INSTRUCTIONS FOR LETTERS TO THE EDITOR

Editorial comment in the form of a Letter to the Editor is invited; however, it should not exceed 800 words, with a maximum of 10 references and no more than 2 figures (submitted as camera ready hard copy per Journal Guidelines) or tables and no subdivision for an Abstract, Methods, or Results. Letters should have no more than 3 authors. Full name(s) and address of the author(s) should accompany the letter as well as the telephone number, fax number, or E-mail address.

Contact. The Managing Editor, The Journal of Rheumatology, 920 Yonge Street, Suite 115, Toronto, Ontario M6J 3G7, CANADA. Tel: 416-967-5155; Fax: 416-967-7556; E-mail: jrheum@jrheum.com Financial associations or other possible conflicts of interest should always be disclosed.

Identity of the Joint Lubricant

To the Editor:

In a recent study, Jay and Cha[1] employed selective enzymatic destruction of joint lubricant to address the important issue of the identity of the vital "active ingredient" in synovial fluid (SF), which imparts effective boundary lubrication to the articular surface. Is it surface active phospholipid (SAPL)[2,3] or their preferred choice[4] of lubricin, which is a macromolecular water soluble glycoprotein? Our study[5] employing trypsin and phospholipase A_2 as the digestive enzymes favored SAPL as the boundary lubricant in bovine SF (BSF) based upon articular cartilage sliding upon glass, whereas Jay and Cha reach the opposite conclusion employing trypsin and phospholipase C (PLC) for glass sliding on rubber (i.e., hydrocarbon). This hydrophilic-hydrophobic combination would appear a strange selection of surfaces if the findings are to have any relevance to the situation *in vivo*.

Jay and Cha[1] conclude in favor of lubricin for two reasons: First, trypsin increases friction, but they then go on to castigate those favoring SAPL, namely ourselves[5,6], as "failing to address repeated reports of the removal of lubricating ability from SF by digestion with trypsin." This remark totally misrepresents the true issue. No one questions a major role for lubricin, but is lubricin the lubricant *per se*, or is lubricin the macromolecular water-soluble carrier for the otherwise highly insoluble SAPL, which is the true lubricant as we have advocated[6,7]? Our analysis[6] has shown how SAPL makes up 12% of lubricin, which would render this macromolecule an ideal carrier for SAPL, while phospholipids also bind to hyaluronic acid[8], which has similar protein chains.

Thus, their trypsin results offer no means of differentiating between boundary lubricants, because by their theory trypsin destroys the lubricant *per se*, while by ours it destroys the carrier for the lubricant with a similar detrimental effect upon lubrication.

Second, Jay and Cha claim that lubricin *per se* is the boundary lubricant because PLC produces $\Delta\mu$ values that do not differ significantly from straight BSF. However, these $\Delta\mu$ values are derived by subtracting from the direct measurement of friction a mean value of saline controls that differ by 27% from each other, and thereby cancel out the difference in values for the primary measurement, i.e., BSF *versus* BSF + PLC, which they list in the first column of Table 1. However, the "controls" would appear to be a thin layer of saline, which, when sandwiched between two nonbiological surfaces such as glass and rubber, should surely give almost the same mean values. It also complicates the direct comparison of two *boundary* lubricants when they state "that only a thin layer of boundary fluid was present" at the interface, implicating *hydrodynamic* lubrication.

If we avoid these questionable "controls" and compare BSF with BSF digested with PLC, i.e., using one as a control for the other, then their results show that PLC *increases* friction 2.8-fold, i.e., from 0.028 to 0.095 (first column, Table 1). Surely this can only occur if it destroys phospholipid, demonstrating how SAPL is the boundary lubricant *per se* and not lubricin. However, the authors point out that their PLC was "contaminated with proteases" but, when adding a protease inhibitor, PLC still increases friction about 2-fold (μ = 0.028 to 0.050). This comparison may not reach statistical significance, but surely it severely undermines any conclusion by Jay and Cha that lubricin *per se* is the boundary lubricant. Their results could even indicate the reverse, as did our study[5] using higher numbers of runs and sliding surfaces, which were far more relevant physiologically, even if our friction apparatus was not as sophisticated.

We still contend that lubricin, and maybe other proteinaceous macromolecules in SF, plays an important role in the joint as the *carrier* for the otherwise highly insoluble SAPL, but is *not* the lubricant *per se*[5].

This view is consistent with the fact that almost all commercial boundary lubricants deposited from adjacent fluids are surfactants, whereas the two major components of lubricin, namely proteins and carbohydrates, tend to be glues. Another factor emphasized elsewhere[2,7] is that boundary lubrication is imparted by the outermost layer of a surface, and so how could binding of such a *hydrophilic*, water-soluble substance as lubricin ever render the articular surface so hydrophobic, as reported by ourselves[2] and others quoted by Jay and Cha? The load-bearing boundary lubricant is surely surface-active phospholipid — the same lubricant found on other sliding surfaces *in vivo*[7].

If this alternative conclusion is correct, it is fortunate clinically because

Table 1. Friction coefficients of bovine synovial fluid (BSF) and phosphatidylcholine (PC) preparations digested with phospholipase C. Data reproduced from Jay and Cha[1].

Test Lubricant	Mean ± SD	Mean ± SD Physiologic Saline	$\Delta\mu$ ± SD	n
Phosphatidylcholine	0.016 ± 0.012	0.149 ± 0.049	−0.133 ± 0.048	6
PC + phospholipase C	0.202 ± 0.107	0.127 ± 0.071	+ 0.075 ± 0.063	10
BSF	0.028 ± 0.013	0.0997 ± 0.032	−0.069 ± 0.026	6
BSF + phospholipase C − PI	0.095 ± 0.044	0.107 ± 0.036	−0.012 ± 0.048	6
BSF + phospholipase C + PI	0.050 ± 0.039	0.123 ± 0.025	− 0.073 ± 0.037	8
BSF + trypsin	0.242 ± 0.083	0.105 ± 0.029	+ 0.137 + 0.082	6

PI: Proteolytic inhibitors leupeptin and aprotinin, BSF: bovine synovial fluid.

exogenous phosphatidylcholine can be injected directly into the joint to replenish the deficiency of SAPL reported in osteoarthritis[9], while preliminary human trials[10] have proven most encouraging.

BRIAN A. HILLS, ScD (Cantab), Paediatric Research Centre, Mater Children's Hospital, South Brisbane, Queensland, Australia.

REFERENCES

1. Jay GD, Cha CJ. The effect of phospholipase digestion upon the boundary lubricating ability of synovial fluid. J.Rheumatol 1999; 26:2454-7.
2. Hills BA. Oligolamellar lubrication of joints by surface-active phospholipid. J Rheumatol 1989;16:82-91.
3. Hills BA.Oligolamellar nature of the articular surface. J Rheumatol 1990;17:349-56.
4. Jay GD, Cha CJ, Haberstroh K, Shaw R. Comparison of the boundary lubricating ability of bovine synovial fluid, lubricin and healon. J Biomed Mater Res 1998;40:414-8.
5. Hills BA, Monds MK. Enzymatic identification of the load-bearing boundary lubricant in the joint. Br J Rheumatol 1998;37:137-42.
6. Schwarz IM, Hills BA. Surface-active phospholipid as the lubricating component of lubricin. Br J Rheumatol 1998; 37:21-6.
7. Hills BA. Boundary lubrication *in vivo*. Proc Inst Mech Eng 2000; 214H:83-94.
8. Pasquali-Ronchetti L, Quaglino D, Mori D, Bacchelli B, Ghosh P. Hyaluronan-phospholipid interactions. J Struct Biol 1997;120:1-10.
9. Hills BA, Monds MK. Deficiency of lubricating surfactant lining the articular surfaces of replaced hips and knees. Br J Rheumatol 1998; 37:143–7.
10. Vecchio P, Thomas K, Hills BA. Surfactant treatment for osteoarthritis [letter]. Rheumatology 1999;38:1020–1.

Dr. Jay replies

To the Editor:

I respectfully submit that the evidence for phospholipid being the actual lubricating moiety of synovial fluid (SF) has been overdrawn. However, both Dr. Hills and I agree that lubrication occurs in the boundary mode, enabling deformable apposed surfaces to slide past one another at a very slow reciprocating speed[1]. The consideration that lubricin is actually a carrier[2] for the lubricant deserves serious inquiry — necessitating our reconciling seemingly disparate observations[3,4]. The conclusion that lubricin carries surface active phospholipid (SAPL) to articular cartilage, a function not unlike that of alveolar surfactant binding proteins (which have been comparatively better characterized) is based on the following evidence: (1) lipid is *posited* to occupy the 9.2–13% (w/w) "undetermined" proportion of bovine lubricin amino acid and glycosylation analyses[2], the purification of which closely followed the procedures of Swann[5]; (2) lipid is a boundary lubricant of both natural and synthetic surfaces *in vitro*, which is not in dispute; and (3) digestion of whole SF with phospholipase A_2(PLA_2) removes lubricating ability[3]. From these and the presence of lipid in SF, Dr. Hills has maintained that lipid is *the* boundary lubricant, transported to articular cartilage by lubricin. This theory was kindled[2] by the observation that 14% of radiolabelled purified bovine lubricin bound to articular cartilage[6]. The possibility that 14% of the radiolabel was delivered to articular cartilage by way of transported lipid is not valid, as the I^{125} radiolabel in these earlier experiments was specifically linked to tyrosine residues.

Jay and Cha[4] showed very clearly that the PLA_2 preparation used by Hills and Monds[3] is contaminated with proteases. Not only was lubrication decreased or eliminated, but also digestion of Nα-benzoyl-L-arginine ethyl ester occurred[4], which is used in calibrating trypsin/protease solutions. Obviously this experimental approach cannot be used to support the notion that lubricin is a lipid carrying molecule. The presence of protease inhibitors (PI) leupeptin and aprotinin partially prevented the loss of lubrication when bovine SF (BSF) was digested with phospholipase C. If SAPL was the sole lubricant then μ values on the order of 0.090 and greater would have been observed. This did not occur; the addition of PI prevented μ from rising past 0.050 from 0.028, the μ value for normal BSF. It is likely that our attempts in antiproteolysis, though diagnostic, were incomplete and typical of the need for multiple PI in state-of-the-art preparative biochemical efforts. Second, Schwarz and Hills[2] give no indication as to the lubricin purity. This would be assessed by means other than single chromatographic peaks arising from a replicated purification[7]. Third, no one has controlled for the quantity of lipid iatrogenically introduced into SF as a result of percutaneous aspiration.

The friction apparatus and bearing system of latex apposed to polished glass used in our study has been used by a number of other investigators[8-10] to study the lubricating ability of SF. In our study[4], each data point of μ for a sample has its own comparative normal saline control (NS), minimizing sample to sample variation of these rubbing surfaces. Data are not grouped and then subtracted *en masse* from the μ for NS as suggested. The artificial test surfaces were chosen since μ values are more reproducible than experimental cartilage containing bearings[10]. One cannot control for the weave of severed collagen fibrils and its resultant effect on surface features.

Trypsin and phospholipase digestion aside, lubricating ability can also be eliminated by galactosidase and neuraminidase digestion[11], removing the penultimate galactose from the α2,3NeuAc-β(1–3)Gal-GalNAc moiety on lubricin. It has been theorized that lubricin interacts with hydrophobic surfaces — articular cartilage and latex[11]. Lubrication may be provided by apposed and pressurized hydrophilic moieties, and it is these mucinous glycoproteins that have amphipathic behavior. Synovial SAPL, if present naturally, may play some role in joint lubrication my rendering articular cartilage hydrophobic, through some as yet undiscovered means. This would enable hydrophobic-hydrophobic attraction (in an aqueous environment) of the lubricant onto its surface.

Resolution of whether lubricin is the lubricant or its carrier will depend on analyses for elemental phosphorus in *purified* lubricin, whose purity is assessed by liquid chromatography mass spectrometry.

GREGORY D. JAY MD, PhD, Department of Medicine, Brown University School of Medicine, Providence, RI 02903, USA.

REFERENCES

1. McCutchen CW. The frictional properties of animal joints. Wear 1962;5:412-5.
2. Schwarz IM, Hills BA. Surface-active phospholipid as the lubricating component of lubricin. Br J Rheumatol 1998;37:21-6.
3. Hills BA, Monds MK. Enzymatic identification of the load-bearing boundary lubricant in the joint. Br J Rheumatol 1998;37:137-42.
4. Jay GD, Cha CJ. The effect of phospholipase digestion upon the boundary lubricating ability of synovial fluid. J Rheumatol 1999; 26:2454-7.
5. Swann DA, Sotman S, Dixon M, Brooks C. The isolation and partial characterization of the major glycoprotein (LGP-1) from the articular lubricating fraction from bovine synovial fluid. Biochem J 1977;161:473-85.
6. Swann DA, Hendren RB, Radin EL, Sotman SL, Duda EA. The lubricating activity of synovial fluid glycoproteins. Arthritis Rheum 1981;24:22-30.
7. Jay GD, Britt D, Cha CJ. Lubricin is a product of megakaryocyte stimulating factor gene expression by human synovial fibroblasts. J Rheumatol 2000; 27:594-600.
8. Wilkins JF. Proteolytic destruction of synovial boundary lubrication. Nature 1968;219:1050-1.
9. Reimann I, Stougaard J, Northeved A, Johnsen SJ. Demonstration of boundary lubrication by synovial fluid. Acta Orthop Scand 1975; 46:1-10.
10. Davis WH, Lee SL, Sokoloff L. A proposed model of boundary lubrication by synovial fluid: structuring of boundary water. J Biomech Eng 1979;185-92.
11. Jay GD. Characterization of a bovine synovial fluid lubricating factor. I. Chemical, surface activity and lubricating properties. Connect Tissue Res 1992;28:71-88.

Joint surgery ("JS" in Figure 1) that was carried out during the study hardly influenced the RA variables since none of the joints involved was severely inflamed and the main indication for surgery was deformity and not pain.

The delayed effect on pain score of time spent outdoors is shown in Figure 2 as the average change in pain score for 30 days following all days that satisfied a certain criterion — as described in A, B, C, and D. The difference between the curves is the number of successive days that met the criterion (1 to 20), as indicated by the length of the first part of the curve (bold line). For example, the bottom line in Figure 2D shows the course of the average change in pain score during (bold line) and after (regular line) 20 successive days with outdoor temperature < 6°C and time outdoors ≥ 2 hours. The change in pain score was always calculated with respect to the pain score on the first of the successive days that met the criteria.

Being outdoors briefly on warmer days (≥ 6°C) increased the pain score as long as the criterion was satisfied (A). Thereafter, the curves level off, which is likely due to the contribution of warm days with time outdoors > 2 hours. Figure 2B illustrates this contribution: staying outdoors for 2 hours or more on days warmer than 6°C hardly affected the pain score.

Being outdoors briefly on cold days had little effect on the pain score (C). After the periods satisfying the criterion (bold line on curve) the curves tend to decrease steeply (thin lines). The explanation may be that cold days with outdoor times > 2 hours did contribute to the averages here as well. A clear decrease in pain score was found for staying outdoors > 2 hours (D). The longer the period of successive days that satisfied the cold criterion, the greater was the decrease.

Depending on the average time outdoors of the preceding 14 days, the ESR data were grouped in 1 hour periods. The mean ESR per period decreased with increasing time outdoors: 0 to 1 h: 18.00 (SD 7.03), 1–2 h: 15.53 (SD 7.03), 2–3 h: 14.60 (SD 4.05), 3–4 h: 12.50 mm/h (SD 2.46). The mean ESR in the last period was significantly lower than in the first ($p < 0.05$, ANOVA with Tukey correction).

The effect of daily time spent outdoors is in accord with the earlier reported effect of outdoor temperature on pain and ESR[9]: a low outdoor temperature as well as being outdoors (on most days in a marine climate) will have a

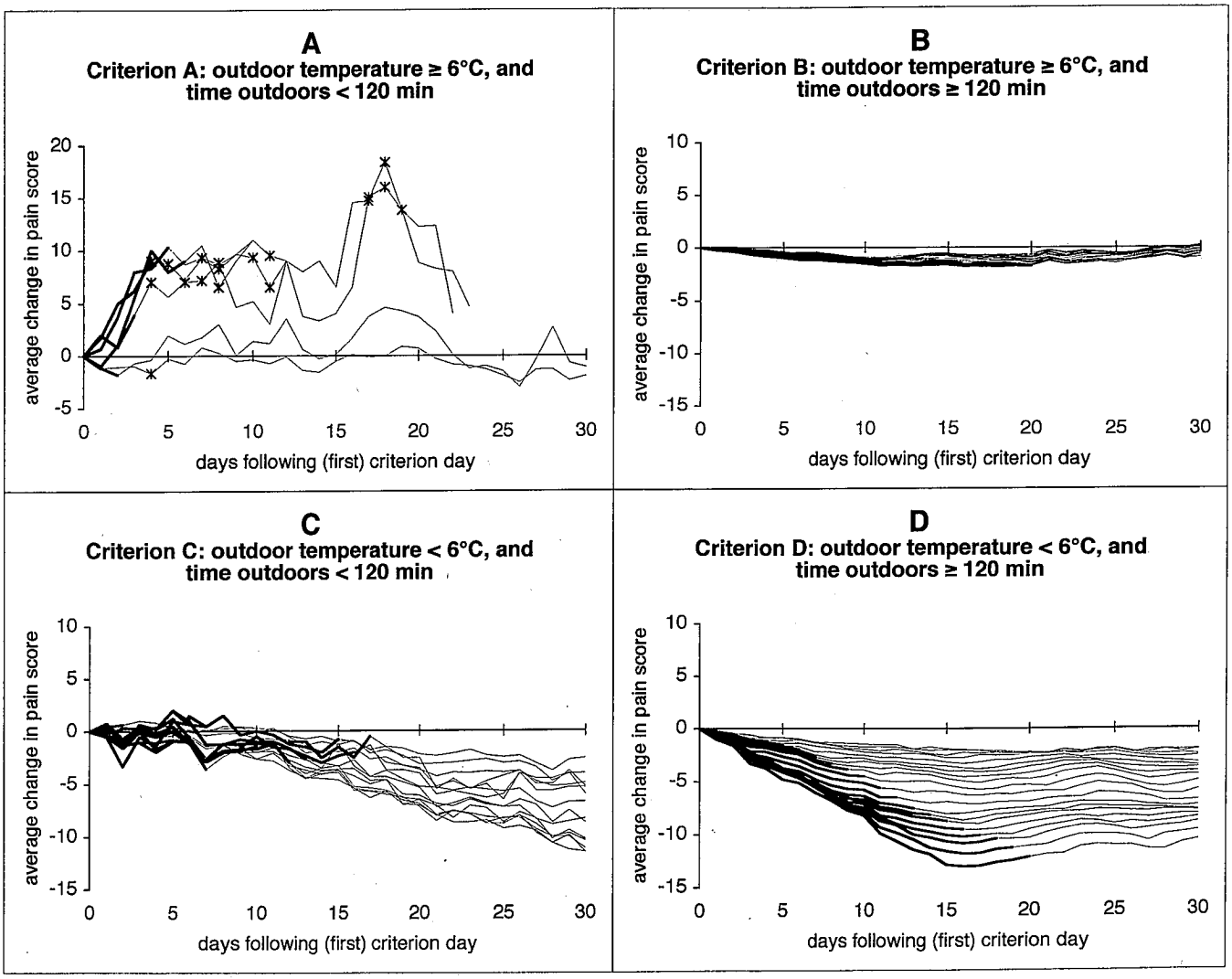

Figure 2. Average change in pain score following one or more successive days (up to 20) meeting 4 different criteria (A to D) of daily outdoor temperature and time outdoors (1995–98). Limited data restricted the analysis to responses following maximally 6 and 17 successive criterion days in A and C, respectively. Curves were truncated when < 3 pain scores contributed to the average (A). Number of data of the averages plotted varies considerably: 3–55 (A), 95–435 (B), 3–74 (C), and 11–160 (D). Differences from zero were tested for significance with Student t test. In A, significant values are indicated with asterisks. In B, significant values occurred beyond Day 4 and before Day 26 (on Days 9 through 13, and on Day 16, all values were significant). In C, most values beyond Day 12 were significant. In D, all values beyond Day 2 were significant.

fracture in patients presenting with unexplained monoarthritis, especially when mechanical factors are present.

CARLOS GARCIA-PORRUA, MD, PhD; JOSE VEGA ANDION, MD; MIGUEL A. GONZALEZ-GAY, MD, PhD, Hospital Xeral-Calde, Lugo, Spain.

REFERENCES

1. Speed CA. Stress fractures. Clin Rheumatol 1998;17:47-51.
2. Zuber M. Ankle pain and swelling with a positive bone scan in the absence of arthritis. J Rheumatol 1999;26:2499-500.
3. Maitra RS, Johnson DL. Stress fractures. Clinical history and physical examination. Clin Sports Med 1997;16:259-74.
4. Reeder MT, Dick BH, Atkins JK, Pribis AB, Martinez JM. Stress fractures. Current concepts of diagnosis and treatment. Sports Med 1996;22:198-212.
5. Bennell K, Matheson G, Meeuwisse W, Brukner P. Risk factors for stress fractures. Sports Med 1999;28:91-122.
6. Buckwalter JA, Brandser EA. Stress and insufficiency fractures. Am Fam Physician 1997;56:175-82.
7. Deutsch AL, Coel MN, Mink JH. Imaging of stress injuries to bone. Radiography, scintigraphy, and MR imaging. Clin Sports Med 1997;16:275-90.

Corrections

Kalden JR, Scott DL, Smolen JS, *et al,* for the European Leflunomide Study Group. Improved functional ability in patients with rheumatoid arthritis — longterm treatment with leflunomide versus sulfasalazine. J Rheumatol 2001;28:1983-91. Table 2, bottom line: the percentage of change over an observation period of 0–24 months should be 59% and 39% for leflunomide and sulfasalazine, respectively, rather than 56% and 45%. Discussion section, left column, page 1990: "...mean HAQ scores were significantly improved with leflunomide compared with sulfasalazine at 24 months (–0.65 vs –0.60; $p < 0.0149$)": the values in parentheses should be (–0.65 vs –0.36; $p = 0.0149$). The value for change in HAQ score from baseline at 24 months in leflunomide cohorts should be 59% (not 56%), and for completers the value should be 59% (not 60%). We regret the error.

Sweeney S, Gupta R, Taylor G, Calin A. Total hip arthroplasty in ankylosing spondylitis: outcome in 340 patients. J Rheumatol 2001;28:1862–6. The title should read "Total hip arthroplasty in ankylosing spondylitis: outcome in 340 hips". We regret the error.