EVALUATION OF AN ORAL SUPPLEMENT ENRICHED WITH GLUCOSAMINE AND CHONDROITINE SULPHATE ON THE JOINT ENZYMATIC BALANCE IN YOUNG HORSES

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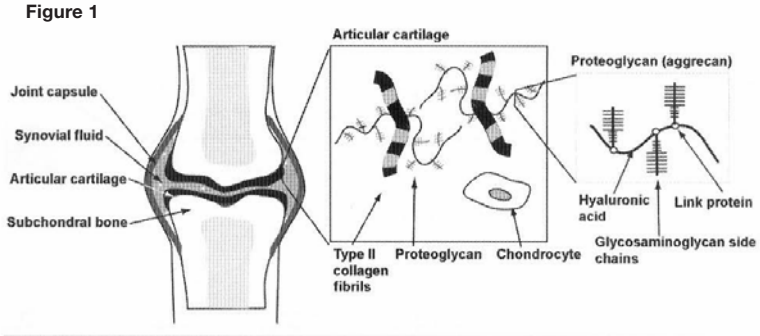
Implication of matrix metalloproteinase on osteoarticular disorder

For the sporting horse, the osteoarticular pathologies represent the major cause of lameness. Joints are complex structures made up of various entities (see figure 1). The neighbouring bones are covered with joint cartilage at the zones of contact to provide good mobility within the joint. This mobility is enhanced by the presence of synovial liquid which acts as a lubricant. The whole joint is enclosed within a synovial membrane and stabilised by ligaments and sometimes muscles which surround it. The structure of the joint cartilage plays a major role in its movement. It includes both chondrocytes and extracellular matrix. The extracellular matrix consists largely of collagen, which supplies the cartilage with its resistance, and proteoglycans and glycoproteins which create the elasticity reducing shocks caused by movement. The term " osteoarticular pathologies " in fact includes a large number of different diseases with a common denominator: the destruction of the joint cartilage extracellular matrix (Van Den Boom, et al., 2005).

Several authors have shown that this destruction of cartilage follows the activation of pro-inflammatory and enzymatic factors among which

Figure 1



Neil et al., JAVMA, 2005

the most important seems to be the matrix metalloproteinases (MMP) (Brama, et al., 2004, Neil, et al., 2005 a).

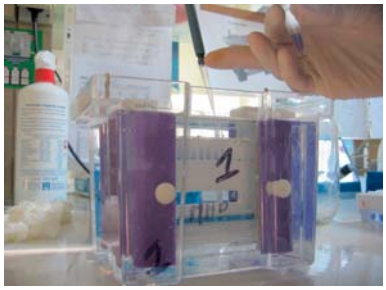
The MMP are zinc-dependent enzymes involved in numerous physiological and pathological processes. These proteinases are able to degrade the extracellular matrix. Their activity is subjected to complex control and notably depends on specific inhibitors: "Tissue Inhibitors of Metalloproteinases" or TIMP. It is largely the balance between the MMP and the TIMP that defines the proteinase activity. Indeed, the enzyme is inactive when bound to its inhibitor. It is only free when its lytic activity expresses itself, so creating the capacity to split the proteins contained in the extracellular matrix. Some pro-inflammatory factors such as cytokines and certain hormones seem capable of activating the MMP and so facilitating the destruction of the extracellular matrix.

These mediators appear to induce a chain reaction, in which the various components join with each other to produce an even more active proteinase (Nagase, et al., 2006). Repeatedly researchers have shown an increase in the activity of the MMP in case of joint pathology (Brama, et al., 2000, Clegg and Cartler, 1999); this increase seems to be the first step in the development of cartilage injury, additionally important and several positive correlations were observed by histological analysis. (Van Den Boom, et al., 2005).

Glycosaminoglycans and their precursors such as glucosamine or chondroitine sulphate seem to be able to modulate the activity of the MMP and facilitate the synthesis of the extracellular matrix (Henrotin, et al., 2002).

In vitro studies carried out with a chondrocyte culture or in vivo with orally supplemented rodents showed the beneficial effect of these products on preventing cartilaginous degradation (Beren, et al., 2001, Neil, et al., 2005 b). A study showed that an oral supplement composed of glucosamine, chondroitine sulphate and manganese ascorbate delayed the appearance of auto-immune arthritis in laboratory rats (Beren, et al., 2001).

In man, there are numerous investigations concerning the effects of



Preparation for MMP activity measurement by zymography

glycosaminoglycans and their precursors on diverse osteoarticular pathologies. Most of these researches result in a decrease of the seriousness and the pain in treated patients. These supplements also seem able to prevent some of the osteoarticular pathologies, both in man and in animals (Henrotin, et al., 2002).

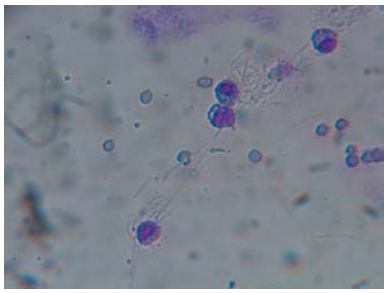
In addition, another study carried out on elderly horses showed that an oral supplement based on a combination of glucosamine hydrochloride and chondroitine sulphate over 12 weeks resulted in a significant increase in the length of stride, joint mobility and the duration of movement. This study seems to confirm the beneficial effect of this supplement on the locomotion of the horse (Forsyth, et al., 2006).

The aim of the present study was to estimate the effect of a feed supplement containing, amongst other products, glucosamine, chondroitine sulphate and harpagophytum on the balance of MMP-TIMP in healthy young horses at rest.

Study presentation

Sixteen healthy ponies with average age 2.5 years, average size of 1.35 m and 300 kg weight were used for this study. The ponies were accommodated on farms at Centres of Ovine research of the University of Namur (Belgium). Their food throughout the study consisted of concentrates given individually once a day and hay twice a day. The ponies had access to a meadow for one hour each day. Two weeks were allowed for the ponies to become acclimatized to their new environment. During this period of acclimatization the first investigation was undertaken (T0). General physical examinations as well as a specific examination of the locomotive system were made to produce a lameness score for each animal. Joint puncture allowed the extraction of synovial liquid. The ponies were divided into two homogeneous groups on the basis of size, weight, sex, age and lameness score. During the following six weeks, the ponies received an individual supply of supplement A* or B **, mixed into their concentrate ration.

Following the six weeks supplementation, a further physical exami-



Chondrocytes in synovial fluid

Figure 2 **MMP2 activity in synovial fluid**

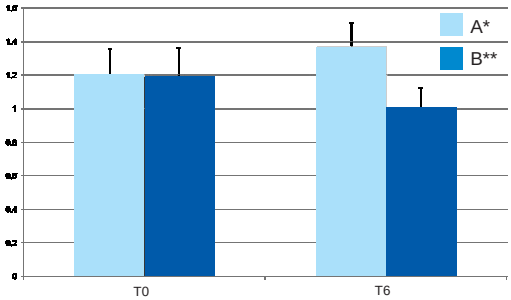
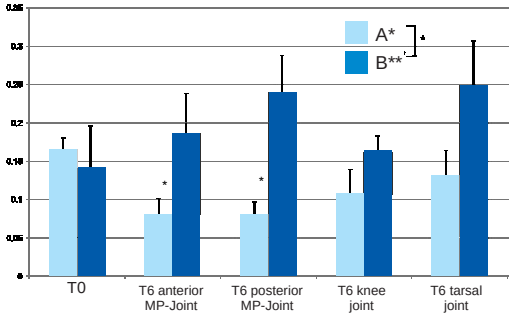


Figure 3 **MMP9 activity in synovial fluid**

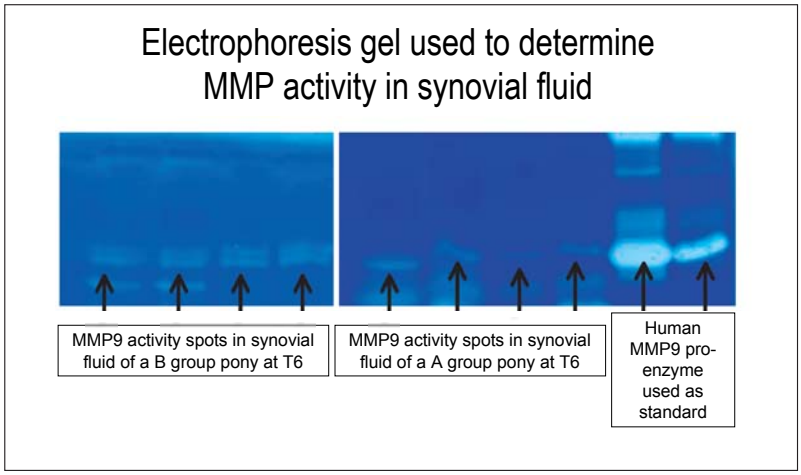


* TWYDIL® ARTRIDIL new formula ** control

nation was undertaken (T6) identical to the first.

The specific examination of the locomotive system produced a new lameness score for each pony. Synovial fluid was analysed for the following markers: activities of MMP2 and MMP9 and the activity of the TIMP as markers of the enzymatic stress. A cytological analysis of the synovial fluid was also undertaken. The investigators were not aware of the identity of supplements A* and B** until after all the analyses were completed.

The study was approved by the local commitee responsible for ethics in animal experimentation.



Results

a) Lameness scores :
At T0, the ponies' lameness scores in both groups were very low suggesting that none of them presented severe lameness. At T6, the lameness scores were similar to the start and showed no difference between groups A* and B**. There was no significant effect of the supplementation on these low lameness scores.

b) MMP2 activity:
At T0 the activity of MMP2 was similar within both groups. This activity did not show any significant difference after the period of supplementation. Groups A* and B ** thus showed comparable activities throughout the experiment (see figure 2).

c) MMP9 activity:
The activity of the MMP9 in the synovial liquid at T6 was significantly lower in the horses in group A* compared to the horses in group B **. Additionally, the activities of MMP9 in the joints of the ponies in group A*measured at T6 were different from their values at T0. In group B **, this activity at T6 was not significantly different from its value at T0 (see figure 3).

d) TIMP2 activity:
At T0 the activity of TIMP2 was similar within both groups. This activity did not show any significant difference after the period of supplementation.

e) Cytology:
The cytological analysis of the synovial liquid revealed no abnormality and no significant difference was noticed between ponies in group A* and group B **.

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In vitro, in a situation of joint enzymatic stress (greater activity MMP) mimicking a developing osteoarticular pathology, supplementation with glucosamine and chondroitine sulphate induced a reduction in the activity of the MMP (Byron, et al., 2003, Fenton, et al., 2002).

In our in vivo study, the MMP activity of synovial liquid was low given that the ponies were healthy animals. Due to individual variability within groups A* and B **, no significant effect of the supplement was evident. However, a significant modulation of the MMP9 activity was present in ponies receiving supplement A* for 6 weeks suggesting a decrease of the protease activity at the articular level.

Conclusion

In this experiment, supplement A* had no significant effect on the MMP2 activity or on TIMP2. A significant decrease of the MMP9 activity was noticed. These results allow us to conclude that supplement A* modulated the articular enzymatic balance.

PERSPECTIVES

Prof. N. Kirschvink

HPH: what does this particular study reveal?

Prof. N. Kirschvink: For the first time, the evaluation of a chondroprotector supplement was carried out in horses by clinical examination including the investigation of joint markers, making it possible to study the effect of the supplement directly on the level of the target structures.

HPH: For years scientists, for lack of tangible proof, have been divided on the proven effectiveness or not of the chondroprotecters that flood the market. Does the original formula tested provide evidence of effectiveness?

Prof. N. Kirschvink: Our study seems to indicate that a preventive effect exists for the formula tested making it possible to maintain joint health. This assumption however remains to be confirmed when young healthy horses are subjected to intense physical exercise - a factor which could not be taken into account in the present study.

HPH: Are you surprised by the absence of effect on the clinical signs used in this study?

Prof. N. Kirschvink: Given that the size of the experimental group was small, that the ponies were clinically healthy and that they showed a very low lameness score before the supplementation, a significant improvement of the clinical signs was far from probable. It would be nevertheless interesting to carry out this type of study on a large scale under field conditions.

References

Beren J., Hill S.L., Diener-West M. and Rose N.R. Effect of pre-loading oral glucosamine HCl/chondroïtine sulphate/manganese ascorbate combination on experimental arthritis in rats. 2001. *Experimental biology and medicine,* **226,** pp 144-151.

Brama P.A.J, Tekoppele J.M., Beekman B., Van El B., Barneveld A., and Van Weeren P.R. Influence of development and joint pathology on stromelysin enzyme activity in equine synovial fluid. 2000. *Annals of the rheumatic diseases,* **59,** pp 155-157.

Brama P.A.J., Van Den Boom R., Degroot J., Kiers G.H. and Van Weeren P.R. Collagenase 1 (MMP1) activity in equine synovial fluid: influence of age, joint pathology, exercise and repeated arthrocentesis. 2004. *Equine Veterinary Journal,* **36,** pp 34-40.

Byron C.R., Orth M.W., Venta P.J., Lloyd J.W. and Caron J.P. Influence of glucosamine on matrix metalloproteinase expression and activity in lipopolysaccharide-stimulated equine chondrocytes. 2003. *American Journal of Veterinary Research,* **11,** pp 1861-1869

Clegg P.D. and Cartler S.D. Matrix metalloproteinases 2 and 9 are activated in joint diseases.1999. *Equine Veterinary Journal,* **31** (4), pp 324-330.

Fenton J.I., Chlebek-Brown K.A., Caron J.P. and Orth M.W. Effect of glucosamine on interleukin-1 conditioned articular cartilage. 2002. *Equine Veterinary Journal Supplement,* **34,** pp 219-223

Forsyth R.K., Brigden C.V. and Northrop A.J. Double blind investigation of the effects of oral supplementation of combined glucosamine hydrochloride (GHCL) and chondroïtin sulphate (CS) on stride characteristics of veteran horses. 2006. *Equine Veterinary Journal Supplement,* **36,** pp 622-625

Henrotin Y., Sanchez C. and Reginster J.Y. The inhibition of metalloproteinases to treat osteoarthritis: reality and new perspectives. 2002. *Expert Opinion on Therapeutic Patents,* **1,** pp 29-43

Nagase H., Visse R. and Murphy G. Structure and function of matrix metalloproteinases and TIMPs. 2006. *Cardiovascular research,* **69,** pp 526-573.

Neil K.M., Caron J.P and Orth M.W. The role of glucosamine and chondroïtine sulphate in treatment for and prevention of osteoarthritis in animals. 2005 a. *Journal of American Veterinary Medical Association,* **7,** pp 1079-1088.

Neil K.M, Orth M.W., Coussens P.M., Chan P.S. and Caron J.P. Effects of glucosamine and chondroïtine sulphate on mediators of osteoarthritis in cultured equine chondrocytes stimulated by use of recombinant equine interleukine-1_. 2005 b. *American Journal of Veterinary Research,* **11,** pp 1861-1869.

Van Den Boom R., Van Der Harst M.R., Brommer H., Brama P.A.J., Barneveld A., Van Weeren P.R. and Degroot J. Relationship between synovial fluid levels of Glycosaminoglycans, hydroxyproline and general activity of Matrix Metallo proteinases and the presence and severity of articular cartilage change on the proximal articular surface of P1. 2005. *Equine veterinary journal,* **37** (1), pp 19-25.

CURRICULUM VITAE PROFESSEUR KIRSCHVINK

Nathalie Kirschvink is a native of the German-speaking part of Belgium. She worked for a brilliant university degree in the FUNDP (university of Namur) then moved to the University of Liège where she undertook a doctoral thesis in veterinarian science, studying physiology under Professor Lekeux (ULg, Belgium). Her thesis was entitled "Study of the role of F2-iso-prostanes as marker and actor of the lung oxidizing stress". This specialisation in oxidative stress then continued with studies in pharmacology and toxicology under Professor Gustin (Ulg, Belgium) where she studied, among other subjects, enzymatic stress. This horse enthusiast has been in charge of the animal physiology department at the University of Namur, Belgium.

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