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A Review of the Literature Relating to Collagen Hydrolysate and Its Potential Clinical Applications

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

The increasing commercial necessity to valorise commodities such as gelatine has led to significant developments in its processing and the outcome of these refinements has resulted in new applications in fields such as pharmaceuticals, medical devices, cosmetics, food and nutraceuticals. This in turn has led to the investigation of alternative sources of compounds with collagen-like properties, other than the conventionally used raw materials from mammalian species. Moreover, the current desire to seek natural, rather than synthetic compounds-especially regarding oral consumption and/or topical application-combined with the ability of gelatine derived products to form gels with varying degrees of flexibility and hydroplasticity has also accelerated research into previously unexplored applications. In the food sector, these include:- use of gelatine derivatives as an encapsulating agent (including the development of micro-beads as carriers of active compounds) foaming agents, emulsifiers, biodegradable films, colloid stabilizers and as nutraceuticals. The latter sector has especially benefitted from developments in enzymatic hydrolysis processes, where specific and highly characterised bioactive peptides often containing the amino acid hydroxyproline are end-products which have been identified to be orally bioavailable and metabolised and hence likely to deliver potential clinical benefits. This review examines manufacturing processes employed to typically produce hydrolysed collagen, evaluates studies examining bioavailability, metabolism and likely health benefits as well as potential clinical applications as a nutraceutical.

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1. INTRODUCTION

Collagen is becoming a popular nutritional supplement for a number of potential applications, ranging from joint and skin health to sarcopenia in the elderly. However, there are a multitude of different product offerings ranging from undenatured collagen to numerous hydrolysates from various sources, often at significantly different retail prices points and frequently claiming contrasting levels of efficacy with disparate suggested dosing regimens. This review examines the literature relating to the use of collagen and its different forms in the three most commonly used situations which are described above. It investigates the data that substantiates use in these applications, identifies the form most probable to be of benefit and the preferred dose required to deliver a degree of optimal efficacy and the time period over which this is most likely to happen. It also offers some further criteria that should be of relevance to clinicians and consumers in terms of selection of product types.

2. STRUCTURE

In mammals, collagen is the predominant protein which provides both mechanical and structural support to many tissues [1,2]. Collagen exists in the body as distinct molecular complexes that deliver various functions such as providing strength to structures like tendons, or occur in large sheet like forms to provide resilience and support to internal organs and the skin. In teeth and bones, collagen can be found complexed with crystals, in the form of hydroxyapatites. The aging process results in collagen becoming increasingly crosslinked, which can ultimately affect the mechanical properties of the tissue of which it forms a part.

Within the family of collagen compounds, 28 variants that are genetically distinct have been identified. The three major classifications are: type I (bone, tendon and skin), type II (cartilage), and type III (vasculature and skin). These various types are typically present as fibrillar structures which are essential components of tissue integrity and architecture.

Generally, collagen chains contain around one thousand amino acids in the form of an α-helix configuration. Within this structure, covalent bonds between atoms hold the individual chains together, whilst the overall triple-helix structure is maintained by weaker bonds [3-5]. A repetitive sequence of three amino acids forms the primary structure. The amino acid glycine appears at every third position, where its' small size allows it to fit neatly within the helix. Generally, the other positions within the chain are occupied by two other amino acids, namely proline and its variant hydroxyproline. The latter is modified from proline after the chain is constructed and confers additional stability to the structure. Within each fibril various intermolecular and intramolecular forces stabilise the collagen, whilst within the triple helix this role is performed by hydrogen bonds. These various electrostatic interactions and the alignment between molecules are key to the definition of the structure and the crosslinks are important in delivering stability to the structure.

Within tendons, the structures are packed in a lateral formation, whilst in tissues like skin, the fibrils are packed closely, but exhibit more fluid like characteristics. The geometry and packing of the fibrillar superstructure is responsible for defining the physical attributes of a ligament or tendon and confers a high level of strength in the tissue along its axis where fibrils are preferentially aligned. Once heated, the chains unravel and the triple helix unwinds. Upon cooling the mass of denatured, tangled chains absorbs any water that surrounds it. This type of denatured collagen, essentially a mix of water-soluble proteins, is known as gelatine. As a partially degraded form of collagen, gelatine typically binds more moisture, due to the availability of a greater number of active groups to be exposed to water and hence the ability to interact through hydrogen bonds.

Because gelatine and collagen have a high degree of application in the body, they are frequently used in the pharmaceutical, medical and cosmetic industries as biomaterials. Gelatine is the cheaper of the two materials and soluble in water, whereas the high degree of cross linking of collagen means it is usually insoluble in oil and water. As a result, to overcome these limitations, collagen is usually reduced to smaller peptides by a process of hydrolysis. Type I, is the most commonly used form of collagen, which is extensively used in a number of applications.

2.1 Hydrolysed Collagen Production

Bovine bone, hide, pig or fish skin or fishbones are the predominant sources used to

manufacture collagen hydrolysates (CH) which occurs in a controlled process of hydrolysis that delivers soluble forms of peptides. These raw materials are washed, homogenized and demineralized in a dilute alkali or acid environment, and then subsequently extracted in a staged process using warm water. Degradation with food grade enzymes produces the collagen hydrolysate [6,7]. As a result of the manufacturing process used, these hydrolysates will vary dependent on the molecular weight of the peptides produced, but generally they tend to be in a range between 2-6 kDa [8]. The product is then purified, concentrated and dried. The most commonly used procedures after drying relate to those that deliver some conformity to molecular size and to reduce or eliminate any bitterness in the final product.

Ultrafiltration is the most effective procedure to remove any remaining unwanted peptides and proteins with higher molecular weights or to minimise the antigen content of formulas that need to be hypoallergenic [9]. Typical quality control procedures will examine and analyse: - the degree of hydrolysis; osmolarity; conformity and distribution of molecular weight; and the total amino acid/nitrogen content.

2.2 Properties and Applications of Hydrolysed Collagen

There is a significant body of evidence demonstrating both collagen hydrolysates and gelatine have a number of beneficial biological functions. In US, the Food and Drug Administration (FDA) have designated hydrolysed collagen as generally recognized as safe (GRAS) for use in food products or as food additives. Although, hydrolysed collagen does not contain a full complement of all the essential amino acids, because of its high level of consumer tolerance, digestibility and variety of applications it is commonly used as a food supplement.

2.3 Bioavailability of Hydrolysed Collagen

Using ^{14}C-labeled material, the profile of enteral absorption over time as well as the subsequent distribution in various tissues of collagen hydrolysate has been investigated [10]. Within 12 hours of administration 95% of collagen hydrolysate was absorbed. Concentration in the skin reached a peak within 12 hours, whilst presence was detected in plasma at relatively high levels for up to 96 hours. Peptides of a molecular weight of 1-10 kDa were detected on the serosal side of the intestine, suggesting that absorption of collagen hydrolysate as large molecules may occur to some extent.

Iwai et al [11] investigated the bioavailability of collagen hydrolysate in healthy human volunteers who consumed the product after 12 hours of fasting and found that a significant amount of hydroxyproline (Hyp) in a peptide form attributable to collagen appeared in blood. Those peptides containing hydroxyproline increased in concentration quickly after intake and reached a peak after two hours, decreasing to 50% of maximum after four hours. There was also a difference between type I and type II collagen structures of the food-derived peptides present in the blood. The small peptide proline-hydroxyproline (Pro-Hyp) was identified as being present in the blood after intake of both the collagen types. The higher concentration in the blood of Pro-Hyp could partly be explained by the relatively greater presence of this dipeptide sequence in the hydrolysate.

Other authors, [12] have compared the presence in human blood of structures and quantity of hydrolysates from three different sources of type I collagen in a single blind crossover study in healthy males. Five volunteers ingested type I collagen hydrolysates after fasting for 12 hours. Over the next period of 24 hours, the quantity of Hyp-peptides derived from the hydrolysate comprised approximately 30% of all the Hyp detected compounds. The authors suggest that structure and quantity peptides containing Hyp derived from hydrolysate in the blood depends on the source of collagen.

Hence, whereas conventional thinking suggests the peptides in collagen are predominantly assimilated in the gastrointestinal tract into free amino acids that are then absorbed and enter into the circulation [13], it is clear there is evidence that suggests intact peptides are also absorbed. For example, hydroxyproline can be absorbed both as a peptide and as a free form amino acid, although dependent upon the collagen source, the amount in the former species can differ. After oral consumption of any source of hydrolysed collagen, the major peptide identified in plasma in humans is proline-hydroxyproline (Pro-Hyp).

Postlethwaite et al used a human dermal fibroblast model to establish that cells in the skin were able to respond to the presence of

collagen-derived peptides derived from supplements and also to quantify the effect [14]. Using this model, they identified that when bacterial collagenase digested collagens of either type I, II, or III collagens, the peptides released as a result of this process attracted fibroblasts to the observed site. Given that at the sites of tissue inflammation and injury, collagen is typically degraded and remodelled, these results indicate supplemental collagen and peptides produced from it might function as a stimulus to attract fibroblasts in vivo to damaged tissue and effect its' repair. It therefore appears that Pro-Hyp derived from oral hydrolysed collagen supplementation might act as a re-organizing influence in the extracellular matrix and as a messenger to initiate and stimulate fibroblast cells.to synthesize new collagen fibres.

2.4 Health Benefits of Hydrolysed Collagen

Given the evidence that collagen hydrolysates appear to be bioavailable, it is logical that oral supplementation delivers clinical benefits as a result of constituent bioactive peptides being able to cross the intestinal barrier and enter the circulation to become available for various cellular processes or satisfy metabolic needs [15]. Hence, collagen hydrolysates are frequently used as medical foods, such as geriatric formulations to benefit sarcopenia, high-energy protein supplements, and in therapeutic, enteric or weight-control formulas. Collagen hydrolysates in the form of dietary supplements are used as agents to improve joint and tendon regeneration, as well as to relieve joint pain [16]. Following intestinal absorption, supplementation with collagen hydrolysate has been shown to accumulate in cartilage and stimulate a significant level of synthesis by chondrocytes of macromolecules in the extracellular matrix [17]. As well as contributing to the synthesis of cartilage matrix [18], collagen hydrolysates have a high degree of safety (1.66 g/kg of body weight per day) as demonstrated by Wu et al [19]. The chemotactic action of the peptides, proline-hydroxyproline-glycine (Pro-Hyp-Gly) and Pro-Hyp to peripheral monocytes, blood neutrophils as well as human fibroblasts has been identified by Zague [20] in cell culture. Peptides degraded from collagen might therefore attract these types of cells and effect the repair of any tissue that is damaged. A key consideration relating to the effectiveness of a supplement containing collagen hydrolysate in the skin is its ability to deliver a gradual enhancement in the absorption of water in the skin. Additional benefits have also been observed for hair and nail quality. Antioxidant activities of peptides isolated from collagen hydrolysates have also been reported as have peptides capable of inhibiting angiotensin-converting enzyme [21].

2.5 Preclinical Studies with Hydrolysed Collagen

Studies have identified that collagen hydrolysate when administered orally is effectively absorbed and circulates in the blood stream, reaching a maximal concentration in plasma within six hours, after which time less than 10% still remains in the GI tract [22]. It is also known that subsequent to oral administration of collagen hydrolysates are not entirely broken down in the digestive tract, and a number of fragments of the parent compounds, including as much as 10% of high molecular form fragments ranging from 1 to ≈10 kD, are absorbed, albeit with a degree of variability [23]. Indeed, in those studies that used radiolabelled collagen hydrolysate have shown a significant quantity of peptides derived from supplements reach cartilage tissue intact within around twelve hours of administration compared to controls ($p < 0.05$).

Cell culture investigations of the efficacy of collagen hydrolysate in the biosynthesis of collagen in articular chondrocytes, have identified that exposure to a concentration of 0.5 mg/mL over a period of eleven days resulted in a statistically significant increase in the synthesis of type II collagen synthesis in the chondrocytes compared with controls ($p < 0.01$) [24]. In contrast, this was not found with native collagens. These results demonstrate the effect that collagen hydrolysate can induce in stimulating synthesis of type II collagen in chondrocytes. Additionally, proteoglycan concentrations also increased significantly after administration of collagen hydrolysate ($p < 0.05$) [25].

Furthermore, it has been found that collagen hydrolysate supplementation results in a non-significant effect on the upregulation of proteases in chondrocytes. This evidence suggests collagen hydrolysate can be absorbed in the GI tract in high molecular weight forms, accumulate preferentially in cartilage [25], and stimulate chondrocyte metabolism. It therefore appears reasonable to conclude that use of collagen hydrolysate as a nutritional supplement in humans will likewise activate biosynthesis of

collagen, especially in those circumstances and situations where cartilage experiences undue stress [24].

3. CLINICAL STUDIES

3.1 Hydrolysed Collagen for Osteoarthritis Pain

A number of open label and double blind placebo controlled studies have been performed to investigate the putative benefits of collagen hydrolysate in this condition [25-34]. A publication in 1979 demonstrated the effect of 5-7g of collagen hydrolysate in degenerative disease of the joints in patients with osteoarthritis of the knee with femoral, retropatellar or tibial involvement, or with degenerative disease of discs in specific spinal areas for one to six months. Of the 56 patients, 24% reported 'very good success' (half identified 'complete freedom from pain' and the remainder reported 'improvement in their general condition'); 44% reported 'noticeable improvement' (two thirds of these identified the 'general situation improved considerably' and six participants noted pain had substantially diminished), and 32% found 'no improvement' [25].

These findings were similarly reflected in a study in 1982 where 60 juvenile patients diagnosed with retropatellar osteoarthritis received 7g collagen hydrolysate, 24 000 units of vitamin A as well as 120 mg of L‑cysteine orally for 3 months [26]. Various parameters were assessed, including soft tissue swelling, knee effusion, retropatellar crepitus and the ability to climb stairs. Upon completion, 75% of participants reported improvement, 45% were free of symptoms and after 3 months symptoms had clearly improved in 30% of patients.

A further open-label trial examined the effect of collagen hydrolysate in 154 subjects with osteoarthritis of the knee, hip, or lower spine [27]. Patients were randomized into three cohorts: therapeutic exercises together with the above formulation of collagen hydrolysate with vitamin A and L‑cysteine taken daily, or the latter supplement without therapeutic exercise, or therapeutic exercises alone. The treatment period for all groups was over three months. In the therapeutic exercise group, 20% reported a 'very good' or 'good' response, whilst in the group using the supplement with exercise, 56% had the same level of response and 69% of the supplement without exercise cohort identified a 'very good' or 'good' response. Further, whereas 43% of subjects receiving only physical therapy were 'unchanged', only 14% of the supplement with physical therapy, and 6% of the group receiving supplement alone reported this outcome.

Adam et al [28] recruited 81 participants in a prospective, placebo-controlled, double-blind, randomised study in patients with osteoarthritis of the hip or knee using a cross- over design to assess four different supplements, including 10g of oral collagen hydrolysate. Compared with 23% of those taking egg albumin, 81% of patients receiving collagen hydrolysate experienced meaningful reduction in pain, and 69% of the latter group also reported a ≥50% reduction in their consumption of analgesics. The authors noted the outcomes of treatments were statistically different according to the Lechmacher test and whereas egg albumin had an 'insignificant' effect, patients receiving collagen hydrolysate supplementation experienced a symptoms reduction that was 'substantial'.

A randomized, double-blind, placebo-controlled, study investigated the effects of collagen hydrolysate in osteoarthritis. Using American College of Rheumatology criteria, 250 adults diagnosed with mild symptoms of osteoarthritis of the knee were recruited. The protocol assessed the effectiveness of 10g of oral collagen hydrolysate daily compared to placebo over 14 weeks. A Biodex Multi-Joint System B2000 was used to assess isometric and isokinetic leg strength [29]. A 50-Foot Walk Test and a 6-Minute Walk Test were used as measures of functional mobility, and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Index, the Lequesne Index, and the Knee Pain Scale of joint pain/stiffness were used to evaluate perceived functional mobility. At the end of the trial, compared to placebo, the group treated with collagen hydrolysate showed statistically significant improvement in three out of six measures of isokinetic leg strength ($p < 0.05$), whilst the remaining assessments approached statistical significance ($p = 0.067$). The greatest benefits were apparent in those tests that generated the highest levels of stress to the joint structure [29]. Hence these findings suggest that collagen hydrolysate may be of benefit in limiting the early changes that might occur in knee cartilage, reflecting the outcomes from preclinical data. The study also suggests objective isometric and isokinetic assessments

might be a more sensitive evaluation of initial improvements in function of the joint than questionnaires about mobility and pain.

A further prospective, double-blind, randomized, placebo-controlled study was conducted by Moskowitz et al. across 20 sites in UK, Germany and US between 1996 and 1998 [30]. 389 patients with osteoarthritis of the knee were recruited, and were randomized to receive either placebo or 10 g of collagen hydrolysate daily over 24 weeks. The measures used to assess primary outcomes were function score, patient global assessment, and WOMAC pain score. Only in the German Arm (112 patients) was a benefit from collagen hydrolysate identified as statistically significant in terms of functional improvement (p = 0.007) and pain reduction (p = 0.016) whist global evaluation (p = 0.074) tended to statistical significance. The reasons for the observed differences between countries was not explained, however dropout rates in the UK and US (respectively, 42% and 37%) were greater than those in Germany (6%). Other contributory factors might have been study conditions, differences in baseline, paracetamol intake, and specialist clinician training.

In 2012 Van Vijven and colleagues performed a systematic review [31] and assessed the results of six studies of collagen hydrolysate, two of gelatine and one on undenatured collagen following an assessment of methodological quality using the criteria of the Cochrane Central Register of clinical trials. It found some studies identified between group differences in pain as statistically significantly when assessed using a visual analogue scale or other objective measures, or when studies compared collagen hydrolysate with glucosamine sulphate.

However, a more recent meta-analysis evaluated the effect of collagen-based supplements on osteoarthritis symptoms. This study searched databases for randomized placebo-controlled trials that assessed the effect of oral collagen hydrolysate on symptoms of osteoarthritis using Visual Analog Scales (VAS) and/or the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scale [32]. Treatment with collagen hydrolysate demonstrated a statistically significant reduction in the total WOMAC index score (p = 0.002). Subgroup analysis of these scores identified a decrease in stiffness that was statistically significant (p = 0.01). Finally, collagen hydrolysate supplementation also resulted in a significant reduction in the VAS score. These outcomes resulted in the authors concluding the meta-analysis demonstrates supplementation with collagen hydrolysate is significantly effective in improving symptoms of osteoarthritis when assessed using both VAS and total WOMAC index scores.

In a double blind, randomized, controlled multicentre study Benito-Ruiz et al. investigated the effects of 10g of collagen hydrolysate (Colnatur) administered orally over 6 months in 250 patients with primary knee osteoarthritis [33]. Knee comfort (as assessed using the WOMAC pain subscale and a visual analogue scale to evaluate pain) was significantly improved. Patients diagnosed with the most severe deterioration in their joints and those habitually consuming an intake of at least one meal containing meat protein experienced most benefit. The authors concluded collagen hydrolysate is effective and safe as a food supplement for the treatment of osteoarthritis.

Kumar et al used a placebo controlled, double-blind, randomised trial to study the effectiveness of oral collagen peptides isolated from bovine bone and pork skin sources in 30 subjects aged between 30 and 65 years with osteoarthritis of the knee over 13 weeks [34]. Assessments included changes in visual analogue scale (VAS) and quality of life (QOL) scores and Western Ontario McMaster Universities (WOMAC) from baseline over the period of the study as well as for tolerability and safety. There was significant improvement in VAS and WOMAC scores as well as those for QOL score in all subjects taking the collagen peptides compared to placebo, with all scores decreasing significantly (P<0.01) in the groups receiving active treatment by the end of the trail.

Mcalinden et al used both magnetic resonance imaging techniques-delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC), or T2 mapping-to determine any short-term alterations in knee hyaline cartilage among participants consuming oral collagen hydrolysate [35]. The study was prospective, double-blind, randomized, and placebo-controlled in 30 patients with mild osteoarthritis of the knee. Outcomes included change in dGEMRIC T1 relaxation time in the cartilage regions of interest after 24 and 48 weeks compared to baseline, changes in T2 relaxation time over the same timeframe, as well as functional and symptom measures taken at each visit, and overall use of analgesics. After 24

weeks, the dGEMRIC score increased in the medial and lateral tibial regions of interest (median increase of 29 and 41 ms respectively) in subjects consuming collagen hydrolysate and decreased (median decline 37 and 36 ms respectively) in those taking placebo, a difference that was statistically significant. These results suggest this magnetic resonance technique may detect changes in the content of proteoglycan in the cartilage of the knee cartilage that occur in individuals taking collagen hydrolysate over 24 weeks.

Lugo et al evaluated the tolerability and efficacy of randomly allocated oral doses of either undenatured type II collagen UC-II compared to 1500mg of glucosamine hydrochloride plus 1200mg chondroitin sulphate (GC) and placebo in relieving pain and associated symptoms in osteoarthritis of the knee in 191 participants over 180 days [36]. Assessments included changes in total Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) scores and subscales, the Visual Analog Scale (VAS) for pain, and the Lequesne Functional Index (LFI) at the end of the trial compared to baseline. At the end of the trial, the collagen group demonstrated a significantly reduced overall WOMAC score compared to both the glucosamine and chondroitin group ($p = 0.04$) and placebo ($p = 0.002$). Collagen supplementation also resulted in significant changes for all three WOMAC subscales: pain ($p = 0.0003$ vs. placebo; $p = 0.016$ vs. glucosamine and chondroitin); stiffness ($p = 0.004$ vs. placebo; $p=0.044$ vs. GC); physical function ($p = 0.007$ vs. placebo). There was no difference in assessments of safety between the groups and the authors concluded collagen was well tolerated and improved symptoms of osteoarthritis of the knee.

In another double-blind, randomized, placebo-controlled trial Schauss et al investigated the efficacy and tolerability of a low molecular weight hydrolysed collagen in 80 patients with symptoms of progressive osteoarthritis of the knee and/or hip joint and with baseline pain present at level 4 or higher as evaluated using Physician Global Assessment scores for at least three months at the time of entering the study [37]. Subjects received either hydrolysed collagen or placebo for 70 days. Outcome measures included Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores and visual analogue scale (VAS) for pain assessed on days 1, 35, and 70. Significant reduction of WOMAC scores were reported on both days 35 ($p = 0.017$) and 70 ($p < 0.001$) and for VAS pain on day 70 ($p < 0.001$) in the collagen treated group with tolerability comparable to placebo. The treated group also experienced a significant improvement in physical activities on days 35 ($p = 0.007$) and 70 ($p < 0.001$) compared to those receiving placebo, thereby suggesting hydrolysed collagen to be effective in managing osteoarthritis associated symptoms over the study period as well as improving patient's daily living activities and hence to be a potential complement to more conventional treatments in this condition.

3.2 Use in Other Populations

The scientific literature also highlights the use of collagen hydrolysate in patient populations other than those diagnosed with osteoarthritis. For example, one observational study in 100 athletes, evaluated the effects of 10g of oral collagen hydrolysate each day over 12 weeks in those not diagnosed with osteoarthritis, but who experienced joint pain in the knee, hip or shoulder [38]. Those with an inflammatory condition and/or in the acute phase of a joint injury were excluded, as was anyone taking any medications other than Non-Steroidal Anti-inflammatory Drugs, corticosteroids, COX-2 inhibitors, or glucosamine or chondroitin or who experienced an interfering concomitant condition. Clinical measures of pain on movement, pain at rest, inflammatory activity and functional limitations were assessed by the clinician at baseline, at 4-6 weeks and at 12 weeks. Of those who completed the study- 88 individuals- 58% presented with knee arthralgia, 22.7% with hip arthralgia and 19.3% with shoulder arthralgia. 78% of patients experienced a reduction in pain after taking collagen hydrolysate for 12 weeks and the use of analgesics and other medications also decreased. Whilst at baseline 27 subjects were taking analgesics, at the end only 12 were using this class of medication and likewise those taking NSAIDs or COX-2 inhibitors reduced from 47 to 13.

3.3 Hydrolysed Collagen and Sarcopenia

In older patients diagnosed with sarcopenia, administration of collagen peptides has been demonstrated to increase the benefits of a resistance training programme over 3 months [39]. Here those consuming a collagen supplement experienced a greater increase in fat-free mass, a higher loss of fat mass and greater muscle strength than those using a

placebo. These results build on observations from an earlier investigation in an ageing population, which demonstrated resistance exercise delivers improved muscle strength, fat-free mass and co-ordination together with enhancing control of posture [40].

Despite the positive effects of this study there is still an ongoing debate regarding whether the anabolic benefits of resistance exercise in the elderly might be further increased as a result of supplementation with additional protein sources [41]. Nevertheless, studies clearly demonstrate that subsequent to resistance exercise the ingestion of dietary protein does stimulate rates of muscle protein synthesis in this group [42,43]. These observations are supported by a meta-analysis [44] which pooled the results of 22 studies in both older and younger subjects of the combined effect of resistance exercise and protein consumption and which indicates that the combined strategy enhances fat free mass gain as well as muscle strength in both groups of subjects. A 2013 review also supports the efficacy of use of protein supplementation, such as collagen hydrolysates, in the treatment of sarcopenia [45]. However, in the Zdzieblik investigation using collagen peptides, the increases in muscle strength and fat free mass as well as the decrease in fat mass appear to be more significant than in other studies [39]. Moreover, it is possible that confounders such as age, nutrition status, participant health, variations in design of the training programmes as well as diversity in the type of dietary protein supplementation intake used in combination with resistance exercise might have resulted in a heterogeneity in observations of efficacy on muscle strength and body composition in the elderly [46,47].

It has also been suggested the timing of intake of any protein supplement and/or its kinetic absorption might also exert an influence on efficacy [48]. Here, some research indicates a rapid digestion and fast kinetic absorption might affect any hypertrophy of muscle anticipated as a result of protein ingestion. Hence, it is suggested an anabolic window of 90–120 minutes exists for optimal effects on anabolism post-exercise which begins to close after that time [49,50]. However, in the study by Zdzieblik et al, collagen peptides were consumed within one hour of training, and it may be that that ingestion at such a short relative time, together with their fast digestibility and rapid absorption might have accelerated post-exercise muscle protein anabolism. This may also be due to the fact that collagen contains significant amounts of glycine and arginine, which are both known to be key substrates in the synthesis of creatine. Additionally, protein supplementation appears to deliver an increased perfusion of the microvasculature, and hence increases delivery of amino acids which can enhance anabolic responses [51]. Given that collagen peptides have been demonstrated to influence microcirculation positively [52,53], this may result in greater effects in enhancing growth of muscle in comparison to other protein sources. Furthermore, since collagen peptides have been demonstrated to reduce both osteoarthritis pain significantly, as well as relieve functional joint pain [54,55], this could result in those supplemented with them experiencing less pain whilst performing resistance exercises, and hence be more responsive to training.

3.4 Collagen Peptides, Bone Mineral Density and Bone Markers in Postmenopausal Women

A twelve month, randomized, placebo-controlled, double-blinded trial demonstrated that collagen peptides significantly improved bone mineral density in postmenopausal women in the femoral neck as well as the lumbar spine in comparison to participants in the placebo group. In comparison to the decrease in bone mineral density that occurred in the placebo cohort, subjects receiving collagen peptides showed a 4.2% greater improvement in this parameter in the spine and a 7.7% benefit in the femoral neck, indicative of a clinical relevance [56]. The anabolic effect of collagen peptide supplementation was also confirmed by a significant increase in amino-terminal pro-peptide of type I collagen (P1NP), a surrogate marker of bone formation.

Two other studies have also reported effects of supplementation with collagen peptides on and bone mineral density and bone markers in humans. In one study, the benefits of collagen peptides, both with and without calcitonin were investigated [57]. The authors suggested their results indicate that collagen peptides together with calcitonin exerted a greater effect on inhibiting breakdown of bone collagen. The second study investigated the effectiveness of collagen and/or vitamin D and calcium. It found that that bone mineral density loss was significantly lower in the group supplemented with collagen than in that using vitamin D and calcium [58]. The rationale to explain these

beneficial observations in bone mineral density may be identified in the findings of experiments in cell-lines as well as preclinical studies. Firstly, collagen peptides are quickly absorbed from the GI tract [59]. Additionally, collagen peptides are significantly absorbed in the small intestine in the peptide form and as a result these entities might act as signalling molecules, that influence anabolic processes in a positive manner [60]. This especially appears to be the case in connective tissue, where this effect has been identified [61,62].

In addition, collagen constitutes the most significant part of bone and pre-clinical studies have established its peptides significantly boost the organic composition of bone [63]. Hence, this increase in the organic fraction together with a subsequent mineralization may result in an enhanced bone mineral density. In a study by Konig et al [64], bone anabolism and collagen synthesis were accompanied by increased levels of the bone marker P1NP in a group receiving collagen peptide supplementation, whereas in a control group there was a significant increase in the bone degradation marker, CTX1. Comparable results have also been found as a result of oral collagen peptide supplementation [65], and in addition, gelatine hydrolysates and/or collagen peptides have been demonstrated to increase the longitudinal bone growth. [66], inhibit bone loss and prevent bone loss in specific situations in other pre-clinical studies, possibly as a result of a reduction in proinflammatory cytokine levels [67]. Together, the data relating to the signalling characteristics and the results of pre-clinical studies of collagen hydrolysates contribute to explain the processes that effect these outcomes, but more human studies are undoubtedly needed.

3.5 Hydrolysed Collagen Ingestion and its Effects on Skin

The effects of collagen hydrolysate ingestion at two different doses was investigated on the extracellular matrix of the Achilles tendon in a pre-clinical study by Minaguchi et al [68] over a period of 56 days. Both doses resulted in a significant increase in collagen fibril diameter as well as a decrease in fibril density compared with control. However, whilst the higher dose resulted in a high percentage increase in collagen fibrils with a diameter of around 160–180 nm, the frequency of increase in fibrils with a diameter over 200 nm was highest for the lower dose. There was also an increase in dermatan sulphate in the high-dose group only. Although these effects occurred in tendons, it is likely the benefits will also extend to the skin, since in both tissues the major component of the extra cellular matrix is type I collagen.

In order to confirm whether the effect of collagen hydrolysate ingestion on skin functionis specific to collagen or simply the result of consumption of protein itself, one group of researchers [69] studied these effects on fibroblast density and diameter as well as the extracellular matrix of the dermis in a controlled study. Diameter and density of fibroblasts and the density of collagen fibrils were greater in the collagen hydrolysate treated group than in controls in a statistically significant manner. This suggests the effect of collagen hydrolysate was not merely dependent on an increased intake of amino acids. Decorin is present on the surface of fibrils as dermatan sulphate and transmits force to other interconnecting collagen fibrils. It also acts to resist compression, to facilitate fibril elongation and regulate the diameter of collagen fibrils and the ratio of dermatan sulfate was largest in the group treated with collagen peptides, suggesting that collagen peptide ingestion induces enhanced formation of collagen fibrils in the dermis and increases fibroblast density in a protein-specific manner.

The effect of daily ingestion of 10g of collagen hydrolysate along with 400mg vitamin C on the hydration of the skin in 20 healthy Japanese women was investigated by Sumida et al [70] and compared to a placebo group. Over sixty days a gradual improvement in the capacity of skin to absorb water absorption capacity was observed only in the group consuming collagen hydrolysate. Here it may be that the effect of collagen was potentiated by vitamin C, but nevertheless results indicate that the unique amino acid and peptide profile of oral supplementation of collagen may be responsible for the positive observations on skin physiology.

The European Food Standards Agency (EFSA) reviewed the data relating to supplementation using collagen and skin elasticity and came to a negative conclusion. However, although the data submitted did not meet the standards of scrutiny demanded by EFSA-because it contained pre-clinical rather than clinical studies-it did further demonstrate the benefits of this intervention in skin health. In one double-blind, randomised, placebo-controlled study, that was considered,114 women received 2.5g of a collagen hydrolysate supplement daily or a

placebo over 8 weeks. The primary favourable outcome was the volume of eye wrinkles, whilst those of a secondary nature were the contents of fibrillin, elastin and fibrillin and type I pro-collagen in suction blister biopsies [71]. In another study of a similar construct, 69 women received either 2.5 or 5 g of a collagen hydrolysate daily or a placebo over 8 weeks. Primary outcomes of the study were skin hydration and elasticity and secondary outcomes were transonychial and trans-epidermal water loss as well as skin roughness. Another preclinical study and one *in vitro* study in fibroblasts were also reviewed. In its final opinion, the EFSA Panel concluded that the pre-clinical and *in vitro* studies could not be used for the scientific substantiation of the claim and an absence of evidence for an effect of collagen hydrolysate on a change in skin elasticity led to an additional failure to establish an improvement in skin function in humans. However, subsequent assessments suggest this decision might now be worth reconsidering [72].

4. DISCUSSION

It is likely that a chronic joint disease such as osteoarthritis might benefit more from nutritional interventions than acute conditions and hence an intervention such as collagen supplementation might deliver positive outcomes to patients who are prepared to adhere to a regimen over a period of months rather than weeks in expectation of a favourable response. Preclinical studies demonstrate the primary mechanism of action of supplementation with undenatured collagen in both rheumatoid and osteo and arthritis is the result of a process of tolerance, whereas that of collagen in the hydrolysed form, potentially may involve stimulation of production of components in the extracellular matrix. Data suggests supplementation with collagen hydrolysate, when administered chronically and at the right dosage to be beneficial in osteoarthritis, and although studies show some efficacy in rheumatoid arthritis compared to placebo its place in comparison to other existing therapies is open to debate [73]. However, in both instances supplementation with collagen hydrolysate stands out in terms of its high degree of tolerability and profile of safety for patients, thereby making it an attractive option to patients. Given these attributes and the patient population affected by both osteo and rheumatoid arthritis its potential in these conditions calls for further research-especially given the pressures today on healthcare costs and the expense and invasiveness of interventions such as joint replacements. Hence, an oral supplement, such as collagen hydrolysate, delivering a degree of efficacy as well as a high safety profile, appears an attractive alternative option.

Much interest has been also focused on the use of hydrolysed collagen either alone or in conjunction with other interventions for treating sarcopenia. Here a recent Systematic Review and Meta-Analysis of Randomized Controlled Studies examined the effectiveness of exercise, medication, nutritional, and combinations of interventions in older people with the condition [74]. It screened 2668 records and included randomised controlled trials that investigated the effects of these interventions on muscle strength, mass, and function in this cohort with the condition. It concluded “(1) exercise interventions may play a role in improving muscle mass, muscle strength, and walking speed in 3 months of intervention; (2) nutritional interventions such as collagen hydrolysates may be effective in improving muscle strength in 3 months of intervention; (3) as drug intervention, selective androgen receptor modulator had no clear effect on muscle mass, strength, and physical function; and (4) a combined intervention of exercise and nutrition may have positive effects in improving the walking speed in 3 months of intervention”. Hence from this review it appears exercise and nutritional interventions, such as collagen hydrolysates have benefits in treating sarcopenia in older people

Over the last decade, many researchers have evaluated the effects of collagen supplements on skin aging and identified that this intervention might improve specific parameters. A new systematic review assessed the literature regarding the effects of collagen supplements on skin health parameters in healthy subjects, focusing on mechanisms of action [72]. The review concluded oral administration of intact or hydrolysed collagen can improve objective measures of skin health and virtually all of the studies that were included reported at least one aspect where supplementation delivered beneficial effects. It identified three possible different mechanisms of action for the beneficial effects of collagen supplementation -“direct effects of collagen peptides on fibroblasts, M2-like macrophages, and oral tolerance-related mechanisms”.

However, it is important to recognise that different collagen sources can vary markedly with respect to both their biochemical composition as

well as their effects on human articular cartilage. One team has recently investigated this, where three collagen hydrolysates were characterized biochemically and pharmacologically using biophysical (MALDI-TOF-MS, NMR, AFM) and fluorescence assays [75]. They revealed marked differences between collagen hydrolysates of fish, and porcine origin with respect to the total number of peptides and common peptides between them. A novel dual radio-labelling procedure identified further differences in gene expression induced by the various forms (for example production of Interleukin (IL)-6, matrix metalloproteinase (MMP)-1, -3 and -13 levels) between the both the different sources and doses of collagen hydrolysates administered. Hence, the heterogeneous peptide composition and disparate possible pharmacological effects between various collagen hydrolysate sources suggest that the effect of a specific preparation cannot always be extrapolated to other formulations. This observation has been verified using different technologies in other studies comparing different source collagen hydrolysates that have similarly demonstrated different CH preparations can differ significantly in their peptide composition [76-78].

5. CONCLUSION

Collagen hydrolysate has been demonstrated in in-vitro, pre-clinical and human studies to be bioavailable and able to deliver an effect in various tissues in molecular forms undegraded by digestive enzymes present in the GI tract. Furthermore, evidence suggests that hydrolysed collagen exerts beneficial effects on joint and skin health and can also deliver improvements in sarcopenia in the elderly, particularly when combined with appropriate exercise. It further appears that, in comparison to unprocessed collagen, the most appropriate form is that of a standardised hydrolysate delivered at a daily dose of 10g over a period of at least three months. There appears to be no evidence that collagen sources claimed to be of vegetarian origin (i.e.supplements rich in the individual amino acids typically characteristic of those found in collagen) have any clinical rationale to support their use, since a described above, collagen needs to be delivered in an amino acid complexed form to deliver its beneficial effects. Moreover, from the perspective of both the clinician and consumer in terms of selecting a specific hydrolysate, given the heterogeneity of the biochemical forms, as well as a possible difference in the biological activity of different forms from various sources, attention needs to be paid to both the manufacturer and their ability to deliver a product of consistent specificity and quality as well as considering a brand that has been clinically evaluated and found to be of benefit. Given that processing of collagen hydrolysates incurs additional expense, this will inevitably result in a higher product cost, but the level of quality control and compositional validation, and indeed investment in trials of clinical efficacy, can be justified by a confidence that the product is of a high repeated consistency that has been demonstrated to be beneficial in the condition for which it being considered. In these circumstances, selection of a product, preferably with manufacturing controlled by the brand owner will address these criteria and deliver a product that is most likely to deliver the desired effect.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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