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EFEITOS DA SUPLEMENTAÇÃO DE COLÁGENO HIDROLISADO NO ENVELHECIMENTO DA PELE: UMA REVISÃO SISTEMÁTICA E METANÁLISE

São Leopoldo - RS
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Dissertação apresentada como requisito parcial para obtenção do título de Mestre em Nutrição e Alimentos, pelo Programa de Pós-Graduação em Nutrição e Alimentos da Universidade do Vale do Rio dos Sinos - UNISINOS

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São Leopoldo – RS

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A Deus pela graça da vida, pelas bênçãos e dons recebidos.

Ao meu marido Jean, pela compreensão, apoio constante, estímulo, amor e paciência.

Aos meus filhos Gabriel e Vitor pela companhia alegre, renovadora e agradável e pela paciência nos momentos em que tenho que me ausentar.

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LISTA DE SIGLAS

ANVISA	Agência Nacional de Vigilância Sanitária
CH	Colágeno Hidrolisado
CP	Peptídeos de Colágeno
GH	Hormônio do Crescimento
IBGE	Instituto Brasileiro de Geografia e estatística
IGF-1	Fator de Crescimento Semelhante à Insulina
MMP	Metaloproteinases
Hyp-Gly	Hidroxiprolina-glicina
Pro-Hyp	Plolil-Hidroxiprolina
ROS	Espécie reativa de oxigênio
RNS	Espécie reativa de nitrogênio
UV	Ultravioleta

SUMÁRIO

1 INTRODUÇÃO

O colágeno é a principal proteína estrutural dos tecidos conjuntivos, como pele, tendões, cartilagens e ossos, constituindo de 25 a 30% de todas as proteínas do corpo. (SQUIRE; PARRY, 2017). No tecido cutâneo, participa da constituição da matriz extracelular, representando até 75% do peso total deste tecido e apresentando a função de sustentação. Juntamente com o ácido hialurônico e as demais fibras encontradas na matriz extracelular como a reticulina e a elastina, o colágeno forma uma rede de sustentação para os fibroblastos, queratinócitos, melanócitos e células especializadas do sistema imunológico cutâneo. (BENSON; WATKINSON, 2012; DINI; LANERI, 2019; RICARD-BLUM, 2011).

A rede de fibras de colágeno dérmico, com a idade, à partir da adolescência e início da idade adulta, torna-se cada vez mais fragmentada com fibras mais curtas e menos organizadas acumulando vários fragmentos de colágeno degradado. (VARANI et al., 2006). Além disso, com o envelhecimento há um aumento das metaloproteinases (MMP) que são enzimas que degradam as fibras de colágeno, diminuindo a síntese de novos componentes da matriz extracelular, incluindo o colágeno produzido por fibroblastos dérmicos. (JIN et al., 2001; QUAN et al., 2013). Os efeitos do envelhecimento intrínseco e extrínseco se sobrepõem levando a alterações estruturais e funcionais na derme com redução de volume, perda de elasticidade, redução da espessura epidérmica, aumento das rugas (CALLEJA-AGIUS; BRINCAT; BORG, 2013) e redução da capacidade de reter a umidade pela pele devido à redução do ácido hialurônico na matriz extracelular que tem a capacidade de reter água nessas estruturas. (CALLEJA-AGIUS; MUSCAT-BARON; BRINCAT, 2007; VERDIER-SÉVRAIN; BONTÉ, 2007).

Uma ampla gama de suplementos alimentares tem sido usada para melhorar a saúde da pele e mostrar benefícios para uma aparência mais jovem. (MADHERE; SIMPSON, 2010; SCHAGEN et al., 2012). Um desses suplementos é o colágeno hidrolisado que tem demonstrado efeitos funcionais e benéficos na pele em diversos estudos científicos, principalmente na melhoria dos sinais clínicos de envelhecimento da pele. (DINI; LANERI, 2019; PROKSCH et al., 2014a, 2014b). Estudos mostraram que a redução da síntese de colágeno dependente da idade e pode ser revertida pela administração oral de peptídeos bioativos de colágeno (SATO, 2017). Estes peptídeos são obtidos por hidrólise enzimática do colágeno

natural. Depois de digeridos, são metabolizados em di- e tri-peptídeos no trato gastrointestinal e, em seguida, são transportados através da corrente sanguínea e se acumulam na pele para formar novas fibras de colágeno. (CHOI et al., 2019; ZAGUE et al., 2011).

Os suplementos de colágeno hidrolisados são ricos nos aminoácidos hidroxiprolina, prolina e glicina. Hidroxiprolina é um componente único do colágeno e diferencia o colágeno de outras proteínas. (LIU et al., 2009). Vários estudos têm demonstrado que os di-peptideos prolilhidroxiprolina (Pro-Hyp) e hidroxiprolilglicina (Hyp-Gly) são absorvidos nesta forma e não como aminoácidos após a ingestão. (ICHIKAWA et al., 2010; IWAI et al., 2005; OHARA et al., 2007; SHIGEMURA et al., 2011; SUGIHARA et al., 2012) e depositados na pele. (OESSER et al., 1999). Esses di-peptídeos aumentam a bioatividade dos fibroblastos dérmicos aumentando a síntese de colágeno e melhorando a hidratação, a elasticidade (OHARA et al., 2009) e diminuindo as rugas. (KOIZUMI et al., 2017).

Com base nas crescentes publicações científicas e estudos clínicos conduzidos para a avaliação da suplementação de colágeno, torna-se evidente a necessidade de realização de estudos que compilem e analisem de forma global esses dados, para auxiliar na tomada de decisão quanto à suplementação. Neste contexto, esse estudo objetivou sumarizar as evidências sobre os efeitos da suplementação de colágeno hidrolisado na pele humana, reportados em ensaios clínicos com enfoque no processo de envelhecimento cutâneo, através de revisão sistemática seguida de metanálise.

2. OBJETIVOS

Os objetivos deste trabalho dividem-se em geral e específicos.

2.1 Objetivo Geral

Sumarizar as evidências sobre os efeitos da suplementação de colágeno hidrolisado na pele humana, reportados em ensaios clínicos com enfoque no processo de envelhecimento cutâneo, através de revisão sistemática seguida de metanálise.

2.2 Objetivos Específicos

a) Avaliar se nos resultados combinados dos estudos houve redução do envelhecimento cutâneo após a suplementação oral de peptídeos de colágeno;
b) Avaliar se houve melhora estatística significativa na redução de rugas nos estudos incluídos;
c) Verificar se houve melhora significativa estatística na hidratação da pele ou umidade da pele nos estudos incluídos;
d) Avaliar se houve melhora significativa estatística na elasticidade da pele nos estudos incluídos;
e) Avaliar o tempo necessário de suplementação do colágeno para obtenção de resultados nos estudos combinados.

3 FUNDAMENTAÇÃO TEÓRICA

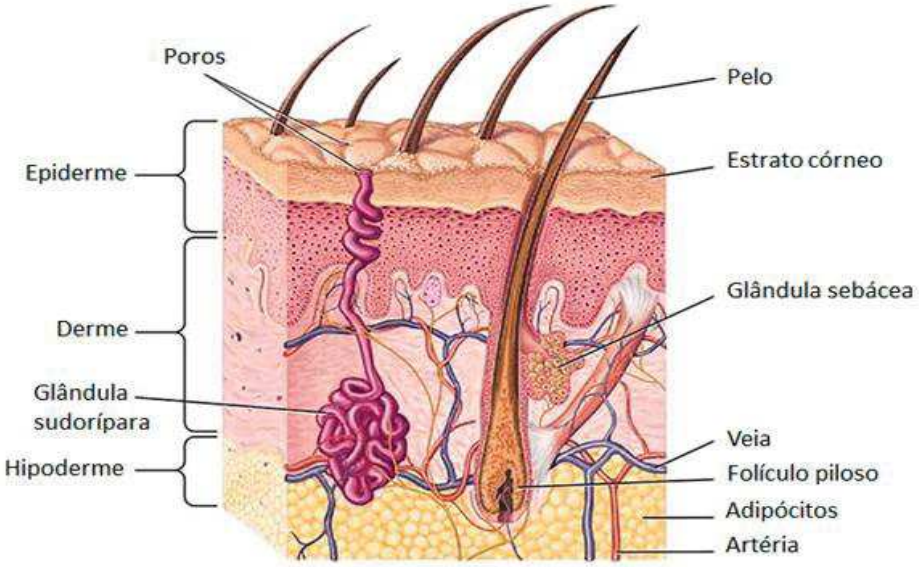
A seguir, será apresentada a fundamentação teórica para a realização dessa pesquisa.

3.1 Envelhecimento cutâneo

Os tecidos do organismo humano passam por mudanças a partir da adolescência e início da idade adulta. Na pele estas alterações são mais facilmente reconhecidas. Os sinais mais aparentes de uma pele senil são: atrofia, presença de rugas e lassidão. O tecido conjuntivo passa por alterações e, como ele atua como alicerce estrutural para a epiderme, estas mudanças são vistas externamente, pois são refletidas no estrato córneo. Algumas modificações que ocorrem nas colágeno-elásticas ao longo da vida estabelecem uma base morfológica substancial para que se possa compreender as alterações bioquímicas e biomecânicas que ocorrem com a pele. A espessura da pele e sua viscoelasticidade vão depender da organização estrutural da epiderme. (ŽMITEK et al., 2020; RODRIGUES, 2009).

A pele é o maior órgão do corpo e possui várias funções como sensorial e barreira de proteção física como fatores do meio externo. É formada por duas camadas principais, que são a epiderme e a derme (Figura 1). A epiderme é formada pela camada basal ou germinativa e as camadas espinhosa, granulosa e córnea e na derme temos os fibroblastos, que produzem elastina e colágeno, principalmente tipo I e tipo III. Os fibroblastos também produzem os outros componentes da matriz extracelular, como o ácido hialurônico, que possui a capacidade de reter água na derme e está diretamente relacionado com a hidratação da pele. (BORUMAND; SIBILLA, 2014).

Figura 1: Estrutura e camadas da pele



Fonte: 2010, Encyclopedya Britannica, Inc.

A integridade e a aparência da pele pioram com a idade devido aos efeitos sinérgicos cronológicos do fotoenvelhecimento, associado a fatores hormonais e do meio ambiente. (INOUE, SUGIHARA et al., 2016). Recentemente, tem sido demonstrado que o cigarro altera os componentes da derme e leva ao envelhecimento precoce dos componentes da pele. O estresse também pode afetar os componentes da pele, pois o altera o ritmo circadiano e a produção de cortisol, o que pode alterar a síntese e degradação do colágeno. (BORUMAND; SIBILLA, 2014).

A exposição excessiva e pronunciada à radiação UV leva a reações fotoquímicas de produção de específicos radicais de oxigênio (ROS) e específicos radicais de nitrogênio (RNS). Isto pode causar eritema e pigmentação da pele, supressão imune e inflamação da pele, que consiste no processo fisiopatológico de envelhecimento cutâneo com degeneração da epiderme e diminuição da expressão de procolágeno tipo I. Esse processo resulta num aumento da fragmentação das fibras colágenas e diminuição na síntese de colágeno. (SCHWARTZ; PARK, 2012).

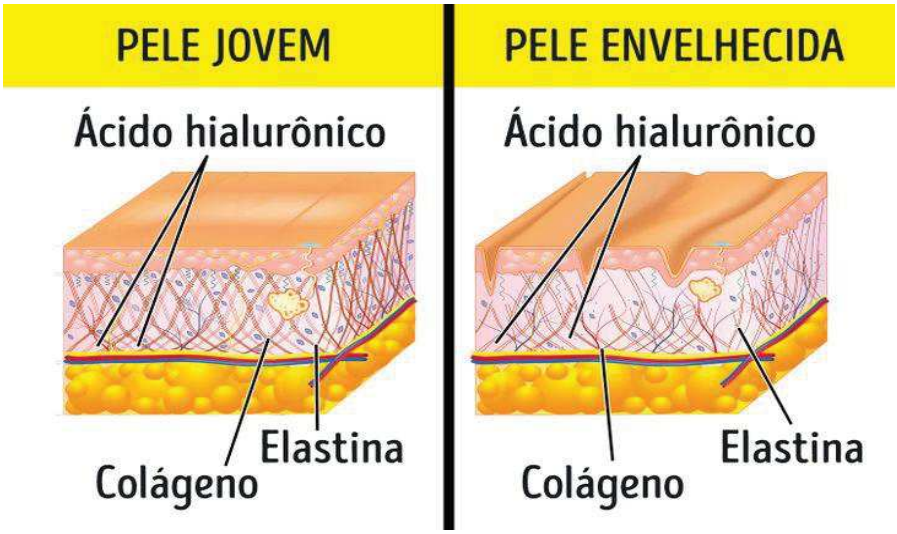
O envelhecimento da pele, principalmente o fotoenvelhecimento, se manifesta como diminuição da espessura da pele e da elasticidade, disfunção na barreira epidérmica e mudanças na pigmentação. Ao longo da vida, a integridade da pele muda e mostra diminuição na elasticidade e umidade e aumento das rugas. A

formação de rugas na pele é o mais proeminente sinal de envelhecimento. (YOON, CHO et al., 2014).

De forma geral, o envelhecimento cutâneo pode ser caracterizado como um processo progressivo de ressecamento, mudanças no micro-relevo da pele, diminuição da firmeza e elasticidade, resultando em rugas e flacidez. (ASSERIN, LATI et al., 2015).

O envelhecimento cutâneo está associado a um aumento na degradação das fibras de colágeno e elastina, que formam a principal rede de sustentação da pele e contribuem para uma aparência suave da pele O colágeno é o componente mais abundante da matriz extracelular dérmica, sendo o principal componente proteico que participa da fisiologia da pele e mantém esta estrutura funcionando. (GENOVESE, CORBO et al., 2017).

Figura 2: Organização das fibras de colágeno na pele jovem e envelhecida



Fonte: deposiphotos

3.2 Suplementação com colágeno

Atualmente, existe uma preocupação das pessoas com a qualidade de vida e isto tem os levado a procurar por produtos alimentares mais saudáveis. As pessoas querem consumir produtos que melhorem suas condições de saúde e que promovam bem-estar. A melhoria nos hábitos alimentares, a prevenção do surgimento de doenças degenerativas e a preocupação com a estética por meio da ingesta de alimentos mais

saudáveis têm contribuído para um aumento de pesquisas nesta área. (SILVA; PENNA, 2012).

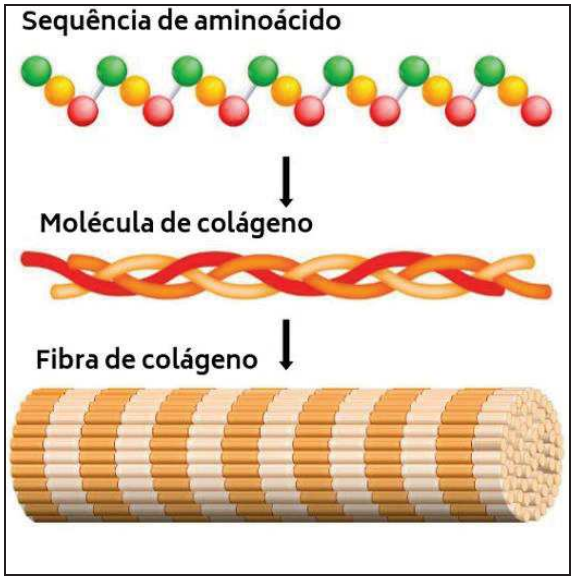
Em virtude do aumento da expectativa de vida, o envelhecimento cutâneo tem sido extensamente estudado com o objetivo de reduzir os seus efeitos. Na tentativa de se atenuar o envelhecimento cutâneo têm sido propostos vários tratamentos, sendo um deles o uso do colágeno hidrolisado. (MACIEL; OLIVEIRA, 2011).

As funções da pele e sua aparência saudável dependem da suplementação suficiente de nutrientes essenciais. A relação entre nutrição e pele tem se tornado um assunto frequente ao redor do mundo. Estudos intervencionistas têm mostrado que é possível modular ou retardar o envelhecimento cutâneo e melhorar a integridade da pele através de ingredientes de suplementação dietética. (PROKSCH et al., 2014).

O colágeno é a proteína mais abundante do organismo compondo cerca de 25 a 30% de toda a proteína corporal (Figura 3). Possui propriedades naturais como baixa resposta imunológica e baixa toxicidade e promove o crescimento celular e a reconstrução de estruturas celulares. Além disso, possui a capacidade de produzir fibras colágenas bastante elásticas e com propriedades hidratantes para a pele. (GONÇALVES et al., 2015).

O conteúdo de aminoácidos do colágeno é formado por glicina (30%), prolina (12%), alanina (11%), hidroxiprolina (10%) e hidroxilisina (1%). Além de pequenas quantidades de pequenos aminoácidos polares e carregados. A unidade básica do colágeno é o tropocolágeno que é formado por 3 cadeias de peptídeos e formam uma molécula linear de 180 nm de comprimento, e aproximadamente 1,4 a 1,5 nm de largura. As três cadeias polipeptídicas estão entrelaçadas formando uma molécula com formato helicoidal. (PRESTES, 2012).

Figura 3: Estrutura do colágeno



Fonte: Biologia Net

Na produção industrial de colágeno, para que tenha uma maior absorção intestinal, o mesmo passa por um processo de hidrólise de suas fibras, onde ocorre uma quebra das moléculas fracionando-as em moléculas menores. O resultado desta quebra molecular é o colágeno hidrolisado. Este suplemento é produzido através da proteína originada de bovinos e suínos por se assemelhar à proteína humana. (VELLY, 2009).

O colágeno hidrolisado é um suplemento alimentar considerado biodisponível e seguro. Possui em sua composição uma mistura de peptídeos de colágeno com massa molecular reduzida. Isto ocorre devido a uma degradação enzimática do colágeno nativo da pele de bovinos, suínos ou peixes. Este processo ocorre em várias etapas transformando o colágeno nativo que é insolúvel em um colágeno digerível e solúvel. (LIU et al., 2015).

O colágeno hidrolisado é um suplemento considerado um nutricosmético. Nutricosmético é um suplemento alimentar ou alimento composto por substâncias com propriedades para atuar na pele modificando características como rugas, celulite, acne, entre outros, podendo ser apresentados na forma de cápsulas, líquidos, pó ou alimentos. (MELLAGE, 2008). Os nutricosméticos atuam melhorando a pele, as unhas, os cabelos, além de atuar na saúde e no bem-estar. Estes suplementos causam uma melhora interna no organismo que é refletida externamente. (RUIZ, 2012).

3.3 Colágeno hidrolisado

Há muito tempo sabe-se que pode haver influência dos nutrientes utilizados para intervir no metabolismo cutâneo e seus efeitos na melhora de sinais de envelhecimento da pele. (BOILLY-GAUTHIER et al., 2010). Nos últimos 10 anos, ocorreu um aumento expressivo de pesquisas nessa área. Isso tem mostrado que os nutrientes e os compostos bioativos de alimentos têm capacidade de modular funções celulares e moleculares da pele. (RAVI SUBBIAH, 2010).

Estudos sobre o colágeno tem trazido conhecimento e proporcionado novas terapias antienvelhecimento, incluindo o uso de suplementos alimentares orais. Estes suplementos têm mostrado efeitos benéficos para a saúde da pele e há um crescente interesse em seus efeitos. (ZAGUE; MACHADO-SANTELLI, 2016).

O colágeno hidrolisado tem sido considerado uma fonte bastante promissora de peptídeos de colágeno com atividade bioativa. Devido aos seus efeitos benéficos para a pele e por apresentar atuação positiva no processo de envelhecimento cutâneo, vem sendo estudado há alguns anos. (SCHADOW et al., 2013).

Estudos têm mostrado que os peptídeos de colágenos hidrolisado possuem atividade bioativa com propriedades que podem ser antioxidantes, anti-hipertensiva e potencial para conseguir estimular o metabolismo de tecidos conjuntivos como a pele, os ossos e as cartilagens. (ZAGUE, MACHADO- SANTELLI, 2016).

O processo de produção do colágeno hidrolisado passa por várias etapas e é complexo. As etapas principais são a degradação térmica, a degradação química do colágeno nativo e depois vem a hidrólise enzimática que resulta em combinações de peptídeos de colágeno, que vão dar origem ao colágeno hidrolisado. (LIU et al., 2015).

Vários estudos realizados em humanos mostraram que 2 peptídeos importantes são encontrados no sangue periférico após a ingestão do colágeno hidrolisado: o prolil-hidroxiprolina (Pro-Hyp) e o hidroxiprolil-glicina (Hyp-Gly). Foi demonstrado que esses peptídeos exercem quimioprofilaxia sobre fibroblastos dérmicos e conseguem aumentar a proliferação de fibroblastos. O Pro-Hyp consegue aumentar a produção de ácido hialurônico dérmico. O achado destes peptídeos no sangue humano mostrou que os mesmos são absorvidos e atingem a corrente sanguínea, sendo importantes para sua ação na derme humana. (INOUE; SUGIHARA; WANG, 2016).

Os efeitos do colágeno têm sido considerados controversos, pois o colágeno nativo na sua forma oral ou parcialmente hidrolisado na forma de gelatina não é absorvido com eficiência. No entanto, quando é hidrolisado em pequenos peptídeos possui alta capacidade de absorção. (GENOVESE, CORBO et al., 2017).

3.3.1 Efeitos biológicos do colágeno hidrolisado

A utilização de enzimas específicas na produção do colágeno nativo vai quebrar pontos específicos da molécula de colágeno, gerando combinações de peptídeos com atividades biológicas diferenciadas. Esse mecanismo é chamado de hidrólise e dá origem a peptídeos que são biologicamente ativos e que têm demonstrado diversos efeitos. (LIU et al., 2015).

O colágeno, quando digerido, assim como outras proteínas que ingerimos, não é absorvido pelo intestino como colágeno. De 10 a 20% da nossa digestão ocorre no estomago, pela ação do ácido clorídrico e pepsina. E a maior parte da digestão proteica, cerca de 80%, ocorre pela ação do suco pancreático no duodeno e jejuno. No intestino delgado ocorre a hidrólise proteica e de aminoácidos livres em pequenos peptídeos. Isto decorre da ação de enteropeptidases, que em pH neutro ativa o tripsinogênio e a tripsina ativando outras enteroptidases do suco pancreático. Esses pequenos peptídeos são absorvidos no jejuno por difusão simples, difusão facilitada ou transferência ativa por co-transporte. Estes peptídeos vão ser destinados a inúmeras funções, inclusive a síntese de colágeno. (PORFÍRIO; BERNADES, 2016).

A biodisponibilidade pode ser definida como sendo a quantidade de compostos bioativos que têm origem na dieta e que conseguem atravessar a barreira gastrointestinal, atingem a circulação sanguínea e participam de processos metabólicos ou são armazenados pelo organismo. (SCHWARTZ; PARK, 2012).

O colágeno hidrolisado não é totalmente quebrado em aminoácidos livres como se acreditava. Evidências cientificas cada vez mais indicam que o colágeno hidrolisado é absorvido na forma de peptídeos. Os estudos mostram que estes peptídeos após serem absorvidos conseguem ser distribuídos pelos diferentes tecidos e ficam acumulados em locais como a pele e as cartilagens. (KAWAGUSHI; NANBU; KUROKAWA, 2012).

No estudo de Watanabe-Kamiyama et al., (2010) foi administrado peptídeos de colágeno e aminoácidos que foram previamente marcados radioativamente. Este estudo concluiu que o colágeno hidrolisado foi parcialmente absorvido na forma de peptídeos. A radioatividade alcançou um pico máximo 3 horas após ter sido administrada e o valor máximo encontrado na pele ocorreu nas próximas 3 horas. Foi realizada nova avaliação em 14 dias e ainda havia 70% da radiação administrada localizada na pele. O que sugere um acúmulo por um prazo longo do colágeno hidrolisado na pele.

Kim et al. (2018) usaram um peptídeo de colágeno com baixo peso molecular uma vez ao dia, comparado com placebo. Com 6 e com 12 semanas foi realizado uma análise clínica da pele a respeito dos níveis de hidratação e elasticidade e das rugas finas. O grau de hidratação da pele foi superior comparado com o grupo placebo com 6 e 12 semanas. Houve uma melhora das rugas também com 6 e 12 semanas. E a elasticidade da pele melhorou no grupo que ingeriu colágeno com 12 semanas.

Asserin et al. (2015) administraram peptídeos de colágeno por 8 semanas a humanos e observaram um aumento da hidratação da pele em 8 semanas à corneometria. Além disso, houve aumento da densidade de colágeno na derme e diminuição na fragmentação de fibras colágenas ao ultrassom da pele e ambos os efeitos se mantiveram após 12 semanas quando foram feitas novas avaliações.

Um estudo proposto por Schunck et al., (2015) compararam a ingestão de colágeno hidrolisado por mulheres por 8 semanas com a ingestão de placebo por outro grupo de mulheres. O estudo concluiu que houve uma melhora na elasticidade da pele das mulheres que ingeriram colágeno e diminuição da perda transepidérmica de água. O que não ocorreu no grupo que ingeriu placebo. E os resultados do grupo que ingeriu colágeno se mantiveram por mais 4 semanas.

Um outro estudo, Alvim, (2015) que administrou colágeno hidrolisado a mulheres na faixa etária de 35 a 65 anos por um período de 90 dias observou que houve um aumento significativo na espessura da derme por meio de ultrassonografia da pele e houve repercussão positiva em questionário subjetivo que avaliou a firmeza, a elasticidade e a hidratação cutâneas.

Inoue el al. (2016) administraram 2 tipos de colágenos compostos por diferentes quantidades de dipeptideos ativos Pro-Hyp e Hyp-Gly para avaliar os efeitos sobre a pele e administrou placebo para um terceiro grupo e após 8 semanas

foi realizado exame de sangue para análise dos peptídeos encontrados. O colágeno hidrolisado com maior quantidade de peptídeos bioativos mostrou efeito superior ao colágeno hidrolisado com menos quantidade de peptídeos bioativos e ao placebo. Uma maior quantidade de Pro-Hyp e Hyp-Gly no colágeno apresenta melhora na umidade, elasticidade e rugas na pele da face.

Proksh et al, (2016) administraram um peptídeo especifico de colágeno bioativo para mulheres de 45 a 65 anos por um período de 8 semanas e avaliaram as rugas da região periorbiral e a produção de procolágeno tipo I , elastina e fibrilina pela pele. Foi observado redução nas rugas periorbitais no grupo que ingeriu colágeno comparado ao grupo que ingeriu placebo e um efeito positivo na melhora das rugas mesmo após 4 semanas de ingestão. Além disso, observou-se um aumento na produção de procolágeno tipo I, elastina e fibrilina pela derme 8 semanas após a última ingestão do peptídeo de colágeno.

4 METODOLOGIA

Os materiais e métodos estão descritos no artigo que se encontra no apêndice A.

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APENDICE A

Effects of hydrolyzed collagen supplementation on skin aging: A systematic review and meta-analysis

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ABSTRACT

Introduction: Skin aging has become a recurring concern even for younger people, mainly owing to increased life expectancy. Appearance is important in aspects such as social attractiveness and health. In this context, the use of nutricosmetics as supplements has increased in recent years. Moreover, numerous scientific studies have shown the benefits of hydrolyzed collagen supplementation in improving the signs of skin aging. **Objective:** The objective of this study was to summarize the evidence on the effects of hydrolyzed collagen supplementation on human skin through a systematic review followed by a meta-analysis of clinical trials focusing on the process of skin aging. **Methods:** A literature search was conducted in the Medline, Embase, Cochrane, LILACS (Latin American and Caribbean Health Sciences Literature), and *Journal of Negative Results in BioMedicine* databases for articles published in English, Spanish, and Portuguese, with no restrictions on publication date. Eligible studies were randomized, double-blind, and controlled trials that evaluated oral supplementation with hydrolyzed collagen as an intervention and reported at least one of the following outcomes: skin wrinkles, hydration, elasticity, and firmness. **Results:** After retrieving articles from the databases, 19 studies were selected, with a total of 1,125 participants aged between 20 and 70 years (95% women). In the meta-analysis, a grouped analysis of studies showed favorable results of hydrolyzed collagen supplementation compared with placebo in terms of skin hydration, elasticity, and wrinkles. The findings of improved hydration and elasticity were also confirmed in the subgroup meta-analysis. **Conclusion:** On the basis of results obtained from the studies, ingestion of hydrolyzed collagen for 90 days is effective in reducing skin aging, as it reduces wrinkles and improves skin elasticity and hydration. Supplements rich in prolylhydroxyproline and hydroxyprolylglycine peptides are effective in reducing wrinkles and improving skin elasticity after 4 weeks of use. Hydrolyzed collagen is a safe food supplement, as evidenced by the absence of adverse effects in all analyzed studies.

Keywords: skin aging, hydrolyzed collagen, supplementation

INTRODUCTION

Collagen is the main structural protein in connective tissues such as the skin, tendons, cartilage, and bones, constituting 25–30% of all proteins in the body (SQUIRE; PARRY, 2017). Collagen is a component of the extracellular matrix of cutaneous tissue, representing up to 75% of its total weight, and its main function is related to providing mechanical support. In association with hyaluronic acid, reticulin, and elastin, which are other fibers found in the extracellular matrix, collagen forms a support network for fibroblasts, keratinocytes, melanocytes, and specialized cells of the skin immune system (BENSON; WATKINSON, 2012; DINI; LANERI, 2019; RICARD-BLUM, 2011).

The dermal collagen fiber network becomes increasingly fragmented (i.e., presenting shorter and less organized fibers that accumulate as several fragments of degraded collagen) with age (VARANI et al., 2006). In addition, aging also increases the generation of metalloproteinases, which are enzymes that degrade collagen fibers, thus decreasing the synthesis of new extracellular matrix components, including the type of collagen produced by dermal fibroblasts (JIN et al., 2001; QUAN et al., 2013). The overlap of intrinsic aging and extrinsic aging leads to structural and functional changes in the dermis, including volume reduction, elasticity loss, decreased epidermal thickness, increased wrinkles (CALLEJA-AGIUS; BRINCAT; BORG, 2013), and decreased capacity to retain moisture through the skin owing to decreased hyaluronic acid (a compound responsible for retaining water in skin structures) in the extracellular matrix (CALLEJA-AGIUS; MUSCAT-BARON; BRINCAT, 2007; VERDIER-SÉVRAIN; BONTÉ, 2007).

A wide range of dietary supplements have been used to improve skin health and achieve a younger appearance (MADHERE; SIMPSON, 2010; SCHAGEN et al., 2012). One of them is hydrolyzed collagen (HC), which has demonstrated functional and beneficial effects on the skin in several scientific studies, mainly by improving the clinical signs of skin aging (DINI; LANERI, 2019; PROKSCH et al., 2014a, 2014b). Some studies have shown that the age-dependent reduction in collagen synthesis can be reversed by the oral administration of bioactive collagen peptides (SATO, 2017). These peptides are obtained from the enzymatic hydrolysis of natural collagen. Once digested, they are metabolized to dipeptides and tripeptides in the gastrointestinal tract, and thereafter transported through the bloodstream and accumulate in the skin to form new collagen fibers (CHOI et al., 2019; ZAGUE et al., 2011).

HC supplements are rich in hydroxyproline, proline, and glycine amino acids. Among these proteins, only hydroxyproline is a component of collagen (LIU et al., 2009). Several studies have shown that prolylhydroxyproline (Pro-Hyp) and hydroxyprolylglycine (Hyp-Gly) are absorbed after ingestion as dipeptides, not as amino acids (ICHIKAWA et al., 2010; IWAI et al., 2005; OHARA et al., 2007; SHIGEMURA et al., 2011; SUGIHARA et al., 201), and become deposited on the skin (OESSER et al., 1999). These dipeptides increase the bioactivity of dermal fibroblasts by increasing collagen synthesis,

thus improving hydration and elasticity (OHARA et al., 2009) and reducing wrinkles (KOIZUMI et al., 2017).

With the increasing number of scientific publications and clinical studies evaluating collagen supplementation worldwide, the need for compiling and analyzing these data, to assist in decision making concerning supplementation, becomes evident. Therefore, the objective of this study was to summarize the evidence on the effects of HC supplementation on the human skin, as reported in clinical trials focusing on the skin aging process, through a systematic review followed by meta-analysis.

1. MATERIALS AND METHODS

2.1 Search strategy, inclusion criteria, and exclusion criteria

The Medline, LILACS (Latin American and Caribbean Health Sciences Literature), Embase, Cochrane, and *Journal of Negative Results in BioMedicine* databases were searched using various combinations of specific terms from the thesaurus of each database, terms used in the titles or abstracts, and free terms associated with the research question. After the search, studies that evaluated the role of HC supplementation in preventing skin aging in humans were retrieved. The retrieved studies were selected by two independent reviewers (RBM and RCR), who initially considered the eligibility of titles and abstracts, and thereafter checked the eligibility of full texts. In case of disagreements, article selection was decided after a consensus was reached between the two evaluators. The databases were last accessed on October 6, 2020, and the studies considered eligible for inclusion were randomized clinical trials conducted in healthy patients aged > 18 years who received oral supplementation with HC. Studies that did not meet the initial criteria and those that did not measure the effect of supplementation on skin aging were excluded.

2.2 Data extraction

Data were independently extracted by the two evaluators (RBM and RCR) and recorded in an electronic spreadsheet. The following data were extracted: number of patients, sex, age, groups evaluated in each study (treatment and placebo), duration of the study, intervention, and primary outcomes (hydration, elasticity, wrinkles, and skin density), as well as baseline data of the measured characteristics in the placebo and treatment groups. In studies that presented secondary outcomes, such as the results of immunohistochemical assays, biopsy findings, levels of hyaluronic acid and enzymes, cutaneous pH, and skin color or presence of erythema, these data were also extracted. All quantitative variables were assessed as means ± standard deviations (SDs).

2.3 Statistical analysis, sensitivity analysis, and bias assessment

The studies were grouped according to outcome similarity, and independent meta-analyses were conducted for each group. Statistical analysis was performed using the random-effect model (inverse variance) to calculate the mean difference and SD for continuous variables. A probability (p) value of < 0.05 was considered statistically significant. The result of the general effect test was reported as a z value corroborating the inference of the 95% confidence interval (CI). The Higgins I (I^2) statistical model was used to assess the heterogeneity of results among the included studies. I^2 values ≤ 50% corresponded to low and moderate heterogeneity, whereas values > 75% indicated high heterogeneity. Subgroup analyses based on result measurement units were performed to identify the sources of heterogeneity. Sensitivity analysis censored by unparalleled measurement units or study size was also conducted to negate the effect of potentially influential studies. The presence of publication bias was graphically presented using a funnel chart. The quality of the included articles was assessed according to the Cochrane guidelines for systematic review and meta-analysis, in which each study was classified according to the five types of bias (selection, performance, detection, attrition, and reporting bias) proposed in the Risk of Bias (RoB)-2 tool (Cochrane, August 22, 2019). All statistical analyses were performed using the RevMan software (version 5.4; The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, 2020).

2. RESULTS

3.1 Research results and study characteristics

The initial search retrieved 365 articles from the databases. After removing studies according to the preestablished criteria in the title and abstract eligibility stage and for being duplicates, 33 articles were considered relevant for a full-text review. Of these, 14 articles were excluded according to the inclusion and exclusion criteria, leaving a total of 19 articles eligible for quantitative analysis. Figure 1 shows the study selection flowchart, divided into the steps of identification, selection, eligibility, and inclusion, according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) recommendations for writing systematic reviews (LIBERATI et al., 2009).

3.2 Study characteristics

A total of 1,125 patients completed the studies. The mean age of the patients was 49.82 ± 5.68 years in the intervention group and 50 ± 5.63 years in the placebo group (p = 0.006). The mean number of female patients was 1,081 (95%) in both groups (p = 0.05). The duration of the studies ranged from 4 to

16 weeks. The forms of intervention and outcome assessment criteria widely varied among studies. The main characteristics of the selected studies are described in Table 1.

With respect to the baseline characteristics of the placebo and treatment groups evaluated before the intervention, a comparative analysis showed no differences in the global means between the two groups for skin elasticity (2.16 ± 0.15 vs. 2.01 ± 0.22 MPa, $p = 0.23$), skin wrinkles (32.1 ± 20.9% area vs. 29.7 ± 16.9% area, $p = 0.06$), and transepidermal water loss (TEWL) (14.7 ± 4.6 vs. 14.6 ± 4 g/m^2/h, $p = 0.72$). However, a difference was observed between groups in dermis density (36.5 ± 3.7 vs. 35.4 ± 5.8, $p = 0.0004$), dermis thickness (1435 ± 110 vs. 1564 ± 217 μm, $p < 0.0001$), and skin moisture content (61.9 ± 19.91% vs. 65.5 ± 15.2%, $p = 0.002$).

3.3 Meta-analysis results

3.3.1 Pooled analysis of selected studies

The 19 selected studies were initially divided into two groups according to the measured outcome (skin hydration or elasticity), and thereafter subjected to a meta-analysis. Figure 2 shows the forest plot of the meta-analysis of nine studies with respect to combined skin hydration estimates comparing the placebo group with the group of patients supplemented with HC. For this outcome, supplementation resulted in significant improvement ($z = 2.58$, $p = 0.010$), as evidenced by the overall effect size of 1.01 (95% CI 0.24, 1.78). In the meta-analysis with 14 studies (Figure 3) evaluating skin elasticity, HC supplementation also significantly improved this outcome ($z = 2.31$, $p = 0.02$) in comparison to the placebo group, with a general effect size of 1.27 (95% CI 0.19, 2.35).

The grouped analysis of the studies also showed positive effects of collagen supplementation in terms of a significantly decreased mean skin wrinkle value (-1.11; 95% CI -1.94, -0.28; $p = 0.009$) and increased cutaneous density (0.48; 95% CI 0.09, 0.88; $p = 0.02$), as shown in Figure 4. No significant differences were identified between the treatment and placebo groups in studies evaluating TEWL secondary outcomes (-0.44; 95% CI -0.99, 0.11; $p = 0.12$), skin rash (-0.27; 95% CI -0.61, 0.08; $p = 0.13$), collagen levels (0.74; 95% CI -0.89, 2.36; $p = 0.37$), and melatonin pattern (-0.17; 95% CI -1.20, 0.86; $p = 0.75$). The forest plots of these outcomes, as well as the studies included in the meta-analysis, are shown in Figure A.1 (Supplementary Material).

For secondary outcomes extracted as visual analog scale photoaging scores, the serum fibronectin, elastin, hyaluronic acid, and carbonylated protein levels reported in the study by Di Cerbo et al. (2014) and skin pH reported by Ito; Seki; Ueda (2018) were not included in the meta-analysis because they were measured in single studies. Comparative data between the HC supplementation group and the placebo group are described in Table A.2 (Supplementary Material).

3.3.2 Subgroup analysis based on corresponding measurements

Subgroup analysis for cutaneous hydration was based on the corresponding measurements in micrometers (five studies), percentage of hydration (two studies), and moisture content (arbitrary units [AU], two studies), and showed no significant global differences between the groups ($p = 0.53$). Additionally, the heterogeneity value decreased from $I^2 = 93\%$ to $I^2 = 0\%$. In this subgroup analysis, only the studies that measured hydration in micrometers showed a significant and favorable difference with HC supplementation (0.73; 95% CI 0.19, 1.27; $p = 0.008$), according to the forest plot presented in Figure 5.

Similar to the grouped analysis (Figure 3), a subgroup analysis for cutaneous elasticity subdivided into MPa, percentage, and AU measurement units demonstrated a significant difference between the HC supplementation group and the placebo group ($p = 0.04$), as shown in Figure 6. This subgroup analysis also showed a decreased heterogeneity value from $I^2 = 98\%$ to $I^2 = 69.1\%$. Separately, only studies that measured elasticity in MPa showed a significant difference in favor of supplementation (4.65; 95% CI 1.11, 8.18; $p = 0.010$). The other studies showed no positive effect of supplementation when measured in percentage (-0.22; 95% CI -1.48, 1.04; $p = 0.73$) and AU (0.28; 95% CI -0.45, 1.01; $p = 0.45$).

3.3 Sensitivity analysis

With respect to cutaneous hydration, the study by Žmitek et al. (2020) measured hydration using a different technique compared with the other studies, resulting in a higher value, as shown in Figure 5. Thus, a sensitivity analysis was used to assess the influence of this study in the subgroup and grouped analyses, as shown in Figure 7. The exclusion of this study resulted in no significant change, and both the corrected global result (1.13; 95% CI 0.28, 1.97; $p = 0.009$) and the intragroup result by measurement unit (0.86; 95% CI 0.26, 1.46; $p = 0.005$) remained favorable to collagen supplementation.

In addition, this sensitivity analysis considered an additional factor because a different measurement instrument was used for hydration and HC was administered in association with coenzyme Q10 in the study by Žmitek et al. (2020). Coenzyme Q10 supplementation had positive effects on skin aging, such as reduced wrinkles and skin smoothing in a clinical trial conducted by the same research group (ŽMITEK et al., 2017). In this context, coenzyme Q10 could act complementarily or synergistically with collagen, mitigating the effects of skin aging, with the sensitivity analysis confirming the direct influence of this study on the overall result.

Of the studies that evaluated the effect of supplementation on skin elasticity, only Genovese; Corbo; Sibilla (2017) expressed the result in millimeters, resulting in a different value compared with the other studies. Thus, a sensitivity analysis was conducted for elasticity, in which this study was removed.

Figure 8 shows that after removing the study, the overall effect remained favorable to collagen supplementation (1.16; 95% CI 0.04, 2.27; p = 0.04).

3.4 Bias

A methodological assessment of the quality of RoB was performed using the RoB-2 tool. The assessment of RoB at the domain level revealed a low RoB for most studies, as shown in Figure 9. At the study level, an RoB was found in the blinding of participants and researchers in the studies by Campos et al. (2015), Choi et al. (2014), and Nomoto; Iizaka (2020), and with respect to incomplete data in the results by Nomoto; Iizaka (2020), Proksch et al. (2014a), and Schwartz et al. (2019), as shown in Figure 10.

3.5 Publication bias

In the visual evaluation, the funnel chart showed symmetry (Figure 11), indicating that the limited dispersion occurred because of sample variation and not because of publication bias. The vertical axis of the graph used standard error to estimate the sample size of the study, plotting large population studies at the top and smaller studies at the bottom. The horizontal expansion showed the power and effect size of the included studies.

3. DISCUSSION

Despite the heterogeneity among studies, which used different collagen peptide concentrations, formulations, origins (pigs, fish, chicken, etc.), and forms of administration (liquid and solid) of the oral supplement, most of the studies reported improved skin hydration and elasticity, increased dermal density, improved collagen dermal fiber structure and organization, and reduced facial wrinkles. The beneficial effects were evident at 60 and 90 days after the start of supplementation and were maintained for 30 days after the end of the intervention.

Several clinical studies evaluated the effects of oral HC, and observed improved dermal collagen synthesis, increased collagen synthesis by fibroblasts, improved skin hydration and elasticity, and decreased wrinkles (POSTLETHWAITE; SEYER; KANG, 1978).

The study by Bolke et al. (2019) showed that a daily intake of 2.5 g collagen peptides increased the level of hydration, skin elasticity, and dermal density, and decreased the area of the wrinkles in women aged > 35 years after 90 days of supplementation. These findings were proven both by objective and subjective assessment methods (questionnaires). In addition, the observed results persisted for 30 days after the intervention. Corroborating these findings, similar results were obtained in clinical trials

that administered 5 g collagen peptides, demonstrating that supplementation of 2.5 g collagen peptides for 90 days is sufficient to obtain beneficial effects. The study by Proksch et al. (2014a) showed no improvement in skin hydration with 2.5 or 5 g HC (Verisol®), and the difference may be attributable to the measurement site (inner side of the arm). As the measurement site is a region that is normally protected from solar radiation, its skin aging process is less accelerated than that of the outer side. That is, positive effects can be more easily observed when evaluating areas that are more exposed to extrinsic factors such as radiation and pollution.

Previous studies have shown that the Pro-Hyp and Hyp-Gly dipeptides have advanced effects on dermal fibroblasts, stimulating their metabolism, migration, and proliferation by producing collagen fibers in the dermis (OHARA et al., 2010; POSTLETHWAITE; SEYER; KANG, 1978; SHIGEMURA et al., 2009). The clinical trial conducted by Koizumi et al. (2017), in which the intervention was ingestion of 3 g collagen peptides derived from tilapia fish scales (high content of Hyp, Gly, and Pro) for 90 days, resulted in effective reduction of periorbital wrinkles and improved skin moisture (hydration) and elasticity in women. Additionally, the study by Sugihara; Inoue; Wang (2015) demonstrated that the ingestion of 2.5 g HC peptides, also derived from fish scales and containing the Pro-Hyp and Hyp-Gly bioactive dipeptides, improved hydration and elasticity, and smoothed facial skin wrinkles in 4 weeks. The effects of the Pro-Hyp and Hyp-Gly concentrations were measured in the study by Inoue; Sugihara; Wang (2016), which showed effectiveness in both groups; however, the clinical improvement of the parameters was more quickly observed in the group supplemented with a higher concentration of dipeptides, reinforcing the presumption that the composition directly affects the results.

Collagen peptides have been shown to be effective in improving the skin, regardless of origin (fish, pigs, cattle, or chickens) but depending on peptide composition and concentration. Some studies that administered type II HC demonstrated effectiveness in improving the structure of dermal collagen, with reduced facial wrinkles and elasticity, and obtained positive results in subjective questionnaires. This shows that both type I and II HC supplements have beneficial effects on the skin.

The study by Nomoto and Iizaka (2020), which included hospitalized older and unhealthy adults, showed that the intake of HC peptide supplement was beneficial and reduced skin vulnerability to traumatic injuries caused by procedures in bedridden older patients. The effects of HC supplementation on skin recovery after laser treatment were evaluated in the study by Choi et al. (2014), and the main results showed improved healing process in supplemented patients.

Most of the selected studies used commercial HC supplementation in ready-to-consume preparations as the intervention (Table 1). These commercial brands had different percentage compositions, containing (in addition to collagen peptides) vitamins, minerals, antioxidants, coenzyme Q10, hyaluronic acid, and chondroitin sulfate. In these studies, the positive outcomes of supplementation were solely attributed to collagen and the effect of the formulation vehicle was not comparatively

evaluated. Thus, the beneficial effects achieved may have occurred owing to the synergism of these substances with collagen. Coenzyme Q10, for example, has an important antioxidant function, neutralizing the damage caused by free radicals generated in the skin aging process (MADHERE; SIMPSON, 2010), thus improving the signs of aging. Other vitamins, such as vitamin C, and hyaluronic acid participate in and stimulate collagen biosynthesis, respectively (LODISH; BERK; ZIPURSKY, 2000). Nevertheless, studies using collagen in its isolated form demonstrated its effectiveness.

None of the studies reported adverse effects related to the dietary supplement. Furthermore, in the evaluated studies, the use of HC in liquid and solid forms (capsules and powder supplements available for resolubilization) showed good patient acceptability owing to easy swallowing and the safety of administration.

4. LIMITATIONS

The limitations of this study were related to the large heterogeneity of the studies, mainly due to the composition of the supplementation, methods used to verify the results, and different measurement units, making it difficult to compare them in terms of both intervention and outcomes. Nevertheless, HC and HC peptides are effective in reducing skin aging and are safe for consumption.

5. CONCLUSION

On the basis of the results of this study, HC supplements or collagen peptides can delay and improve the signs of skin aging by decreasing facial wrinkles and improving skin hydration and elasticity. The time required to delay skin aging in most studies was 90 days, and the result was maintained for 4 weeks after the end of supplement administrations. Studies using supplements with higher concentrations of Pro-Hyp and Hyp-Gly dipeptides showed visible improvements of the evaluated parameters after 4 weeks. Supplement intake is effective and safe because no adverse effects were reported in any of the analyzed studies. Further studies are needed to evaluate the long-term use of HC peptides, as the longest intervention among the studies lasted for 90 days, with 120 days of evaluation of the effects. Further studies are needed to evaluate the effect of the vehicle and of other substances co-administered with collagen, mainly vitamins and coenzyme Q10, which can act in association or synergistically with collagen to significantly improve the measured effects.

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TABLES

Table 1. Description of the included studies

Study	Author (year)	Participants	Time	Intervention	Placebo	Outcome extracted
1	Bolke et al. (2019)	72 healthy women aged > 35 years	16 weeks / 12 weeks of intervention	2.5 g collagen peptides (Elasten®)	Yes	Hydration / elasticity / wrinkles / skin density / subjective questionnaire
2	Genovese; Corbo; Sibilla (2017)	111 healthy women and 9 men aged 40–60 years	12 weeks	5 g HC (Colágeno de Ouro Forte®)	Yes	Elasticity / biopsies / subjective questionnaire
3	Koizumi et al. (2017)	71 healthy women aged 30–60 years	12 weeks	3 g collagen peptides	Yes	Wrinkles / moisture / elasticity / blood tests
4	Proksch et al. (2014a)	60 healthy women aged 35–55 years, Fitzpatrick* I to IV	12 weeks / 8 weeks of intervention	2.5 g HC / 5 g HC (Verisol®)	Yes	Elasticity / hydration / TEWL / wrinkles
5	Žmitek et al. (2020)	31 Caucasian women aged 40–65 years, Fitzpatrick II and III	12 weeks	4 g HC	Yes	Dermal density and thickness / viscoelasticity / hydration / TEWL / wrinkles / moisture / dermal microrelief
6	Sugihara; Inoue; Wang (2015)	53 healthy Chinese women aged 35–55 years	8 weeks	2.5 g HC (Wellnex®)	Yes	Hydration / elasticity / wrinkles
7	Yoon et al. (2014)	44 healthy women aged > 44 years	12 weeks	3 g HC	Yes	RT-PCR for procollagen type 1, fibrillin 1, metalloproteinases 1 and 12 / biopsies / immunohistochemical staining
8	Schwartz et al. (2019)	113 healthy white women aged 36–59 years, Fitzpatrick I to IV	12 weeks	0.6 g HC (Bio Cell®)	Yes	Erythema / hydration / TEWL / elasticity / wrinkles / dermal collagen / subjective questionnaire
9	Laing et al. (2020)	60 healthy women aged 40–70 years	12 weeks	2.5 g collagen peptides (Elasten®)	Yes	Dermal collagen fragmentation / subjective questionnaire

10	Czajka et al. (2018)	120 healthy people aged 21–70 years	12 weeks	4 g HC (Colágeno de Ouro Ativo®)	Yes	Elasticity / biopsies / self-perception questionnaire
11	Di Cerbo et al. (2014)	30 healthy women	4.5 weeks of intervention	372 mg HC (Viscoderm®)		Cutaneous pH, hydration, sebum, elasticity and skin tone / ELISA for elastin, elastase 2, fibronectin, hyaluronic acid, and carbonyl proteins
12	Proksch et al. (2014b)	107 healthy women aged 45–65 years	12 weeks / 8 weeks of intervention	2.5 g collagen peptides	Yes	Wrinkles / biopsy / ELISA procollagen type 1, elastin, and fibrillin
13	Campos et al. (2015)	60 healthy women aged 40–50 years	12 weeks	9 g HC	Yes	Corneal stratum hydration / skin viscoelasticity / dermal echogenicity / high-resolution photography
14	Asserin et al. (2015)	134 healthy Japanese women aged 40–59 years and Caucasian women aged 40–65 years	8 weeks / 12 weeks	1) 10 g pig Peptan 10 g fish Peptan 2) 10 g Peptan	Yes	Skin moisture / TEWL / dermal density / dermal echogenicity / dermal collagen fragmentation
15	Ito; Seki; Ueda (2018)	21 healthy Japanese people aged 30–50 years	8 weeks	10 g fish collagen peptides	Yes	Elasticity / moisture / TEWL / skin pH / spots, wrinkles, skin pores, texture / ultrasound density and collagen score / Gh, IGF-1
16	Choi et al. (2014)	32 women undergoing fractional laser treatment	5 weeks	Collagen peptides	Yes	Skin hydration / TEWL / erythema / satisfaction questionnaire
17	Inoue; Sugihara; Wang (2016)	80 people aged 35–55 years	8 weeks	2.5 g Collagen peptides with different Pro-Hyp and Hyp-Gly concentrations	Yes	Skin moisture / elasticity / wrinkles
18	Sangsuwan; Asawanonda (2020)	36 healthy women aged 50–60 years	8 weeks / 4 weeks of intervention	5 g HC	Yes	Elasticity
19	Nomoto; Iizaka (2020)	39 people aged > 65 years hospitalized for < 5 months	8 weeks	12 g collagen peptides	No	Stratum corneum hydration / Elasticity

* Fitzpatrick skin phototype; HC: hydrolyzed collagen; TEWL: transepidermal water loss; RT-PCR: reverse transcriptase polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay; IGF-1: insulin-like growth factor 1.

FIGURES

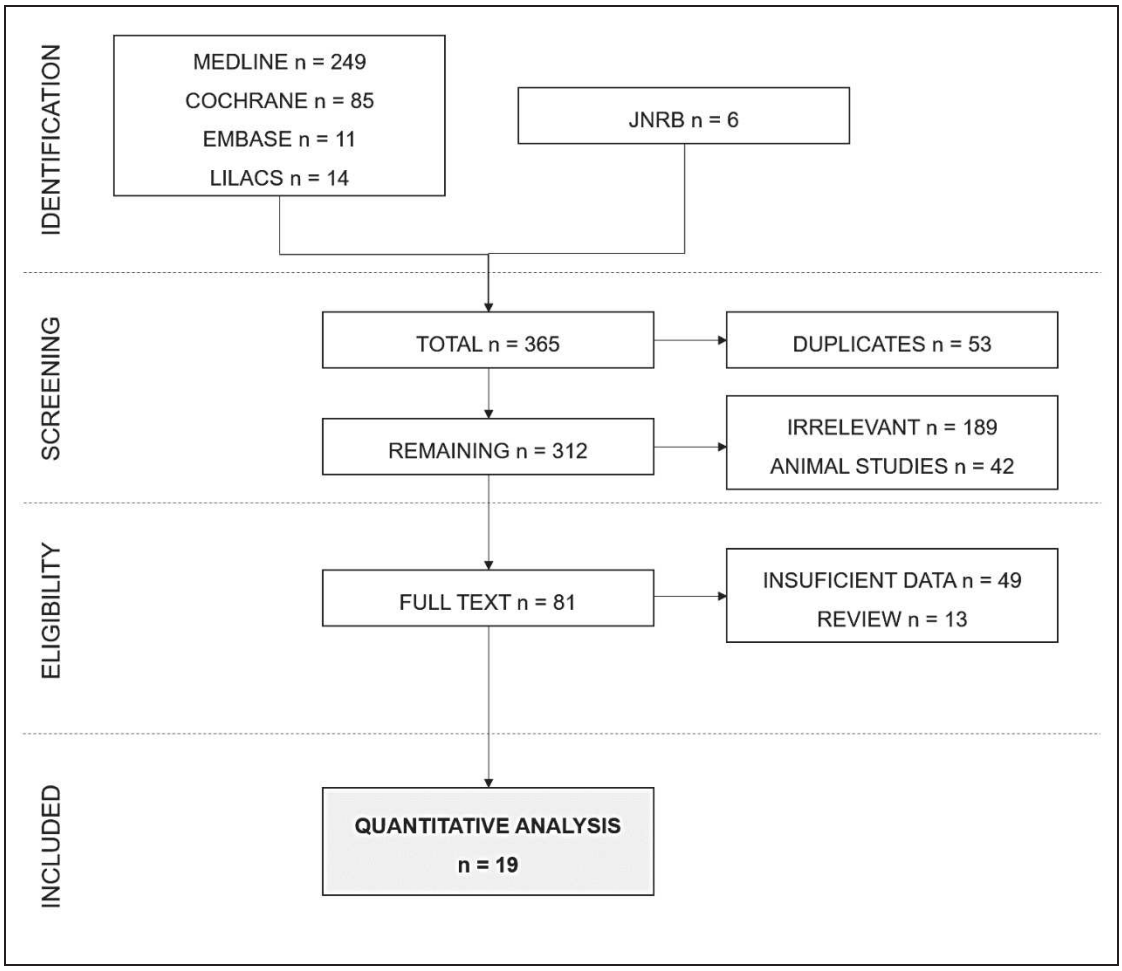

Figure 1: Flowchart of the included studies.

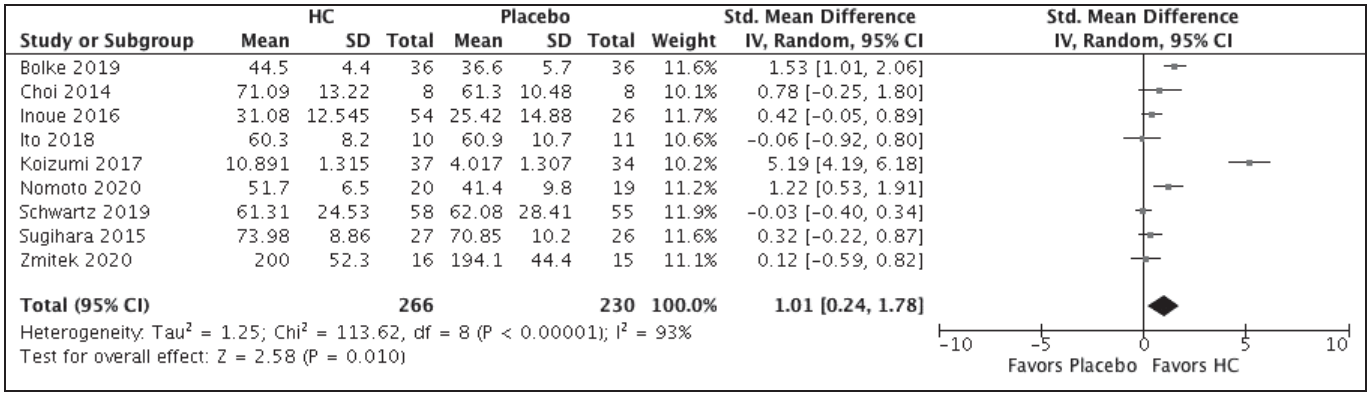

Figure 2: Forest plot for the combined estimate of the included studies evaluating skin hydration in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamond.

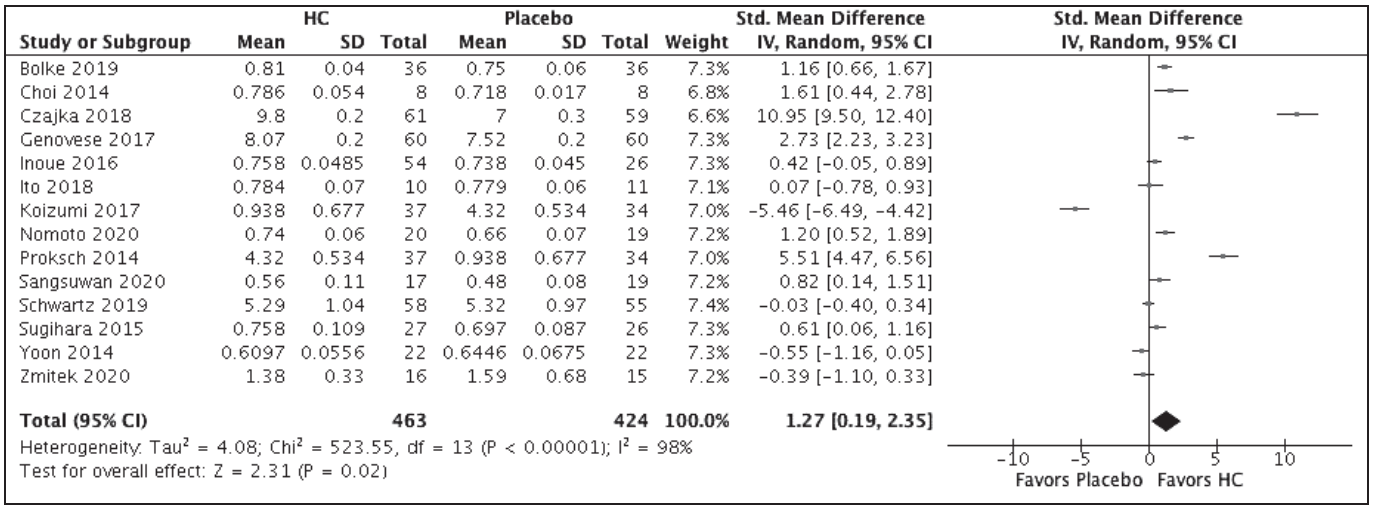

Figure 3: Forest plot for the combined estimate of the included studies evaluating skin elasticity in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamond.]

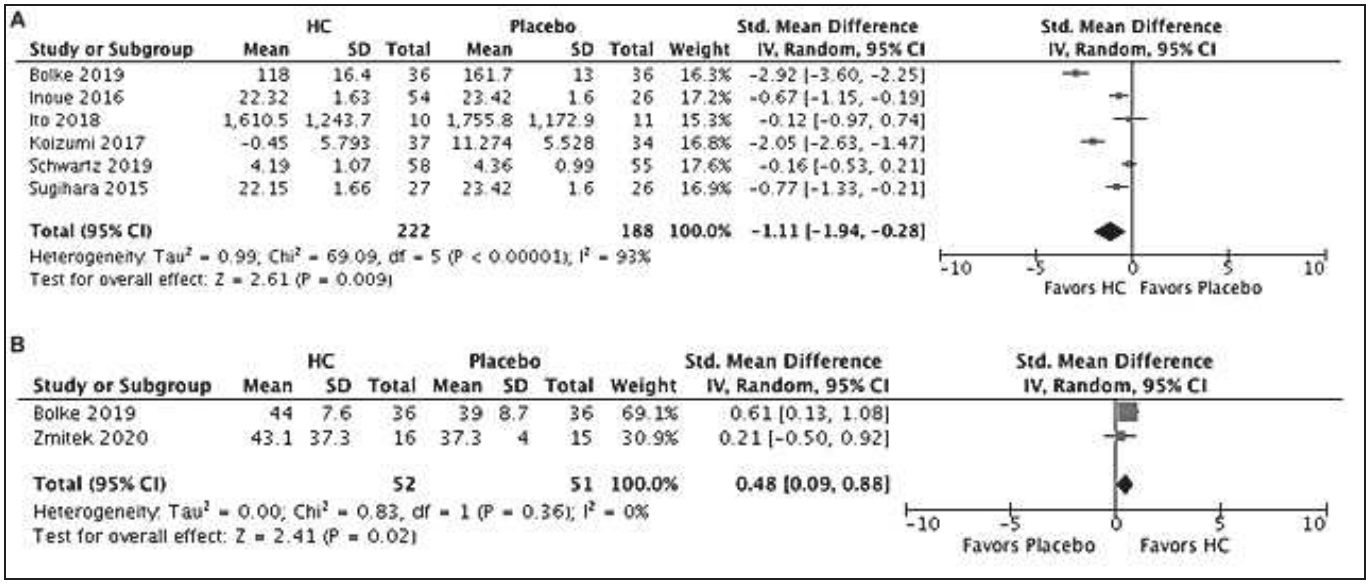

Figure 4: Forest plot for the combined estimate of the included studies evaluating skin roughness in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamond.

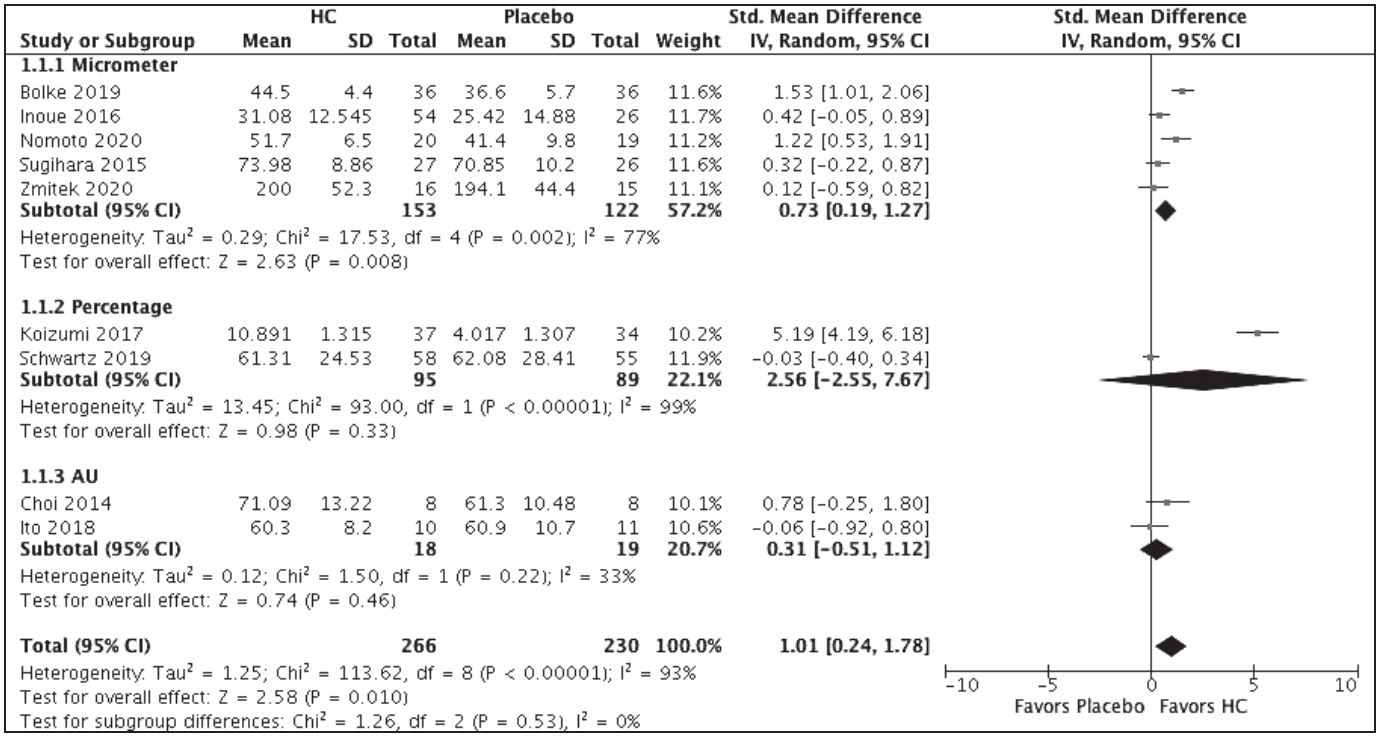

Figure 5: Forest plot for subgroup analysis of skin hydration expressed as micrometers, percentage, and arbitrary units (AU) in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamonds.

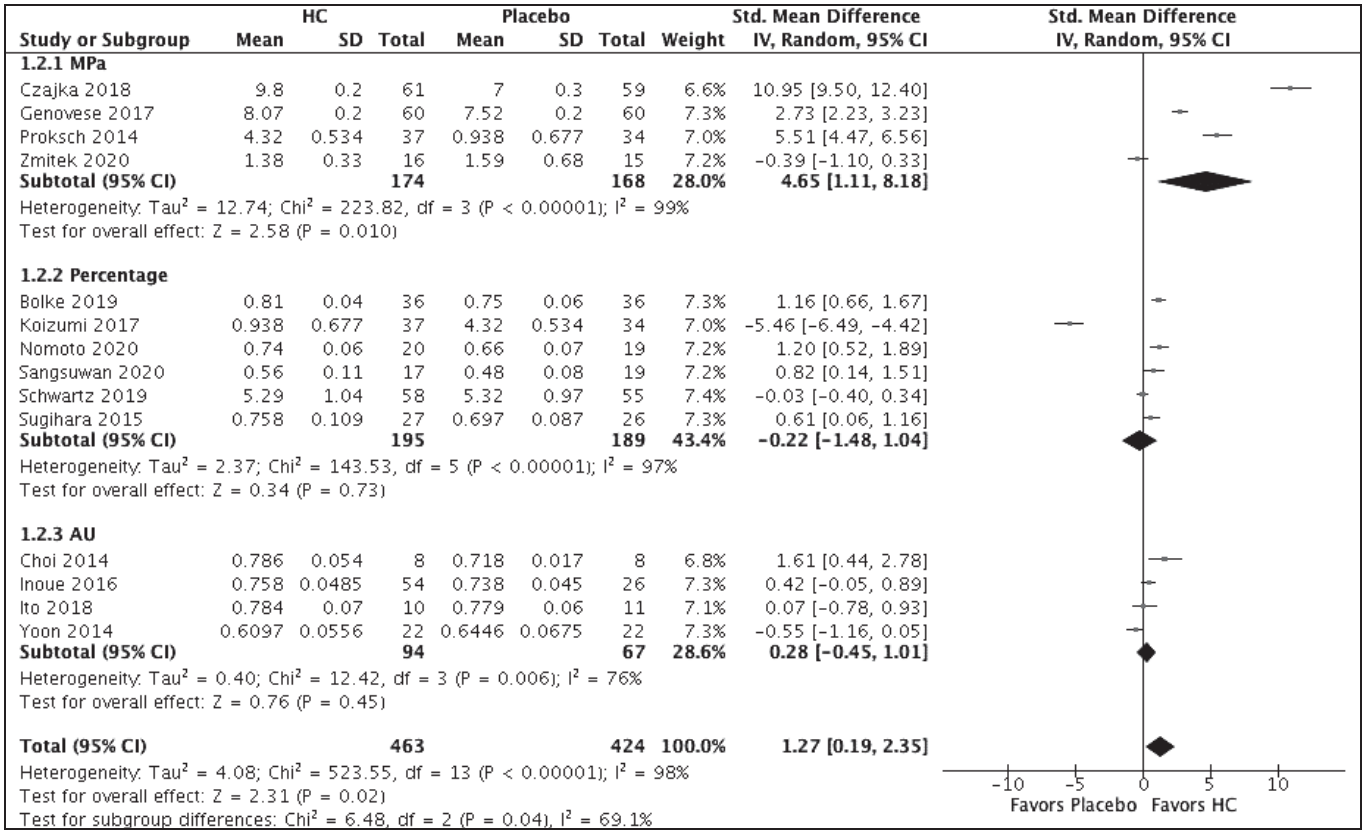

Figure 6: Forest plot for subgroup analysis of cutaneous elasticity expressed as MPa, percentage, and arbitrary units (A.U.) in patients supplemented with hydrolyzed collagen (HC) and patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamonds.

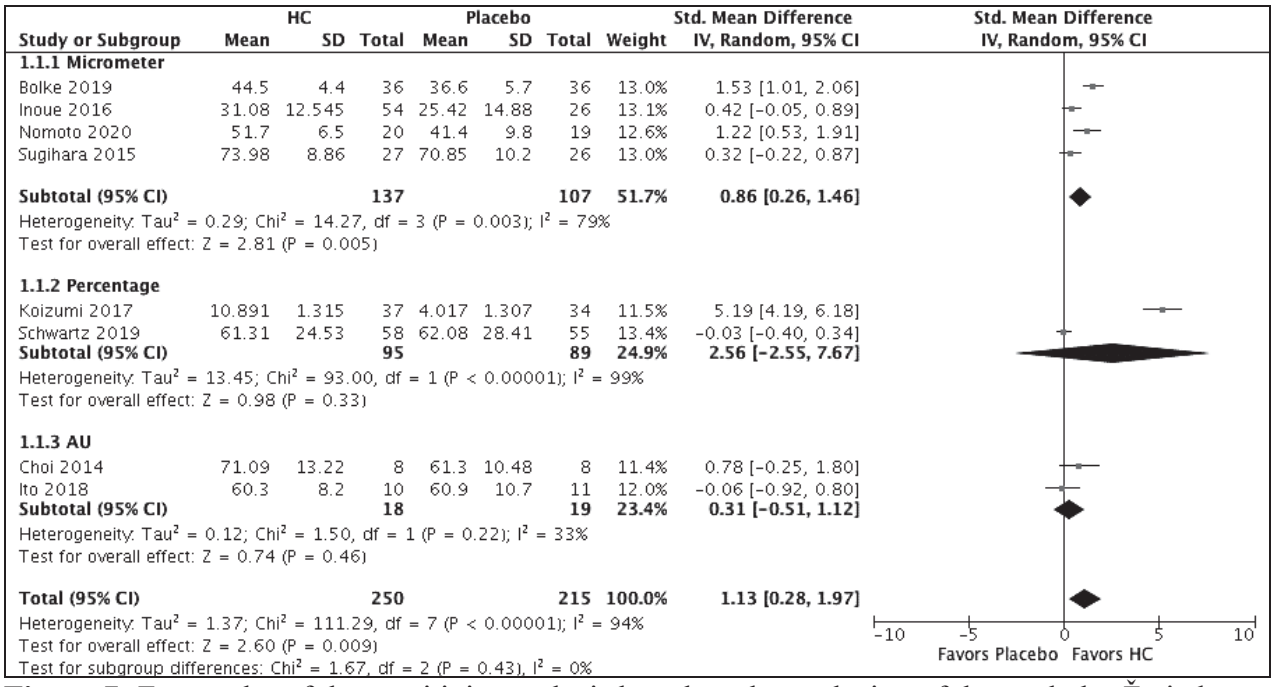

Figure 7: Forest plot of the sensitivity analysis based on the exclusion of the study by Žmitek et al. (2020) for cutaneous hydration: patients supplemented with hydrolyzed collagen (HC) vs. patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamonds.

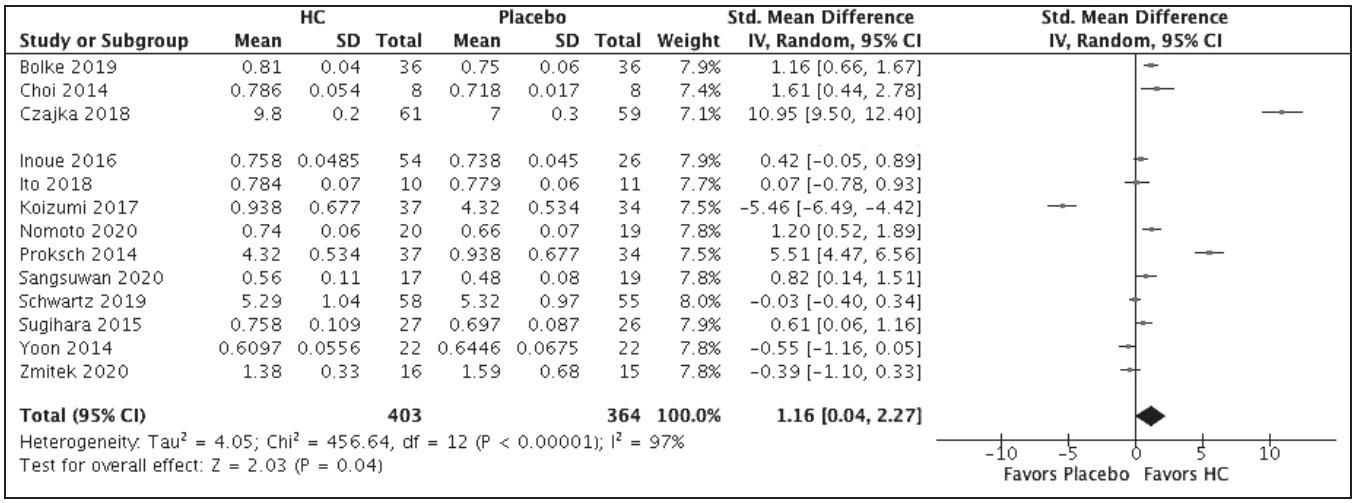

Figure 8: Forest plot of the sensitivity analysis based on the exclusion of the study by Genovese; Corbo; Sibilla (2017) for cutaneous elasticity: patients supplemented with hydrolyzed collagen (HC) vs. patients in the placebo group. The horizontal lines represent the effect size ± confidence interval (95% CI). The summary effect size is represented by the diamonds.

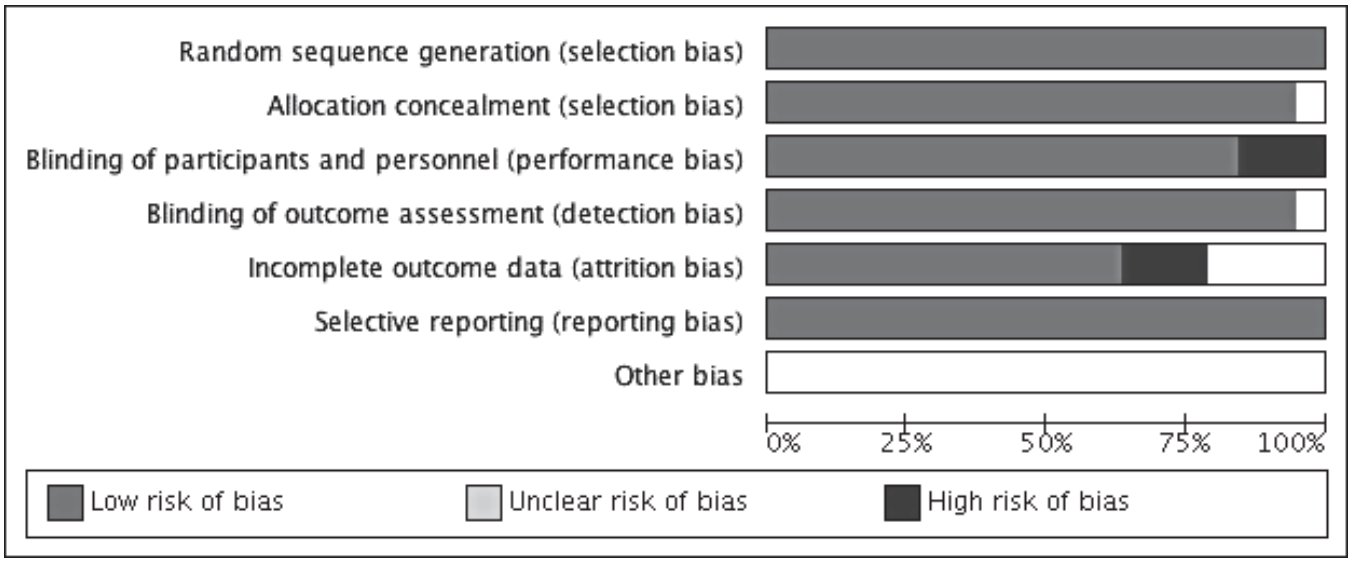

Figure 9: Graph of risk of bias for each study according to the five domains defined by RoB-2.

Random sequence generation (selection bias)
Allocation concealment (selection bias)
Blinding of participants and personnel (performance bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective reporting (reporting bias)
Other bias
Asserin 2015
Bolke 2019
Campos 2015
Cerbo 2014
Choi 2014
Czajka 2018
Genovese 2017
Inoue 2016
Ito 2018
Koizumi 2017
Laing 2020
Nomoto 2020
Proksch(2) 2014
Proksch 2014
Sangsuwan 2020
Schwartz 2019
Sugihara 2015
Yoon 2014
Zmitek 2020

Figure 10: Graph of risk of bias at the study level.

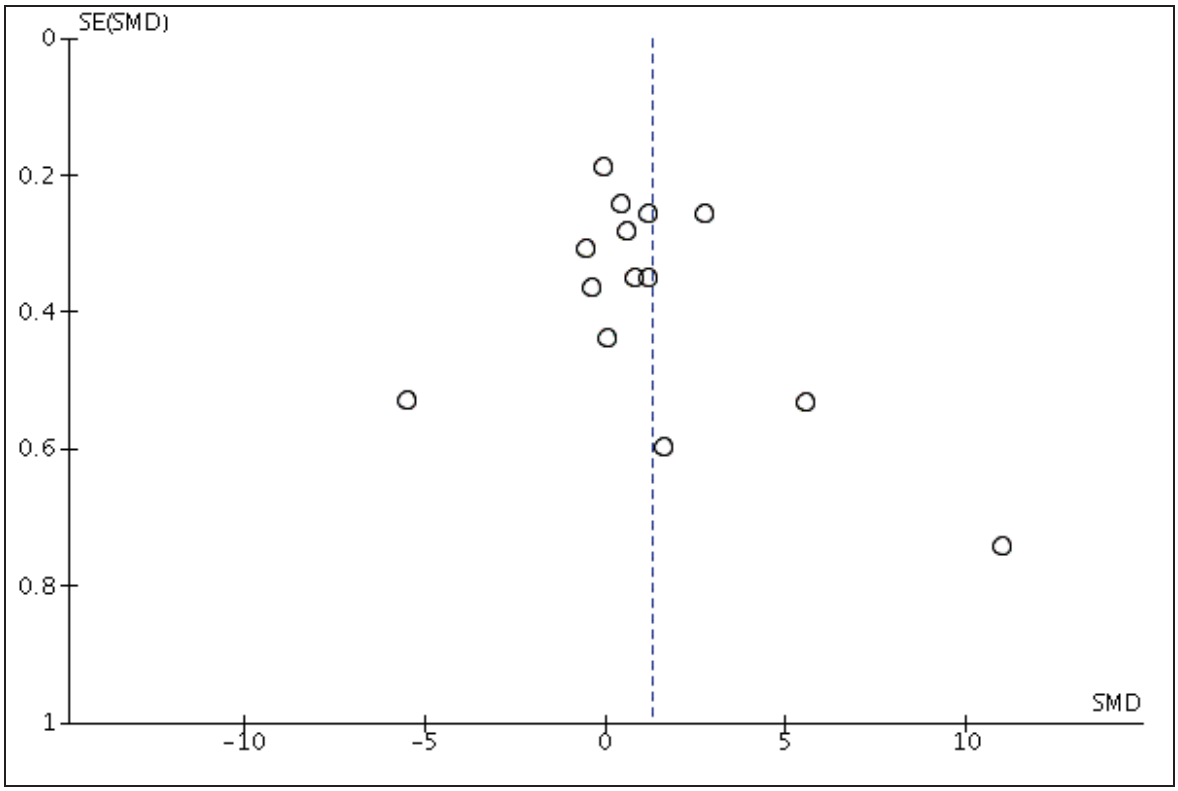

Figure 11: Funnel chart for publication bias.

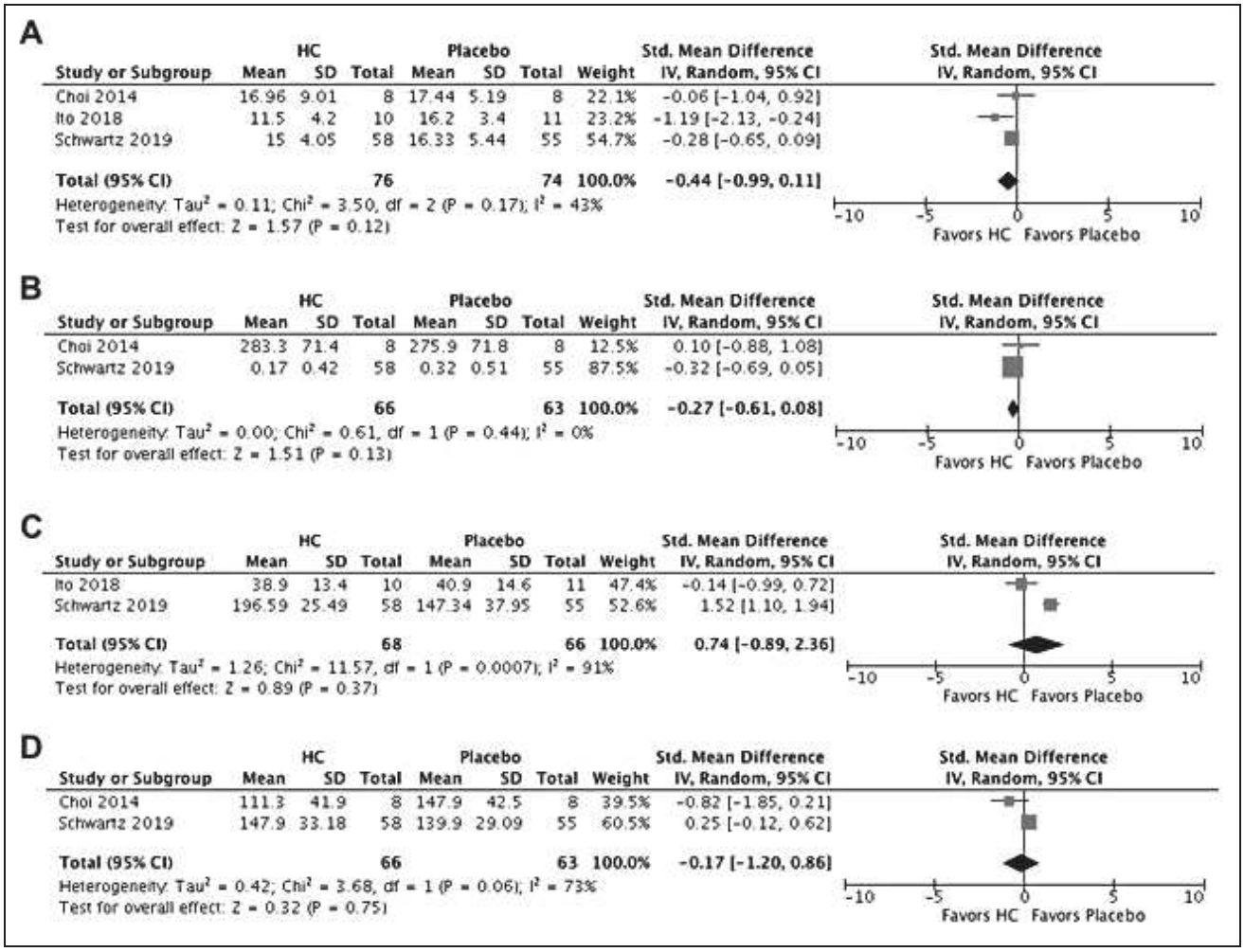

Figure A.1: Forest plots for combined estimate of included studies evaluating **(A)** transepidermal water lost (TEWL), **(B)** cutaneous erythema, **(C)** collagen levels, and **(D)** melatonin pattern of patients supplemented with hydrolyzed collagen (HC) and patients of placebo group. The horizontal lines represent the effect size ± the confidence interval (CI 95%). Summary effect size is represented by the diamond.