A ^{1}H NMR STUDY OF REGENERATED SYNOVIAL FLUID AND BOVINE CALF SERUM

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Statement of Purpose: To date, bovine calf serum (BCS) represents the most acceptable lubricant in laboratory wear simulations of artificial joints [1]. Although BCS is recommended by ASTM and ISO standards, its rheological properties are significantly different to human regenerated synovial fluid (RSF) [2]. Moreover, the biomolecular composition of BCS, and how it differs to RSF have seen few studies. Therefore, to develop improved lubricants to mimic RSF, we need full information regarding the compositions of these biofluids.

In this study we employed the ^{1}H NMR technique to investigate, for the first time, the characteristics and metabolic status of RSF and BCS. The test hypothesis was that RSF will show elevated lactate and ketone levels compared to BCS.

Methods: Samples of human knee-joint RSF (~5 ml) were collected from four consenting patients during revision surgery. The samples were stored in plastic tubes at −20 °C prior to NMR. Samples of fresh newborn calf serum (Sigma, UK) were taken after dilution with deionised water to achieve a 15 mg/ml protein content. RSF samples were centrifuged to remove debris. ^{1}H NMR measurements were conducted (Bruker-Avance 600) at 600.13 MHz for ^{1}H for all samples [3]. The Carr-Purcell-Meiboom-Gill technique was employed to suppress broad protein resonances.

Results / Discussion: Compared to healthy SF, the RSF metabolic profiles showed elevated levels of lactate, elevated ketone bodies (such as acetone and 3-D-hydroxybutyrate) and 'acute-phase' glycoproteins, plus diminished levels of lipoprotein triacylglycerol and glucose. These observations support the hypothesis that a significant hypoxic, inflammatory environment exists in the artificial joints, a characteristic previously noted in studies of rheumatoid SF [3].

All RSF samples showed phospholipid loss, and was likely attributed to the reduced levels of hyaluronate. This modification may have a sizable effect on joint lubrication of artificial joints. To support this theory, a recent laboratory wear study of UHMWPE hip cups reported a 3-fold reduction in wear with increased phospholipids concentration of BCS [4]. Further studies in this area are warranted.

Comparisons of ^{1}H NMR profiles of RSF and BCS samples revealed marked differences (Figure 1), particularly elevated lactate and ketone body concentrations in RSF. As expected, resonances ascribable to 'acute-phase' glycoproteins and lipoprotein-associated triacylglycerols were notably higher in BCS and lower in RSF, respectively. Therefore, our hypothesis was proved. Clearly, a wide range of signals were present in the spectrum for BCS, and were attributable to low-molecular-mass components, together with the more mobile portions of macromolecules.

Conclusions:

1) RSF shares important ^{1}H NMR-recognisable characteristics with rheumatoid SF, i.e. its metabolic status indicated a hypoxic, inflammatory environment at the prosthesis-joint interface.
2) There are significant differences between the ^{1}H NMR metabolic profiles of RSF and BCS, particularly elevated lactate and ketone body concentrations in RSF.
3) There are substantial grounds for the development of a more physiologically-relevant test lubricant in laboratory wear simulations compared to BCS.

References: [1] Wang *et al*, JBMR, 68(B), 45-52, 2004. [2] Yao *et al*, 27th Ann Mt of Soc Biomat, 34, 2001. [3] Naughton et al, FEBS Letters, 332, 221-225, 1993. [4] Bell *et al*, Proc Inst Mech Engrs [H], 214, 513-8, 2000.

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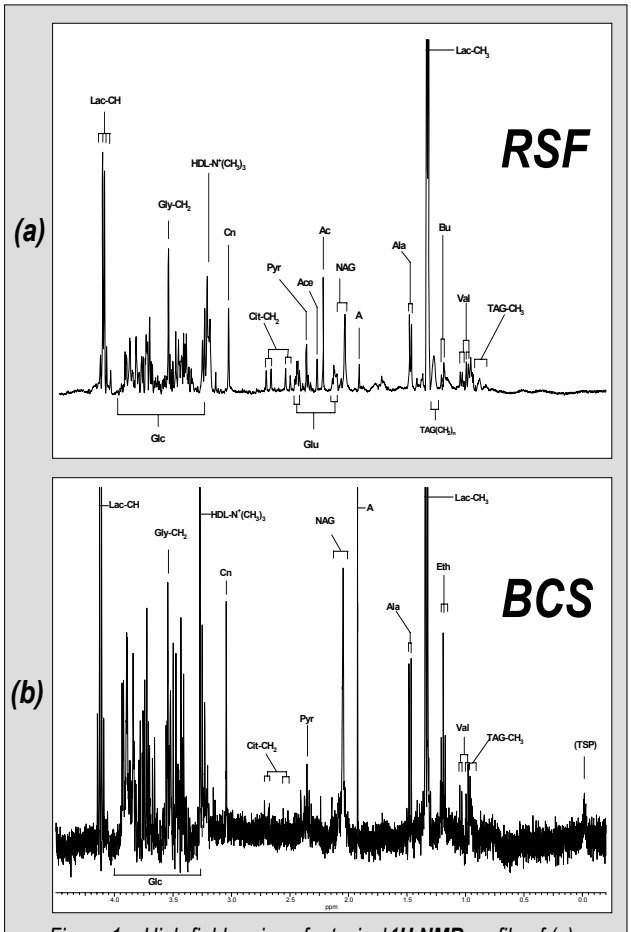

*Figure 1. High-field region of a typical **^{1}H NMR** profile of (a) regenerated knee synovial fluid, (b) 15 mg/ml newborn calf serum.*
***Abb.** Lac-CH3 and –CH = lactate-CH3 and –CH groups*
NAG = both free and carbohydrate side-chain N-acetyl sugars