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ORIGINAL RESEARCH

Efficacy and Safety of Topical or Oral Hydrolyzed Collagen in Women with Dermatoporosis: A Randomized, Double-Blind, Factorial Design Study

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ABSTRACT

Background: Dermatoporosis defines the progressive chronic cutaneous insufficiency syndrome. Stage I is characterized by cutaneous atrophy, senile purpura, and stellate pseudoscars.
Objective: To assess clinical, histologic, quality of life, and biophysical effects of oral and/or topical hydrolyzed collagen (HC) on forearm skin of postmenopausal women with Dermatoporosis stage I.
Methods: Double-blind randomized placebo-controlled factorial design study. Two groups of menopausal women with stage I dermatoporosis on forearms were randomized to oral HC 5 g/day or matching placebo, and also to topical serum 2.5% HC or matching placebo once a day, for 6 months.
Results: A total of 56 women, age range 60–93 years (mean 69.5 ± 7.3 years) were included. Comparing data from baseline and after 6 months, no significant difference was observed for each intervention nor their comparison, for all efficacy parameters: clinical and quality of life scores, dermal elasticity, thickness and echogenicity, and histologic and immunohistochemical markers ($p > 0.1$).
Limitations: Larger studies to confirm our findings are warranted.
Conclusions: In menopausal women with stage I dermatoporosis, oral or topical collagen peptides used alone or in combination do not have benefits on forearm skin after 6 months of intervention, and therefore should not be used routinely in this population.

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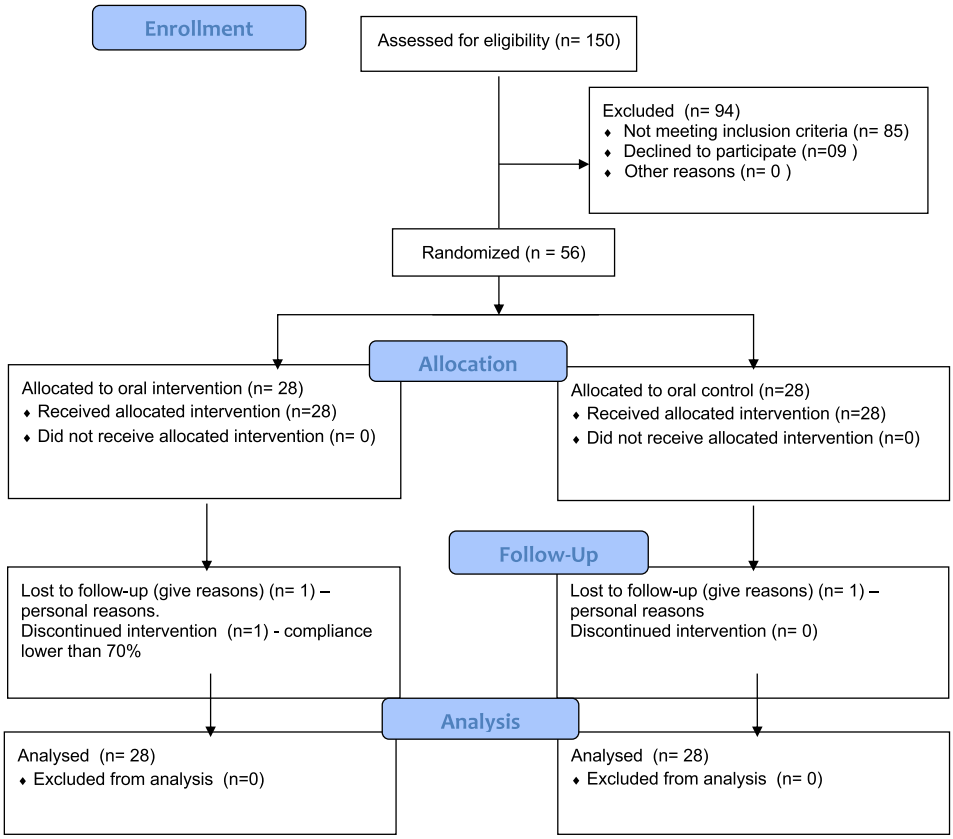
Graphical Abstract: CONSORT flow chart



Keyword: Dermatoporosis; Hydrolyzed collagen; Cutaneous atrophy; Senile purpura; Collagen peptides; Photoaging

Key Summary Points

Why carry out this study?

Dermatoporosis defines the chronic cutaneous insufficiency syndrome.

To date, there is no proven oral or topical treatment for dermatoporosis.

The aim of the study was to assess clinical, histologic, quality of life, and biophysical effects of oral and/or topical hydrolyzed collagen (HC) on forearm skin of postmenopausal women with dermatoporosis.

What was learned from the study?

In postmenopausal women with stage I dermatoporosis, oral or topical collagen peptides used alone or in combination have no benefits on forearm skin after 6 months of intervention.

The use of oral supplements is growing worldwide, and besides the lack of robust evidence of benefit, its use might cause adverse events and have a financial impact on patients' lives.

The negative result of this study might contribute to sparing patients from ineffective treatments.

INTRODUCTION

Skin aging was considered for years an aesthetic concern but with the increase in life expectancy, the functional impact of aging has been emphasized [1, 2]

Bateman's purpura was described in 1836 as a marker of photoaging and is characterized by hemorrhagic areas, purpuric lesions, confluent petechiae or ecchymoses, stellate scars, and thinning skin [3]. The conception of dermatoporosis (DP) includes functional consequences of skin aging and the potential risks associated with skin atrophy and fragility [4]. The term DP was proposed by Saurat in 2007 and defines the chronic skin failure syndrome, which resembles osteoporosis, such as structural weakness due to aging, the occurrence of complications, and the need for preventive measures [2].

Clinical manifestations of DP include morphological alterations (cutaneous atrophy, senile purpura, and stellate pseudoscars) and functional consequences of skin fragility resulting from minimal trauma (lacerations and dissecting hematoma), which compromises the quality of life of these patients (Fig. 1).

In vivo high frequency ultrasound (20 MHz), and reflectance confocal microscopy are useful tools to evaluate the degrees of DP, instead of skin biopsy, in clinical practice [5].

Advanced age is the main risk factor for DP and patients older than 85 years have a more than twofold increased risk versus younger patients [5]. A cross-sectional study conducted in France revealed a prevalence of DP in 32% of patients older than 60 years [6], and Saurat el al. [4] estimated the prevalence at 37.5% in patients aged 65 years and older, reaching up to 51.9% of the population aged over 80 years.

Other risk factors include chronic actinic damage or photoaging, genetic susceptibility or intrinsic aging, and chronic use of topical or systemic corticosteroids [7]. Chronic renal failure is also a relevant factor and increases the risk of DP by up to five times, regardless of age [6]. Other factors, such as diabetes, anticoagulant

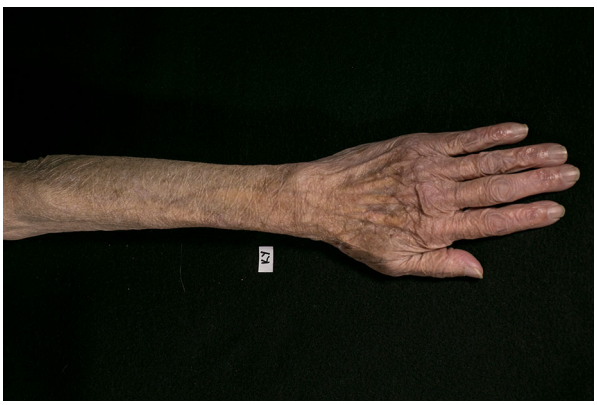


Fig. 1 General aspect of forearms skin and lesions characteristic of dermatoporosis

use, and solar exposure have been associated with DP and cutaneous atrophy [4]. In regard to diet, higher intake of carbohydrates is capable of damaging the skin structure through nonenzymatic glycation, the covalent attachment of sugar to a protein with production of advanced glycation end products (AGEs) [8].

Currently, there are no specific measures adopted in dermatological practice that resulted as highly efficient to prevent or revert DP [2]. The ideal intervention should be well tolerated, inexpensive, and with few adverse events. There is no robust evidence of the benefit of oral treatment for DP. In postmenopausal women, some studies have shown an increase in skin thickness after hormone replacement therapy [9–12]. Clinical trials have shown benefits of oral hydrolyzed collagen (HC) in skin aging, such as improved elasticity, hydration, density of the dermis, and collagen content [13–18].

Hydroxyproline is a collagen-specific amino acid, and the main peptide found in plasma after ingestion of HC is proline-hydroxyproline, which functions as a trigger for collagen synthesis and extracellular matrix reorganization. The mechanism of action of HC is a chemotactic stimulus for fibroblasts with attraction of cells to repair tissue damage [14].

Oral treatments for skin aging control, such as oral isotretinoin, hyaluronan and "senolytics" or mTOR inhibitors (metformin, rapamicin) have been discussed; despite that, the studies are not conclusive. [19–22]

To date, there are no studies concerning topical HC formulations. Topical hyaluronic acid creams (50–400 kDa) improved skin atrophy [23], and the combination of hyaluronic acid and retinaldehyde also reduced senile purpura [24–27]. A randomized comparative trial between tretinoin cream 0.05% and oral isotretinoin showed increase in epidermal thickness, reduction of elastosis, increase in dermal collagen I, and decreased protein p53 expression, with no difference between treatments. [28]. Vitamin C 5% cream improved elasticity and skin thickness in elderly patients with Bateman's purpura [03].

To date, there is no proven oral or topical treatment for DP. Retinoids and glycolic acid are useful topical treatment to improve skin atrophy and fragility [28].

The aim of this study was to assess the efficacy and safety of oral and/or topical HC on clinical, quality of life, histological, immunohistochemical, viscoelastic, and ultrasound parameters.

IRB approval status: Reviewed and approved by UNiFESP IRB (848/2015) – approval # 1.282.133. Standardized photographs were taken by Lais RS Guadanhim.Dr Fernanda Caramella participated in histologic and immunohistochemistry evaluation. Independent photographic evaluation was performed by Drs Gisele Jacobino Nunes, Juliana Mossini Nicoliello. This studied reviewed and approved by UNIFESP. IRB (848/2015) – approval # 1.282.133 and was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. All subjects provided informed consent to participate in the study.

METHODS

This was a randomized, double-blind, placebo-controlled, parallel, and factorial study of therapeutic intervention to assess the efficacy and safety of topical and/or oral HC in postmenopausal women with DP.

The study population included eligible women aged over 60 years with mild DP (stage I) of the forearms. Exclusion criteria were procedures, topic or oral treatments for photoaging of the forearms in the past 3 months, patients with secondary DP or with causes for its aggravation (smoking, chronic renal failure, diabetes, chronic corticosteroid use, use of anticoagulant medication, even acetylsalicylic acid), solid organ transplantation, presence of photodermatosis, inflammatory or infectious skin disease in the forearms, chemotherapy, and immunosuppression and hormone replacement therapy. The study was conducted in the outpatient clinic of a public hospital in São Paulo, Brazil, from June 2016 to April 2018.

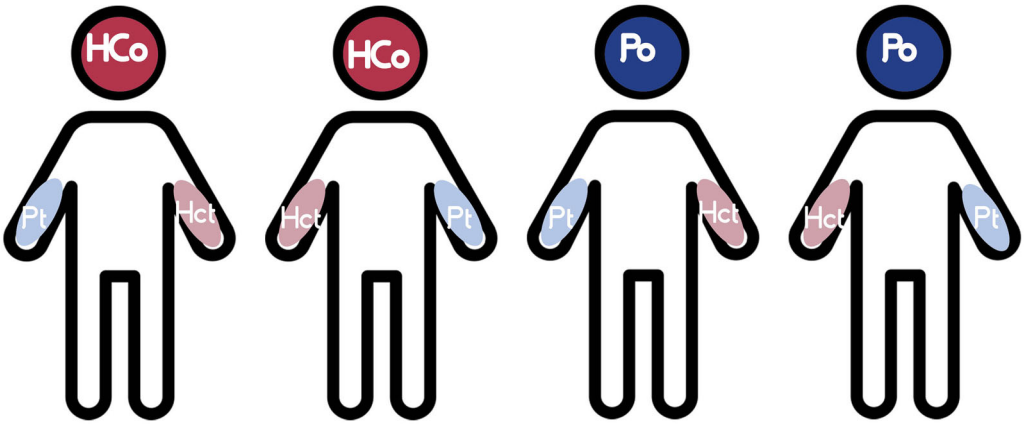


Fig. 2 Randomized groups for oral and topical HC versus placebo

Randomization and Blinding

The study was double blinded, and all patients were randomized using a computer software in a factorial manner. The allocation was concealed from the investigator and participants through numbered packs with the treatments inside.

Intervention

Patients were randomized to receive either oral HC (HCo, collagen peptides, 5 g) or placebo (Po, maltodextrin) once a day for 6 months and also randomized to receive either topical HC (HCt, hydrolyzed collagen 2.5% serum) or placebo (Pt, vehicle serum), three pumps at night on each forearm for 6 months (Fig. 2). All patients were instructed to use physical photoprotection and to avoid sun exposure during the trial.

The HCo was a combination of different and specific collagen peptides, derived from the hydrolysis of type I collagen of bovine and porcine origin, with molecular weight of 2 kDa. The HCt was developed by the Institute of Environmental Chemical and Pharmaceutical Sciences–UNIFESP and used the same collagen peptides as the oral version in a serum containing polyacrylamide & C13-14 isoparaffin & laureth-7 (3.5%), vegetable glycerin (5%), isononyl isonanoate (3%), phenoxyethanol, and parabens (0.5%).

The participants underwent anamnesis and dermatological examination monthly, as well as photos at baseline (V1) and after 24 weeks (V7). Skin biopsies were performed at V1 and V7 with a 2 mm punch in a standardized area on the dorsal surface of each forearm (7 cm from the antecubital fold on the midline) for histological (hematoxylin and eosin, picrosirius red) and immunohistochemical (collagen I and elastin) analysis. For immunohistochemistry, we used elastin clone EPR20603 dilution 1:2000 and collagen I clone 2Q276 dilution 1:500. The skin fragments were stained and images were obtained with a high resolution camera attached to a microscope, in 400× magnification and measurements were performed using T. Capture 4.3 software. Each fragment was photographed an average of six times (three upper dermis fields, three deep dermis fields) and the images were then evaluated by color deconvolution using Image J (Image processing and analysis in Java, imagej.nih.gov) with the color deconvolution plugin.

Skin elasticity was measured using the Cutometer equipment (Courage & Khazaka, Germany). Measurements were performed in triplicate at standardized points: 5, 7, and 10 cm from the antecubital fold, on the dorsal surface of the forearms, in the median line. We chose to analyze the values of R2, R5, R6, and R7, which mean:

- R2: Gross elasticity
- R5: Liquid elasticity
- R6: Viscous component
- R7: Viscoelastic component of the relaxation curve

A high frequency ultrasound equipment (20 MHz, DermaScan-C, Cortex Technology, Denmark) was used to obtain cross-sectional skin images (B-mode). The ultrasound was performed in standardized areas at baseline and after 12 and 24 weeks. The transducer was positioned perpendicularly to the skin surface in an area of 2 cm^2 at standardized points, 7 and 10 cm from the cubital fossa, on the midline of the dorsal face of the forearms. We chose to use the 50–255 pixel measurements, because they allow better visualization of dermal collagen with minimal interference from water.[14]

The skin elasticity measurement and ultrasonography were performed in standardized areas on the forearms, at baseline (V1), and after 12 (V4) and 24 weeks (V7).

The main investigator and two independent dermatologists performed a clinical blinded evaluation by comparing standardized photos of both forearms at baseline (V1) and after 24 weeks (V7) using a 5-point scale (1, very worsened; 2, worsened; 3, unaltered; 4, improved; and 5, very improved). The same scale was used to assess participants' opinion about the treatments.

Outcomes

The primary outcome was increase of dermal collagen I and elastin content and epidermal thickness after 24 weeks, increase in dermal echogenicity, thickness and pixel intensity of total and upper dermis in ultrasound images and in skin viscoelastic measures, as well as reduction of DLQI scores after 12 and 24 weeks. Reported or observed adverse effects were also considered outcomes.

STATISTICAL ANALYSIS

The unit of analysis for this study was each forearm. The data were tested for normality using the Shapiro–Wilk test. The variables were represented as mean and standard deviation (SD). The analysis was conducted in a factorial manner, that is, the effect of the oral, the topic, and whether there was interaction between oral and topic was measured. Data were compared between groups and according to visits. The data were compared according to time and groups over time, using a linear mixed effects (hierarchical) model with a robust covariance matrix, and probability adjustment for each distribution. Post hoc analysis was performed using the Šidák correction. Statistical analysis was performed blindly to the groups, using the software IBM SPSS 25v.

The data were analyzed as intention to treat, and missing data was imputed through the mixed model. Significance was defined as two-tailed $p < 0.05$.

Significant changes ($p < 0.05$) were noted by bold letters.

To estimate sample size we assumed mean baseline histologic collagen density as 41% (SD 7%) as the main outcome and expected an increase over 10% for one of the groups, with a power of 90% and an alpha of 5%, and thus expected a sample size of 56.

RESULTS

A total of 56 patients, aged from 60 to 93 years with DP stage I were included. The mean age was 69.53 years (SD 7.33) and 46/56 (82.14%) of patients were phototype III. No participant developed activities under direct sun exposure. There were three dropouts, two from the placebo group, due to reasons not related to the treatment.

Regarding histological findings (Table 1), there were no increases promoted by topical or oral treatments in the thickness of the epidermis and density of collagen and elastin. On the contrary, total collagen was reduced in the oral HC groups. All groups but oral and topical HC saw the upper dermal elastin reduced. By using the high frequency ultrasound images and digital analysis, it was observed that the average measurements of echogenicity, thickness and pixel intensity of total and upper dermis did not show an increase between the observed times. None of the groups obtained a consistent temporal gain in the parameters in the observed periods, but a reduction in pixel intensity and echogenicity were evidenced in the oral HC groups (Table 2).

Table 1 Mean (SD) measures of forearms epidermal thickness, dermal collagen, collagen type 1, and elastin, at 0, 90, and 180 days, according to the treatment groups

Variable	Visit	PoPt	PoHCt	HCoPt	HCoHCt
EpidTot (μm)	D0	93.16 (36.21)	92.57 (27.98)	93.27 (36.98)	94.67 (36.99)
	D180	96.88 (33.18)	90.79 (30.25)	87.2 (30.12)	91.21 (20.44)
EpidViable (μm)	D0	50.74 (16.8)	50.17 (13.54)	52.48 (18.99)	53.32 (25.37)
	D180	53.66 (15.57)	51.67 (16.4)	46.77 (13.08)	55.95 (12.56)
ColTot (%)	D0	45.25 (5.03)	44.28 (6.52)	47.04 (3.14)	47.23 (4.61)
	D180	44.24 (4.37)	45.28 (4.26)	**44.30 (3.61)***	**44.36 (4.06)***
Col1	D0	41.4 (8.07)	40.54 (6.57)	41.37 (7.15)	40.51 (6.01)
	D180	40.9 (5.67)	38.66 (7.86)	39.47 (6.82)	39.82 (6.27)
Elastin	D0	19.62 (8.44)	19.72 (11.78)	21.13 (9.22)	17.03 (7.86)
	D180	**16.40 (6.72)***	**15.34 (8.66)***	**14.43 (7.03)***	16.44 (7.55)

EpidTot: Thickness of total epidermis; EpidViable Thickness of the viable epidermis, ColTot (%) Dermal Collagen, *Col*1 Collagen type 1

*$p < 0.05$ (bold values)

The measures of viscoelastic properties of the skin, according to groups, did not show increase sustained until V7 (Table 3).

A low impact in the QoL was detected at baseline (mean DLQI 2.2). All treatments reduced the DLQI score (mean 0.6; $p < 0.01$), with no difference among the groups ($p = 0.65$).

The clinical blinded evaluation performed by the main investigator and two independent dermatologists observed no difference in general aspect of the skin and lesions characteristics of DP by comparing standardized photos of both forearms (at baseline and after 24 weeks) (Fig. 2).

The perception of skin improvement was reported by 70% to 89% of the patients within the groups, without difference among the treatments ($p > 0.48$). According to individual reports, there were improvements in skin hydration, smoothness and wrinkles, nail fragility, hair strength, genital lubrication, and articular pain. No adverse effects were reported or observed.

DISCUSSION

There is a progressive reduction in collagen production and an increase in its degradation due to higher expression of metalloproteinases (MMPs) by intrinsic aging. This process is aggravated by extrinsic aging caused mainly by chronic and uncontrolled exposure to UVA and UVB radiation, smoking, hormones, chronic diseases, diet, and pollution, among others [29, 30].

Previous studies suggested that oral hydrolyzed collagen (HC) supplementation can promote collagenesis and elastogenesis in the skin. Preclinical data demonstrated potential benefits of HC on the metabolism of extracellular matrix proteins, as well as positive impact on barrier function, hydration, and improvement of skin elasticity [13, 31–33].

HC is considered a safe ingredient, with good tolerability profile and, to date, there are no studies on its topical use.

DP is a prevalent condition affecting up to 35% of the elderly population, and there are still no evidence-based specific strategies for its prevention and treatment. The impact of chronological aging appears to be lower than

Table 2 Mean (SD) measures of forearms skin thickness, echogenicity and pixel intensity of the upper and total dermis at 0, 90, and 180 days, according to the treatment groups

Parameter	Visit	PoPt	PoHCt	HCoPt	HCoHCt
UdTh (mm)	D0	0.55 (0.09)	0.55 (0.08)	0.56 (0.09)	0.56 (0.10)
	D90	0.55 (0.09)	0.55 (0.08)	0.57 (0.09)	0.57 (0.10)
	D180	0.54 (0.10)	0.54 (0.09)	0.56 (0.10)	0.55 (0.10)
UdEcho	D0	52.59 (15.27)	51.11 (16.55)	55.91 (13.03)	59.27 (14.27)
	D90	53.46 (13.66)	52.21 (14.01)	55.29 (15.37)	56.25 (15.93)
	D180	54.96 (13.25)	53.39 (13.48)	**52.69 (13.21)***	**57.94 (14.07)***
UdIntens	D0	37.86 (12.02)	37.07 (11.67)	39.43 (9.45)	41.89 (10.86)
	D90	37.86 (10.05)	36.77 (9.81)	39.21 (11.25)	40.10 (11.99)
	D180	**39.06 (9.86)***	**37.33 (9.51)***	**36.35 (8.98)***	**40.46 (9.40)***
TdTh (mm)	D0	1.16 (0.17)	1.14 (0.16)	1.17 (0.18)	1.16 (0.20)
	D90	1.17 (0.18)	1.15 (0.18)	1.20 (0.19)	1.19 (0.21)
	D180	1.14 (0.19)	1.14 (0.17)	1.18 (0.22)	1.17 (0.20)
TdEcho	D0	61.34 (12.50)	61.48 (12.98)	65.16 (7.93)	67.59 (9.52)
	D90	62.59 (11.60)	61.77 (9.90)	64.02 (10.55)	65.44 (11.61)
	D180	64.70 (9.03)	62.81 (9.83)	**63.13 (8.61)***	**65.79 (10.14)***
TdIntens	D0	45.02 (11.35)	45.38 (11.23)	47.39 (7.58)	49.32 (9.19)
	D90	45.59 (9.94)	44.79 (8.77)	47.37 (9.6)	48.27 (10.61)
	D180	47.31 (8.13)	45.63 (8.81)	**45.52 (7.33)***	**47.90 (8.22)***

UdTh upper dermis thickness, *UdEcho* upper dermis echogenicity, *UdIntens* upper dermis pixel intensity, *TdTh* total dermis thickness, *TdEcho* total dermis echogenicity, *TdIntens* total dermis pixel intensity

*$p < 0.05$ (bold values)

that caused by extrinsic factors such as chronic sun exposure, use of corticosteroids, anticoagulants, and the presence of renal failure. In this study, the presence of comorbidities and the use of drugs that potentially aggravate DP were exclusion criteria.

Despite the longer supplementation time (24 weeks) and the higher dose of HC (5 g/day), the histological and immunohistochemical parameters did not reveal an increase in the amount of total collagen, collagen I, and elastin biomarkers, which differs from a previous study where HC supplementation (2.5 g/day) for just 8 weeks increased procollagen I by 65% and elastin by 18% [15]. It should be highlighted that the authors performed these measurements by using the ELISA test in the fluid of skin blisters obtained by suction in periocular area. That methodology is not comparable with ours as we have used punch biopsy in forearms of all patients and immunohistochemical biomarkers for detection of tissue collagen and elastin. Moreover, we have included older patients in our study (older than 60 years versus 45–65 years old) which may contribute to poorer intestinal absorption and more advanced fibroblast senescence.

Table 3 Mean (SD) measures of forearms skin elasticity parameters at 0, 90, and 180 days, according to the treatment groups

Parameter	Visit	PoPt	PoHCt	HCoPt	HCoHCt
R2	D0	0.60 (0.10)	0.65 (0.10)	0.62 (0.11)	0.62 (0.11)
	D90	0.63 (0.08)	0.63 (0.10)	**0.67 (0.11)***	**0.67 (0.09)***
	D180	0.65 (0.08)	0.65 (0.09)	0.66 (0.11)	0.64 (0.10)
R5	D0	0.68 (0.19)	0.73 (0.25)	0.69 (0.21)	0.70 (0.21)
	D90	0.70 (0.13)	0.76 (0.25)	**0.80 (0.26)***	**0.78 (0.19)***
	D180	0.74 (0.17)	0.77 (0.17)	0.74 (0.24)	0.71 (0.25)
R6	D0	0.85 (0.22)	0.88 (0.28)	0.80 (0.20)	0.80 (0.17)
	D90	**0.87 (0.20)***	**0.95 (0.27)***	0.88 (0.18)	0.88 (0.18)
	D180	0.87 (0.17)	0.87 (0.17)	0.83 (0.23)	0.87 (0.23)
R7	D0	0.36 (0.09)	0.38 (0.08)	0.37 (0.11)	0.39 (0.10)
	D90	0.37 (0.07)	0.39 (0.09)	0.42 (0.11)	0.41 (0.10)
	D180	0.39 (0.08)	0.41 (0.10)	0.39 (0.11)	0.37 (0.11)

*$p < 0.05$ (bold values)

Proksch et al. [14] evaluated skin elasticity after 8 weeks of supplementation with HC and found an average increase of 7%, with better results in elasticity parameters in patients aged over 50, which motivated our evaluation of patients over 60 years old. However, the authors did not mention if this result was sufficient for clinical observation of improvement in skin elasticity. In the present study, some viscoelastic parameters showed an increase after 12 weeks in patients treated with HCo, but returned to baseline values at week 24. In parameter R2, the positive impact seemed greater in patients treated with HCo associated or not with HCt. In parameter R5, the use of HCt had an additional positive effect on patients using HCo or Po after 12 weeks. In parameter R6, patients in the Po group performed better when treated with HCt at 12 weeks, with return to baseline values at week 24. These findings may be explained by the possibility of irregular use of study products after a period of time, which is understandable when participants are unable to see improvement. They also illustrate the limitations of noninvasive instrumental measures, particularly in skin viscoelastic properties.

There is still no well-established consensus on the meaning of ultrasound changes as a parameter for pre- and post-treatment assessment. Different studies suggest variable interpretations for improvement, such as increase in total, upper, or lower dermis echogenicity, reduction in echogenicity, and in the rate of low echogenicity pixels of the upper and lower dermis [35, 36].

In contrast to what was described in the literature, i.e., an increase of 8.8% of dermal echogenicity [13] and collagen density in the dermis after 12 weeks, no difference in the average measurements of echogenicity and thickness and pixel intensity of upper and total dermis after 24 weeks of supplementation was detected in our study. None of the groups showed a temporal gain in these parameters. Furthermore, the high heterogeneity of the results and publication bias were indicated by a recent meta-analysis in oral HC [37].

There was improvement in the QoL and in the general appearance of the skin in the opinion of patients in all groups, which is probably

due to regular monitoring and the hydration effect promoted by the use of the serum. Patients' acceptance of supplementation was good and, according to their opinions, there was improvement in several aspects, such as skin hydration and smoothness, reduction of wrinkles, improvement in nails, hair quality, genital lubrication, and joint pain, despite no difference among the groups.

Throughout the follow-up, the patient who reported the greatest clinical improvement was the oldest patient (aged 93 years). This patient reported benefits in dryness and thinning of the skin of both forearms. At the end of the study, we found out that this patient had received just oral placebo and there was no difference between the forearms, which reinforces the role of the placebo effect in clinical trials and the importance of using moisturizers (even only a topical serum vehicle) in fragile skin. The so-called Hawthorne effect could also explain this fact, in which the intensive follow-up of the clinical trial helps patients evolve better, regardless of the intervention [38].

Larger studies to confirm our findings are warranted.

CONCLUSIONS

In postmenopausal women with stage I dermatoporosis, oral or topical collagen peptides, used alone or in combination, have no benefits on forearm skin after 6 months of intervention, and therefore should not be used routinely in this population.

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Author Contributions. Lilia RS Guadanhim participated in all the steps of the study, including design patient consultation and follow up, skin biopsies, histologic and immunohistochemistry evaluation, data analysis and manuscript writing. Hélio A Miot participated in concept and design, histologic and immunohistochemistry evaluation,statistical analysis and manuscript writing. Juliana LM Soares participated in patient follow up and performed high frequency ultrasound and skin elasticity measurements. Silas MA Silva and Gislaine R Leonardi developed the topical creams used in the study. Renato D Lopes participated in manuscript writing.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. We thank all the patients that kindly took part in the study and collaborated greatly to our findings.

Disclosures. Lilia RS Guadanhim, Hélio A Miot, Juliana LM Soares, Silas MA Silva, Gislaine R Leonardi, Renato D Lopes and Ediléia Bagatin declare that they have no competing interest in this work..

Compliance with Ethics Guidelines. IRB approval status: Reviewed and approved by UNiFESP IRB (848/2015) – approval # 1.282.133. Standardized photographs were taken by Lais RS Guadanhim.Dr Fernanda Caramella participated in histologic and immunohistochemistry evaluation. Independent photographic evaluation was performed by Drs Gisele Jacobino Nunes, Juliana Mossini Nicoliello. This studied reviewed and approved by UNIFESP. IRB (848/2015) – approval # 1.282.133 and was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. All subjects provided informed consent to participate in the study.

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