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CHANGES IN CUTANEOUS AGEING PARAMETERS FOLLOWING 6 WEEKS SUPPLEMENATION WITH HYDROLYSED COLLAGEN

Dr Riekie Smit; University of Pretoria. Department of Pharmacology Promoter: Prof JR Snyman.

ABSTRACT

Objective: A 6 week trial evaluating the efficacy of Hydrolyzed Collagen supplementation on cutaneous ageing.

Study design: A randomised clinical trial of 100 women between the ages of 50 and 65 years of age receiving either the hydrolyzed collagen supplement for 6 weeks or placebo tablets for 6 weeks. The women had baseline and 6 weeks after supplementation cutaneous ageing parameters analysis with a standardised device testing skin moisture, wrinkle size, keratin count, porosity and sebum.

Results: Statistically significant benefits were shown for skin moisture, porosity and wrinkles. The results demonstrated that there was an average increase in skin moisture in the hydrolyzed collagen group compared to the placebo group. There was a statistically significant difference (P-value >0.05) between group comparisons in skin moisture. Decrease in wrinkle width after treatment with hydrolyzed collagen (p value < 0.0001) compared to no difference in the placebo group. There was a major decrease in the change in the average difference in pore size in the hydrolyzed collagen group compared to the placebo group. The between-group comparison was deemed statistically significant (P<0.05).

Conclusion: In this double blind placebo controlled study, 6 weeks of hydrolyzed collagen supplementation (Purelogicol®) led to an improvement in skin wrinkle scores, skin moisture content as well as skin porosity compared to the placebo group. Multiple previous studies have shown the benefits of using hydrolyzed collagen for the prevention of osteo-degenerative diseases. Now, this study indicates the potential benefit of using hydrolyzed collagen for the prevention of skin aging in female patients. This phenomenon warrants further studies.

INTRODUCTION

Skin rejuvenation and the anti-ageing market is part of the fastest growing industries in the past few years. Unfortunately many supplementation and products come on to the market making medical claims and promising years off the face or body without any evidence.

The medical field has also embraced this industry and many physicians have incorporated anti-ageing or aesthetic medicine in their practices. Products, supplements or devices making medical claims without scientific proof in the form of double blind placebo controlled studies should not be embraced by medical personnel. The industry should also be putting more pressure on companies to provide proper safety data and research on the indications and benefits of products.

Collagen supplementation does not only fall into the aesthetic and anti-ageing industry, but also provides benefits for certain arthritic and dermatological conditions. Therefore companies manufacturing collagen supplementation should provide sufficient data to convince physicians to use it for pathological conditions as well as for aesthetic indications.

This study evaluated the benefits of Purelogicol® a hydrolyzed collagen supplementation, in the aesthetic field. This collagen supplement has been researched for joint and skin ageing, osteoarthritis and rheumatism. This study specifically evaluated the effect that Purelogicol® supplementation has on the skin of the face and the hand. These areas are more prone to aging due to increased time of sun exposure in almost all climatic conditions.
A unique collagen formulation, rich in essential amino acids and proven to be readily absorbed into the body for optimum results.

Various clinical trials have proven the effects of daily ingestion of hydrolysed collagen

- 43.3% DECREASE IN WRINKLE WIDTH
- 25.8% INCREASE IN SKIN MOISTURE
- 34.5% DECREASE IN SKIN PORES
- STRENGTHENS HAIR DIAMETER & NAIL QUALITY
- PREVENTIVE TREATMENT FOR OSTEO-DEGENERATIVE DISEASES

The effects of Purelogical® collagen supplement

Collagen is protein that accounts for up to 33% of the protein in the human body and makes up to 75% of the skin. Collagen is one of the major factors contributing to a plump, smooth appearance of young healthy skin and breaks down at a rate of 1.5% every year after the age of 25.

Purelogical® uses a pharmaceutical grade collagen hydrolysate with a low molecular weight which facilitates absorption into the body. The recommended treatment is 3 to 6 months.

Results:

<table>
<thead>
<tr>
<th>Skin Moisture</th>
<th>Skin Porosity</th>
<th>Skin Wrinkles</th>
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</thead>
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<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
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</tbody>
</table>

Skin analysis following 6 weeks of supplementation with 2.4 g of hydrolysed collagen (Purelogical®).
BACKGROUND ON COLLAGEN SUPPLEMENTATION

Collagen is a protein that accounts for up to 33% of the protein in the human body. Collagen is the primary structural protein found in the connective tissues in the body (including the skin, bones, cartilage, tendons, and ligaments). Collagen makes up 75% of the skin and is one of the major factors contributing to a plump, smooth appearance of young healthy skin. Collagen's major role in the skin is the support system in the extra cellular matrix surrounding the cells. During skin ageing, collagen breakdown and reduced collagen production, contributes to wrinkle formation and decreased skin elasticity.

Fibroblasts produce collagen which is then integrated into the extra-cellular matrix where its major function is tensile strength and support. Hydrolyzed collagen is simply a modified form of collagen that has been enzymatically broken down to increase the protein's digestibility and increase the amino acids' absorbability. Hydrolyzed collagen protein (HCP) is often used as a general protein source in body building products because it is a relatively inexpensive protein source.

HCP has most recently been used to promote joint health, nourish cartilage and bones and help athletes recover from exercise and sports-related injuries. Hydrolyzed collagen protein (HCP) is also known as purified gelatin or collagen hydrolysate.

Hydrolyzed collagen consists of water-soluble peptides which are rich sources of the amino acids glycine, L-proline and L-hydroxyproline. Nutritional supplements containing hydrolyzed collagen are typically marketed for osteoporosis, osteoarthritis, rheumatoid arthritis, weight loss, and to assist in recovery from exercise and sports-related injuries, although scientific data in these areas is sparse.

Collagen capsules contain collagen - a protein. When ingesting protein, the metabolic process of digestion breaks it down to amino-acids, used to build and maintain various tissues. Thus, when taking a collagen supplement, the body does not move the collagen as it is to the skin to replace collagen there. New collagen has to be built by the fibroblasts in the skin. It makes sense that ingesting collagen may provide essential building blocks for the production of new collagen without doing any harm.

Collagen supplements that contain hydrolyzed collagen claims to also be directly integrated into the extra-cellular matrix and not only stimulate the fibroblast to produce new collagen. This is contrary to normal physiological reasoning as stated above.

LITERATURE REVIEW SUMMARY

In summary, hydrolyzed collagen intake seems to manipulate fibroblast production of collagen and possible supplementation of the extracellular matrix. Effects mimic benefits of proper fibroblast functioning, supporting the postulated mechanisms of action as shown in table below.

Postulated mechanisms of hydrolyzed collagen supplementation on altering cutaneous ageing

1. Improved fibroblast production of type II collagen
2. Improved quality fibroblast and production of hyaluronic acid
3. Enhanced collagen quantity and quality in the extra cellular matrix.
4. Anti-inflammatory effect and anti-oxidant effect

Table 1

STUDY OBJECTIVE

The objective of this study was to examine the changes in cutaneous ageing parameters after 6 weeks of supplementation with hydrolyzed collagen and compare the results with a placebo control group.

STUDY METHODS

Study Design:

The analysis of the skin ageing parameters following 6 weeks supplementation was a double blind, randomised, placebo controlled study. Supplementation with either 2.4g per day of hydrolyzed collagen or placebo was given to the subjects with pre- and post supplementation testing of cutaneous aging signs.

The supplement was in the form of 2.4g of hydrolyzed collagen daily. Supplied by Purelogical (Pty) Ltd. Unit 15, Ladywell Business Centre, 94 Duke Street, Glasgow. United Kingdom. G4 0UW.

This contains g/100g protein Amino Acids:

<table>
<thead>
<tr>
<th>Amino Acid</th>
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<tr>
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</tr>
<tr>
<td>Arginine</td>
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<td>Hydroxylysine</td>
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<tr>
<td>Isoceusine</td>
<td>1.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.2</td>
</tr>
</tbody>
</table>
Methionine 0.9
Phenylalanine 2.2
Proline 13.4
Serine 3.2
Threonine 1.6
Tyrosine 1.6
Valine 2.4

Cutaneous tests were performed with the Antiageing SD Integrated Dermatological Analysis System®. The system evaluates moisture, elasticity, keratine, sebum, porosity, wrinkles, spots and skin health and balance.

Somatologist, Mrs Elmarie van Biljon, evaluated the females by doing the following on the Anti-ageing SD Cutaneous Analysis:
- Moisture;
- Elasticity;
- Keratine;
- Sebum;
- Porosity;
- Wrinkles;
- Spots;
- Skin Health and balance.

Subjects:
100 post-menopausal females age 50 - 65 years were involved.

The subjects had stabilised and controlled treatment of conditions.

Exclusion criteria included Diabetes Mellitus, Connective tissue disorders, Collagen related illnesses, skin conditions such as psoriasis, eczema, etc; Systemic or topical corticosteroids.

Study objectives:
Primary objective:
The primary objective was to assess the change in skin ageing parameters with collagen supplementation.

Secondary objective:
The secondary objective is to assess patient satisfaction and the safety of the product in the subjects.

Hypothesis:
Nil-hypothesis: There will be no improvement in the skin parameters of post-menopausal females after 6 weeks hydrolyzed collagen supplementation.

Alternative-hypothesis: There will be improvement in the skin parameters of post-menopausal females after 6 weeks hydrolyzed collagen supplementation.

Data analysis:
Data analysis was be done by the statistician of the University of Pretoria.

RESULTS

Patient characteristics and demographics:
100 Women were enrolled in the study. 47 Completed the hydrolyzed collagen supplementation for 6 weeks (mean age 56.2) and 45 completed the 6 weeks of placebo trial (mean age 55.9). Skin analysis was performed before commencing supplementation and 6 weeks after finishing supplementation. The mean age of the subjects was years and 100% were Caucasian. There were no statistically significant differences in patient demographics (age, race, physical examination, or facial wrinkle scale score) between the treatment groups at baseline. Of the eligible subjects, 65% to 95% had baseline wrinkle scores rated as severe by skin analysis. A total of 92 (92%) completed the double-blind trial; 2 withdrew consent, and 6 were lost to follow-up.

SKIN AGEING ANALYSIS

The primary measures were wrinkles and hydration with the hypothesis that the hydrolyzed collagen supplementation group would show improvement in the measures compared to the placebo group. Baseline comparisons were conducted with the Anti-ageing SD complexion analysis system that evaluated the following: age, moisture, elasticity, keratine, sebum, porosity, wrinkles, spots, skin health and balance.

The paired-difference t test was used to test for a significant pre- to post supplementation change for each sample. p Values >0.05 were deemed statistically significant for all between-group and within-group comparisons. Statistical analyses were performed on an intent-to-treat basis. Last-observation carried-forward data were used in cases in which the middle point data were not available. If a subject had exited the study, it was assumed that she had a zero change from baseline in all following time points.

Changes in skin moisture:

Figure 1 a & b: Comparison between the percentage changes in skin moisture of Group A, who received the hydrolyzed collagen, compared to Group B, who received the placebo.
Figure 1 a & b represents the percentage change in skin moisture of Group A and B after 6 weeks of supplementation of either hydrolyzed collagen or placebo. There was a slight increase in the percentage skin moisture in the group that received the hydrolyzed collagen, compared to a slight decrease in the placebo group although there was no significant difference between baseline measurement (pre-test) and measurements taken after 6 weeks. P-value of both Group A and B < 0.05.

Figure 2: Comparison between the average differences in changes in skin moisture of the group that received the hydrolyzed collagen compared to the placebo group.

Figure 2 represents the mean average of the participants after treatment minus the mean average of the participants before treatment. For the hydrolyzed collagen group the average difference increased indicating that the percentage change in skin moisture after 6 weeks is increased compared to the percentage change in skin moisture before 6 weeks of treatment. Thus there was an average increase in skin moisture in the hydrolyzed collagen group compared to the placebo group. There is a statistically significant difference (P-value >0.05) between group comparisons in skin moisture.

Figure 3 a & b: Comparison between the percentage changes in skin moisture T-zone of Group A, who received the hydrolyzed collagen, compared to Group B, who received the placebo.

Figure 4: Comparison between the average differences in changes in skin moisture T-zone of the group that received the hydrolyzed collagen compared to the placebo group.
Figure 4 represents the mean average of the participants after treatment minus the mean average of the participants before treatment.

For the hydrolyzed collagen group the average difference increased dramatically indicating that the percentage change in skin moisture T-zone after 6 weeks is increased compared to the percentage change in skin moisture before 6 weeks of treatment. Thus there was an average increase in skin moisture T-zone in the hydrolyzed collagen group compared to a decrease in the placebo group.

There is a statistically significant difference (P-value >0.05) between the average percentage difference change in skin moisture between the two groups.

Figure 5 a & b: Comparison between the percentage changes in skin moisture U-zone of Group A, who received the hydrolyzed collagen, compared to Group B, who received the placebo

Changes in skin keratin:

Figure 6 represents the average of the participants after treatment minus the mean average of the participants before treatment. For the hydrolyzed collagen group the average difference increased dramatically indicating that the percentage change in skin moisture U-zone after 6 weeks is increased compared to the percentage change in skin moisture before 6 weeks of treatment. Thus there was an average increase in skin moisture U-zone in the hydrolyzed collagen group compared to the placebo group. There is a statistically significant difference (P-value >0.05) in the between group comparison.

Figure 6: Comparison between the average differences in changes in skin moisture U-zone of the group that received the hydrolyzed collagen compared to the placebo group

Figure 7 a & b: Comparison between the percentage changes in skin over-keratin of Group A, who received the hydrolyzed collagen, compared to Group B, who received the placebo

Figure 7 a & b: Comparison between the percentage change in skin over-keratin of Group A and B after 6 weeks of supplementation of either hydrolyzed collagen or placebo. There was a significant difference between baseline measurement (pre-test) and measurements taken after 6 weeks. P-value of both Group A and B > 0.05.
Changes in skin sebum:

In figure 16a there is an increase in the change in percentage in the sebum T-zone of the hydrolyzed collagen treated group compared to a decrease in the percentage change in the placebo treated group in figure 16b. The within-group comparison for the hydrolyzed collagen group was statistically significant (P>0.05) compared to the placebo treated within-group comparison that was not statistically significant (P<0.05).

Figure 8 represents the mean average of the participants after treatment minus the mean average of the participants before treatment. For the hydrolyzed collagen group there was only a slight increase in the average difference in change in skin over-keratin after 6 weeks is compared to the percentage change in skin over-keratin before 6 weeks of treatment. Compared to the hydrolyzed collagen group the average difference in skin over-keratin increased dramatically. The between group comparison is statistically insignificant (P-value <0.05).

Figure 17 represents the mean average of the participants after treatment minus the mean average of the participants before treatment. For the hydrolyzed collagen group the average difference increased indicating that the percentage change in sebum T-zone after 6 weeks is increased compared to the percentage change in sebum T-zone before 6 weeks of treatment.
Thus there was an average increase in sebum T-zone in the hydrolyzed collagen group compared to the placebo group, which in fact decreased. There is a statistically significant difference (P-value >0.05) in the between group comparison.

**Figure 18 a & b:** Comparison between the changes in sebum U-zone of Group A, who received the hydrolyzed collagen, compared to Group B, who received the placebo.

**Figure 19** represents the mean average of the participants after treatment minus the mean average of the participants before treatment. For the hydrolyzed collagen group the average difference increased indicating that the percentage change in sebum U-zone after 6 weeks is increased, compared to the percentage change in sebum T-zone before 6 weeks of treatment. But the increase was not a marked as the increased compared to the placebo treated group. There is no statistically significant difference (P-value <0.05) in the between group comparison.

**Changes in skin porosity:**

**Figure 9 a & b:** Comparison between the changes in skin porosity of Group A, who received the hydrolyzed collagen, compared to Group B, who received the placebo.

**Figure 19:** between the average differences in changes in the percentage sebum U-zone of the group that received the hydrolyzed collagen compared to the placebo group.

**Figure 9 a & b** represents the percentage change in pore size (μm2) of Group A and B before and after 6 weeks of supplementation of either hydrolyzed collagen or placebo. Although there was a larger decrease in the pore size on the experimental group compared to the placebo group, the with-in group comparison insignificant. P-value of both Group A and B < 0.05.

There is a major decrease in the change in the average difference in pore size in the hydrolyzed collagen group compared to the placebo group. The between-group comparison is deemed statistically significant (P<0.05).
**Figure 10:** Comparison between the average differences in changes in skin pore size ($\mu m^2$) of the group that received the hydrolyzed collagen compared to the placebo group.

**Group B:**

![Graph showing comparison between Group B: Placebo](image)

**Changes in skin wrinkles:**

**Group A:**

![Graph showing changes in skin wrinkles](image)

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<td>Column B</td>
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Table 3: No significant difference in the wrinkle width before and after treatment with the placebo group

**C: Comparison between Group A and Group B**

Decrease in the wrinkle width after treatment with the hydrolyzed collagen compared to no difference in the placebo group. There is a significant difference between the 2 groups.

![Graph showing comparison between Group A and Group B](image)

Table 2: A significant decrease in the mean wrinkle width before and after treatment with hydrolyzed collagen.
Changes in skin health:

Figure 14 a & b: Comparison between the changes in skin health of Group A, who received the hydrolyzed collagen, compared to Group B, who received the placebo

Group A had a higher percentage of skin health before treatment compared to the placebo group. The within group comparisons were deemed statistically insignificant (P=0.05)

Figure 15 represents the mean average of the participants after treatment minus the mean average of the participants before treatment. The between group comparison indicates that the changes in the difference the skin health in the hydrolyzed collagen group increased compared to the placebo group that decreased in the difference in change in skin health. The difference between the hydrolyzed collagen and placebo group is significant (P>0.05)

Changes in skin balance:

Figure 13 a & b: Comparison between the changes in skin balance of Group A, who received the hydrolyzed collagen, compared to Group B, who received the placebo

Figure 13 a & b represents the change in skin balance before treatment compared to after treatment. In both figure a and b, there is a decrease in the change in skin balance after treatment, although the within group comparison for the group that received the hydrolyzed collagen was deemed statistically significant (P-value >0.05), compared to the placebo group.

For all within group comparisons, the Wilcoxon-rank sum test was used.
For all between group comparisons, the Wilcoxon-Mann-Whitney test was used.
Confidence-level used was 95%.
Data was analyzed using Graphpad Prism demo version 4.0.
CLINICAL STUDY

DISCUSSION:

In this study supplementation with hydrolyzed collagen showed statistical improvement in the skin ageing parameters. In particular the wrinkle scores and moisture content improved with the collagen group compared to the placebo group.

Hydrolyzed collagen intake seems to manipulate fibroblast production of collagen and possible supplementation of the extracellular matrix. Effects mimic benefits of proper fibroblast functioning, supporting the postulated mechanisms of action.

Postulated mechanisms of hydrolyzed collagen supplementation on altering cutaneous ageing include improved fibroblast production of type II collagen, improved quality fibroblast and production of hyaluronic acid, enhanced collagen quantity and quality in the extra cellular matrix and an anti-inflammatory effect and anti-oxidant effect.

Other reports have concluded the benefits of hydrolyzed collagen supplementation in the health and appearance of the hair and nails, cartilage and skeletal structures of the human body. This study now clearly demonstrates the ability of hydrolyzed collagen supplementation to alter the cutaneous ageing parameters in ageing females. The improvement of the moisture total and the wrinkle scores in the skin of the face could be attributed to an improved fibroblast producing glycol-amino glycans. This supports the postulated theories that hydrolyzed collagen supplementation improves fibroblast production of type II collagen, improved quality fibroblast and improved production of hyaluronic acid and collagen quantity and quality.

CONCLUSION

In this double blind placebo controlled study, 6 weeks of hydrolyzed collagen supplementation (Purelogico®) led to an improvement in skin wrinkle scores, skin porosity and skin moisture content compared to the placebo group. Multiple previous studies have shown the benefits of using hydrolyzed collagen for the prevention of osteo-degenerative diseases. Now, this study indicates the potential benefit of using hydrolyzed collagen for the prevention of skin ageing in female patients. This phenomenon warrants further studies.

REFERENCES

1. Merck UK Calbiochem: Cytoskeletal, 2006
28. Monash; Women with silicone implants have elevated levels of autoantibodies to collagen, in a manner highly similar to women with lupus and rheumatoid arthritis. Current Topics in Microbiological Immunology 210 [1996], 307-316.
35. Nagler-Anderson C; Suppression of Type II Collagen-Induced Arthritis by Intragastric Administration of Soluble Type II Collagen. PNAS,1986; 83(19)7443-7446.