



A pendulum test as a tool to evaluate viscous friction parameters in the equine fetlock joint

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ABSTRACT

An equine fetlock joint pendulum test was studied and the influence of post mortem time and intra-articular lipid solvent on the viscous frictional response examined. Fresh equine digits (group 1, $n = 6$ controls; group 2, $n = 6$ lipid solvent) were mounted on a pendulum tribometer. Assuming that pendular joint damping could be modelled by a harmonic oscillator fluid damping (HOFD), damping time (τ), viscous damping coefficient (c) and friction coefficient (μ) were monitored for 5 h under experimental conditions (400 N; 20 °C). In all experiments, pendular joint damping was found to follow an exponential decay function ($R^2 = 0.99714$), which confirmed that joint damping was fluid.

The evolution of τ , c and μ was found to be significantly ($P < 0.05$) different in the two groups, with a decrease in τ and an increase in c and μ that was faster and more prominent in digits from group 2. It was concluded that pendular joint damping could be modelled by a HOFD model. The influence of post mortem time on results suggested that, ideally, joint mechanical properties should only be tested on fresh cadavers at the same post mortem time. Moreover, the addition of lipid solvent was found to be responsible for upper viscous friction parameters and for a reduced damping time, which suggested that articular lubricating ability was compromised. This equine pendulum test could be used to test the efficacy of various bio-lubricant treatments.

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Introduction

The synovial joint is a complex tribological structure, which supports and redistributes joint contact forces and facilitates low friction and low wear. The connective tissue of the synovial joint, a triplex of cartilage–synovial fluid–cartilage, is essential to function and provides a sophisticated lubricated system.

In recent years, the cartilage lubrication mechanism has been shown to depend significantly on the pressure of the cartilage interstitial water, which supports most of the joint contact load and helps to shift it away from the collagen–proteoglycan matrix, thereby producing a low frictional response (Ateshian, 1997, 1998; Krishnan et al., 2004; Caligaris and Ateshian, 2008). Frictional forces would be significant only over the portion of the load transferred across the solid matrices of the opposing articular layers; thus, as long as the interstitial fluid pressure remains elevated, supporting most of the load, the friction should remain low (Atesh-

ian, 2009). This mechanism, called biphasic boundary lubrication or self-pressurised hydrostatic lubrication, is related to cartilage composition.

Articular cartilage consists of water (68–85% weight), fibrillar matrix of type II collagen (10–20%), proteoglycans (5–10%) and chondrocytes. Due to the negative polarity of proteoglycans, which attracts more cations than anions into the tissue to maintain electroneutrality, a net electrolytic imbalance is created between the cartilage interstitial fluid and the surrounding tissue bath. This phenomenon produces a difference in osmotic pressure whereby the interstitial fluid exhibits a higher pressure than the external solution (Ateshian, 2009). A complementary approach, called boundary lubrication, has however been proposed, which describes the lubricating ability of the components of synovial fluid (SF). The boundary lubricants are adherent to apposed surfaces and thereby prevent adhesion and wear.

Three types of boundary lubricant are considered to be involved, individually or in combination, in synovial joints, namely, hyaluronan (HA), lubricin and surface-active phospholipids (SAPLs) (Jay, 1992; Schwarz and Hills, 1998; Elsaid et al., 2005; Bell et al.,

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2006). Hyaluronan is an un-branched high-molecular-weight (500–3800 kDa, 1–4 mg/mL) anionic disaccharide consisting of alternating units of glucuronic acid β (1–3) and N-acetylglucosamine β (1–4) (Forsey et al., 2006). HA is very hydrophilic (50,000 H₂O molecules for 1 HA), which gives SF a high viscosity (Trunfio Sfarighiu, 2006) and it has been recognised as the key molecule responsible for the rheological properties of SF (Tadmor et al., 2002).

Lubricin is a mucinous glycoprotein (240 kDa, 0.02 mg/mL) that consists of four components: (1) a vitronectin-like domain which confers a molecular adhesive role; (2) a mucin-like domain in which the abundance of negatively charged sugars creates strong repulsive hydration forces that enable the protein to act as a boundary lubricant; (3) a haemopexin-like domain that confers a lipidic antioxidant role, and (4) a linkage protein (Schvartz et al., 1999; Tolosano and Altruda, 2002; Rhee et al., 2005). Lubricin is now recognised as playing a major protective role in preventing cartilage wear and synovial cell adhesion and proliferation (Rhee et al., 2005).

SAPLs are amphiphilic molecules (0.75 kDa, 3 mg/mL) that are composed of a hydrophobic domain consisting of two fatty acid chains and a hydrophilic domain (Sarma et al., 2001). According to their hydrophobic domain, SAPLs are classified into two types: (1) unsaturated species (57%) and (2) saturated species (43%) (Sarma et al., 2001). Lipids are not miscible in SF water and SAPLs are therefore organised into 3–7 phospholipid bilayers on the surface of cartilage in joints, causing SF gel to be imprisoned between them (Schwarz and Hills, 1998). By attaching SAPLs on the SF gel and on the cartilage, and by performing its lipidic antioxidant role, lubricin would provide SAPLs stability (Trunfio Sfarighiu, 2006).

The impact of SAPL organisation and composition on boundary lubrication is important since the frictional response will decrease significantly when the number of phospholipid bilayers increases (Higaki et al., 1997), while the degree of un-saturation within fatty acid chain residues will reduce friction even further (Foy et al., 1999).

The role of SAPLs is debatable due to their instability. Indeed, unsaturated SAPLs are present as bilayers in an antioxidant and hydrophilic environment, such as the joint (Trunfio Sfarighiu, 2006), where the tension of oxygen (O₂) is low (7%) (Grimshaw and Mason, 2000; Schneider et al., 2007). When SAPLs are present in ambient air (O₂ 21%), the phospholipid bilayers are transformed into hydrophobic phospholipid mono-layers and the unsaturated connections are oxidised in oxirane (oxygen fixation) and then in diol (H₂O fixation) (Trunfio Sfarighiu, 2006). Singly or in combination, these phenomena act to decrease the role of SAPLs in boundary lubrication.

It is therefore apparent that to investigate the properties of articular cartilage we require a realistic joint model. Some studies have maintained conditions found within the joint to test the boundary lubricating ability of novel intra-articular treatments (El-said et al., 2005; Teeple et al., 2007, 2008), although not SAPLs. There is even less information known about the properties of articular cartilage when the joint atmosphere is conserved and a joint viscous friction model used.

The present study was the first attempt to measure viscous friction parameters on a whole equine metacarpophalangeal joint (MPJ), conserving the structural integrity of the joint and testing the influence of post mortem time and intra-articular lipid solvent.

Materials and methods

Specimen preparation

Twelve warmblood horse carcasses from a local abattoir were used in this study. The bodyweight (BW) ranged from 350 to 450 kg (mean 400 kg), and we collected the right distal limbs (cut at the middle carpus) immediately after slaughter. These were classified as group 1 ($n = 6$) or group 2 ($n = 6$). For each specimen, the

skin and the musculature were left intact, the hoof was trimmed in order to have a linear pastern-coffin axis and, using a power saw, the metacarpus was cut transversally 2 cm distal to the carpometacarpal joint.

The specimen was then fixed on the pendulum apparatus, completely immobilising the interphalangeal joints and leaving free the MPJ (Fig. 1). The hoof was screwed using six screws and the interphalangeal joints were clamped onto the static portion of the apparatus. The pendulum was fixed and adjusted on the metacarpus using eight compression screws. Biomechanical tests commenced within 90 min of slaughter.

Description of the pendulum test

A lateral-view of the pendulum apparatus is shown in Fig. 1. The apparatus was used to measure the pendular damping time (τ) or the time duration from onset angle to resting angle, the viscous damping coefficient (c) and the friction coefficient (μ).

The pendulum was designed to swing the metacarpus relative to the fixed pastern-hoof segment while a compressive load of 400 Newtons (N) was applied across the MPJ. The motions of the MPJ were recorded using a camera coupled to light emitting diodes (LEDs). The acquisition system was ImageJ,¹ an open-source program that was developed by the National Institutes of Health.² Four LEDs were rigidly mounted on the pendulum to document MPJ motion. The wire attachments to the LEDs were maintained free of tension. The locations of the LEDs were referenced to a terrestrial based coordinate system. Within this coordinate system, the metacarpus was identified as a rigid body and pendulum motion was described relative to the fixed terrestrial referential. The MPJ is a condylar joint, and pendulum motion was characterised as a flexion–extension motion.

Description of mechanical measurements and calculations

After immobilising the MPJ in 5° extension using an attached cord between the horizontal load bar and the vertical beam, we cut the cord using a flame so that the metacarpus was released, fell and oscillated freely between flexion and extension until it stopped. The inherent viscoelastic properties of the joint and the surrounding tissues, coupled to the mass of the moving loaded metacarpus, caused the metacarpus to come to rest close to the equilibrium position (static).

As shown in Fig. 2, several variables could be derived from the kinematics of each trial of the pendulum test. We measured the following displacement and timing parameters: the angle at the start of the test (onset angle), the peak angle of the cycle n (θ_n), the time duration from onset angle to resting angle or pendular damping time (τ), and the period of the cycle (T).

Assuming that pendular joint damping acted as a harmonic oscillator with fluid damping (HOFD), the MPJ kinematic data of each trial were used to compute the variations of viscous friction parameters for the duration of each experiment. The viscous damping coefficient (c) and friction coefficient (μ) were calculated using the following equations as reported by Lin and Rymer (1991) and Crisco et al. (2007):

$$\zeta = \frac{c}{2\sqrt{JK}} = \sqrt{\frac{(\ln D)^2}{4\pi^2 + (\ln D)^2}} \quad (1)$$

where J is the sagittal moment of inertia applied to the metacarpus complex rotation around the MPJ axis;

$$K' = K + \frac{mgl}{2}$$

and m is the metacarpus complex mass, g is the gravity acceleration and l is the distance of the metacarpus complex centre of mass from the MPJ axis; $D = \frac{\theta_n}{\theta_{n+1}}$ is the ratio of the peak angle of one cycle (θ_n) to the peak angle of the following cycle (θ_{n+1}).

$$\omega = \sqrt{\frac{K'}{J}} = \frac{2\pi}{T} \quad (2)$$

where T is the period of one cycle (see Fig. 2).

The estimations of J and of mass characteristics (m and l) were obtained for each subject according to Huygens' theory:

$$J_A = J_{GA} + md^2$$

where solid inertial moment around an axis A is equal to the inertial moment around an axis going through the solid inertial centre of mass (J_{GA}), added to the solid mass multiplied by the second power of the distance between the two axes (md^2).

$$\theta_n = \theta_0 \left[e^{-2n\zeta} - \delta \frac{1 + e^{-\zeta}}{1 - e^{-\zeta}} (1 - e^{-2n\zeta}) \right] \quad (3)$$

$$\delta = \frac{\mu m g r T^2}{4\pi^2 J \theta_0} \quad (4)$$

¹ <http://rsbweb.nih.gov/ij/>.

² <http://www.nih.gov/>.

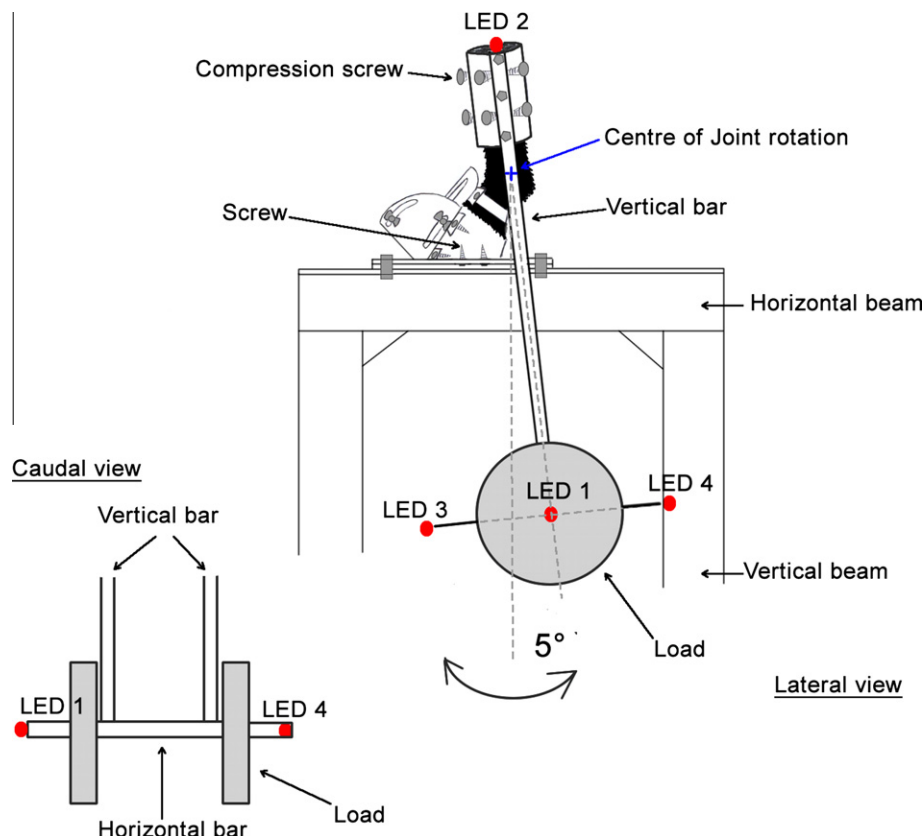


Fig. 1. The pendulum tribometer apparatus. The pendulum was designed to swing the metacarpus relative to the fixed pastern-hoof segment while a compressive load of 400 N was applied across the metacarpophalangeal joint (MPJ). After immobilising the MPJ in 5° extension, the metacarpus was released, fell and oscillated freely between flexion and extension until it stopped. The motions of the MPJ were recorded using a camera coupled to light emitting diodes (LEDs). Finally, kinematic data were calculated to estimate viscous frictional response.

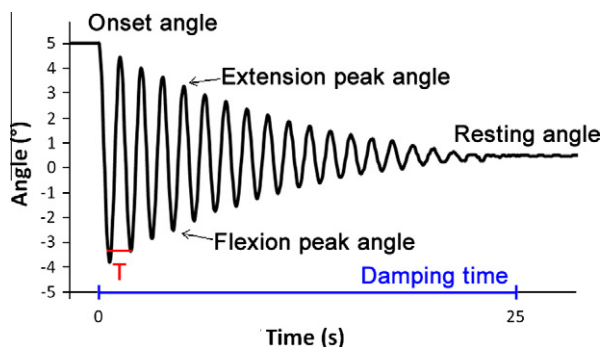


Fig. 2. Typical metacarpophalangeal joint flexion–extension response showing onset angle, resting angle, the period of the first cycle (T) and damping time.

Using Eqs. (1) and (2), the values of the viscous damping coefficient were obtained as follows:

$$c = 2 \cdot \zeta \cdot \omega \cdot J \quad (5)$$

Using Eqs. (3) and (4), the values of the friction coefficient were obtained as follows:

$$\mu = \frac{\delta 4 \pi^2 J \theta_0}{m g r T^2} \quad (6)$$

Tested parameters

All experiments were performed at room temperature (20 °C). Two sets of experiments were defined in this study (Table 1). Experiment E1 investigated the evolution of the joint mechanical properties after slaughter and we measured τ , c and μ in group 1 ($n = 6$), for four trials (at 90, 190, 290 and 390 min post mortem). For each trial, c and μ were averaged from the first 10 oscillation peaks. In order to

disorganise SAPLs in experiment E2, we investigated the evolution of the joint mechanical properties before and after an injection of 2.5 mL Norvanol D (denatured ethanol 95%, VWR international PROLABO) given in the palmar recess of each MPJ using a syringe with a 21 GA needle. We measured τ , c and μ in group 2 ($n = 6$), for five trials (at 90 min before and after alcohol addition, 190, 290 and 390 min post mortem). For each trial, c and μ were averaged from the first 10 oscillation peaks.

Between every trial, each specimen remained fixed on the apparatus to avoid any adjustment error. The MPJ was relaxed using a hydraulic jack to support the loaded horizontal bar. All MPJs were cut open just after the experiments to reveal the articular surface (smooth or rough, stiff or compressible with a bluish or yellowish tint) and the synovial fluid. We then undertook a macroscopic examination.

Statistical analysis

The experimental values of pendular decay, corresponding to the peak values of the LED displacements in direction x , were fitted using two different models, one exponential and the other linear. An F -test was then applied to find out which model was the best fit for the same pendular decay (OriginPro 8). The statistical significance of the differences in τ , c and μ for experiment E2 before and after the addition of alcohol were determined using one-way analysis of variance (ANOVA) with repeated measurements to compare values (two levels) in group 2. The statistical significance of the differences in τ , c and μ for experiments E1 and E2 was determined using two-way analysis of variance (ANOVA) with repeated measurements to compare values of the two groups (two levels) between the time configurations (four levels). In all cases, α was set to 0.05 and statistical significance was accepted for $P < 0.05$, with the Bonferroni correction applied post hoc.

Results

Experiments

At the 0.05 significance level, the exponential model ($R^2 = 0.99714$) was more likely to be correct than the linear model

Table 1

List of experiments and details of testing protocols.

Experiment	Lubricant	n	Test	Extension angle at the start (°)	Load (N)	Post mortem time of test (min)			
						t1 90	t2 190	t3 290	t4 390
Experiment 1	Synovial fluid	6	Pendulum test	5	400	+	+	+	+
Experiment 2	Synovial fluid	6	Pendulum test	5	400	+	+	+	+
Experiment 2	Synovial fluid + alcohol		Pendulum test	5	400	+	+	+	+

Each experiment was performed at room temperature (20 °C). Damping time, viscous damping coefficient and friction coefficient were followed until 6.5 h post slaughter.

($R^2 = 0.85849$) to fit joint pendular decay (Fig. 3a and b). This confirmed that pendular joint damping is fluid and that the HOFD model provided the best representation of the whole joint. Measurements of τ , c and μ that were performed in experiments E1 and E2, are presented in Table 2 and in Fig. 4a–c.

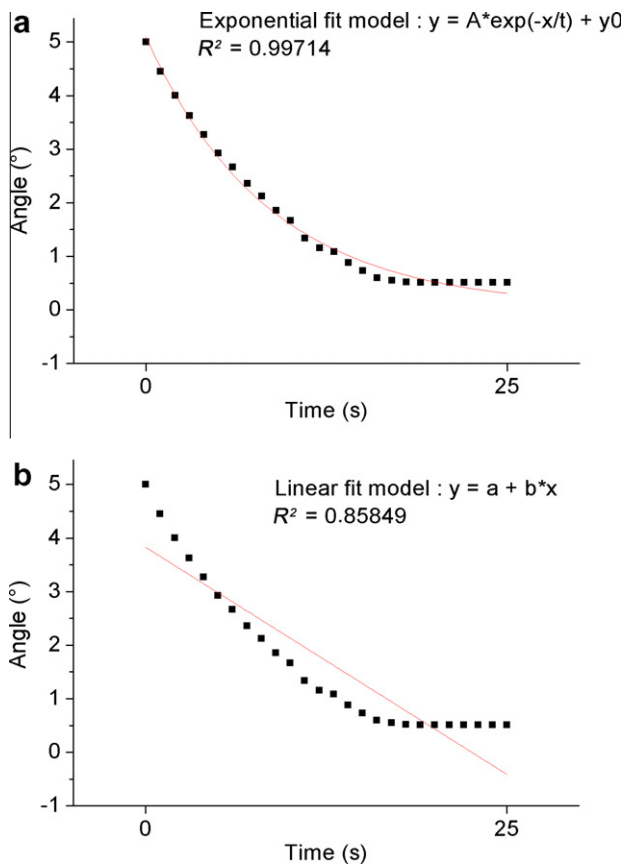


Fig. 3. Fitted joint pendular decay using an exponential model (a) and using a linear model (b).

Table 2Mean values of friction coefficient (μ) viscous damping coefficient (c) and damping time (τ), which were collected in experiments E1 and E2.

Experiments Parameters	Experiment 1			Experiment 2		
	μ (10^{-3})	c	τ	μ (10^{-3})	c	τ
t1 (90 min)	6.9 (1.69)	0.862 (0.174)	24.47 (1.45)	5.69 (1.26) 5.49 (1.25)	1.069 (0.132) 0.945 (0.133)	22.56 (2.00) 23.23 (2.04)
t2 (190 min)	7 (1.57)	0.914 (0.186)	23.99 (1.33)	7.23 (1.68)	1.6 (0.257)	19.72 (1.74)
t3 (290 min)	7.3 (1.64)	0.972 (0.132)	22.44 (1.23)	7.76 (1.84)	1.951 (0.400)	17.97 (1.58)
t4 (390 min)	7.9 (1.69)	1.095 (0.151)	21.46 (1.00)	14.10 (5.58)	2.313 (0.509)	17.49 (1.56)

Experiments 1 and 2 were performed under an applied load of 400 N at room temperature 20 °C. For Experiment 2, alcohol was injected, the grey sections corresponding to the measured parameters after injection of alcohol. μ is a dimensionless measurement (~ 0 –1), which describes the ratio of frictional force and the normal force between two surfaces. The units for c is $\text{kg m}^2/\text{s}$ and for τ is second. The SE is shown in brackets.

Effects of time and treatment

In group 2, the comparison of τ , c and μ before and just after the alcohol injection showed that τ increased and that c and μ decreased, but not significantly. In group 2, following alcohol injection, and in group 1, the effect of time resulted in a significant ($P < 0.05$) decrease in τ , a significant ($P < 0.05$) increase in c and a significant ($P < 0.05$) increase in μ . Moreover, we found that the evolution of τ , c and μ were significantly ($P < 0.05$) different in group 1 compared to group 2. Indeed, τ decreased, c and μ increased more rapidly and to a higher level in group 2 than in group 1.

Interaction of time and alcohol injection

For τ , the effect of alcohol injection within time was found to be significant ($P < 0.05$) except within t1. Furthermore, the effect of time within group 1 was found to be significant ($P < 0.05$) in the t1–t3, t1–t4 and t2–t4 pair wise comparisons, whereas the effect of time within group 2 was found to be significant ($P < 0.05$) in the t1–t2, t1–t3, t1–t4 and t2–t4 pair wise comparisons. For c , the effect of time within group 2 was found to be significant ($P < 0.05$) in the t1–t3 and t1–t4 pair wise comparisons. For μ , the effect of alcohol injection within time was found to be significant ($P < 0.05$) only in t4 and the effect of time within group 2 was found to be significant ($P < 0.05$) only in the t1–t4 pair wise comparison.

Macroscopic examination

In all the experiments, the articular surfaces were smooth, and stiff with a bluish tint. None showed an arthritic aspect that could influence the results on joint mechanical properties. The SF was found to be slightly clearer in group 2 than in group 1.

Discussion

The main objective of our study was to measure joint viscous friction parameters (τ , c and μ) using an equine pendulum test, and to investigate the influence of post mortem time and of the injection of alcohol on the evolution of τ , c and μ .

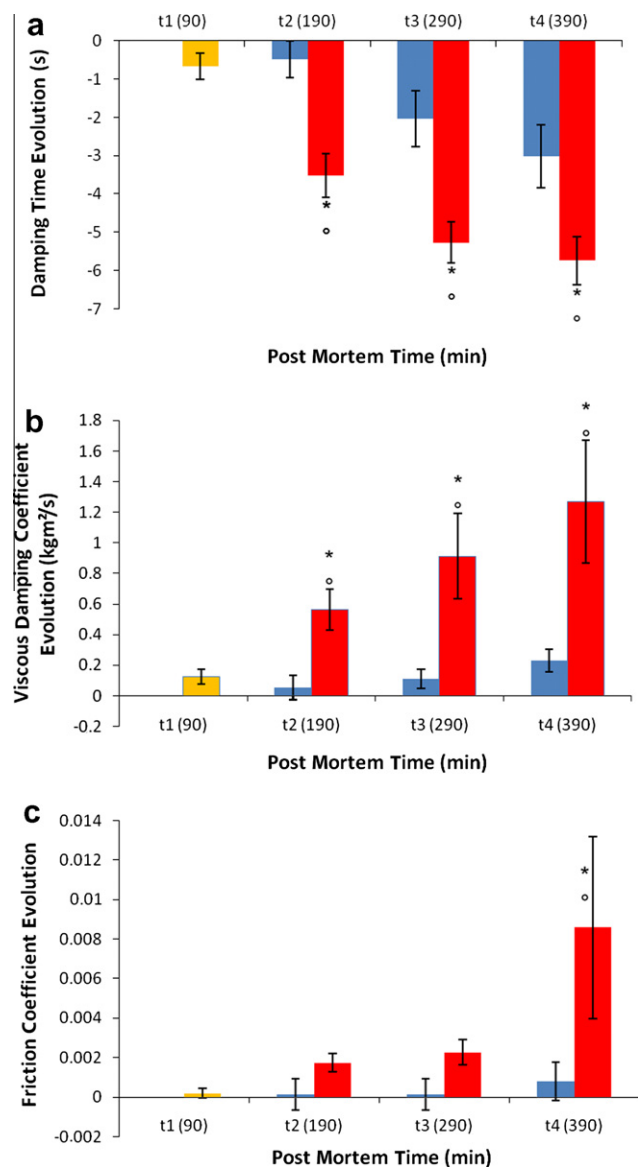


Fig. 4. Effect of alcohol on the damping time (a); on the viscous damping coefficient (b); on the friction coefficient (c) of the whole fetlock joint. The control group is shown in blue and the alcohol group is shown in red representing before alcohol injection and in red representing after alcohol injection. Each point represents the mean \pm SE; * $P < 0.05$, significant difference within group; † $P < 0.05$, significant difference between control and lipid solvent groups. The unit for τ is second, the units for c is $\text{kg m}^2/\text{s}$ and μ is dimensionless (~ 0 – 1).

It was not possible to compare the current results with other reports because this was the first time that a pendulum apparatus has been used as an equine MPJ model. Nevertheless, in comparing our results with joint pendular studies carried out in other species, our mean μ (~ 0.006) was similar to reports in other studies (~ 0.001 – 0.05), while our mean c ($\sim 0.96 \text{ kg m}^2/\text{s}$) was not ($\sim 3 \times 10^{-5}$ – $1.4 \times 10^{-3} \text{ kg m}^2/\text{s}$) (Crisco et al., 2007; Drewniak et al., 2009). This difference may be due to the substantially different load (ratio: 1/1000) used in the calculations.

The joint pendular decay was exponential, which agreed with other reports (Crisco et al., 2007; Drewniak et al., 2009), and demonstrated that the exponential decay function (including both frictional and viscous damping) dramatically increased the accuracy of measuring the frictional response in a pendulum test of modelled whole joints.

However, our experimental procedure had several limitations, including the fact that the joint pendulum test was performed at

room temperature (20°C) and specimens were not perfused (hypoxia). The conditions were not physiological and this may be responsible for a change in the rheological properties of the joint. Indeed, whereas our results clearly established that τ , c and μ changed during post mortem time in both groups, this could be explained by the decrease in temperature, the deteriorating hydration status and post mortem autolysis. The results highlight the importance of rigorous and consistent specimen preparation when evaluating joint mechanical properties, which should only be tested on fresh cadavers at the same post mortem time.

In group 2, the comparison of τ , c and μ before and just after alcohol injection showed that τ increased, and that c and μ decreased, but not significantly. This result could be explained by the fact that the ratio of the volume of alcohol (constant) to the MPJ volume (variable) was randomised, and so the dilution effect was not significant. A comparison of the control group (group 1) and the alcohol group (group 2) showed a significant difference between the evolution of τ , c and μ , where τ decreased and c and μ increased more rapidly and to a higher level in the alcohol group than in the control group.

Assuming that the addition of alcohol had only a lipid solvent effect on the SF, the results of our experiments suggest that SAPLs contribute to boundary lubrication of the joint. However, Schmidt et al. (2007) demonstrated that SF friction coefficients were not significantly affected by the addition of the dipalmitoyl-phosphatidylcholine (DPPC) at physiological concentrations. So, while the cartilage samples in that experiment were completely immersed in the test lubricant for 24 h prior to lubrication testing, SAPL organisation was probably disturbed by the ambient air condition. Moreover, Chen et al. (2007) showed that the individual relative percentage of DPPC on all SAPLs was only 8%, and its ability to reduce the frictional response was lower than other SAPLs (Chen et al., 2005). Finally, it should also be noted that the lipid boundary lubricant of synovial joints is constituted by a combination of SAPLs rather than a single SAPL (Sarima et al., 2001).

A further consideration is that the triplex (cartilage–SF–cartilage) is linked structurally and functionally and the frictional response of cartilage is not limited to a surface phenomenon and is greatly influenced by the degree of tissue degradation (Basalo et al., 2005). Indeed, experimental cartilage damage has suggested a molecular level of matrix degradation associated with a deterioration in the functional physical properties of the tissue, such as a decrease in the compressive stiffness (Bonassar et al., 1995), a decrease in Young's modulus (Lyrra et al., 1999), and alterations in the frictional response (Basalo et al., 2005).

Our previous work has suggested the same conclusion regarding cartilage ageing (Noble et al., 2009). Therefore, taking into consideration the fact that the injection of alcohol not only had a lipid solvent effect on the SF but also dehydration and an oxidant effects on the triplex, these experiments suggested that the lubrication mechanism of the alcohol group could have been compromised. A combination of SAPL disturbance, dehydration and oxidant effects could have limited the function of HA and lubricin, so destabilising SF rheology since antioxidant effects and boundary lubrication did not occur. The biphasic boundary lubrication theory would suggest that the cartilage lost its matrix integrity so that the interstitial pressure could have fallen and the viscous frictional response could have increased, although further studies are required to confirm this.

Conclusions

This was the first time that viscous friction parameters have been measured using an equine pendulum. Experimental data con-

firmed that pendular joint damping can be modelled by a harmonic oscillator with fluid damping. In addition, lipid solvent treatment was found to be responsible for upper viscous friction parameters and for a reduced damping time, which suggests that the articular lubricating ability was compromised. Although the pendulum is a simplified tribological model, it may be useful for testing the efficacy of various bio-lubricant treatments.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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