

Effect of Reducing Pigmentation by Collagen Peptide Intake: A Randomized, Double-Blind, Placebo-Controlled Study

Ai Himeno ^{1*}, Masaya Tsujikami ¹, Seiko Koizumi ¹, Toshiro Watanabe ², Michiya Igase ³

¹ Nitta Gelatin Inc., Osaka, Japan; a-himeno@nitta-gelatin.co.jp, ma-tsujikami@nitta-gelatin.co.jp, se-koizumi@nitta-gelatin.co.jp

² Sonoda Women's University, Hyogo, Japan; watnb-ts@sonoda-u.ac.jp

³ Department of Anti-aging Medicine, Ehime University Graduate School of Medicine, Ehime, Japan; migase@m.ehime-u.ac.jp

*Correspondence: a-himeno@nitta-gelatin.co.jp; Tel.: +81-72-948-8252 (A.H.); Nitta Gelatin Inc. R&D center 2-22, Futamata, Yao-city, Osaka, Japan

Supplementary Material 1. Participant background

		All age				Aged under 55 years			
		Baseline		3 months		Baseline		3 months	
CP group	Number	14		12		14		12	
	Sex (male/female)	13/1		11/1		13/1		11/1	
	Age (years old)	41.4 ± 8.8	*	40.6 ± 9	*	41.4 ± 8.8		40.6 ± 9	
	Oil content	19.6 ± 16.4		13.2 ± 7.7		19.6 ± 16.4		16.6 ± 10.4	
	Water content	61.1 ± 14.7	#	53.5 ± 8.4	#	61.1 ± 14.7	#	53.5 ± 8.4	#
FCP group	Number	15		13		13		11	
	Sex (male/female)	13/2		12/1		11/2		10/1	
	Age (years old)	46.3 ± 8.4		46.8 ± 8.9		44.5 ± 7.7		44.8 ± 8.2	
	Oil content	25.2 ± 15.2		15.5 ± 9.9		24.0 ± 14.3		13.2 ± 7.7	
	Water content	66.1 ± 10.2	#	52.8 ± 16.0	#	67.0 ± 10.5	#	55.7 ± 14.8	#
PL group	Number	15		13		12		10	
	Sex (male/female)	10/5		10/3		8/4		8/2	
	Age (years old)	49.9 ± 8		51.5 ± 7.7		47.3 ± 6.7		48.8 ± 6.6	
	Oil content	15.5 ± 7.8		15.2 ± 14.5		16.4 ± 7.9		18.3 ± 15.2	
	Water content	59.6 ± 8.8	##	47.6 ± 12.5	##	59.5 ± 9.2	#	48.4 ± 13.4	#

Mean ± standard deviation

Intergroup comparison: Tukey test by analysis of variance, * P < 0.05, ** P < 0.01 (vs. PL group)

Intragroup comparison: Wilcoxon signed-rank test, # P < 0.05, ## P < 0.01 (vs. baseline)

CP, collagen peptide; FCP, fermented collagen peptide; PL, placebo

Supplementary Material 2. Improved skin pigmentation and redness by CP or FCP supplementation

CP group		N	Baseline	N	1 month	N	2 months	N	3 months	
Pigmented macules	Number	14	232 ± 75	14	203 ± 56	13	227 ± 58	12	233 ± 92	
	Area (mm ²)	14	18.0 ± 6.8	14	18.4 ± 7.4	13	21.8 ± 7.2	12	21.5 ± 8.3	
Pigmented patches	Lv1	Number	14	64.6 ± 38.1	14	64.8 ± 33.0	13	71.2 ± 38.4	12	65.8 ± 36.1
		Area (mm ²)	14	203.1 ± 114.7	14	174.9 ± 123.4	13	166.5 ± 91.4	12	163.1 ± 111.4
	Lv2	Number	14	13.1 ± 7.9	14	9.1 ± 7.0	13	10.8 ± 7.3	12	11.1 ± 7.3
		Area (mm ²)	14	61.4 ± 63.6	14	28.1 ± 25.2	13	32.2 ± 22.2	12	29.5 ± 21.8
	Lv3	Number	14	4.8 ± 3.9	14	2.1 ± 2.4	13	2.8 ± 2.2	12	2.7 ± 2.0
		Area (mm ²)	14	30.4 ± 47.5	14	4.9 ± 4.9	13	8.2 ± 7.4	12	6.3 ± 5.6
Redness	Lv3	Number	14	3.9 ± 6.3	14	3.2 ± 8.1	13	2.7 ± 3.4	12	1.9 ± 4.5
		Area (mm ²)	14	1.5 ± 2.5	14	0.8 ± 1.6	13	1.1 ± 2.4	12	0.4 ± 1.1
FCP group		N	Baseline	N	1 month	N	2 months	N	3 months	
Pigmented macules	Number	15	20.1 ± 6.4	15	18.2 ± 6.8	15	16.7 ± 5.4	13	18.2 ± 8.6	
	Area (mm ²)	15	17.5 ± 4.8	15	15.1 ± 5.4	15	15.2 ± 5.3	13	15.0 ± 5.8	
Pigmented patches	Lv1	Number	15	48.6 ± 15.8	15	45.3 ± 18.4	15	42.1 ± 12.9	13	45.5 ± 16.2
		Area (mm ²)	15	148.6 ± 92.1	15	134.3 ± 101.1	15	111.5 ± 65.1	13	104.8 ± 70.6
	Lv2	Number	15	8.5 ± 6.7	15	6.7 ± 6.2	15	5.8 ± 4.9	13	5.9 ± 3.7
		Area (mm ²)	15	26.3 ± 28.9	15	27.7 ± 51.5	15	17.3 ± 20.6	13	20.0 ± 25.1
	Lv3	Number	15	3.5 ± 3.8	15	2.3 ± 2.8	15	1.3 ± 2.0	13	2.5 ± 2.4
		Area (mm ²)	15	9.2 ± 10.8	15	9.6 ± 19.2	15	4.8 ± 7.8	13	7.7 ± 8.5
Redness	Lv3	Number	15	0.9 ± 1.3	15	2.0 ± 2.7	15	2.1 ± 2.7	13	0.8 ± 1.2
		Area (mm ²)	15	0.7 ± 1.1	15	0.2 ± 0.4	15	1.9 ± 3.9	13	0.9 ± 2.0
PL group		N	Baseline	N	1 month	N	2 months	N	3 months	
Pigmented macules	Number	15	20.0 ± 9.4	15	20.2 ± 10.1	15	17.6 ± 8.7	13	21.8 ± 8.6	
	Area (mm ²)	15	16.6 ± 7.7	15	15.7 ± 6.7	15	15.3 ± 5.8	13	15.7 ± 7.5	
Pigmented patches	Lv1	Number	15	51.7 ± 24.6	15	60.8 ± 30.7	15	57.7 ± 27.6	13	59.3 ± 20.9
		Area (mm ²)	15	135.6 ± 89.9	15	157.0 ± 107.2	15	149.7 ± 128.8	13	229.8 ± 282.7
	Lv2	Number	15	9.9 ± 5.6	15	9.1 ± 6.7	15	8.3 ± 6.1	13	11.0 ± 7.8
		Area (mm ²)	15	34.9 ± 33.9	15	29.9 ± 28.7	15	35.9 ± 45.0	13	85.4 ± 185.7
	Lv3	Number	15	4.3 ± 2.3	15	2.9 ± 2.2	15	2.7 ± 1.8	13	5.2 ± 4.5
		Area (mm ²)	15	10.0 ± 9.5	15	5.7 ± 4.7	15	7.9 ± 9.8	13	30.4 ± 75.4
Redness	Lv3	Number	15	2.7 ± 3.7	15	3.8 ± 7.3	15	4.7 ± 7.1	13	2.4 ± 3.3
		Area (mm ²)	15	1.9 ± 3.5	15	0.7 ± 2.2	15	1.2 ± 2.4	13	1.8 ± 3.7

Mean \pm standard deviation

Intergroup comparison: Tukey test by analysis of variance, * $P < 0.05$, ** $P < 0.01$ (vs. PL group)

Intragroup comparison: Wilcoxon signed-rank test, # $P < 0.05$, ## $P < 0.01$ (vs. baseline)

CP, collagen peptide; FCP, fermented collagen peptide; PL, placebo

Supplementary Material 3. Improved skin pigmentation and redness by CP or FCP supplementation (below 55 years of age)

CP under 55 years old		N	Baseline	N	1 month	N	2 months	N	3 months	
Pigmented macules	Number	14	232 ± 7.5	14	203 ± 5.6	13	227 ± 5.8	12	233 ± 9.2	
	Area (mm ²)	14	18.0 ± 6.8	14	18.4 ± 7.4	13	21.8 ± 7.2 *	12	21.5 ± 8.3	
Pigmented patches	Lv1	Number	14	64.6 ± 38.1	14	64.8 ± 33.0	13	71.2 ± 38.4	12	65.8 ± 36.1
		Area (mm ²)	14	203.1 ± 114.7	14	174.9 ± 123.4	13	166.5 ± 91.4	12	163.1 ± 111.4
	Lv2	Number	14	13.1 ± 7.9	14	9.1 ± 7.0 #	13	10.8 ± 7.3	12	11.1 ± 7.3
		Area (mm ²)	14	61.4 ± 63.6	14	28.1 ± 25.2	13	32.2 ± 22.2	12	29.5 ± 21.8
	Lv3	Number	14	4.8 ± 3.9	14	2.1 ± 2.4 ##	13	2.8 ± 2.2	12	2.7 ± 2.0 #
		Area (mm ²)	14	30.4 ± 47.5	14	4.9 ± 4.9 ##	13	8.2 ± 7.4 #	12	6.3 ± 5.6 ##
Redness	Lv3	Number	14	3.9 ± 6.3	14	3.2 ± 8.1	13	2.7 ± 3.4	12	1.9 ± 4.5 #
		Area (mm ²)	14	1.5 ± 2.5	14	0.8 ± 1.6	13	1.1 ± 2.4	12	0.4 ± 1.1
FCP under 55 years old		N	Baseline	N	1 month	N	2 months	N	3 months	
Pigmented macules	Number	13	20.5 ± 6.7	13	17.8 ± 6.9	13	15.8 ± 5.0 ##	11	17.3 ± 8.0	
	Area (mm ²)	13	17.0 ± 4.6	13	13.8 ± 4.0 ##	13	14.8 ± 5.4	11	13.4 ± 4.7 ##	
Pigmented patches	Lv1	Number	13	45.6 ± 14.8	13	44.4 ± 19.6	13	39.1 ± 10.9 #	11	41.5 ± 14.1 ##
		Area (mm ²)	13	129.0 ± 80.5	13	106.2 ± 57.9	13	91.2 ± 36.6	11	82.3 ± 29.6 #
	Lv2	Number	13	7.2 ± 6.3	13	4.8 ± 3.9	13	4.6 ± 4.0	11	5.1 ± 3.4
		Area (mm ²)	13	17.2 ± 16.5	13	11.8 ± 13.7	13	10.5 ± 11.8	11	11.3 ± 7.9
	Lv3	Number	13	3.1 ± 3.9	13	1.6 ± 2.2	13	1.0 ± 1.8 #	11	2.4 ± 2.6
		Area (mm ²)	13	6.9 ± 9.8	13	3.8 ± 6.8	13	2.8 ± 6.4 #	11	5.7 ± 7.5
Redness	Lv3	Number	13	0.9 ± 1.4	13	2.2 ± 2.8	13	2.2 ± 2.9	11	0.6 ± 0.9
		Area (mm ²)	13	0.8 ± 1.1	13	0.2 ± 0.4	13	2.1 ± 4.2	11	1.1 ± 2.1
Placebo under 55 years old		N	Baseline	N	1 month	N	2 months	N	3 months	
Pigmented macules	Number	12	18.0 ± 8.5	12	18.3 ± 9.7	12	16.5 ± 9.2	10	20.8 ± 7.7	
	Area (mm ²)	12	15.6 ± 8.2	12	14.5 ± 6.3	12	14.8 ± 5.5	10	14.4 ± 5.5	
Pigmented patches	Lv1	Number	12	47.8 ± 25.9	12	55.4 ± 31.3	12	52.5 ± 26.7	10	57.1 ± 21.0
		Area (mm ²)	12	119.8 ± 79.2	12	137.1 ± 106.7	12	130.3 ± 133.9	10	217.0 ± 314.4
	Lv2	Number	12	8.8 ± 4.1	12	7.5 ± 5.8	12	6.8 ± 5.5	10	9.7 ± 7.6
		Area (mm ²)	12	34.1 ± 36.4	12	25.5 ± 30.2	12	30.7 ± 48.7	10	95.9 ± 210.6
	Lv3	Number	12	4.5 ± 2.4	12	2.5 ± 2.3	12	2.3 ± 1.6	10	5.4 ± 5.1
		Area (mm ²)	12	11.1 ± 10.3	12	4.8 ± 4.8	12	7.2 ± 10.3	10	37.0 ± 84.9
Redness	Lv3	Number	12	3.3 ± 3.9	12	4.6 ± 8.0	12	5.8 ± 7.6	10	2.9 ± 3.6
		Area (mm ²)	12	2.3 ± 3.8	12	0.9 ± 2.5	12	1.5 ± 2.6	10	2.3 ± 4.1

Mean \pm standard deviation

Intergroup comparison: Tukey test by analysis of variance, * $P < 0.05$, ** $P < 0.01$ (vs. PL group)

Intragroup comparison: Wilcoxon signed-ranked test, # $P < 0.05$, ## $P < 0.01$ (vs. baseline)

CP, collagen peptide; FCP, fermented collagen peptide; PL, placebo

Supplementary Material 4. Di- and tri-peptide compositions of CP and FCP

	CP	FCP
Pro-Hyp	8	14
Hyp-Gly	3,447	2,942
Ala-Hyp	73	0
Ala-Hyp-Gly	436	306
Glu-Hyp	82	0
Glu-Hyp-Gly	4	3
Phe-Hyp	203	16
Gly-Ala	3,867	2,916
Gly-Pro	2,379	3,431
Gly-Pro-Hyp	36	4
Leu-Pro/Ile-Pro	81	1,682

Units: ppm