THE SCIENCE BEHIND

REPAIR

Repair shot: good for tendons, ligaments, and joints

Key Points:

- Collagen is a major constituent of ligament, tendon, bone, and cartilage.
- The ECM is important in imparting movement from muscle to bone.
- Collagen is important in the myotendinous junction linking muscle to bone.
- Collagen is high in the amino acids glycine, hydroxyproline, and proline.
- When ingested the peptides and amino acids from collagen are readily absorbed by the gut and accumulation in the ECM
- Taking collagen supplements daily results in less joint pain and improvements in joints of athletes and those with osteoarthritis.
- Combination of training and collagen supplementation can strengthen the myotendinous junction and improve muscle-joint interaction
- Collagen supplementation should be seriously considered for athletes recovering from joint injuries and joint surgery.
- Glucosamine and chondroitin are also (on balance) considered useful in alleviating joint pain
- Vitamin C, together with collagen, has been shown to promote collagen synthesis in joints
- Copper and zinc also are important in the formation of collagen
- Overall, a nutrition product which contains collagen, glucosamine, chondroitin, vitamin C, copper, and zinc would be efficacious in promoting joint recovery from injury, trauma and surgery, and indeed in alleviating joint pain too.
  NutritionX Repair shots have these constituents.

Introduction

Connective tissues and joints are important for the health of any person and not just athletes since these tissues can cause muscles to tear, and joints can be affected by arthritis and impact injuries. An understanding of the function of connective tissue and joints, as well as the importance of collagen is required in order to prevent or recover from such types of injuries. Collagen is the most abundant protein in connective tissue – it forms the mechanical backbone not just of tendons and ligaments but also intramuscular connective tissue (Baar, 2015). This article briefly explores the function of connective tissue in muscle and joints before examining the role of collagen in enhancing tendon and ligamentous tissue, as well as those of glucosamine, chondroitin, vitamin C, vitamin D, vitamin E, zinc, and copper. Selected studies will demonstrate the efficacy of taking collagen as a supplement in offsetting joint pain, and indeed in promoting repair of connective tissue.

Basics of soft tissue

Musculoskeletal soft tissue injuries (strains and sprains) are the most common injuries suffered by athletes. In the English Premier league, nearly 60% of all injuries are sprains or strains (Hawkins et al., 2001). While musculoskeletal injuries are common in elite sports, there have been few attempts to understand the problem, and so slow progress has been made in studies investigating the prevention and treatment of these injuries. However, with the competitive and financial cost to professional teams when games are missed due to injury, this issue is starting to attract greater attention.

One of the reasons why musculoskeletal injuries are common in elite sport is that in order to optimize performance, athletes have to maximize the stiffness of the musculoskeletal system. In endurance athletes, stiffness is directly related to the economy of movement. The same can be said for power athletes or those engaged in most team sports since sprinting speed is directly related to leg stiffness. It appears that the greater the stiffness of the musculoskeletal system, the better the performance. The downside to this is that as musculoskeletal stiffness increases, so does exercise-induced muscle injury. In essence, when the tendon is stiffer than the muscle is strong, the protective effect of the tendon is lost and the muscle ruptures.

Muscles and tendons form a single unit that transfers the force produced in the muscle to the target bone. In order for this system to function properly, it requires the integration of the contractile proteins of the muscle, the force transfer apparatus inside the muscle, the intramuscular connective tissue, and the tendon. Each of these separate systems plays an essential role in the development of

force and power as well as the health of the muscle, and a weakness in any of these components can result in an injury.

Most people believe that muscle develops force by the sequential shortening of sarcomeres in the muscle (in other words transmitting force longitudinally or along the muscle) when in fact, greater than 80% of the force developed in a muscle fibre is transmitted laterally to the intracellular connective tissue (Ramaswamy et al., 2011). Lateral transmission has two main functions, (a) to transfer the force out of the working muscle fibre so that it can continue to shorten, and (b) to bind adjacent fibres together to protect them from injury. These two functions make lateral transmission essential to performance since this determines the strength and power of a muscle as well as the likelihood of injury.

The most abundant protein in connective tissue is collagen. Collagen forms the mechanical backbone of the intramuscular connective tissue and also of the tendons and ligaments within our bodies, and so the strength and stiffness of connective tissue and tendon is determined by the amount of collagen they contain. However, long thin collagen molecules are not be able to transfer force without the chemical crosslinks that bind them together (Baar, 2015). Crosslinks between collagen molecules are required for a stiff connective tissue that transmits force effectively. In general, there are two kinds of crosslinks: enzymatic and non-enzymatic. The enzymatic crosslinks are mostly made by lysyl oxidase that links lysine (an amino acid) residues of adjacent collagen fibres, whilst non-enzymatic crosslinks (also called advanced glycation end products - AGEs), are formed when sugar molecules bind one collagen molecule to another.

At each end of the muscle, the intramuscular connective tissue and the last sarcomeres of the muscle come together to form what is known as the myotendinous junction i.e. the connection between the muscle and tendon (Figure 1). This structure is one of the most important for determining the health of the muscle. A major component of this junction in its function to protect a muscle from injury is the presence of invaginations. The interface between the muscle and the tendon is not a smooth or flat transition, instead finger-like invaginations are present at the interface. These invaginations serve two important functions that are responsible for the strength of this tissue. Firstly, the invaginations increase the surface area that connects the muscle and tendon, and secondly, the invaginations mean that this interface is loaded in shear and not tension.

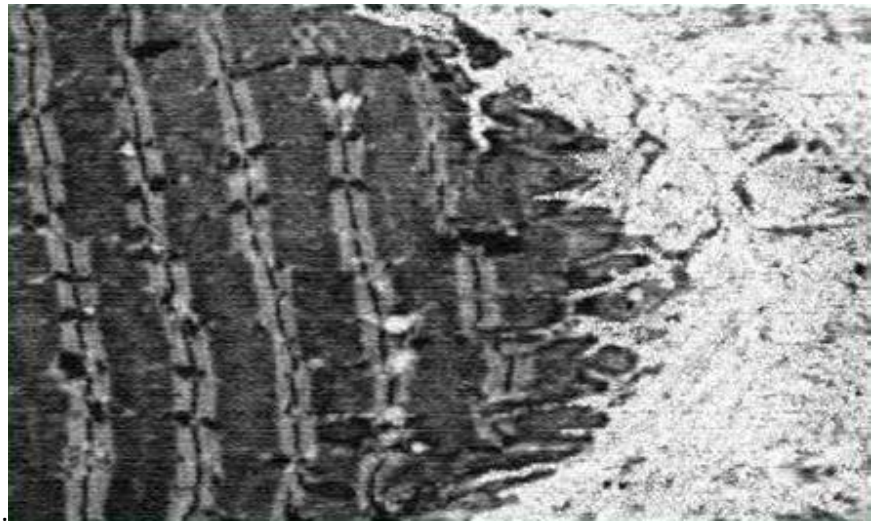

Figure 1. The myotendinous junction. On the left (dark area) are the end of the sarcomeres whilst on the right (lighter area) is the collagen rich tendon. The finger like projections of the sarcomere can be seen in the middle (from Baar, 2015)

Once the force from the muscle reaches the tendon, a healthy tendon transfers this force to the bone. Since muscle and bone are different in their mechanical properties (compliant vs stiff), this simple job (transferring force) is actually quite complex. To transfer the force from a compliant muscle to stiff bone without injury, the tendon has to act as a variable mechanical tissue (Figure 2A). This means that the tendon is compliant near the muscle and slowly becomes stiffer along its length until it nears the bone (Arruda et al., 2006). The reason that tendons are stiffer near the bone is because in this region there are more crosslinks (Curwin et al., 1994). Crosslinking increases stiffness and since there are more crosslinks near the bone than the muscle, stiffness increases as you move from muscle to bone. Interestingly, when a joint is inactive (such as when in a cast), the tendons that cross that joint lose the compliant region (Figure 2B) and become stiffer (Arruda et al., 2006), probably because the crosslinks increase.

The result of the stiffer tendon is that athletes often suffer injuries to that muscle if they return to play too quickly after immobilization.

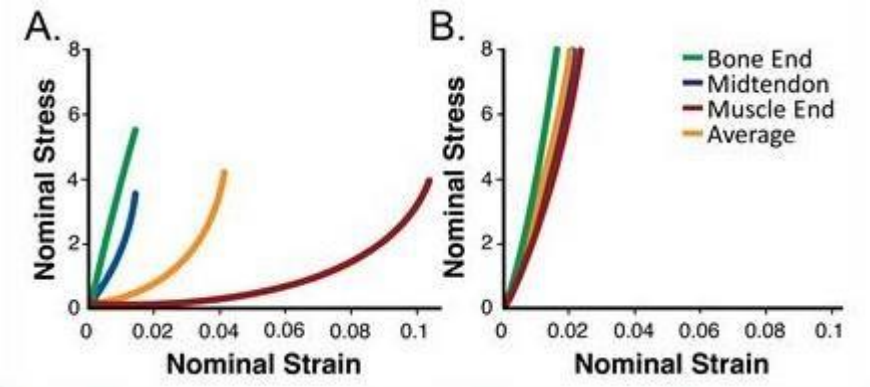

Figure 2. Mechanics of a healthy tendon (A) and one immobilised for 5 weeks in a cast. The muscle end (red) in the healthy tendon stretches more than the bone end (green). Following immobilisation all parts have become stiffer (after Arruda et al., 2006).

The key points are:-

- stiffer tendons are better for performance but increase the risk of injury,
- lateral transmission of force protects individual muscle fibres from injury by linking them to their neighbours,
- the compliant region of the tendon acts as a shock absorber and protects the whole muscle from injury.

If you wish to read more detail, please refer to Baar (2015).

Extracellular Matrix and Bone

Bones are constantly remodelled throughout life to maintain robust structure and function. Dysfunctional remodelling can result in pathological conditions such as osteoporosis (bone loss) or osteosclerosis (bone gain). Bone contains hundreds of extracellular matrix (ECM) proteins, and the ECM of the various bone tissue compartments plays essential roles directing the remodelling of bone through the activity of osteoclasts (which resorb bone) and osteoblasts (which produce new bone). One important role for the ECM is to serve as a scaffold upon which mineral is deposited. This scaffold is mainly type I collagen, but other ECM components are involved in binding of mineral components. In addition to providing a mineral scaffolding role, the ECM components provide structural flexibility for a tissue that would otherwise be too rigid.

So we can note that collagen plays a crucial role in human movement since it is important in the structure of bone, tendon, ligament, and cartilage too. Figure 3 is a schema showing (once again) the myotendinous junction between muscle and bone.

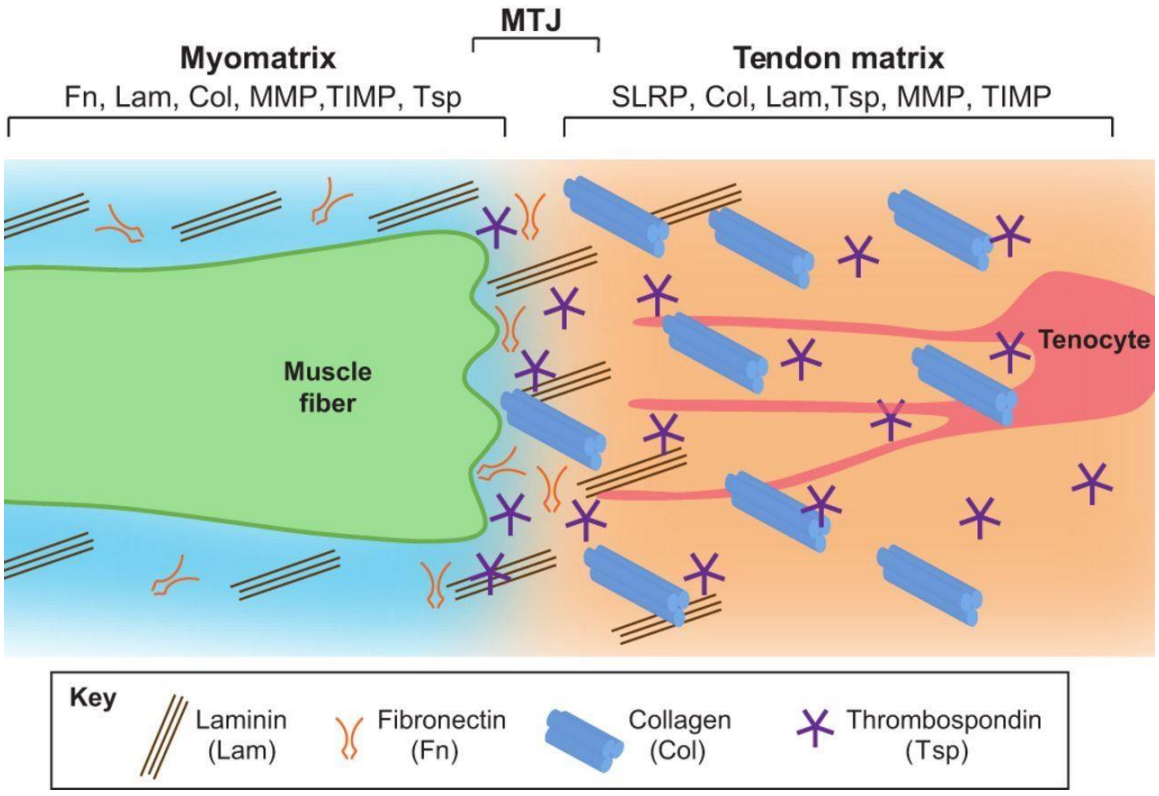

Figure 3. The Myotendinous Junction.

If collagen is the major protein in connective tissue, one question surely is regarding the likely effect (if any) of taking collagen-containing products on joints from a health perspective or a prevention of injury perspective or indeed from a recovery from injury perspective.

Collagen

Collagen is a major protein in the ECM – it constitutes more than 80% of tendons and ligaments as well as being a significant component of bone. The amounts of amino acids that make up collagen are different to those found in whey protein, casein, and soy (see Table 1). Collagen contains greater amounts of glycine, hydroxyproline and proline than the other proteins, and is lower in the BCAAs (leucine, isoleucine, and valine). Since this is the case, it is quite likely that taking a collagen supplement should confer an advantage over whey, casein, and soy as a product of choice for improving joint function. Is there any evidence regarding the impact of collagen on joints?

Table 1. The amino acid constituents of collagen, whey, casein, and soy proteins in a typical 20g serving.

Amino Acids	Collagen	Whey	Casein	Soy
Alanine	1.3	0.88	0.48	0.8
Arginine	1.6	0.48	0.64	1.36
Aspartic Acid	1.1	1.76	1.12	2.1
Cysteine	0.00	0.32	0.08	0.24
Glutamic Acid	2.0	2.88	3.52	3.44
Glycine	**4.0**	0.32	0.4	0.72
Histidine	0.2	0.32	0.48	0.48
Hydroxyproline	**2.7**			
Isoleucine	0.2	1.12	0.96	0.88
Leucine	0.5	1.84	1.44	1.44
Lysine	0.81	1.52	1.28	1.12
Methionine	0.08	0.4	0.4	0.24
Phenylalanine	0.38	0.56	0.8	0.96
Proline	**2.95**	1.12	1.76	0.96
Serine	0.55	0.88	0.96	0.96
Threonine	0.30	0.8	0.73	0.64
Tryptophan	0.00	0.24	0.16	0.16
tyrosine	0.08	0.56	0.96	0.64
Valine	0.38	0.8	1.12	0.88

Where does ingested collagen end up?

Collagen supplementation results in an increase in blood hydroxyproline-glycine (Sugihara et al., 2012). Figure 4 illustrates the effect of taking 8g of collagen hydrolysate on blood – note the significant increase in hydroxyproline, reaching a peak at 1-h and then return to baseline after 4-h. Similar results were obtained when ingesting 2, 10, and 25g of collagen hydrolysate (Shigemura et al., 2014) – with the higher dose providing highest plasma concentrations. Gelatin (an animal source of collagen) administration also produces similar findings (Ichikawa et al., 2010).

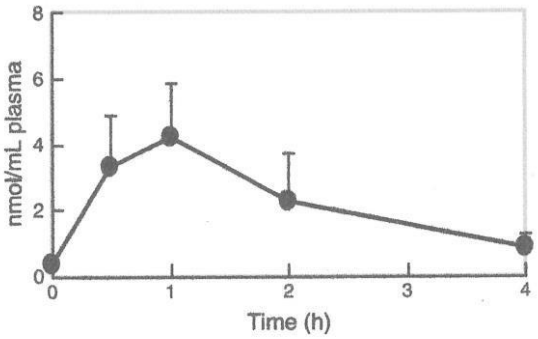

Figure 4. Plasma concentration of hydroxyproline after ingestion of collagen hydrolysate (after Sugihara et al., 2012).

An earlier study (albeit on mice) has shown that taking collagen, which was radiolabelled with an isotope, was found to be incorporated into the mouse cartilage (Oesser et al., 1999). More recently, a study using humans and MRI scanning showed that products from collagen hydrolysate were taken into the knee cartilage of osteoarthritic participants (McAlindon et al., 2011). From these findings we can realise that the constituents of collagen are absorbed across the gut as constituents such as peptides and amino acids, and that these are then directed to joints.

Collagen intake and joints

Since collagen is a major protein in joints, many investigations over the past 10 years have explored the efficacy of taking a collagen supplement for joint pain in athletes and also for osteoarthritic participants. Most of these studies have resulted in positive findings with regard to pain and also to movements of the joints (usually the knee joint is examined).

In one large study which examined taking 10g of collagen daily in 97 university and club athletes over a 24 week period, found that those who ingested the collagen hydrolysate had (a) less joint pain at rest, (b) less joint pain when walking, (c) less joint pain when standing, (d) less joint pain when lifting, and (e) less joint pain when carrying objects (Clark et al., 2008). When examination was made for those athletes with knee arthralgia, the findings were even more significant – here there were significantly less joint pain when participants ran in a straight line or indeed when running and changing direction.

The fact that collagen (specifically collagen hydrolysate) is readily absorbed across the gut and accumulates in cartilage, and that collagen hydrolysate stimulates ECM molecules, suggest that ingestion would be advantageous for those persons with osteoarthritis (Bello & Oesser, 2006). To highlight the benefit of taking collagen hydrolysate, a study using 250 osteoarthritic participants were

given 10g daily for 6 months (Benito-Ruiz et al., 2009). Those on collagen showed a significant improvement in pain (75% showed a 30mm reduction on a visual analogue pain scale) compared with those on placebo (53% showed reduction on the pain scale). A very similar, more recent study using 12g of collagen hydrolysate on 200 osteoarthritic patients also over a 6 month period found 56% improved clinical responses compared with 36.5% for a placebo (Bruyere et al., 2012).

Collagen supplementation and training

Research using rodents and humans have shown that short loading protocols (5 and 40 loads) separated by about 6 hours of rest are enough to significantly increase bone synthesis rates. Likewise, collagen synthesis in ligaments can be maximised by short periods (5-10 minutes) of exercise separated by 6 hours of rest (Baar, 2017).

These data suggest that, unlike muscle that continues to adapt as long as we exercise, the ECM only gets the signal to adapt for 5-10 minutes before the cells start shutting down. Everything after that causes mechanical fatigue and damage without giving a further stimulus to adapt and get stronger.

This means that to strengthen the ECM short periods of loading (5- minutes) that target the tendons/ligaments/bones/cartilage should be employed e.g. rope jump for runners, bench step ups for basketball players, rotator cuff exercises for baseball/water polo/cricket players and so on. These training sessions should be performed at least 6 hours separate from other training (where possible), and can be seen as protective sessions which serve to stimulate ECM production and decrease the likelihood of repetitive stress injuries to bone, ligament, tendon, and cartilage.

Shaw et al., (2017) used intermittent exercise in combination with ingesting gelatin (a food rich in collagen). Subjects consumed either placebo, 5g, or 15g of gelatin in ~500 ml of vitamin C rich blackcurrant juice. The appearance rate of amino acids and the production of collagen over the first 4 hours of the intervention were measured. To increase collagen synthesis, subjects jumped a rope for 6 minutes one hour after taking the supplements. Consistent with the short loading periods on collagen synthesis, the 6 minutes of rope jumping doubled collagen synthesis in the placebo and 5g gelatin groups. However, when the subjects consumed the higher gelatin load (15g) a further 2-fold increase in collagen synthesis was observed.

For coaches and athletes, this means that an athlete could add a 5-minute protective session for ligaments, tendons, and bone by consuming gelatin an hour before the session and at least 6 hours

before or after their other training. Such a combination of supplementation, short training, recovery, and then more prolonged training should prevent injuries or accelerate return to play.

Collagen supplementation and strength

In the articles on Big Whey and on Casein clear evidence is presented with regard to the positive effects of training and ingesting whey or casein on strength. Since collagen contains a different amino acid profile than whey and casein (the BCAAs – notably leucine are low in collagen products), is collagen helpful in stimulating muscle protein synthesis? There is very little evidence to suggest the beneficial effects of collagen on muscle protein synthesis, although one recent study appears to show benefits to an elderly population (Zdzieblik et al., 2017). In this study 53 males of an average age of 72 years underwent a 12 week strength training course with or without a daily collagen intake of 15g. Those participants who were taking collagen peptides increased muscle strength and also muscle mass significantly more than those on placebo. Figure 5 illustrates the effects of collagen and training on fat free mass (FFM – in effect muscle and bone mass) and on leg quadriceps muscle strength. The graphs show an increase in FFM of around 4kg and an increase in leg muscle strength of 17 Nm (Newton metres force) in the collagen group.

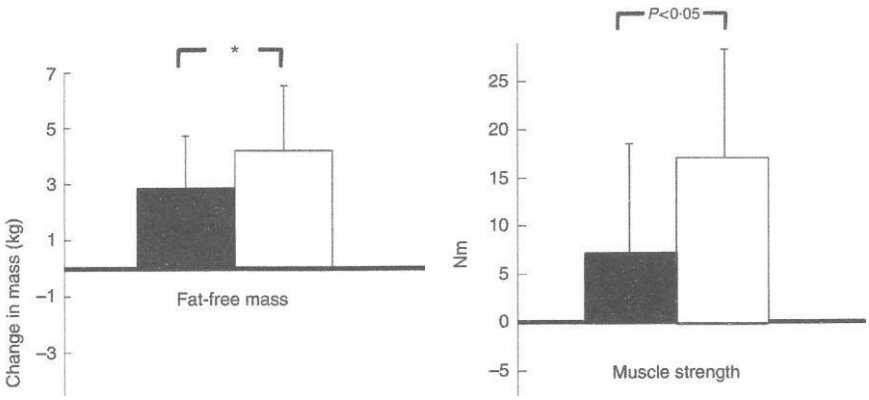

Figure 5. Changes in fat free mass and in strength after 12 weeks of resistance training in elderly men with collagen supplementation or placebo (after Zdzieblik et al., 2017)

Glucosamine and Chondroitin

Glucosamine or 2-amino-2-deoxy-D-glucose is an amino monosaccharide. It is synthesized from glucose in almost every human tissue and is most abundant in connective tissue and cartilage. In cartilage, it is important for the formation of hyaluronic acid, chondroitin sulfate as well as keratin sulfate, which are, other than collagen fibres, the most important components of the extracellular matrix of the articular cartilage and the synovial fluid.

Due to its basic role in cartilage and synovial fluid synthesis, glucosamine, administered as glucosamine sulfate or hydrochloride, has been tested in many clinical osteoarthritis trials, and the effects have been summarized in reviews and meta-analyses (Hochberg et al.,2014; McAlinden et al., 2000; Zeng et al., 2015).

In vitro studies on isolated chondrocytes, or cartilage explants from healthy or OA patients, have provided much evidence for the proposed mechanisms regarding how glucosamine supports joint health. It has been shown that glucosamine enhances the production of cartilage matrix components in chondrocyte culture, such as aggrecan and collagen type II. Further experiments have shown that glucosamine prevents collagen degeneration in chondrocytes.

In a meta-analysis of 54 studies which used a total of 16,427 patients, Zeng et al.(2015) found that glucosamine alone and in combination with chondroitin were more effective for pain relief and joint function when compared with placebo. This conclusion was similar to an earlier meta-analysis (McAlinden et al., 2000) which examined clinical trials from 1966 to 1999 on the use of glucosamine and chondroitin, in which the authors stated that trials of glucosamine and chondroitin preparations for osteoarthritis demonstrated moderate to large positive effects.

Chondroitin

Chondroitin sulfate (CS) is one of the natural glycosaminglycans (GAG) composed of the alternating sugars D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine (GalNAc). It is an important component of the extracellular matrix (ECM). CS is the most frequent GAG in the aggrecan molecule of the cartilage. Due to the negative charge of CS, it is responsible for the water retention of the cartilage, which is important for pressure resistance.

In the European League Against Rheumatism (EULAR) recommendation concerning knee OA, CS achieved the highest evidence grade. The first effects of CS treatments become noticeable after 2 to 3 weeks of regular intake and has a prolonged effect that remains for up to several months. CS influences the symptoms of OA such as pain and inflammation, but also acts as a structure-modifying drug. It may retard OA progression and could modify the course of OA.

In a recent review and meta-analysis, Singh et al.(2016) concluded that the improvements in joint pain with CS (alone or in combination with glucosamine) in patients with osteoarthritis was clinically meaningful and statistically significantly better than placebo.

Vitamin C

Vitamin C is needed for collagen formation (Van Robertson & Schwartz,1953). Figure 6 presents a schema showing that collagen formation requires the key amino acids proline, hydroxyproline, and glycine for its basic structure, and that the actual formation of the triple helix structure requires glucosamine and vitamin C. Vitamin C is also important in stimulating crosslinking of collagen due to activating the key enzymes which are responsible for crosslinking i.e. lysyl oxidase, and prolyl and lysyl hydroxylases (Levene et al.,1972). This means that vitamin C is not only necessary for the formation of collagen per se but also in enhancing crosslinking in the collagen matrix to promote strength.

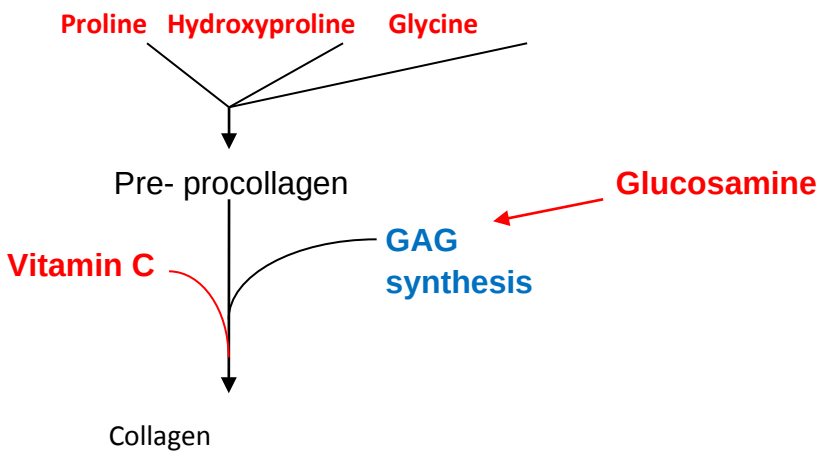

Figure 6. Collagen 1 has a triple helix structure of repeated amino acids proline, hydroxyproline, and glycine. These 3 amino acids form pre-procollagen, which then forms collagen using glucosamine and vitamin C.

Recently, Shaw et al. (2017) highlighted the efficacy of a combination of gelatin (a form of collagen) and vitamin C given 1-h prior to an intermittent exercise regime. They observed that 15g of collagen with 45mg of vitamin C resulted in significantly greater collagen formation than placebo or a lower dose of gelatin (i.e. 5g). The accelerated increase in collagen synthesis was observed 5-h after ingestion and continued for a further 72-h. Repair shots contain 20g of collagen and 96mg vitamin C.

Finally, Omeroglu et al. (2009) found that providing rats with vitamin C compared with placebo resulted in significantly enhanced recovery from Achilles tendon healing. In part this faster recovery was due to greater amounts of type 1 collagen formation leading to enlarged collagen fibres in the healing tissue.

Copper

The synthesis of collagen, as mentioned previously, is quite complex. A key enzymatic reaction involves copper and the enzyme lysyl oxidase. Lysyl oxidase is an extracellular copper enzyme that catalyzes the formation of aldehydes from lysine residues in collagen and elastin precursors. Being highly reactive, they undergo spontaneous chemical reactions with other lysyl oxidase-derived aldehyde residues, or with unmodified lysine residues. This action forms the basis of the crosslinking of collagen and elastin, an essential process for the stabilization of collagen fibrils, and for the integrity and elasticity of mature elastin. The absence of crosslinking results in lathyrism, which is characterized by poor bone formation and strength, and weak ligaments. Studies clearly show that the synthesis of mature elastin and collagen can be controlled by the availability of copper (Harris et al.,1980; Rucker et al., 1998).

Zinc

Zinc is an essential trace element with quite a wide range of functions.Many studies have clearly demonstrated the negative effects of zinc deficiency on a range of issues – particularly growth, development and immune system function. Among chronic zinc deficiency problems there have been observations of a cause in delaying reparative processes - and the rate of repair is normalized by the administration of zinc. Trauma (including sports related injury) lowers serum zinc levels, and intramuscular zinc administration before trauma results in an increased collagen accumulation (Tengrup et al., 1980). More recently a study found that collagen formation was promoted in a dose dependent manner with higher levels of zinc being administered (Seo et al., 2010).

The NutritionX product Repair shot contains 20g of collagen hydrolysate with additional glucosamine, chondroitin, vitamin C, copper, and zinc. We recommend that this product could be of significant benefit for those athletes who wish to strengthen their ECM as well as being beneficial for those athletes with joint pain or indeed those recovering from surgery to joints. Furthermore, elderly athletes and gym goers could benefit from Repair shot due to its amelioration of osteoarthritic pain.

For those athletes who wish to help support muscle growth and ECM, Repair shot taken in combination with Leucine or Big Whey could have a 'double whammy' effect.

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