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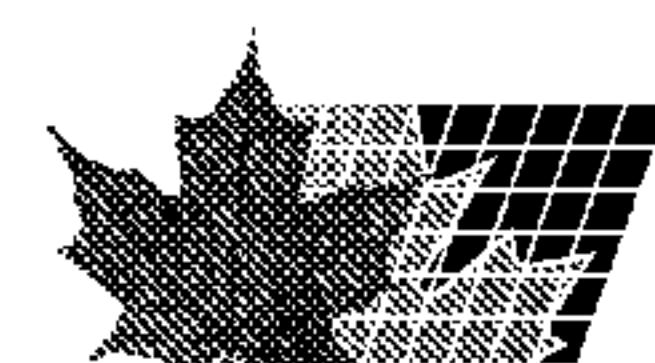
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(57) **Abrégé/Abstract:**

An oxidizable metal ion, such as ferrous ion in the form of ferrous sulphate, and an ascorbate, such as ascorbic acid, have a synergistic effect on cartilage development. Therapeutic compositions comprising an oxidizable metal ion and an ascorbate are therefore useful in the treatment of osteoarthritis. The addition of a glucosamine, such as glucosamine hydrochloride, to the composition has a further synergistic effect on cartilage production. Therapeutic compositions comprising an oxidizable metal ion, an ascorbate and a glucosamine derivative are even more useful in the treatment of osteoarthritis.



Abstract of the Description

An oxidizable metal ion, such as ferrous ion in the form of ferrous sulphate, and an ascorbate, such as ascorbic acid, have a synergistic effect on cartilage development. Therapeutic compositions comprising an oxidizable metal ion and an ascorbate are therefore useful in the treatment of osteoarthritis. The addition of a glucosamine, such as glucosamine hydrochloride, to the composition has a further synergistic effect on cartilage production. Therapeutic compositions comprising an oxidizable metal ion, an ascorbate and a glucosamine derivative are even more useful in the treatment of osteoarthritis.

COMPOSITIONS USEFUL IN THE TREATMENT OF DISEASES OF CONNECTIVE TISSUES

Field of the Invention

This invention relates to therapeutic compositions for use in treating diseases of connective tissues in animals, more particularly, for use in treating osteoarthritis in mammals, such as humans.

Background of the Invention

Arthritic diseases, characterized by the pain, inflammation and stiffness of the joints leading to reduced range of mobility, are due to the degradation of connective tissue (mainly cartilage) in joints. Such diseases particularly affect weight-bearing joints such as the hips, knees, spine, ankles and feet and those joints with frequent movement such as hands, arms and neck.

Osteoarthritis (OA) in particular is a degenerative disease of the joint cartilage resulting in narrowing of the joint space and changes in the underlying bone (Barclay, et al., *The Annals of Pharmacotherapy*, (May, 1998) 32: 574-79). OA is the most common form of arthritis among people and it affects approximately one in ten people in North America. People of all ages can get OA, but it more often affects older people and women. For example, 85% of the age group 70 years or older is affected by OA (*Arthritis Society Newsletter*, January, 2000). OA is not limited to humans, but occurs in other mammals such as horses, dogs, cats, mice and guinea pigs as well, making OA one of the most common sources of chronic pain seen by veterinarians.

The cause of OA could be one or more of the following: nutritional deficiencies, aging, long-term stress on joints (e.g. athletes, manual workers), old joint injuries and genetic factors. The tissue that is directly affected is the cartilage covering the end of long bones in joints that provide cushioning for the bones during movements. In normal cartilage, chondrocytes (cartilage cells) maintain a balance between the synthesis and degradation of cartilage matrix. However, when the degradation of cartilage matrix exceeds that of synthesis, it leads to OA. When the disease progresses further, bone underlying the articular cartilage in joints becomes exposed in certain places. In addition, irregular bone growth occurs in the place of degenerating cartilage resulting in rough bony alterations. As a result, the joint loses its smooth functioning leading to joint pain, stiffness and swelling thus limiting mobility.

Cartilage is a unique tissue having cells (chondrocytes) embedded in their own secretions which forms the cartilage matrix. The cartilage matrix is composed of a meshwork of collagen fibrils and proteoglycan aggregates filling the space between collagen. Collagen fibrils provide high tensile strength and proteoglycan aggregates provide internal swelling pressure due to their hydrophilic nature. Cartilage cells are remarkable in that they have the ability to proliferate while synthesizing and remodeling the matrix around them. These two features provide the cartilage the ability to repair itself during damage and replenish wear and tear.

Collagen fibrils are a major component of the cartilage matrix. Collagen is made from amino acids, particularly lysine, proline and glycine. Fibrillar collagen are triple helical molecules. The three α -chains of each collagen molecule are initially produced as

individual peptides which are further processed by the hydroxylation of proline and lysine residues bound to the peptides. The hydroxyproline and hydroxylysine so produced facilitate hydrogen bonding between the three α -chains, this being essential for the formation of the triple helical structure (Linsenmayer, *Collagen*, Chapter 1 in Cell Biology of Extracellular Matrix, Second Edition, Elizabeth D. Hay, ed. Plenum Press, N.Y. (1991)).

Unlike individual collagen peptides (α -chains) that become easily digested by proteolytic enzymes, triple helical collagen is extremely stable to proteolytic enzymes, heat and variations of pH. Therefore, the most important step in collagen synthesis is the formation of the triple helical structure by hydroxylation of amino acids in collagen α -chains. While the Linsenmayer reference suggests that ascorbic acid and ferrous ions are cofactors in the hydroxylation of proline and lysine to hydroxyproline and hydroxylysine respectively, Linsenmayer does not suggest that a therapeutic composition comprising ascorbic acid and ferrous ions would be useful in the treatment of osteoarthritis.

Proteoglycan aggregates are the other major component of the cartilage matrix. Cartilage proteoglycans are macromolecules comprised of glycosaminoglycan (GAG) chains, such as chondroitin sulphate and keratan sulphate, that are made up of repeating disaccharide units containing aminosugars, attached to a core protein. Proteoglycans are in turn attached to a backbone of hyaluronic acid, which is yet another GAG. Among GAGs of cartilage, hyaluronic acid is unique in that it is an extremely large molecule with about 25,000 repeating disaccharide units (in comparison, chondroitin sulphate and keratan sulphate have only about 250 and 80 repeating disaccharide units respectively). About 50% of hyaluronic acid and keratan sulphate are glucosamine.

Rheumatoid arthritis (RA) is a disease that has some similar symptoms to osteoarthritis, but whose cause is considerably different. RA is known to be an autoimmune disease (Maini, et al., *Aetiopathogenesis of Rheumatoid Arthritis*, in Mechanisms and Modes of Rheumatoid Arthritis, (1995) Academic Press Ltd. pp. 25-46), in which the immune system attacks body tissues as if they were foreign invaders, culminating in inflammatory and destructive responses in joints as well as other tissues. Although the exact cause of RA is not completely understood, contributing factors are believed to include food allergies, pathogens, leaky gut syndrome and hereditary factors. Because of the difference in cause of RA as opposed to diseases of the connective tissues such as osteoarthritis, it is not necessarily expected that treatment for RA would be effective against osteoarthritis and the like.

A number of treatments for osteoarthritis and like diseases are commonly used. Most of the treatments currently available are aimed towards reducing symptoms but do not deal with the underlying tissue degradation. The use of steroids, corticosteroids and other anti-inflammatory agents, such as non-steroidal anti-inflammatory drugs (NSAIDs), for example, aspirinTM, relieve symptoms and reduce pain but also do not deal with the underlying tissue degeneration. NSAIDs may even speed up the progression of OA (Rashad et al., *The Lancet*, (September, 1989) 2: 519-521, and, Herman et al., *The Journal of Rheumatology*, (1986) 13: 1014-1018).

Therapies based on the regeneration of connective tissue, particularly cartilage, are attractive long-term solutions to the problem of osteoarthritis. To this end, there have been a number of disclosures of therapeutic compositions for the treatment of arthritic diseases.

United States Patent Serial Number 3,683,076 issued on August 8, 1972 to Rovati discloses pharmaceutical compositions comprising the glucosamine salts - glucosamine sulphate and glucosamine hydroiodide - for the treatment of osteoarthritis and rheumatoid arthritis.

United States Patent Serial Number 5,587,363 issued on December 24, 1996 to Henderson discloses therapeutic compositions comprising a synergistic combination of certain aminosugars (glucosamine and its salts) with GAG's (chondroitin and its salts) for the repair and replacement of connective tissue. Henderson suggests that Zn, Mn and Vitamin C play a role in the synthesis of procollagen which is a building block of collagen and that Cu, Fe and Vitamin C play a role in the synthesis of collagen from procollagen. However, Henderson does not disclose synergistic compositions of oxidizable metal ion, such as ferrous ion, and an ascorbate. Henderson further suggests that glucosamine is a building block in the synthesis of procollagen and that procollagen is a building block in proteoglycan synthesis. However, it is generally accepted that glucosamine is a direct building block of proteoglycan while amino acids are the building blocks of procollagen which becomes further processed to give rise to collagen.

Great Britain application Serial Number 2,317,109 published on March 16, 1998 discloses a therapeutic composition for the treatment and repair of connective tissue in mammals comprising glucosamine, chondroitin sulphate and one or both of ascorbic acid and zinc sulphate. This application teaches that ascorbic acid and zinc sulphate serve as catalysts in the metabolic pathways whereby cartilage and related tissues are produced from the chondroitin sulphate and glucosamine building blocks. It further teaches that

one of ascorbic acid and zinc sulphate may be omitted from the composition. This application does not teach a role for ascorbic acid and oxidizable metal ion in the production of collagen nor does it teach a synergistic combination of oxidizable metal ion and ascorbic acid in the production of connective tissue.

Barclay (Barclay, et al., *The Annals of Pharmacotherapy*, (May, 1998) 32: 574-79) teaches the use of glucosamine derivatives, such as the sulphate, hydrochloride and chlorhydrate salts as well as N-acetylglucosamine, for the treatment of osteoarthritis. Barclay suggests that glucosamine can be used in combination with herbs, vitamins and minerals including the salts of magnesium, potassium, copper, zinc and selenium and vitamins A and C. There is no disclosure of a therapeutic combination of metal ions and ascorbic acid.

Levenson (Levenson, G.E., *Experimental Cell Research*, (1969) 55: 225-228) teaches the effect of ascorbic acid on chondrocytes. Levenson suggests that ascorbic acid plays a role in the production of cartilaginous material but does not disclose a combination of ascorbic acid and metal ions.

Deal (Deal and Moskowitz, *Rheum. Dis. Clin. North. Am.*, (May, 1999) 25(2): 379-95) discloses nutraceuticals as therapeutic agents in osteoarthritis comprising glucosamine and chondroitin sulphate. Glucosamine derivatives have been shown to be as effective as NSAIDs in relieving the symptoms of OA without having the adverse side effects of NSAIDs.

Sandy (Sandy, et al., *Biochem. J.*, (1998) 335: 59-66) discloses the inhibitory effect of glucosamine on aggrecanase, an enzyme that breaks down aggrecan in cartilage. Test formulations also contain

ascorbic acid but there is no discussion of its role. Sandy does not teach the combination of metal ions and ascorbic acid in a therapeutic composition for the treatment of osteoarthritis.

European Patent Serial Number 25,721 published on March 25, 1981 discloses an oral medication for the treatment of rheumatoid arthritis comprising a mixture of a variety of metals including ferrous ions in the form of ferrous sulphate. There is no discussion of the role of ferrous ions, nor is there any suggestion that ascorbic acid may be used in combination, nor is there any indication that the medication is effective against diseases of the connective tissue such as osteoarthritis. As has been discussed previously, rheumatoid arthritis is a different disease and it is not necessarily expected that medications useful against rheumatoid arthritis would be effective against diseases like osteoarthritis.

While the aforementioned compositions have been successful to varying degrees, none have proven to be entirely satisfactory in the treatment of diseases of the connective tissue like osteoarthritis. In particular, there is still a need for therapeutic compositions that further facilitate the production of collagen alone or in combination with the production of GAGs.

The disclosures of all previously mentioned patents, patent applications and non-patent references are hereby incorporated by reference.

Summary of the Invention

It is therefore an object of this invention to provide compositions and methods useful in treating a disease of connective tissue, particularly treating osteoarthritis, in animals, preferably

mammals, more preferably humans.

It has now been found that a combination of an oxidizable metal ion and an ascorbate is surprisingly effective at facilitating the production of connective tissue and is thus useful in treating diseases of connective tissue. It has also been found that a glucosamine derivative in combination with an oxidizable metal ion and an ascorbate is even more surprisingly effective.

In accordance with the teachings of the present invention, there is provided a composition for treating a disease of connective tissue comprising an effective amount of an oxidizable metal ion and an effective amount of an ascorbate.

There is also provided a composition for treating a disease of connective tissue comprising an effective amount of an oxidizable metal ion, an effective amount of an ascorbate and an effective amount of a glucosamine derivative.

There is further provided a composition for treating a disease of connective tissue comprising an effective amount of ferrous ion and an effective amount of an ascorbate.

There is yet further provided a composition for treating a disease of connective tissue comprising an effective amount of ferrous ion, an effective amount of an ascorbate and an effective amount of a glucosamine derivative.

There is still further provided a use of an effective amount of an oxidizable metal ion and an effective amount of an ascorbate for treating a disease of connective tissue or for preparing a medicament for treating a disease of connective tissue.

There is yet still further provided a method of treating a disease of connective tissue comprising administering to a patient suffering from the disease, effective amounts of an oxidizable metal ion and an ascorbate.

Brief Description of the Drawings

Figure 1 is a graph showing the dose dependent effect of ascorbic acid on cartilage development.

Figure 2 is a graph showing the combined effect of ferrous sulphate (FeSO_4) and ascorbic acid (AA) on cartilage development and showing the individual effect of ferrous sulphate on cartilage development at a particular dose of ascorbic acid.

Figure 3 is a graph showing the combined effect of ferrous sulphate (FeSO_4), ascorbic acid (AA) and glucosamine hydrochloride (GS-HCl) on cartilage development.

Figure 4 is a graph showing the dose dependent effect of zinc sulphate (ZnSO_4) on cartilage development.

Description of Preferred Embodiments

The compositions of the present invention comprise an oxidizable metal ion and an ascorbate which, surprisingly, act synergistically in the development of cartilage. Without being limited to any particular mode of action, it is thought that the oxidizable metal ion and the ascorbate influence the production of collagen.

While it is thought that ascorbate and oxidizable metal ions

enhance cartilage development by enhancing collagen synthesis, it is thought that glucosamine is a building block for glycosaminoglycans of proteoglycans in the cartilage matrix. The presence of the two types of cartilage enhancing agents further enhances total cartilage development. The addition of a glucosamine derivative to the metal ion/ascorbate composition provides a further synergistic effect on activity.

The term "treating" is used in a broad sense to encompass the amelioration of both the cause and the symptoms of a preexisting disease or condition, and the prevention or prophylaxis of the disease or condition.

An oxidizable metal ion is any ion from the transition metal, rare earth metal or lanthanide metal series which can be oxidized to form an ion of higher oxidation state. Examples of such ions are V^{+2} , Cr^{+2} , Mn^{+2} , Fe^{+2} , Co^{+2} , Ni^{+1} , Ni^{+2} , Cu^{+1} , Ru^{+2} , Os^{+2} . Fe^{+2} , also called ferrous ion, is preferred. Oxidizable metal ions are preferably provided in the form of an inorganic or organic acid salt wherein the metal ion is accompanied by a counter-ion to balance the charge. Examples of inorganic counter-ions are sulphate, phosphate, nitrate, carbonate and halide. A preferred inorganic counter-ion is sulphate. Ferrous sulphate is a preferred inorganic form for ferrous ion. Examples of organic counter-ions are fumarate, gluconate, ascorbate, tartarate, succinate, lactate, citrate and maleate. Two preferred organic counter-ions are fumarate and gluconate. Ferrous fumarate and ferrous gluconate are two preferred organic forms for ferrous ion.

Oxidizable metal ion is present in the composition in an amount effective for promoting the development of connective tissue in the body. The actual amount is not critical provided it is sufficient

to promote such development. The daily dosage is preferably in the range of about 2 mg to about 200 mg, more preferably in the range of about 10 mg to about 18 mg, and most preferably is about 15 mg.

An ascorbate is any species capable of providing the ascorbate ion. Examples include ascorbic acid (Vitamin C) and salts of ascorbic acid including the potassium, sodium, calcium, ferrous and manganese salt. Ascorbic acid is a preferred ascorbate.

Ascorbate is present in the composition in an amount effective for promoting the development of connective tissue in the body. The actual amount is not critical provided it is sufficient to promote such development. The daily dosage is preferably in the range of about 40 mg to about 1000 mg, more preferably in the range of about 100 mg to about 1000 mg, and most preferably is about 100 mg.

Glucosamine is an aminosugar. Examples of glucosamine derivatives are glucosamine itself, glucosamine hydrochloride, glucosamine hydroiodide, glucosamine chlorhydrate, glucosamine sulphate and N-acetyl glucosamine. Glucosamine hydrochloride is a preferred glucosamine derivative.

The glucosamine derivative is present in the composition in an amount effective for promoting the development of connective tissue in the body. The actual amount is not critical provided it is sufficient to promote such development. The daily dosage is preferably in the range of about 500 mg to about 3000 mg, more preferably in the range of about 1000 mg to about 2000 mg, and most preferably is about 1500 mg.

The dosage ranges described above are typically for humans. One skilled in the art can readily determine appropriate doses for

other animals.

The compositions of the present invention may also include other factors that may be useful in treating a disease of connective tissue. These include glycosaminoglycans (GAGs) such as chondroitin.

Other minerals and vitamins which have other therapeutic indications may be present in the compositions. These include: zinc (in the form of zinc sulphate for example), potassium, sodium, calcium, magnesium, vitamin D and vitamin E.

The compositions are preferably formulated together with a pharmaceutically acceptable excipient or diluent. Such excipients or diluents as well as the methods of formulating the compositions are well known to those skilled in the art. Cellulose and water are preferred.

The compositions are generally formulated in a dosage form. Dosage forms include tablets, capsules, solutions, suspensions, emulsions and other forms that are readily appreciated by one skilled in the art. The compositions may be administered orally, parenterally, intravenously or by any other convenient method. Capsules for oral administration are preferred.

There is also provided a method for treating osteoarthritis comprising administering an effective amount of an oxidizable metal ion and an effective amount of an ascorbate to a patient suffering from osteoarthritis. The method may further comprise administering an effective amount of a glucosamine derivative.

In such methods, the patient preferably receives from about 2 mg to

about 200 mg of ferrous ion per day and from about 40 mg to about 1000 mg of the ascorbate per day. More preferably, the patient receives from about 10 mg to about 15 mg of ferrous ion per day and from about 100 mg to about 1000 mg of the ascorbate per day. When a glucosamine derivative is also administered, it is preferably administered in an amount from about 500 mg to about 3000 mg per day, more preferably in an amount from about 1000 mg to about 2000 mg per day.

One skilled in the art will understand that, in a method for treating diseases of connective tissue, daily dosage can be given all at once in a single dose or can be given incrementally in several smaller dosages. Thus, the compositions of the present invention can be formulated such that the recommended daily dose is achieved by the administration of a single dose or by the administration of several smaller doses.

It is apparent to one skilled in the art that the compositions of this invention can be included in a commercial package together with instructions for its use against a disease of the connective tissue such as osteoarthritis. Such a package may be in the form of a bottle or blisterpack but is not limited to such. Instructions are normally in the form of a written material but are not limited to such.

Example 1: Cell Culture Preparation

Cell cultures were prepared substantially as described in the prior art (S. Ekayanake and B.K. Hall, *Int. J. Dev. Biol.*, 38: 683-694 (1994)), the entire disclosure of which is hereby incorporated by reference. In general, cartilage precursor cells from the developing limb bud of normal chick embryos were isolated and a single cell suspension containing 2×10^7 cells/ml was prepared. For the production of micromass cultures, cells were plated as 10 μ l drops on to bottoms of 24-well plastic tissue culture plates and incubated for 1 hour in a tissue incubator to allow cells to attach to the culture plate. Once cells are attached, cultures were flooded with liquid culture medium comprising:

- a 3:1 mixture of Ham's F12 (from Gibco™) and BGJb (from Gibco™),
- 10% fetal bovine serum (from Gibco™),
- about 75 μ g/ml of ascorbic acid, and
- other normal ingredients known to those skilled in the art.

This is the basic culture medium. Generally, the basic culture medium contains about 0.6 μ g/ml ferrous sulphate due to its presence in the commercial medium. As can be appreciated from this example, the basic cell culture medium also contains about 75 μ g/ml of ascorbic acid that was added during the cell culture preparation as described above. Cells were maintained in culture for up to 10 days. The culture medium that cells grew in was changed daily. Under these conditions, cartilage precursor cells produced cartilage tissue within 4 days, and the amount of cartilage present in cultures gradually increased with time.

Example 2: Effect of Ascorbic Acid on Cartilage Development

Cultured cartilage cells were dosed with varying amounts of ascorbic acid. The amount of cartilage produced was quantified using standard procedures such as the number of cartilage nodules present per culture, the amount of cartilage matrix present per culture as measured by alcian blue spectrophotometry, and microphotography. Figure 1 shows that ascorbic acid significantly enhances cartilage development in a dose-dependent manner. The Control bar in Figure 1 represents the situation where no ascorbic acid was added during the cell culture preparation. It is thought that cartilage development in the Control experiment occurs because the cartilage cells themselves have a basal level of ascorbic acid in them. The T₁ bar represents the results obtained when the basic culture medium is used, that is, when 75 µg/ml of ascorbic acid is added during cell culture preparation as described in Example 1. The T₂ bar represents the results obtained when an additional 75 µg/ml of ascorbic acid is added to the basic culture medium to bring the total ascorbic acid content to 150 µg/ml.

Example 3: Effect of Ferrous Sulphate on Cartilage Development

Cultured cartilage cells prepared as described in Example 1 were dosed with varying amounts of additional ferrous sulphate (test group) or no additional ferrous sulphate (control group). It is thought that the control group shows cartilage development due to the basal level of ferrous sulphate (about 0.6 µg/ml) and ascorbic acid (about 75 µg/ml) normally present in the basic culture medium. The amount of cartilage produced was quantified using standard procedures as outlined in Example 2. As shown in Figure 2, the results indicate that, at a particular dose of ascorbic acid,

ferrous sulphate significantly enhanced cartilage development in a dose-dependent manner.

Example 4: Synergistic Effect of Ferrous Sulphate and Ascorbic Acid on Cartilage Development

Cultured cartilage cells were dosed with a combination of ferrous sulphate and ascorbic acid at different concentrations. As shown in Figure 2, the results indicate that when the dose of ascorbic acid is increased, there is a greater than expected increase in cartilage development over the same dose range of ferrous sulphate. The results indicate that the combination of ferrous sulphate and ascorbic acid exert a synergistic effect on cartilage development and that the effect is dose dependent.

Example 5: Synergistic Effect of Ferrous Sulphate, Ascorbic Acid and Glucosamine Hydrochloride on Cartilage Development

Cultured cartilage cells were dosed with varying concentrations of glucosamine hydrochloride in combination with optimal doses of ferrous sulphate and ascorbic acid. The amount of cartilage produced was quantified using standard procedures as outlined in Example 2. As shown in Figure 3, the results indicate that the combination of glucosamine hydrochloride, ascorbic acid and ferrous sulphate have a synergistic effect on cartilage development and that this effect was greater with increasing doses of glucosamine hydrochloride. The amount of ferrous sulphate represented in Figure 3 (2 µg/ml) is the amount of additional ferrous sulphate added and does not account for the basal level of ferrous sulphate present in the basic culture medium. The amount of ascorbic acid (150 µg/ml) represents the total amount of ascorbic acid including

the 75 µg/ml normally added during the cell culture preparation as described in Example 1.

Example 6: Effect of Zinc Sulphate on Cartilage Development

The effect of zinc sulphate on cartilage development was also tested. Cultured cartilage cells prepared as described in Example 1 were dosed with varying concentrations of zinc sulphate. The results are shown in Figure 4. There was no statistically significant increase in cartilage development between the control group that contained no zinc sulphate and the test groups that contained varying amounts of zinc sulphate. Therefore, zinc sulphate does not have the same cartilage enhancing effect as ferrous sulphate.

Example 7: Effect of Potassium Sulphate on Cartilage Development

A similar experiment to that of Example 6 was conducted using potassium sulphate instead of zinc sulphate. No statistically significant difference was observed between the control group and the test groups.

It is apparent to one skilled in the art that many variations on the present invention can be made without departing from the scope or spirit of the invention claimed herein.

Claims:

1. A composition for treating a disease of connective tissue comprising: an effective amount of an oxidizable metal ion and an effective amount of an ascorbate.
 2. The composition according to claim 1, wherein the metal ion is selected from the group consisting of V^{+2} , Cr^{+2} , Mn^{+2} , Fe^{+2} , Co^{+2} , Ni^{+1} , Ni^{+2} , Cu^{+1} , Ru^{+2} , Os^{+2} .
 3. The composition according to claim 2, wherein the metal ion is provided in the form of an acid salt.
 4. The composition according to claim 2 or 3, wherein the metal ion is Fe^{+2} .
 5. The composition according to claim 4, wherein Fe^{+2} is provided in the form of ferrous sulphate.
 6. The composition according to any one of claims 1 to 5, wherein the ascorbate is selected from the group consisting of ascorbic acid, sodium ascorbate, potassium ascorbate, calcium ascorbate, ferrous ascorbate and manganese ascorbate.
 7. The composition according to claim 6, wherein the ascorbate is ascorbic acid or calcium ascorbate.
 8. The composition according to claim 7, wherein the ascorbate is ascorbic acid.
 9. The composition according to any one of claims 1 to 8 further comprising a glucosamine derivative.
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10. The composition according to claim 9, wherein the glucosamine derivative is selected from the group consisting of glucosamine sulphate, glucosamine hydrochloride, glucosamine hydroiodide and N-acetyl glucosamine.
11. The composition according to claim 10, wherein the glucosamine derivative is glucosamine hydrochloride.
12. The composition according to any one of claims 1 to 11 further comprising a pharmaceutically acceptable excipient or diluent.
13. The composition according to any one of claims 1 to 12, wherein the disease is osteoarthritis.
14. The composition of any one according to claims 1 to 13, wherein the metal ion is present in an amount from about 2 mg to about 200 mg.
15. The composition according to any one of claims 1 to 14, wherein the ascorbate is present in an amount from about 40 mg to about 1000 mg.
16. The composition according to any one of claims 9 to 11, wherein the glucosamine derivative is present in an amount from about 500 mg to about 3000 mg.
17. A composition for treating osteoarthritis comprising:
 - (a) an effective amount of ferrous ion;
 - (b) an effective amount of an ascorbate; and,
 - (c) an effective amount of a glucosamine derivative.

18. The composition according to claim 17, wherein the ferrous ion is provided in the form of ferrous sulphate, the ascorbate is ascorbic acid and the glucosamine derivative is glucosamine hydrochloride.
19. The composition according to claim 18, wherein:
 - (a) the ferrous ion is present in an amount from about 2 mg to about 200 mg;
 - (b) the ascorbic acid is present in an amount from about 40 mg to about 1000 mg; and,
 - (c) the glucosamine hydrochloride is present in an amount from about 500 mg to about 3000 mg.
20. The composition according to any one of claims 17 to 19 further comprising a pharmaceutically acceptable excipient or diluent.
21. The composition according to any one of claims 1 to 20 in the form of a capsule.
22. The composition according to any one of claims 1 to 20 in the form of a tablet.
23. The composition according to any one of claims 1 to 20 in the form of a solution, suspension or emulsion.
24. Use of a composition as defined in any one of claims 1 to 23 for treating a patient having a disease of connective tissue.
25. The use according to claim 24, wherein the disease is osteoarthritis.

26. Use of a composition as defined in any one of claims 1 to 23 for the preparation of a medicament for treating a disease of connective tissue.
 27. The use according to claim 26, wherein the disease is osteoarthritis.
 28. Use of a composition for treating a patient having a disease of connective tissue, wherein the composition comprises ferrous ion, an ascorbate and a glucosamine derivative, and, wherein the patient receives from about 2 mg to about 200 mg of the ferrous ion per day, from about 40 mg to about 1000 mg of the ascorbate per day, and from about 500 mg to about 3000 mg of the glucosamine derivative per day.
 29. The use according to claim 28, wherein the patient receives from about 10 mg to about 18 mg of the ferrous ion per day, from about 100 mg to about 1000 mg of the ascorbate per day, and from about 1000 mg to about 2000 mg of the glucosamine derivative per day.
 30. The use according to claim 29, wherein the patient receives about 15 mg of the ferrous ion per day, about 100 mg of the ascorbate per day, and about 1500 mg of the glucosamine derivative per day.
 31. The use according to any one of claims 28 to 30, wherein the ascorbate is ascorbic acid and the glucosamine derivative is glucosamine hydrochloride.
 32. The use according to any one of claims 28 to 31, wherein the disease is osteoarthritis.
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33. A commercial package comprising a composition as defined in any one of claims 1 to 23 together with instructions for its use in treating a disease of connective tissue.
34. The commercial package according to claim 33 wherein the disease is osteoarthritis.
35. A method for treating osteoarthritis comprising administering an effective amount of an oxidizable metal ion and an effective amount of an ascorbate to a patient suffering from osteoarthritis.
36. The method of claim 35, wherein the oxidizable metal ion is administered in the form of an acid salt.
37. The method of claim 35, wherein the metal ion is ferrous ion.
38. The method of claim 35, further comprising administering an effective amount of a glucosamine derivative.
39. The method of claim 38, wherein the metal ion is ferrous ion.
40. The method of claim 39, wherein the patient receives from about 2 mg to about 200 mg of ferrous ion per day, from about 40 mg to about 1000 mg of the ascorbate per day, and from about 500 mg to about 3000 mg of the glucosamine derivative per day.
41. The method of claim 40, wherein the patient receives from about 10 mg to about 15 mg of ferrous ion per day, from about 100 mg to about 1000 mg of the ascorbate per day, and from

about 1000 mg to about 2000 mg of the glucosamine derivative per day.

42. The method of claim 41, wherein the ferrous ion is administered in the form of ferrous sulphate, the ascorbate is ascorbic acid, and the glucosamine derivative is glucosamine hydrochloride.

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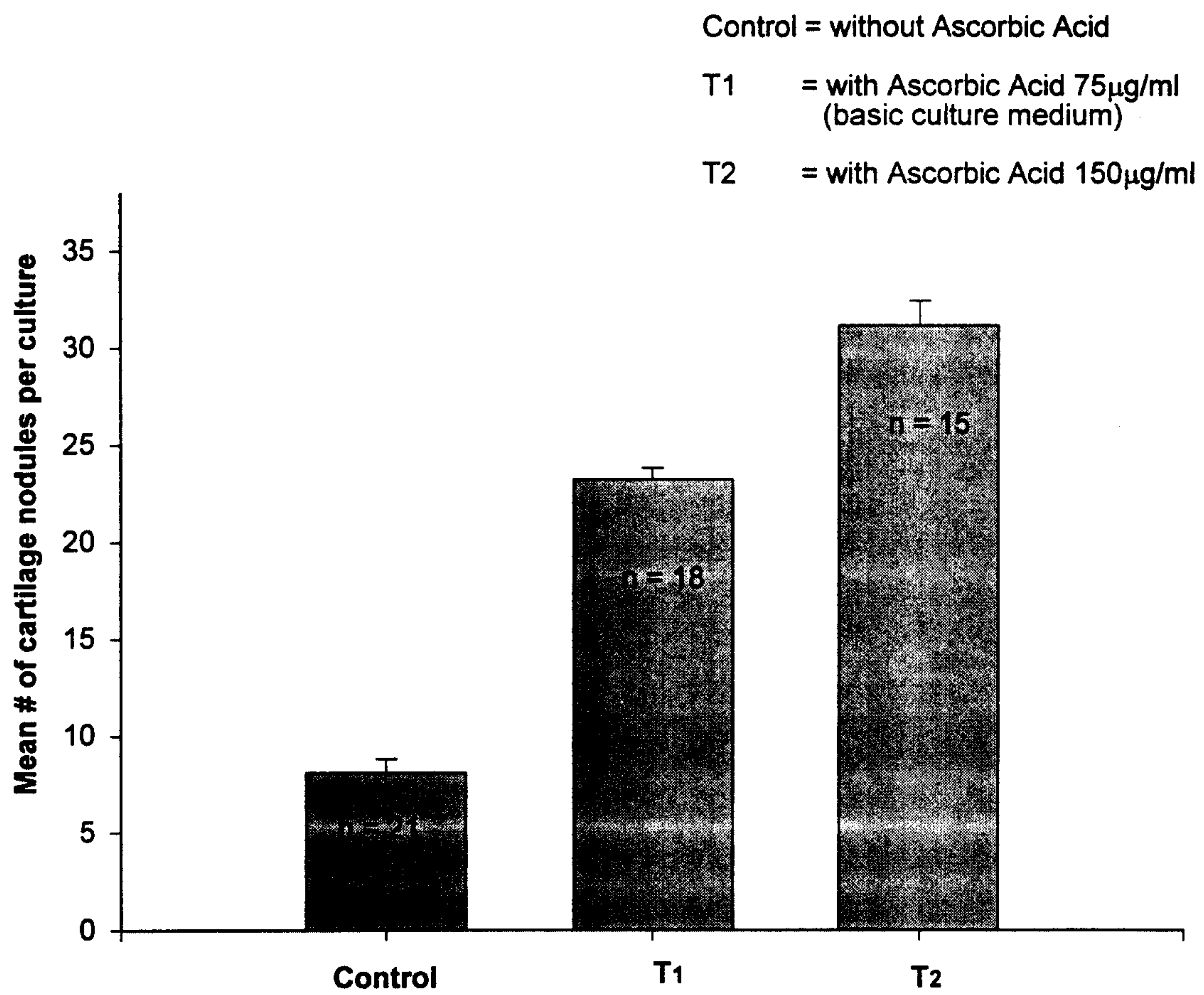
Figure 1

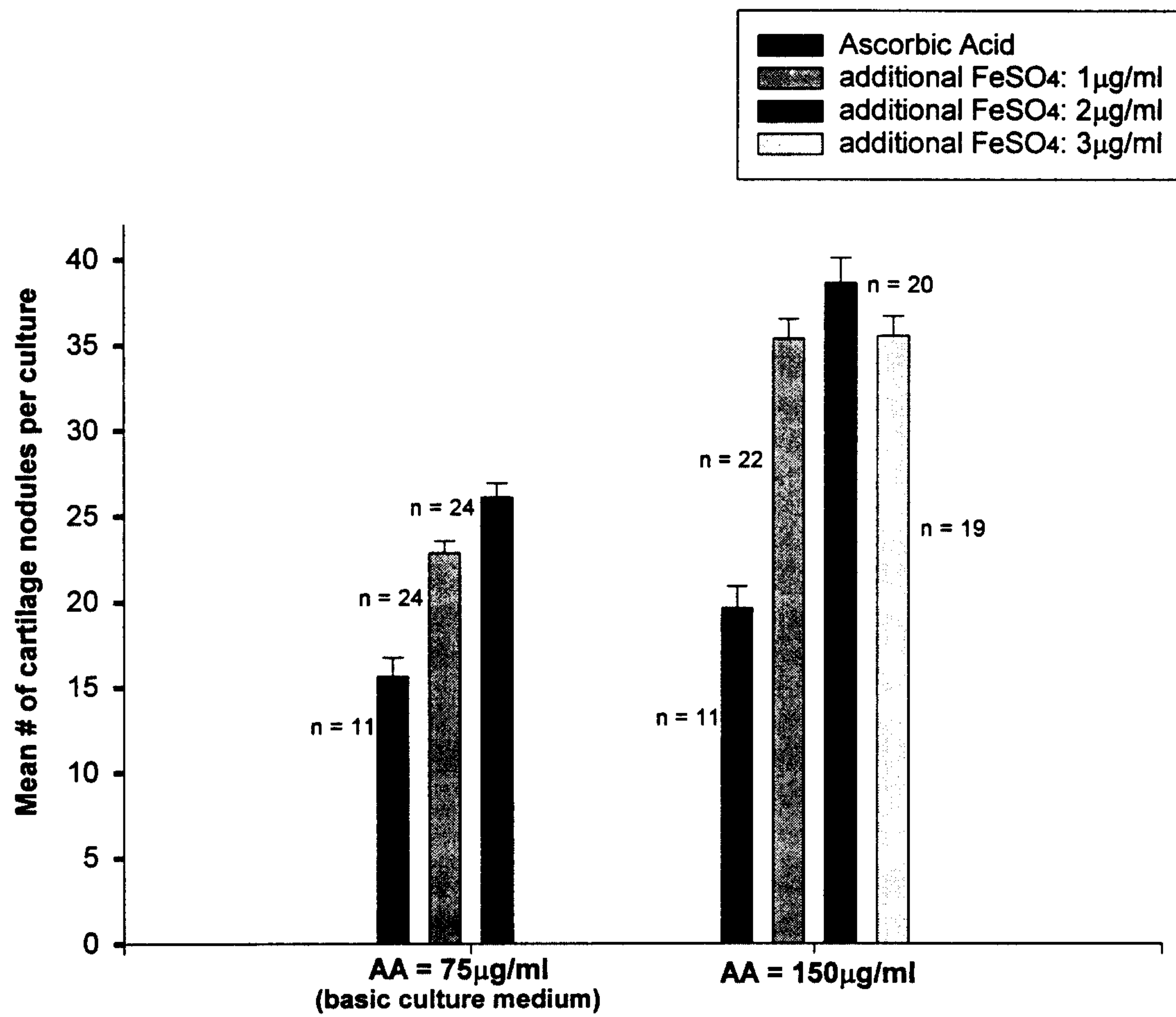
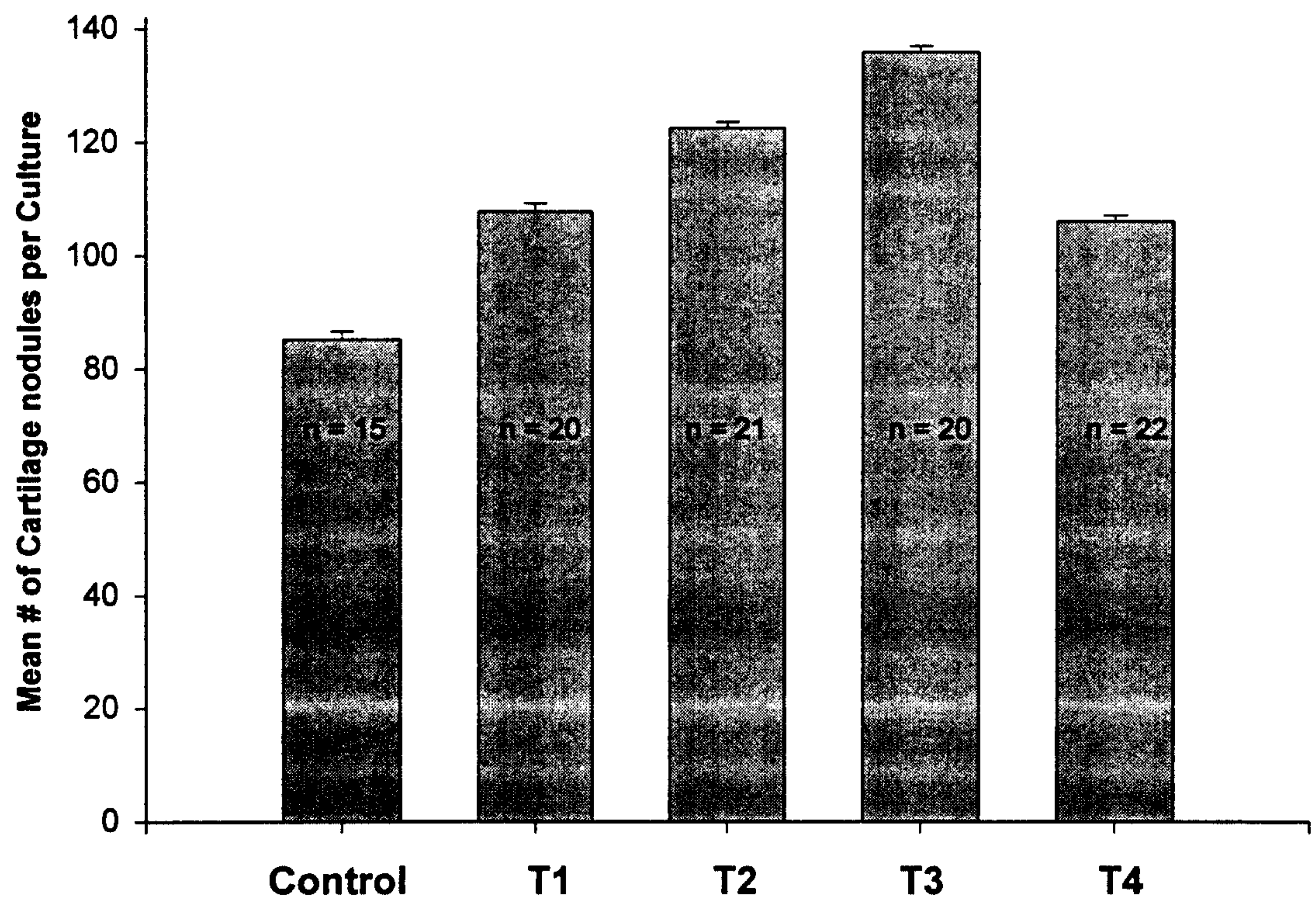
Figure 2

Figure 3

Control = FeSO₄(20 μ g/ml) + AA (150 μ g/ml total)

T1 = Control medium + GS-HCl (20 μ g/ml)

T2 = Control medium + GS-HCl (40 μ g/ml)

T3 = Control medium + GS-HCl (60 μ g/ml)

T4 = Control medium + GS-HCl (100 μ g/ml)

Figure 4