collagen on all skin properties from literature review which are skin elasticity, hydration, transepidermal water loss, melanin index, smoothness and number of wrinkle along with anti-aging effect on knee joint in the same time.

Most studies reported that collagen peptide effects were seen at 4 weeks after ingestion. Koyama et al. performed a study demonstrated that women after ingestion of 5 or 10 g of pig skin collagen perceived improvement of their skin already after just 3 weeks and at the end of the treatment after 7 weeks. So we design to measure more frequent to see if the VHPCC can be significantly faster than 3 weeks or how fast the result will show.

Materials and Methods

Investigation Products

The test product used in this study was a collagen peptide composed of very high content of selectively-manufacturing amino acids group, called “proline complex”- proline, glycine and hydroxyproline- with about 500 g/kg of product. The product was provided by DERMOFIX (THAILAND) co., ltd (Bangkok, Thailand), commercially available under the name DERMOFIX®. The average molecular weight is 500 Da and the average size is 2 nanometer particle. The commercially available collagen was a collagen peptide purchased from modern trade stores in Thailand. The placebo was inulin fiber. Each 10 g test sample was packed in identical aluminium sachet and could not be distinguished by the subjects or investigators.

Study design

This study was carried out as a randomized, placebo-control, and double-blind experiment study on the anti-aging effects of VHPCC on 6 skin properties (primary interest) as well as anti-aging effects on knee joint and other collagen-containing organs (secondary interest) compare to CAV and placebo after 8 weeks. The protocol was approved by the Institutional Review Board, Faculty of Applied Science, Dhurakij Pundit University, Bangkok, Thailand. All participants received detailed information and signed consent form.

A total of 50 Thai women participants were enrolled at the beginning of the study and were randomized into 3 treatment groups: VHPCC, CAV, and placebo in a 2:2:1 ratio to receive a daily dose of 10 g of either VHPCC, CAV, or placebo. There were no differences between the treatment and the placebo groups (table 1) with regard to age.

The participants took the products orally at home once daily with water or any other liquid for 8 weeks. Data including demographic, health, skin health and Oxford knee score questionnaire, to evaluate the signs, symptoms and severity of osteoarthritis, were collected and assessed prior to the beginning of oral treatment. Skin properties measurement was done at 0, 1, 2, 4, 8 weeks of the study at test areas- both cheeks and forearms. Each visit every participant had to do the questionnaire to assess the subjective improvement on skin, knee joint and other collagen-containing organs. Blood test for CBC, creatinine were collected before and after the study to test safety of the products together with blood test for *sirt1* gene expression. The *sirt1* gene expression was associated with anti-aging and powerful anti-oxidant system. The more *sirt1* gene expresses, the longer longevity one have. Moreover, all participants were photo taken before and after the study by professional photographer having light control

During the trial, the subjects had to refrain from using new skin care products, taking new supplements, medicine, doing treatment, laser, or anything affected skin condition on test areas. The study participants were not allowed to change their usual skin care routine and not allowed to apply the skin care on test areas. Moreover, ablative laser, botox, filler treatment on the test areas were not allowed within 6 weeks prior to the start of the study. In addition to that, changes in living or dietary habits, intravenous vitamin injection, losing weight more than 5% of baseline body weight, and intensive exposure to sun or UV light were prohibited during the study.

Inclusion criteria

The inclusion criteria was: healthy female, 30-45 years old, Fitzpatrick skin type III-IV, general good health and mental condition, and has willingness and capability to follow the study rules and a fixed schedule.

Exclusion criteria

The exclusion criteria was: dermatological disease or disorder on test areas, abnormal collagen-producing dermatological disorder (e.g. scleroderma), skin malignancy or precancerous lesion, hormonal replacement therapy, food allergic to test products, no protein or amino acids intake restriction conditions, no current skin-affecting medication (e.g. Roaccutane, tranxenamic acid), no other collagen-containing supplement intake, scurvy, heavy smoking, ablative laser treatment within 3 months prior to the study, botox injection within 6 months prior to the study, filler, fat or cell injection within 1 year prior to the study, and pregnancy or breastfeeding.

Discontinuation criteria

The discontinuation criteria was: pregnant during the study, forget to take the test products more than one time in 2 weeks, treatment or laser on test areas, all aesthetic facial injection (e.g. botox, filler), weight loss more than 5% of baseline body weight, start new skin care product or change routine skin care, take new supplement or change eating habit, change in lifestyle, acute stress, acute stress (e.g. moving, heavy exercise), intensive sun or artificial UV exposure (solarium) on the test areas or participant want to discontinue the study.

Skin Properties Measurement

Test areas

The test areas were 4 points: right and left sides of the face, 2.5 cm below the lateral canthus, and right and left sides at the middle of inner aspects of both forearms.

Measurement times

There were 5 measurement times: 0, 1, 2, 4, and 8 weeks of treatment for skin elasticity, hydration, melanin index and TEWL. The skin smoothness, wrinkle, blood tests and photo shooting were done at 0, 8 weeks.

Measurement of skin properties

The subjects had to wash the test areas and acclimatized for 15 minutes before skin measurement. There were 2 medical dermatological measurement device: Cutometer® Dual MPA 580 and Visioscan® VC 98. 6 aging-related skin properties were skin elasticity, skin hydration, melanin index, transepidermal water loss, smoothness, and number of wrinkles. The first 4 were performed by using Cutometer® Dual MPA 580. Skin elasticity was performed by using Cutometer probe. Skin hydration was performed by using Corneometer probe. Mean melanin index was performed by using Mexameter. Transsepidermal water loss was performed

by using Tewameter. Visioscan VC 98 was used to assess skin smoothness and wrinkle. Skin smoothness includes acne scar, acnes and skin scaling. The measurement of each test area was repeated 3 times then calculated for the average.

Sirt1 Gene Expression

Famous sirtuin 1, which was a protein encoded by *Sirt1* gene, is an enzyme that deacetylates proteins contributing to cellular regulation such as reaction to stressors. *Sirt1* helps protecting human cells from inflammation and oxidative stress which are the cause of aging, and results in healthy and longevity. The more *Sirt1* gene expresses, the longer age one lives, and the more anti-aging effect one have. Herein, *Sirt1* gene expression level was measured to assess the systemic anti-aging effect of tested products. Isolation of human peripheral blood mononuclear cells (PBMC) was performed by using density gradient centrifugation with Lymphoprep™. The process was done at Nanomedicine Research Unit, Department of Anatomy, Faculty of Medicine, Chulalongkorn University.

Blood test

Since the VHPCC and CAV are nutritionally defined as protein group. 10 g per day of VHPCC is considered as safe and the VHPCC product was already approved by Thai FDA. Concerning safety of this kind of product, we focused on kidney function, so creatinine was measured together with CBC.

Statistical analysis

Difference among three groups was analyzed by using One-Way Analysis of Variance (ANOVA). Also, statistical analysis in each group was performed using paired *t*-test. Statistical significance was considered when $P < 0.05$.

Results

Demographic characteristics

Four participants dropped out. One was pregnant and others had difficulty to follow up on fixed schedules. None of which were related to the adverse effect of product intake or the study procedure. There was no significant difference in age between the groups. Blood test of all participants showed no adverse effect and there was no adverse reaction reported.

Table 1. Panel demographics						
Group	Number of subjects			Age		
	Baseline	Dropout	Week 8	Minimum	Maximum	Mean age at week 8*
Placebo	10	2	8	30	41	34.75 ± 4.37
VHPCC	20	0	20	30	42	33.45 ± 3.65
CAV	20	2	18	30	43	33.83 ± 3.60
Total	50	4	46			
*Data are expressed in mean ± SD						
VHPCC, Very high proline complex collagen; CAV, Commercially available collagen						

Skin elasticity

There was no significant difference in skin elasticity levels between the VHPCC, CAV and placebo groups prior to trial in all test areas, right face, left face, right arm and left arm ($P=0.856, 0.939, 0.339, 0.300$). At week 8, the VHPCC and CAV groups showed statistically significant improvement in skin elasticity at both cheeks and forearms compared to placebo

($P < 0.05$ in all cases). VHPCC showed statistically significantly higher skin elasticity improvement than CAV in all test areas ($P < 0.05$ in all cases). Moreover, VHPCC showed statistically significantly faster skin elasticity improvement than placebo and CAV groups since the first week of the study ($P < 0.05$ in all cases). Changing rate (%), which is a change from week 0 to 8, VHPCC and CAV showed statistically significant enhancement of skin elasticity ($P < 0.05$) compared to placebo group. Moreover, changing rate of VHPCC group showed 3-fold more than CAV group in both cheeks and forearms. The results are summarized in table 2.

Table 2. Skin elasticity (R2, Cutometer)		Mean \pm SD (*10 ⁻⁴)				
Group	Week 0	Week 1	Week 2	Week 4	Week 8	Changing rate (%), week 8
Right-Face						
Placebo (n=8)	6858 \pm 413.96	6780 \pm 386.31	6848 \pm 397.28	6885 \pm 326.47	7034 \pm 427.87	2.57 \pm 1.38
VHPCC (n=20)	6938 \pm 677.59	7455 \pm 638.73 ^{1 2}	8442 \pm 608.32 ^{1 2}	8763 \pm 587.75 ^{1 2}	9120 \pm 499.47 ^{1 2}	32.219 \pm 10.22 ^{1 2}
CAV (n=18)	6989 \pm 441.91	7018 \pm 415.32	6951 \pm 356.88	7536 \pm 339.29 ¹	7787 \pm 342.53 ¹	11.585 \pm 3.62 ¹
Left-Face						
Placebo	7052 \pm 525.70	6994 \pm 461.26	7090 \pm 428.31	7005 \pm 453.76	6940 \pm 449.33	-1.494 \pm 2.56
VHPCC	7078 \pm 706.61	7749 \pm 661.73 ^{1 2}	8398 \pm 651.73 ^{1 2}	8969 \pm 552.17 ^{1 2}	9246 \pm 467.88 ^{1 2}	31.542 \pm 11.40 ^{1 2}
CAV	7129 \pm 402.49	7230 \pm 381.35	7597 \pm 388.33	7656 \pm 360.76 ¹	7991 \pm 319.91 ¹	12.251 \pm 4.26 ¹
Right-Arm						
Placebo	7634 \pm 397.58	7520 \pm 305.52	7611 \pm 311.88	7704 \pm 367.73	7557 \pm 400.14	-0.977 \pm 3.01
VHPCC	7712 \pm 498.09	8218 \pm 323.61 ¹	8885 \pm 269.72 ^{1 2}	9354 \pm 235.36 ^{1 2}	9596 \pm 262.60 ^{1 2}	24.817 \pm 7.00 ^{1 2}
CAV	7899 \pm 495.08	7964 \pm 520.06 ¹	8355 \pm 384.19 ¹	8304 \pm 409.56 ¹	8464 \pm 389.12 ¹	7.344 \pm 4.81 ¹
Left-Arm						
Placebo	7874 \pm 288.51	7802 \pm 217.39	7795 \pm 227.82	7878 \pm 186.65	7739 \pm 192.36	-1.671 \pm 1.70
VHPCC	7936 \pm 462.47	8506 \pm 348.77 ^{1 2}	9311 \pm 249.66 ^{1 2}	9666 \pm 193.02 ^{1 2}	9840 \pm 141.50 ^{1 2}	24.392 \pm 7.48 ^{1 2}
CAV	8105 \pm 373.19	7708 \pm 315.00	8151 \pm 274.30	8536 \pm 291.48 ¹	8804 \pm 300.54 ¹	8.762 \pm 4.51 ¹
All data in week 0-8 are expressed in mean \pm SD (*10 ⁻⁴)						
¹ Intergroup comparison (p < 0.05, vs. Placebo)						
² Intergroup comparison (p < 0.05, VHPCC vs. CAV)						
"Changing rate (%)" shows changing rate in % figures before vs. 8 weeks after ingestion						
VHPCC, Very high proline complex collagen; CAV, Commercially available collagen						

Skin hydration

There was no significant difference in skin hydration levels between the VHPCC, CAV and placebo groups prior to trial in all test areas except left face ($P = 0.009$). So, left face hydration levels were not able to do intergroup comparison. At week 8, the VHPCC showed statistically significant improvement in skin hydration at right face and both forearms compared to placebo ($P < 0.05$) and CAV ($P < 0.05$). CAV showed significantly enhanced skin hydration only at right face and right arm ($P < 0.05$) compared to placebo at week 8. VHPCC showed statistically significantly faster skin hydration improvement than placebo and CAV since the second week of the study ($P < 0.05$ in all cases). Like skin elasticity, changing rate of VHPCC and CAV showed statistically significant enhanced skin elasticity ($P < 0.05$ in all cases) compared to placebo group and VHPCC changing rate was significantly greater than CAV group in right face and both forearms. The left face was analyzed intragroup comparison, VHPCC and CAV were both statistically significant improved skin hydration since the first week until the end of the study. However, VHPCC group showed 2.8 times more in skin hydration enhancement than CAV. The results are summarized in table 3.

Table 3. Skin hydration (Corneometer)			Mean \pm SD			
Group	Week 0	Week 1	Week 2	Week 4	Week 8	Changing rate (%), week 8
Right-Face						
Placebo (n=8)	49.67 \pm 4.58	50.43 \pm 4.09	51.95 \pm 4.02	52.49 \pm 4.07	51.33 \pm 3.03	3.74 \pm 6.15
VHPCC (n=20)	52.33 \pm 6.63	58.11 \pm 7.09 ^{1,2}	68.43 \pm 7.58 ^{1,2}	75.56 \pm 7.88 ^{1,2}	79.49 \pm 7.77 ^{1,2}	52.902 \pm 13.61 ^{1,2}
CAV (n=18)	51.26 \pm 4.09	52.61 \pm 3.73	55.47 \pm 3.41	58.27 \pm 3.40	62.90 \pm 3.24 ¹	23.082 \pm 6.7 ¹
Left-Face						
Placebo	55.33 \pm 4.77	51.87 \pm 1.97	54.67 \pm 2.80	53.43 \pm 2.13	55.67 \pm 3.56	0.811 \pm 3.20
VHPCC	50.75 \pm 5.34	54.73 \pm 5.87 *	64.83 \pm 7.02 *	71.44 \pm 7.20 *	75.13 \pm 7.72 *	48.535 \pm 12.56
CAV	54.96 \pm 3.15	55.83 \pm 3.33 *	56.45 \pm 3.52 *	60.61 \pm 2.97 *	64.44 \pm 2.96 *	17.412 \pm 5.11
Right-Arm						
Placebo	36.87 \pm 3.61	36.70 \pm 3.44	37.34 \pm 3.44	37.49 \pm 3.15	36.79 \pm 3.24	-0.121 \pm 1.86
VHPCC	35.68 \pm 3.98	37.97 \pm 3.79	43.03 \pm 3.79 ^{1,2}	46.52 \pm 3.73 ^{1,2}	48.17 \pm 3.61 ^{1,2}	35.72 \pm 8.57 ^{1,2}
CAV	36.34 \pm 3.59	36.68 \pm 3.59	37.04 \pm 3.66	39.38 \pm 3.66	41.15 \pm 3.70 ¹	13.402 \pm 4.01 ¹
Left-Arm						
Placebo	38.15 \pm 4.15	37.20 \pm 3.38	38.12 \pm 4.26	38.55 \pm 3.93	38.05 \pm 3.82	-0.165 \pm 2.59
VHPCC	37.04 \pm 3.50	39.51 \pm 3.44 ²	45.15 \pm 3.37 ^{1,2}	47.44 \pm 3.37 ^{1,2}	49.09 \pm 3.98 ^{1,2}	32.922 \pm 8.27 ^{1,2}
CAV	35.74 \pm 2.91	36.02 \pm 2.97	37.90 \pm 3.04	38.10 \pm 3.09	39.97 \pm 2.93	11.971 \pm 3.78 ¹
All data in week 0-8 are expressed in mean \pm SD						
* Intragroup comparison (p < 0.05, vs. before)						
¹ Intergroup comparison (p < 0.05, vs. Placebo)						
² Intergroup comparison (p < 0.05, VHPCC vs. CAV)						
"Changing rate (%)" shows changing rate in % figures before vs. 8 weeks after ingestion						
VHPCC, Very high proline complex collagen; CAV, Commercially available collagen						

Skin melanin index

There was no significant difference in mean melanin index between the VHPCC, CAV and placebo groups prior to and after the trial in all test areas ($P > 0.05$ in all cases) due to wide standard deviation. Instead, intragroup comparison was analyzed. VHPCC group showed significant decrease of melanin pigment volume in week 1 to 8 ($P < 0.05$ in all cases) and changing rate in VHPCC group was significantly less than CAV and placebo group in all test areas ($P < 0.05$ in all cases). Although the VHPCC group showed greater decrease in mean melanin index in all test areas among the three groups, it failed to show statistical significance. The results are summarized in table 4.

Table 4. Melanin index (Mexameter)		Mean melanin index \pm SD				
Group	Week 0	Week 1	Week 2	Week 4	Week 8	Changing rate (%), week 8
Right-Face						
Placebo (n=8)	210.338 \pm 21.40	206.450 \pm 20.71	210.088 \pm 20.82	211.625 \pm 20.61	212.413 \pm 19.66	1.115 \pm 3.71
VHPCC (n=20)	200.155 \pm 35.68	196.020 \pm 33.30 *	191.535 \pm 33.24 *	187.135 \pm 32.62 *	183.330 \pm 31.21 *	-8.179 \pm 4.79 ^{1 2}
CAV (n=18)	198.244 \pm 33.93	201.817 \pm 33.56	201.694 \pm 34.88	198.806 \pm 34.68	195.528 \pm 32.80 *	-1.264 \pm 3.43
Left-Face						
Placebo	199.413 \pm 21.45	200.663 \pm 19.76	202.413 \pm 19.94	203.163 \pm 21.01	201.650 \pm 20.27	1.194 \pm 1.95
VHPCC	193.550 \pm 35.08	191.240 \pm 33.85	185.555 \pm 31.19 *	183.870 \pm 35.40 *	179.650 \pm 32.23 *	-6.901 \pm 5.61 ^{1 2}
CAV	203.389 \pm 38.83	204.656 \pm 38.39	203.444 \pm 37.48	199.861 \pm 36.55	198.511 \pm 36.03 *	-2.117 \pm 4.00
Right-Arm						
Placebo	206.413 \pm 18.95	207.75 \pm 22.79	205.963 \pm 17.14	206.375 \pm 15.38	207.163 \pm 16.83	0.655 \pm 7.01
VHPCC	203.060 \pm 48.29	199.665 \pm 46.71 *	195.360 \pm 45.67 *	186.660 \pm 46.31 *	182.850 \pm 45.98 *	-10.004 \pm 5.99 ^{1 2}
CAV	202.456 \pm 46.64	204.289 \pm 46.08	205.417 \pm 48.89	207.050 \pm 48.29	206.722 \pm 47.65	2.4 \pm 7.84
Left-Arm						
Placebo	208.713 \pm 16.67	209.038 \pm 20.50	207.625 \pm 16.72	206.125 \pm 13.01	205.788 \pm 14.79	-1.23 \pm 5.19
VHPCC	202.885 \pm 50.90	198.410 \pm 49.73 *	194.440 \pm 46.72 *	184.755 \pm 45.92 *	180.165 \pm 44.82 *	-11.053 \pm 6.19 ^{1 2}
CAV	206.700 \pm 47.52	208.311 \pm 48.45	206.894 \pm 51.53	207.411 \pm 49.31	208.483 \pm 47.79	1.241 \pm 8.90
All data in week 0-8 are expressed in mean \pm SD						
* Intragroup comparison (p < 0.05 vs. before)						
¹ Intergroup comparison (p < 0.05 vs. Placebo)						
² Intergroup comparison (p < 0.05, VHPCC vs. CAV)						
"Changing rate (%)" shows changing rate in % figures before vs. 8 weeks after ingestion						
VHPCC, Very high proline complex collagen; CAV, Commercially available collagen						

Skin transepidermal water loss

There was no significant difference in transepidermal water loss (TEWL) between the VHPCC, CAV and placebo groups prior to the study. The TEWL at both face and arm in VHPCC group was statistically significantly decreased compared to placebo group ($P < 0.05$) and CAV group ($P < 0.05$) from week 4 to the end of the study. A significant reduction of TEWL in VHPCC group was greater than CAV group since week 2 ($P < 0.05$). The changing rate of VHPCC was significantly far more than CAV and placebo at both face and arm ($P < 0.05$ in all cases). However, CAV group did not show the significant improvement of TEWL compared to the placebo. The results are concluded in Table 5.

Table 5. Transepidermal water loss (TEWA meter)		Mean transepidermal water loss \pm SD				
Group	Week 0	Week 1	Week 2	Week 4	Week 8	Changing rate (%), week 8
Face						
Placebo (n=8)	13.54 \pm 2.02	13.15 \pm 1.74	13.08 \pm 1.68	13.46 \pm 1.71	13.13 \pm 1.66	-2.471 \pm 8.17
VHPCC (n=20)	13.31 \pm 2.65	11.85 \pm 2.88	10.74 \pm 2.18 ²	9.88 \pm 1.66 ^{1 2}	9.46 \pm 1.44 ^{1 2}	-27.779 \pm 9.22 ^{1 2}
CAV (n=18)	12.88 \pm 2.94	12.23 \pm 2.86	13.01 \pm 2.70	12.66 \pm 2.56	12.32 \pm 2.64	-3.594 \pm 12.48
Arm						
Placebo	10.61 \pm 1.52	10.31 \pm 1.26	10.50 \pm 1.37	10.88 \pm 1.27	10.40 \pm 1.01	-1.112 \pm 9.78
VHPCC	10.82 \pm 1.93	9.66 \pm 1.56	9.13 \pm 1.40 ²	8.72 \pm 1.21 ^{1 2}	8.41 \pm 1.14 ^{1 2}	-21.26 \pm 8.72 ^{1 2}
CAV	10.77 \pm 2.42	10.52 \pm 2.36	10.96 \pm 2.22	11.32 \pm 2.16	11.40 \pm 2.33	6.981 \pm 15.77
All data in week 0-8 are expressed in mean \pm SD						
¹ Intergroup comparison (p < 0.05, vs. Placebo)						
² Intergroup comparison (p < 0.05, VHPCC vs. CAV)						
"Changing rate (%)" shows changing rate in % figures before vs. 8 weeks after ingestion						
VHPCC, Very high proline complex collagen; CAV, Commercially available collagen						

Skin smoothness and skin wrinkle

There was no significant difference in skin smoothness and wrinkle at face between the VHPCC, CAV and placebo groups prior to the study. Only VHPCC group showed significantly

improved skin smoothness ($P<0.05$) at the end of the study compared to CAV and placebo groups. Furthermore, VHPCC group showed a significantly ten-fold difference in changing rate of smoothness improvement ($P<0.05$) more than CAV group. Regarding number of wrinkles, though there was no significant difference between VHPCC, CAV and placebo group after the study due to wide standard deviation, every group showed significantly improvement when compared internally between baseline and week 8. However, only VHPCC group showed marked significantly greater changing rate ($P<0.05$) than CAV and placebo group. VHPCC changing rate was three times more than CAV group. However, there was no significant difference of skin smoothness, wrinkle, and their changing rate between CAV and placebo group. The results are shown in Table 6.

Table 6. Skin smoothness and wrinkle (VisioScan)		Mean \pm SD	
Group	Before	After	Changing rate (%)
Smoothness			
Placebo (n=8)	224.638 \pm 30.22	230.100 \pm 33.32	2.389 \pm 4.68
VHPCC (n=20)	225.025 \pm 41.32	312.040 \pm 53.66 ^{1 2}	41.484 \pm 30.08 ^{1 2}
CAV (n=18)	236.367 \pm 56.67	245.639 \pm 57.75	4.149 \pm 8.24
Number of wrinkles			
Placebo	65.381 \pm 9.79	63.634 \pm 9.18*	-2.569 \pm 3.35
VHPCC	72.254 \pm 13.54	62.098 \pm 11.37*	-13.748 \pm 5.66 ^{1 2}
CAV	68.226 \pm 15.06	65.081 \pm 15.31*	-4.542 \pm 8.86
All data are expressed in mean \pm SD			
* Intragroup comparison (p < 0.05, vs. before)			
¹ Intergroup comparison (p < 0.05, vs. Placebo)			
² Intergroup comparison (p < 0.05, VHPCC vs. CAV)			
"Changing rate (%)" shows changing rate in % figures before vs. 8 weeks after ingestion			
VHPCC, Very high proline complex collagen; CAV, Commercially available collagen			

Subjective feeling of all subjects were get along well with the objective measurement. Almost all VHPCC group reported improved skin qualities since the first week which went well with the objective results that significantly improved since the first week. Moreover, VHPCC subjects reported that mostly they perceived improved skin change after 3 days of ingestion. CAV group perceived skin improvement at week 4, same as the objective measurements that improved at week 4. In VHPCC group, not only 6 measured skin qualities improved, but other skin qualities were also reported: faster wound healing (100%), stretch mark improvement (66.7%), less oily face (85%), less hair loss and hair qualities improvement (90%) and nail strengthening (70%). Atrophic acne scar improvement was seen in skin smoothness parameter.

Blood test

Blood test for *Sirt1* gene expression results demonstrated that no significant improvement among the VHPCC, CAV and placebo group. The complete blood count (CBC) and creatinine level at baseline and after 8 weeks of ingestion was within the limits of standard values. Furthermore, no adverse effect was reported throughout the study. Blood test analysis results are shown in Table 7.

Table 7. CBC Blood test									
Item	Unit	Placebo (n=8)		High Proline Collagen (n=20)		Available Collagen (n=18)		All Groups (n=46)	
		Before	After	Before	After	Before	After	Before	After
WBC	$\times 10^3/\mu\text{L}$	8.11 ± 2.543	7.91 ± 1.997	7.07 ± 2.278	6.91 ± 1.980	7.93 ± 1.839	7.05 ± 1.724	7.59 ± 2.165	7.14 ± 1.879
RBC	$\times 10^6/\mu\text{L}$	4.92 ± 0.421	4.99 ± 0.335	4.58 ± 0.494	4.57 ± 0.482	4.74 ± 0.461	4.69 ± 0.384	4.70 ± 0.476	4.69 ± 0.440
Hb	g/dl	12.85 ± 1.180	13.05 ± 1.138	12.51 ± 0.999	12.46 ± 0.960	12.80 ± 1.353	12.69 ± 1.255	12.68 ± 1.164	12.65 ± 1.110
Hct	%	39.18 ± 3.149	39.85 ± 3.221	38.44 ± 2.590	37.73 ± 2.586	39.02 ± 3.744	38.54 ± 3.300	38.79 ± 3.126	38.42 ± 3.022
PLT	$\times 10^3/\mu\text{L}$	272.25 ± 52.462	273.63 ± 55.688	283.5 ± 76.146	283.75 ± 72.635	307.28 ± 59.866	298.61 ± 70.326	290.85 ± 66.511	287.80 ± 68.324
Cr	mg/dl	0.73 ± 0.152	0.7 ± 0.122	0.66 ± 0.096	0.64 ± 0.109	0.7 ± 0.097	0.69 ± 0.096	0.69 ± 0.109	0.67 ± 0.107

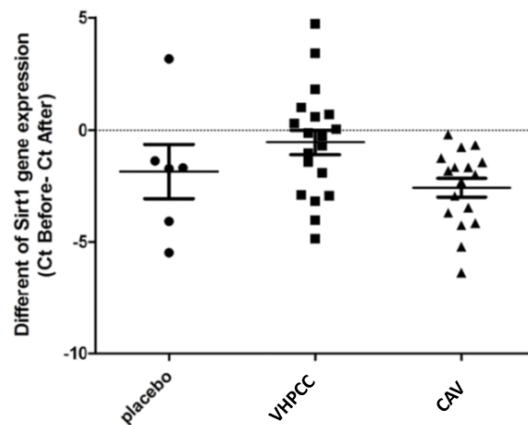


Figure 1. Difference of Sirt1 gene expression level between before and after the study. No group was able to improve Sirt1 gene expression level.



Figure 2. Before(left) and after(right) pictures of VHPCC participant (frontal, at rest). The after picture showed facial skin looking more volumized, rejuvenated and hydrated with brighter and smoother appearances, and decreasing wrinkles. Under eyes area was brighter. Besides, nasolabial folds were better. This participant stated that she felt like she had received Botox, filler injection and laser treatment.



Figure 3. Before(left) and after(right) pictures of VHPCC participant (right oblique). The after picture showed facial skin looking more volumized especially at forehead and cheeks. Facial skin looked rejuvenated and hydrated with brighter and smoother appearances. Besides, nasolabial folds were better. Acnes and acne redness spots were much improved without permission of using any drugs or treatments.



Figure 4. Before(left) and after(right) pictures of VHPCC participant (left oblique, at rest). The after picture showed facial skin looking more volumized especially at forehead and cheeks. Facial skin looked rejuvenated and hydrated with brighter and smoother appearances. Besides, nasolabial folds were better. Acnes and acne redness spots were much improved without permission of using any drugs or treatments.



Figure 5. Before(left) and after(right) pictures of VHPCC participant (frontal, smile) showed less wrinkles around eyes area, crow's feet, less dark circles under eyes. Besides, nasolabial folds were improved. Facial skin looked rejuvenated and hydrated with brighter and smoother appearance. The face also looked more volumized.



Figure 6. Before(left) and after(right) pictures of VHPCC participant (right oblique, smile) showed less wrinkles, crow's feet, and improved under eye area. Besides, nasolabial folds were improved. Acne redness spots and scar on the cheek were much improve without permitted using any drug or treatment. The face looked volumized, rejuvenated and hydrated with brighter and smoother appearance.



Figure 7. Before(left) and after(right) pictures of VHPCC participant (left oblique, smile) showed less wrinkles, crow's feet, and improved under eye area. Besides, nasolabial folds were improved. Acne redness spots and scar on the cheek were much improve without permitted using any drug or treatment. The face looked volumized, rejuvenated and hydrated with brighter and smoother appearance.



Figure8. Before(left) and after(right) picture set of VHPCC participant. The after picture showed pigmented spots were less and pigmented spot color was lighter.

Secondary objective was to evaluate anti-aging effect on knee joint. Prior to the study, every participant was evaluated by Oxford knee score and it was found that no one was likely diagnosed as osteoarthritis (OA) of knee. Then 7 signs and symptoms of OA knee were evaluated before and after the study. In VHPCC group, 5 subjects had joint pain before the trial and all of them reported joint pain decrease (100%) at week 8 of the study. 5 subjects had joint sound on movement, 4 of 5 improved, 1 was same as before. 2 subjects had joint stiffness, one had better result, the other had symptom at same level. On the other hand, in CAV group, 4 had joint pain prior to the study, only 1 had joint pain improvement, the others stayed the same. One had joint sound and another had limited range of motion, yet none had improved result. For placebo group, 1 had joint pain and another had joint stiffness, none had better outcome. The effects on OA knee are summarized in Table 8.

Table 8. Sign & symptom of osteoarthritis knee							
Sign & symptoms	Placebo (n=8)		High Proline Collagen (n=20)		Available Collagen (n=18)		
	Before	Better after	Before	Better after	Before	Better after	
Joint pain	1	0	5	5	4	1	
Joint sound on movement	0	0	5	4	1	0	
Joint stiffness	1	0	2	1	0	0	
Joint deformity	0	0	0	0	0	0	
Joint edema	0	0	0	0	0	0	
Limit ROM* of knee joint	0	0	0	0	1	0	
Joint instability	0	0	0	0	0	0	
ROM, range of motion							

Besides that, VHPCC group also reported other improvement in collagen-containing organs: faster muscle and/or tendon recovery after injury or exercise (77.78%), improve myofascial pain (66.67%), more body flexibility (10%), cellulite reduction (15%) and feeling energetic (50%) after taking VHPCC.

From aforementioned study data, it can be concluded that VHPCC was proved safe and gave far faster and better anti-aging effects than CAV essentially on skin properties, improve sign and symptoms of osteoarthritis of knee joint and has benefit on other collagen-containing organs.

Discussion

To the best of our knowledge from literature reviews, the present study is the first clinical trial demonstrating the efficacy and safety of oral VHPCC on skin properties and knee joint in the same trial, and is the world's most numbers of dermatological testing which consists of 6 skin properties measurement so far.

The clinical study demonstrates that ingestion of VHPCC, which contains a selectively-manufacturing high content of the free-formed Proline, Hydroxyproline and Glycine called "Proline complex", was proved safe and gave much faster and more effective anti-aging effects than CAV essentially on skin properties, improve sign and symptoms of osteoarthritis of knee joint and has benefit on other collagen-containing organs. CAV was also effective but only on skin elasticity and hydration and results were lesser and slower, seen at week 4 and 8 respectively. CAV effects from present study are consistent with many previous studies of collagen peptide that effects were mostly seen in 4 weeks. Many participants from VHPCC group have increase in *Sirt1* gene expression level and VHPCC group overall tend to have increase in *Sirt1* gene expression level but fail to show significant difference. If number of subjects increase, probably significant difference may be seen. The CBC and creatinine blood tests are within normal standard of level. This can assure and answer the consumers' frequently asked problem if taking collagen supplement badly affected the kidney and health or not.

The faster and more effective results of VHPCC than CAV may result from 1) very high content of “proline complex”- a group of amino acids that is specific for collagen synthesis in human body 2) very low average molecular weight – 500 Da, which is pretty lighter than other collagen peptide that has weight range 500-8000 Da 3) very small molecule size – about 2 nanometer, as nanoparticle can be absorbed not only by intracellular but also intercellular way from previous study but CAV rarely have small size in nanometer unit 4) highest amount of collagen peptide permitted by Thai FDA was used, 10 gram per day 5) the purity of VHPCC raw material is more than 95% but the purity of CAV is unknown depending on each factory 6) the purity final product was not diluted by any powder or fiber while manufacturing in OEM. So, consumers get highly pure product.

The quickly significant result may result from mechanism of acute inflammation which takes just a first few days meanwhile normal collagen synthesis process takes 6 weeks. This hypothesis are get along well with the study of Postlethwaite et al. which reported that hydroxyproline-containing peptides resulted chemotactic for fibroblasts and both collagen and collagen derived peptides might function as chemotactic stimuli for fibroblasts in vivo and attract these cells for the repair of damaged tissues. It means that there was preexisting damaged tissue and hydroxyproline-containing peptide or collagen peptide come to repair damaged tissue via acute inflammation process.

From previous studies, mechanisms of action of VHPCC in dermis are 1) act as substrate of collagen fiber 2) Pro, Hyp, Gly act as signal transducer on dermal fibroblast 3) stimulate fibroblasts growth, proliferation, motility, metabolism to repair damaged tissue 4) induce an increase in collagen fibers' density and diameter in the dermis 5) increase hyaluronic acid production 6) activate protection against UVB radiation. Increased number of collagen fibers leads to increased skin elasticity. Hyaluronic acid increases hydration of the extracellular matrix and reduces transepidermal water loss and helps fibroblast proliferation process. Increased in number of collagen fibers, hyaluronic acid create skin smoothness appearance, reduce number of wrinkles, increase skin hydration and reduce transepidermal water loss. Activation against UVB help decreasing mean melanin index. Increase in number of fibroblasts, collagen fiber and extracellular matrix component make dermal thickness thicker and lead to more skin integrity and youth. Moreover, previous studies reported that Pro-Hyp inhibited the loss of chondrocytes and thinning of the articular cartilage layer and increased glycosaminoglycan in the extracellular matrix. By all those mechanisms of action, skin properties, knee joint and other collagen-containing organs can be anti-aged by VHPCC.

Other collagen-containing organs improved simultaneously, so this is to confirm that the orally dietary VHPCC supplement (DERMOFIX®) has benefit on not only skin but also knee joint, bone, ligament, tendon and other collagen-containing organs simultaneously.

Conclusion

Though the present study demonstrates that both VHPCC and CAV are safe and effective supplements for the skin properties improvement but there is a significant difference between conventionally commercially available collagen peptide and a new type of collagen peptide with very high proline complex content. VHPCC demonstrates a much greater and faster improvement in anti-aging effects on all skin properties since the first week and showed improved knee joint degeneration sign and symptoms. Beneficial effects on other collagen-containing organs were reported. DERMOFIX®, a new very high selectively-manufacturing ‘proline complex’-containing-collagen peptide (VHPCC), has proved safe and effective in quickly and greatly reverse unfavorable aging skin properties, knee joint degeneration problems and improve other collagen-containing organs simultaneously. It is suitable for who needs to quickly rejuvenating their skin to reveal beauty from within and keep their health to the optimum sustainably.

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