Equine vet. J., Suppl. (2006) **36** 00-00

Double blind investigation of the effects of oral supplementation of combined Glucosamine hydrochloride (GHCL) and chondroitin sulphate (CS) on stride characteristics of veteran horses

R. K. FORSYTH, C. V. BRIGDEN* and **A. J. NORTHROP**

Animal and Equine Science Department, Myerscough College, St. Michaels Road, Bilsborrow, Preston, PR3 0RY Lancashire, UK.

Keywords: horse; veteran; chondroitin; chondroprotective; Glucosamine; joint disease.

Summary

Reasons for performing study: **Oral chondroprotective supplements are commercially popular for veteran (and other athletic or arthritic) horses prone to joint degeneration, yet lack conclusive scientific support.**

Objectives: **To quantify the effects of an oral joint supplement (combination glucosamine hydrochloride (GHCL), chondroitin sulphate (CS) and N-acetyl-D-glucosamine) *in vivo* on stride parameters of veteran horses.**

Methods: **Twenty veteran horses were randomly assigned to a treatment (n = 15) or placebo group (n = 5). Pre-treatment gait characteristics were recorded at trot using digital video footage (50 Hz). The range of joint motion, stride length, and swing and stance duration were assessed using 2-dimensional motion analysis. Treatment (or placebo) was administered daily for 12 weeks at the manufacturer's recommended dosage. Gait was reassessed every 4 weeks using the pre-treatment protocol. Double blind procedure was implemented throughout. Relationships between variables were analysed using General Linear Model.**

Results: **Differences occurred in the treated horses by week 8. Range of joint motion increased significantly in the elbow ($P<0.05$), stifle and hind fetlock ($P<0.01$). Stride length increased significantly ($P<0.05$) with treatment. Swing duration was significantly increased at week 12 ($P<0.05$), whilst stance duration remained constant.**

Conclusion: **The oral chondroprotective offered symptomatic relief to veteran horses, evidenced by improved stride characteristics.**

Potential relevance: **Oral GHCL and CS supplementation may improve welfare by alleviating symptoms of degenerative joint disease.**

Introduction

Joint disease poses a significant threat to the health of equines as a common cause of lameness (Wallin *et al.* 2000). This is particularly prevalent in older working horses (Brama *et al.* 1999; Leblond *et al.* 2000; Clayton *et al.* 2002) as a result of wear and tear, strain caused by intensive athletic demands (Pool 1996) and reduced efficiency of the repairing process with ageing. Limited effectiveness of surgery for degenerative joint disease means that aggressive application of preventative methods and early treatment using rest, therapy, supplements and appropriate medication is desirable (McIlwraith and Vachon 1988). Long-term use of medication has been shown to cause further degradation of the cartilage matrix (Clayton *et al.* 2002), and there is therefore a need for a noninvasive treatment without side effects that can be used in the early stages, or prior to, the development of joint disease. Anecdotal evidence indicates that the joint degeneration process can be slowed, and symptoms improved, using joint supplements that may increase athletic performance, improve quality of life and reduce pain without the associated side effects of medical treatments.

Glucosamine hydrochloride and CS are frequently included in joint supplements due to their perceived chondroprotective properties. These ingredients have been shown to enhance the protective metabolic response of chondrocytes to mechanical stress, improving their repair and regeneration capabilities (Lippiello 2003). Decrease in glycosaminoglycan (GAG) content is the most significant alteration in degenerating joint cartilage, with the GAG loss directly proportionate to the severity of joint disease (Clark 1991). *In vitro* research demonstrates that GHCL stimulates GAG production, whilst CS inhibits cartilage matrix degradation, leading to the suggestion that they are more effective when used in combination (Orth *et al.* 2002). These changes may improve joint health and contribute to increased joint mobility. GHCL and CS also demonstrate anti-inflammatory properties. Interleukin-1β is a proinflammatory cytokine observed locally during the arthritic process; GHCL and CS appear to inhibit Interleukin-1β induced cyclooxygenase-2 (COX-2), and other key inflammatory regulators such as nuclear factor kappa B (Largo *et al.* 2003). These properties would be expected to reduce the pain and inflammation associated with joint degeneration.

There is limited quantitative *in vivo* evidence of the performance of specific chondroprotective ingredients, individually or in combination, especially in the older horse (Jarvis *et al.* 2005). Studies undertaken specifically in horses show conflicting results (Hanson *et al.* 1997; Dorna and Guerrero 1998; Clayton *et al.* 2002; White *et al.* 2003; Dechant *et al.* 2005) highlighting the need for the present study.

*Author to whom correspondence should be addressed.

It was hypothesised that GHCL and CS supplementation would improve range of joint motion and stride length of veteran horses. The objectives of this study were to quantify the effects of an oral joint supplement (Synequin)[1] and to provide a better understanding of the therapeutic effects of joint supplements in the veteran horse.

Materials and methods

Horses

Twenty veteran horses, 9 mares and 11 geldings, (age 15–35 years, mean ± s.d. 20.9 ± 6.68) of varying breeds and heights were used. Selection of subjects was based on age criteria alone, with no consideration of medical or athletic history to allow a larger sample size, desirable for more reliable results. Hanson *et al*. (1997) indicated that 3 weeks without medication prior to a joint supplement trial was sufficient to negate any effects; the present study followed this protocol by excluding any subject receiving medication or supplements within the 4 weeks prior to the trial. All horses were kept on a similar routine regarding exercise, turnout and feeding as these factors may influence mobility. This study was conducted under the ethical guidelines set by the Biological Sciences Department, University of Central Lancashire and received ethical approval from the University Ethics Committee.

Double blinding procedure

Uptake of CS and GHCL is highly variable (Dechant *et al*. 2005) and to account for this a larger treatment group was used. Five horses were randomly assigned to a placebo group and 15 horses a treatment group. All received treatment or placebo in the daily feed according to the manufacturers' recommendations. Placebo and treatment supplements had identical physical appearances and were distributed in identical containers labelled numerically. The coding remained in a secure location until after all measurements had been recorded to ensure that both horse owners and the researchers were not aware of the identity of the treated horses.

Treatment

The treatment contained 2000 mg purified CS (95% purity) and 5000 mg GHCL (99% purity) and 500 mg N-acetyl-D-glucosamine/10 g. The placebo consisted of the nonactive filler element of the treatment. Dose was calculated according to bodyweight, as recommended by the manufacturer (Table 1).

Videographic recording procedure

Assessment took place immediately prior to treatment and 3 times during treatment at intervals of 4 weeks. This allowed establishment of pre-treatment kinematic parameters and monitoring of the treatment effects regularly with minimum stress to the horses. The manufacturer recommended that 12 weeks was the minimum time in which effects would be seen and was considered an appropriate time scale for this investigation.

TABLE 1: Supplement dosage

	Dosage for horses <500 kg bwt (g/day)	Dosage for horses >500 kg bwt (g/day)
Loading phase		
Day 1–35	20	30
Transition phase		
Day 36–60	10	15
Maintenance		
Day 61 onwards, alternate days	10	10

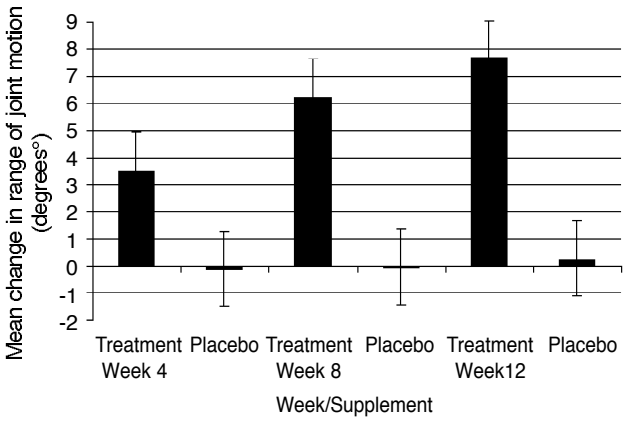

Fig 1: Summary of collective mean change (± s.d.) in range of joint motion after 4, 8 and 12 weeks receiving an oral chondroprotective supplement (n = 15) or placebo (n = 5).

Skin markers (13 mm) were applied by the same well-trained researcher to relevant anatomical locations adapted from the model described by Leach and Dyson (1988); hoof at the coronary band (1), distal metacarpus (2), proximopalmar quadrant of the ulnar carpal bone (3), distal humerus at the lateral epicondyle (4), proximal humerus at the caudal greater tubercle (5), proximal scapular spine (6), hoof at the coronary band (7), distal metatarsus (8), centre of the lateral aspect of the tarsus (9), distal femur at the lateral epicondyle (10), and proximal femur at cranial greater trochanter (11).

Horses were trotted in-hand by an experienced handler, at a speed comfortable for each horse, in a straight line twice in each direction to record a minimum of 3 strides for each limb. Assessment occurred on a flat, concrete surface and was recorded by a digital video camera (Sony Handycam, DCR-HC18E)[2] recording at 50 Hz, placed on a standard tripod 6 m away and perpendicular to the trot-up line. The trotting speed for each trial was assessed using the computer software and any trials with markedly different trotting speeds (± 0.1 m/sec) were discounted from the study. In hand assessment was used as many subjects were retired, and ridden or treadmill work may have been unethical, as well as interfering with natural gait.

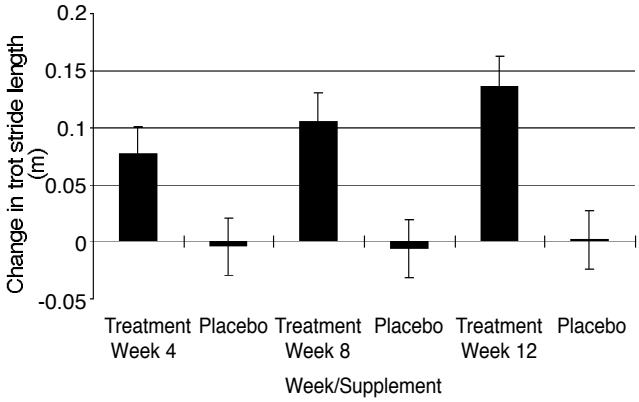

Fig 2: Summary of collective mean change in trot stride length after 4, 8 and 12 weeks receiving an oral chondroprotective supplement (n = 15) or placebo (n = 5).

TABLE 2: Mean and F values of change in range of joint motion from pre-treatment for individual joints of 20 veteran horses receiving an oral chondroprotective supplement (n = 15) or placebo (n = 5). Significance levels denoted by asterisks (*P <0.05, **P <0.01, ***P <0.001)

		Week 4		Week 8		Week 12	
Joint	Group	(Mean°)	F	(Mean°)	F	(Mean°)	F
Elbow	Treatment	2.48	4.45	6.88	4.94*	7.41	5.11*
	Placebo	-2.60		-4.02		-1.45	
Knee	Treatment	4.38	0.23	8.43	2.09	9.28	2.04
	Placebo	2.93		3.56		4.71	
Fetlock	Treatment	4.16	1.28	6.48	4.22	8.34	3.45
	Placebo	3.16		0.58		3.69	
Stifle	Treatment	2.06	4.47	4.25	10.55**	5.42	11.61**
	Placebo	-1.54		-0.14		-1.80	
Hock	Treatment	4.22	3.19	4.25	3.71	6.00	3.79
	Placebo	-0.74		-0.09		0.05	
Hind fetlock	Treatment	3.80	3.52	6.99	10.72**	9.51	19.81***
	Placebo	-1.88		-0.24		-3.57	

Analysis of gait

Gait analysis was performed using a 2-dimensional motion analysis system (Equinalysis)[3] for each assessment. Range of motion in 6 joints (elbow, knee, fetlock, stifle, hock and hind fetlock) was calculated for 3 strides at the trot by manually digitising each joint marker and subtracting the minimum joint angle from the maximum angle in each stride. Three strides allowed sufficient repetition to calculate an accurate mean without repeating strides excessively and replicate the methods used by Hanson *et al*. (1997) and White *et al*. (2003). Stride length, swing and stance duration were also measured.

Statistical analysis

Means of all measured parameters were calculated for individual horses pre- and post treatment. The data was pooled for the treatment and placebo group to assess the efficacy of the treatment. Prior to analysis the data was examined using a Kolmogorov-Smirnov normality test and found to be highly homoscedasic. Any potential significant differences between the group values were highlighted using a general linear model and Minitab (version 14)[4].

Results

There were no significant differences in the measured kinematic variables of the subjects at week 4. At week 8, significant differences were evident within the treatment group. Range of joint motion for all joints collectively increased with treatment (Fig 1). This effect was significant in the elbow (P<0.05), stifle (P<0.01) and hind fetlock (P<0.01) at week 8, when compared to the placebo group, and continued to be significant at week 12 (Table 2).

Stride length measurements had increased significantly with treatment in trot at week 8 (P<0.05) and week 12 (P<0.01) (Fig 2). The treatment had no effect on stance duration compared to the placebo, but did have a significant effect on swing duration at week 12 in both the fore- and hindlimb (P< 0.05) (Table 3).

Discussion

The present study demonstrated the positive effect of combined GHCL/CS supplementation on the movement of veteran horses, by increasing use of joints (seen as increased range of joint motion). This is thought to have led to the increased stride length, also associated with increased swing duration as the horse protracts the limb further forward. These improvements in kinematic parameters indicate that the supplement was improving horse comfort and wellbeing.

The mechanism by which these substances improve joint movement requires further consideration. GHCL and CS are included in joint supplements for their ability to enhance the protective metabolic response of chondrocytes (Lippiello 2003) and stimulate GAG production, whilst inhibiting cartilage matrix degradation (Orth *et al*. 2002). It was suggested that these changes would improve joint health and contribute to increased joint mobility; however, this may require long-term exposure to a supplement. The improvements seen in range of joint movement and stride length after 8 weeks of treatment may be explained by the anti-inflammatory properties of GHCL and CS (Largo *et al*. 2003). The reduction in inflammation and subsequent pain relief is likely to occur more quickly than significant improvements in joint health and may account for the initial improvements in mobility seen in this study.

The dose rate of GHCL and CS appear to be important (Dechant *et al*. 2005). High quantities of combined ingredients are suggested to be more effective than lower doses and separate treatments (Dechant *et al*. 2005). The product investigated in the present study contained large quantities of high purity GHCL and CS (95–99%) and so extrapolation of data to other products is only viable with products of similar composition. Dechant *et al*. (2005) also recognise the importance of *in vivo* factors, such as oral absorption, tissue concentrations and biotransformation that limit the extrapolation of *in vitro studies* to the live animal. The present study, again, successfully addressed this limitation and can be applied to a general population of living veteran horses.

Variations in the joint status of the subjects may have led to variations in individual horse's responses, therefore affecting results. There were, however, measurable improvements in the majority of the treated subjects by the end of the trial. Significant increases in stride lengths and range of joint motion demonstrated that this supplement has the potential to improve the movement of all veteran horses, regardless of a formally diagnosed joint disease. These results implicate a potential preventative role for this supplement, which could help to reduce the incidence of lameness caused by joint degeneration.

This study appears to be the first to investigate the effects of an oral chondroprotective supplement in a general population of veteran horses; therefore some variation in results is to be expected when compared to trials investigating horses suffering from specific joint disease. Significant improvements in stride parameters were consistent with studies by Hanson *et al*. (1997)

TABLE 3: Mean and F values of the change in swing and stance duration from pre-treatment for 20 veteran horses receiving an oral chondroprotective supplement (n = 15) or placebo (n = 5). Significance levels denoted by asterisks (*P <0.05, **P <0.01, ***P <0.001)

			Week 4		Week 8		Week 12	
Limb	Phase	Group	Mean duration (secs)	F	Mean duration (secs)	F	Mean duration (secs)	F
Forelimb	Stance	Treatment	-0.011	2.15	-0.015	2.94	-0.018	1.07
		Placebo	0.004		0.004		0.004	
	Swing	Treatment	0.016	1.22	0.025	0.72	0.036	14.96**
		Placebo	0		0.012		-0.02	
Hindlimb	Stance	Treatment	-0.013	0.55	-0.011	0.76	-0.013	0.73
		Placebo	-0.004		0		-0.004	
	Swing	Treatment	0.016	2.41	0.025	3.84	0.027	13.8**
		Placebo	0		-0.004		-0.016	

and Clayton *et al*. (2002), although significance was attained much earlier in those studies compared to the eight weeks taken in this study. This is likely to be due to the variation in horses' age and joint health status for this study where other studies used horses with diagnosed joint disease.

Skin markers are likely to displace, particularly in areas where the skin slides over bony areas. Correction models have been devised (Van Weeren *et al*. 1992) but were not utilised for this study, as they may not be accurate when applied to horses that differ in conformation to the Dutch Warmbloods used in the original investigation. Gait irregularities, likely to occur in the veteran horses used in this trial, might also affect locomotion to a point where correction factors are not reliable. Back *et al*. (1993) deemed correction models unnecessary if an animal was used as its own control, as in this study.

Limited research in this area may be a result of reliance upon artificially-induced models of degenerative joint disease to provide sufficient subjects with similar joint pathology. If methods that prove to be both effective and ethical, such as those used in this study, become more popular, further research is more likely to be considered and should be encouraged. This study has contributed to the current gap in research; however, if chondroprotective supplements are going to be considered a viable alternative to other, long proven treatments, more evidence is required. Further work would need to investigate whether beneficial effects are maintained in the long-term or if medical intervention is inevitable in horses suffering degenerative joint disease. The consequences of supplement withdrawal should also be considered in order to determine whether or not the beneficial effects are reversible.

The positive effect of oral chondroprotectives illustrated with this group of veteran horses supports suggestions that mobility is reduced with increasing age and can be improved prior to formal diagnosis of a joint condition. There are implications for a preventative role; if veteran horses are routinely administered with oral chondroprotectives, this may prevent or slow progression of joint degeneration, therefore prolonging working life and reducing the potential need for more extensive medical intervention at a later stage. This may also eliminate the need for costly veterinary intervention as well as reducing suffering and wastage.

The significant improvements in stride characteristics observed in this trial suggest treatment with an oral chondroprotective has improved joint mobility over the short term. The potential improvements to welfare by alleviation of degenerative joint disease and removing the need for extreme or invasive veterinary intervention suggest that this is an important area of research.

Acknowledgements

This study was sponsored and supported by Myerscough College in association with the University of Central Lancashire. Many thanks to the Veteran Horse Society and the owners of all the horses used in the trial. The authors also wish to thank Dr Jaime Martin for editorial and statistical advice and support.

Manufacturers' addresses

[1]VetPlus Ltd, Docklands, Dock Road, Lytham, UK.
[2]Sony United Kingdom Ltd, Pipers Way, Thatcham, Berks, UK.
[3]Equinalysis, Ty-Freeman Lane, Gwehelog Usk, Monmouthshire, UK.
[4]Minitab Version 14, Brandon Court, Unit E 1 Progress Way, Coventry, UK.

References

Back, W., Van Den Bogert, A.J., Bruin, G. and Barneveld, A. (1993) Quantification of the locomotion of Dutch Warmblood foals. *Acta Anat*. **146**, 141-147.

Barrey, E (1999) Methods, applications and limitations of gait analysis in horses. *Vet. J*. **157**, 7-22.

Brama, P.A.J., TeKoppele, J.M. and Bank, R.A. (1999) Influence of site and age on biochemical characteristics of the collagen network of equine articular cartilage. *Am. J. vet. Res.* **60**, 341-345.

Clark, D.M. (1991) The biochemistry of degenerative joint disease and its treatment. *Comp. cont. Educ. pract. Vet*. **13**, 275-281.

Clayton, H.M., Almeida, P.E., Prades, M., Brown, J., Tessier, C. and Lanouaz, J.L. (2002) Double blind study of the effects of an oral supplement intended to support joint health in horses with tarsal degenerative joint disease. *Proc. Am. Ass. equine Practnrs*. **48**, 317-324.

Dechant, J.E., Baxter, G.M., Frisbie, D.D., Trotter, G.W. and McIlwraith, C.W. (2005) Effects of glucosamine hydrochloride and chondroitin sulphate, alone and in combination, on normal and interleukin-1 conditioned equine articular cartilage explant metabolism. *Equine vet. J*. **32**, 227-231.

Dorna, V. and Guerrero, R.C. (1998) Effects of oral and intramuscular use of chondroitin sulphate in induced equine aseptic arthritis. *J. equine vet. Sci*. **18**, 548-555.

Goodrich, L.R and Nixon, A.J. (2004) Medical treatment of Osteoarthritis in the Horse - A Review. *Vet. J*. Article in press.

Hanson, R.R., Smalley, L.R., Huff, G.K., White, S. and Hammad, T.A. (1997) Oral treatment with a glucosamine-chondroitin sulphate compound for degenerative joint disease in horses: 25 Cases. *Equine Practice* **19**, 16-20.

Henrotin, Y. Sanchez, C. and Balligand, M. (2005) Pharmaceutical and nutraceutical management of canine osteoarthritis: present and future perspectives. *Vet. J*. **170**, 113-123.

Jarvis, N., Harris, P.A. and Lockyer, C. (2005) How to: Feed the older horse. In: *Proceedings of the BEVA Congress 2005*. Harrogate. British Equine Veterinary Association, 337-338.

Largo, R., Alvarez-Soria, M.A., Diez-Orlego, I., Calvo, E., Sanchez-Pernaute, O., Egido, J. and Herrero-Beaumont, G. (2003) Glucosamine inhibits IL-1-beta-induced NFKappaB activation in human osteoarthritic chondrocytes. *Osteoarthritis Cartilage* **11**, 290-298.

Leach, D.H and Dyson, S. (1988) Instant centres of rotation of equine limb joints and their relationship to standard skin marker locations. *Equine vet. J., Suppl*. **6**, 113-119.

Leblond, A., Villard, I. and Leblond, L. (2000) A retrospective evaluation of the causes of death of 448 insured french horses in 1995. *Vet. Res. comm*. **24**, 85-102.

Lippiello, L. (2003) Glucosamine and chondroitin sulphate: biological response modifiers of chondrocytes under simulated conditions of joint stress. *Osteoarthritis Cartilage*. **11**, 335-342.

McIlwraith, C.W. and Vachon, A. (1988) Review of pathogenesis and treatment of degenerative joint disease. *Equine vet. J., Suppl.* **6,** 3-11.

Orth, M.W, Peters, T.L and Hawkins, J.N (2002) Inhibition of articular cartilage degradation by glucosamine HCL and chondroitin sulphate. *Equine vet. J., Suppl*. **34**, 224-229.

Peek, S.F., Semrad, S.D. and Perkins, G.A. (2003) Clostridial myonecrosis in horses (37 Cases 1985-2000). *Equine vet. J*. **35**, 86-92.

Pool, R. (1996) Pathologic manifestations of joint disease in the athletic horse. In: *Joint Disease in the Horse,* Eds. C.W. McIlwraith and G.W. Trotter, Saunders, London, p 87.

Van Weeren, P.R., Van Den Bogert, A.J. and Barneveld, A. (1992) Correction models for skin displacement in equine kinematic gait analysis. *Equine vet. Sci*. **12** (3), 178-192.

Vigre, H., Chriél, M., Hesselholt, M., Falk-Rønne, J. and Ersbøll, A.K. (2002) Risk factors for the hazard of lameness in Danish Standardbred trotters. *Prev. vet. Med.* **56**, 105-117.

Wallin, L., Strandberg, E., Philipsson, J. and Dalin, G. (2000) Estimates of longevity and causes of culling and death in Swedish Warmblood and Coldblood horses. *Livestock Production Science* **63**, 275-289.

White, G.W., Stiles, T. Jones and W, Jordan, S (2003) Efficacy of intramuscular chondroitin sulphate and compounded acetyl-d-glucosamine in a positive controlled study of equine carpitis. *Veterinary Review* **23**, 295-300.