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An investigation of the abnormal metabolic status of synovial fluid from patients with rheumatoid arthritis by high field proton nuclear magnetic resonance spectroscopy

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The ^{1}H Hahn spin-echo NMR profiles of rheumatoid synovial fluids have been investigated and compared with those of matched serum samples. In addition to markedly elevated lactate and diminished glucose concentrations, inflammatory synovial fluids contained (i) substantially lower levels of NMR-detectable chylomicron- and very-low-density-lipoprotein-associated triacylglycerols which appear to have a shortened mean chain-length, and (ii) high concentrations of ketone bodies (predominantly 3-D-hydroxybutyrate), relative to those of corresponding paired serum samples. These observations confirm the abnormal metabolic status of the inflamed rheumatoid joint and provide evidence for an increased utilisation of lipids for fuel therein.

Synovial fluid; Rheumatoid arthritis; NMR spectroscopy

1. INTRODUCTION

It has been recognised for many years that impairment of the vascular supply to and/or an increase in the metabolic rate of the inflamed rheumatoid joint gives rise to the markedly abnormal metabolic profile of the intra-articular environment. Previous investigations of the physiological status of the inflamed joint have established an extremely low synovial fluid oxygen tension [1,2], increased carbon dioxide tension, diminished glucose concentrations, substantially elevated lactate levels and an associated acidosis [3–6]. Indeed, Falchuck et al. [3] have demonstrated that joints exhibiting severe microvascular destruction in the synovial membrane had the lowest pO_2, were substantialy hypercapnic (high pCO_2), and contained high levels of lactate.

The recent development of high field nuclear magnetic resonance (NMR) spectrometers with increased sensitivity, resolution and dynamic range has permitted the rapid, simultaneous investigation of complex mixtures of endogenous or exogenous components present in biological materials. The technique is largely non-invasive since it has little or no requirement for pre-treatment. Previous high field proton NMR studies of human whole blood, plasma, serum and urine [7–10] have provided much useful biochemical information about the molecules present in these samples. The broad overlapping resonances which arise from the large number of macromolecules present are routinely suppressed by spin-echo pulse sequences [11] resulting in spectra which contain many well-resolved signals attributable to a wide range of low-molecular-mass (non-protein-bound) components and the mobile portions of macromolecules.

In the present study we have employed proton Hahn spin-echo NMR spectroscopy to assess the abnormal metabolic status of knee-joint synovial fluid obtained from patients with rheumatoid arthritis. The ^{1}H NMR profiles of paired serum samples were also evaluated to allow detailed metabolic comparisons of the two body fluids for each patient investigated.

2. EXPERIMENTAL

2.1. *Synovial fluid and serum samples*

Patients with moderately severe rheumatoid arthritis and associated knee effusions (n = 17, age range 40–67 years) were rested for a period of 30 min prior to the aspiration of paired serum and synovial fluid samples. Sterile knee-joint synovial fluid samples were drawn into plastic tubes and transported to the laboratory on ice immediately after collection. These samples were centrifuged at 3,000 r.p.m. for a period of 15 min to remove cells and debris. These samples were then stored at −70°C for a maximum duration of 8 days prior to ^{1}H NMR analysis. Control experiments established that none of the criteria investigated changed significantly during these periods of storage. Non-heparinized blood was drawn from these rheumatoid patients at the same time points as the synovial fluid samples. These samples were allowed to clot and the resulting serum was immediately centrifuged and stored as described above.

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2.2. *NMR measurements*

Proton NMR measurements were conducted on a JEOL JNM-GSX 500 (University of London Intercollegiate Research Service, Biomedical NMR Centre, Birkbeck College, London, UK) spectrometer operating at 500 MHz for ^1H. All spectra were recorded at ambient probe temperature (22 ± 1°C). Typically, 0.60 ml of serum, or the supernatant obtained from centrifuged synovial fluid was placed in a 5-mm diameter NMR tube, and 0.07 ml of ^2H_2O was added to provide a field frequency lock. The broad protein resonances were suppressed by the Hahn spin-echo sequence [12] (D[90° x–τ–180°y–τ-collect]), which was repeated 128–312 times, with τ = 60 ms. The intense water signal was suppressed by continuous proton irradiation at the water frequency, or by presaturation with gated decoupling during the delay between pulses. Chemical shifts were referenced to external sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)-propionate (TSP, δ = 0.00 ppm). The methyl group resonances of alanine (δ = 1.487 ppm), lactate (δ = 1.330 ppm) or valine (δ = 1.050 ppm) served as secondary internal references.

Single pulse spectra of putative synovial fluid components were obtained using a pulse angle of 30–40° and a total delay between pulses of 5 s to allow full spin-lattice (T_1) relaxation of the protons in the samples investigated. These spectra were also recorded at ambient probe temperature and referenced to TSP.

3. RESULTS

The identities of components responsible for the resonances present in proton Hahn spin-echo NMR spectra of rheumatoid knee joint synovial fluid and serum samples were routinely assigned by a consideration of their characteristic chemical shift values, coupling patterns and coupling constants obtained from single-pulse standard reference spectra recorded under similar experimental conditions. Where appropriate, standard additions of putative biomolecules were made to the biofluids to confirm identity assignments.

Fig. 1 shows the high field regions of typical 500 MHz proton Hahn spin-echo NMR spectra of paired serum and synovial fluid samples obtained from a patient with rheumatoid arthritis. As expected, the proton NMR profiles of synovial fluid are markedly different from those of paired serum samples. The major differences consist of (1) highly intense lactate -CH_3 and -CH proton resonances in the synovial fluid spectra reflecting very high synovial fluid lactate levels (ca. 7–12 × 10^{-3} mol · dm^{-3}), and (2) the relatively weak intensity of the synovial fluid glucose resonances (measured as the normalised intensities of the α- or β-anomeric proton signals located at 5.3 and 4.7 ppm, respectively) which were on average ca. 20% less intense than those present in corresponding spectra of paired serum samples. These observations are consistent with the markedly hypoxic status of the inflamed rheumatoid joint.

In addition, the spectra of all synovial fluid samples examined contained fatty acid acyl chain terminal -CH_3 and bulk (-CH_2-)$_n$ resonances (predominantly attributable to chylomicron- and very-low-density-lipoprotein (VLDL)-associated triacylglycerols [20]) which were of markedly weaker intensity than those present in corresponding spectra of matched serum samples (Fig. 1). These resonances represent the molecularly mobile

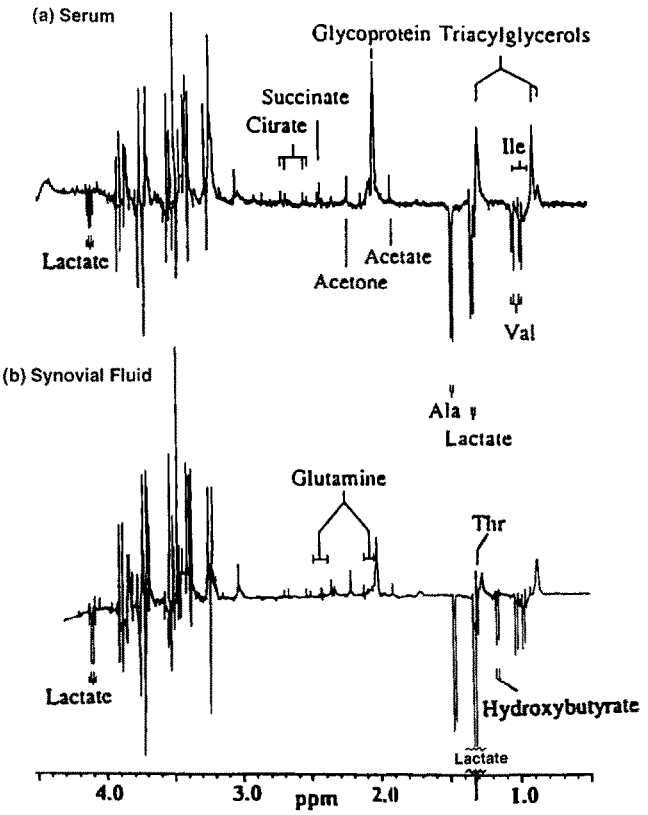

Fig. 1. Partial ^1H Hahn spin-echo NMR spectra of matched samples of (a) serum and (b) knee-joint synovial fluid obtained from a patient with rheumatoid arthritis. Typical spectra are shown. Ile, isoleucine -CH_3; Thr, threonine -CH_3; Val, valine -CH_3; Glycoprotein represents -$NHCOCH_3$ groups of the molecularly mobile carbohydrate side-chains of 'acute-phase' glycoproteins (predominantly α_1- acid glycoprotein).

components of the above fatty acids [13]. Moreover, the synovial fluid chylomicron and VLDL triacylglycerol acyl chain terminal -CH_3: bulk (-CH_2-)$_n$ resonance intensity ratio was substantially higher than those observed in spectra of corresponding paired serum samples. Indeed, in 8 of the patients investigated, the bulk (-CH_2-)$_n$ resonance was barely detectable. These observations indicate that the molecularly mobile components of the above lipoprotein triacylglycerols in rheumatoid synovial fluid have a significantly lower chain length relative to those of paired serum samples, and further indicate an increased utilisation of fats for energy in the intra-articular environment, despite its hypoxic status. Consistent with this hypothesis, resonances attributable to the -CH_3 group protons of the ketone bodies acetone and 3-D-hydroxybutyrate were present in spectra of all synovial fluid samples investigated. Although the acetoacetate -CH_3 group signal was also present in spectra of approximately 60% of synovial fluid samples examined, 3-D-hydroxybutyrate was the predominant ketone body detectable in these samples. Although acetone and 3-D-hydroxybutyrate reso-

nances were also detectable in some of the matched serum samples examined, the intensity of the 3-D-hydroxybutyrate -CH_3 group signal, when present, was always far lower than that of corresponding synovial fluid spectra.

The above NMR-detectable differences between matched serum and synovial fluid samples were observed in virtually all rheumatoid patients investigated (n = 15 out of a total of 17).

Several of the synovial fluid spectra examined contained only very weakly intense glucose proton resonances (a typical example of which is exhibited in Fig. 2), demonstrating a markedly hypoglycaemic intra-articular environment present in some rheumatoid arthritis patients. Within the inflamed rheumatoid joint, glucose levels are often diminished and an enhanced oxidative consumption of fatty acids occurs for purposes of energy utilisation, as previously reported by Dunham et al. [21].

4. DISCUSSION

The major advantages of high field proton NMR spectroscopy in the analysis of human body fluids is that it generally requires no knowledge of sample composition prior to analysis and permits rapid assessments of the nature and levels of a large number of biomolecules simultaneously. In addition to the markedly elevated lactate and diminished glucose concentrations present in rheumatoid synovial fluid (characteristic of the hypoxic intra-articular environment), the experiments performed here provide evidence for low synovial fluid levels of chylomicron- and VLDL-associated triacylglycerols which appear to have a reduced mean chain length. Moreover, high levels of ketone bodies (predominantly 3-D-hydroxybutyrate) were detectable in ^{1}H Hahn spin-echo spectra of all synovial fluid samples investigated. Taken together, these observations indicate an increased intra-articular utilisation of fats for energy, consistent with previous observations [21]. Unfortunately, the procurement of normal synovial fluid samples for high field ^{1}H NMR analysis was perturbed by the very small volumes (< 0.2 ml) obtainable from normal knee-joints, and hence we have not been able to compare and contrast the metabolic profiles of matched normal serum and synovial fluid samples at this stage. To our knowledge, no such investigations have been previously reported in the literature.

Lipid-derived metabolites are respired aerobically, whilst conditions within the joint are largely hypoxic. An explanation for this phenomenon is that during the course of inflammation, mobilisation of metabolites from the blood to the tissues is diminished in view of the increased diffusion distance. Hence, there is a decrease in the availability of glucose attributable to an increased metabolic rate and the inability of this sugar to overcome the barrier presented by the inflamed joint space.

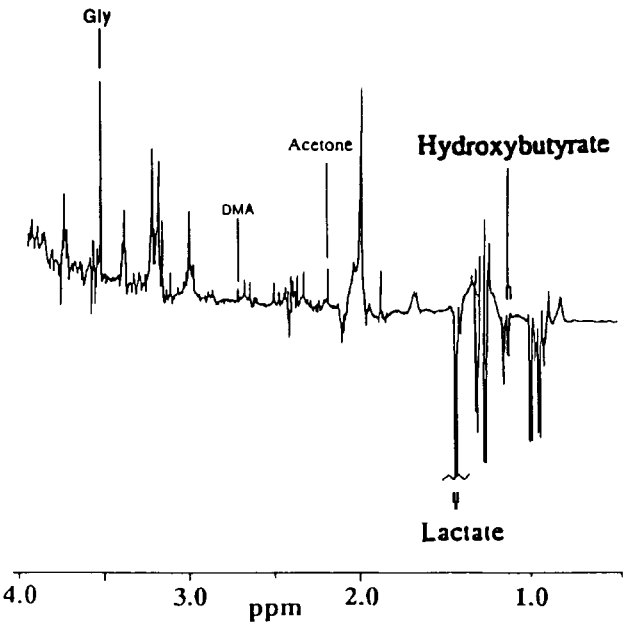

Fig. 2. Partial ^{1}H Hahn spin-echo NMR spectrum of a typical hypoglycaemic rheumatoid synovial fluid sample. DMA, dimethylamine -$\dot{N}(CH_3)_2$; Gly, glycine -CH_2-.

An initial rise in glycogenolysis is anticipated, followed by an increase in lipolysis. However, high localised concentrations of free fatty acids exert deleterious toxic effects [15].

The above hypothesis is particularly appealing in view of the chemical nature and physical properties of ketone bodies. The major compounds produced are acetoacetate, 3-D-hydroxybutyrate and small quantities of acetone. With the notable exception of diabetes mellitus, the 'glucose fatty acid cycle' dictates that ketogenesis only occurs in starvation. Lipolysis is expected to be carefully regulated by ketone body formation since it has been demonstrated that high levels of these species inhibit lupolysis [16], and oxidative metabolites can be generated in the liver and transferred to tissues that have an increased requirement for them. Ketone bodies have a relatively high solubility in aqueous environments, facilitating their ease of transport to sites of starvation. This is of much significance in the inflamed joint where a large diffusion gradient must be overcome. Indeed, a long chain fatty-acid would be expected to have an unacceptably high diffusion rate under these conditions [17].

An alternative source of ketone bodies in the inflamed joint may be derived from the presence of an immunological infiltration. Indeed, a recent investigation has shown that lymphocyte metabolism is largely attributable to the oxidation of ketone bodies [14,18]. Starvation has been found to enhance glucose utilisation and concomitantly to have no influence on the rate of ketogenesis [19]. It has previously been proposed that this unusual behaviour results from the ability of the lymphocyte to function as a rapidly activated cell.

Ketone body utilisation serves to increase the cellular pool of acetyl-coenzyme A (Ac-CoA) which is readily available for oxidation when an antigenic stimulus signals proliferation of the clone.

In conclusion, high field ^1H NMR analysis of paired synovial fluid and serum samples obtained from patients with rheumatoid arthritis provides much detailed metabolic information regarding the characteristically 'unnatural' physiological status of the inflamed intra-articular environment.

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