# Synovial Fluid Fatty Acid Composition in Patients with Rheumatoid Arthritis, Gout and Degenerative Joint Disease.* (31407)

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Recent reports(1,2,3) on the occurrence of chylous synovial effusions in patients with rheumatoid arthritis have re-emphasized the need for a better understanding of changes in the synovial fluid lipid content in various articular diseases. However, this aspect of synovial fluid analysis received scant attention until Chung and associates(4) reported on the lipid contents of pathologic synovial fluids obtained from patients with rheumatoid arthritis and degenerative joint disease and Bole(5) compared the concentration of the lipids in normal human and rheumatoid synovial fluids. One of us reported a detailed lipid analysis including the fatty acid composition of a chylous effusion occurring in a patient with longstanding rheumatoid arthritis(2). Some differences between the relative amounts of fatty acids in serum and synovial fluid were suggested in this study. A review of the literature at that time revealed no systematic study of the fatty acid profile of synovial fluid in various articular diseases. Since the introduction of gas-liquid chromatography(6) has greatly simplified the hitherto technically difficult fatty acid analysis, we have undertaken an analysis by this method of the fatty acid composition of synovial fluids of patients with rheumatoid arthritis, degenerative joint disease and gouty arthritis with the following purposes: (1) to compare the synovial fluid fatty acid composition of each of these joint diseases with one another and (2) to compare synovial fluid fatty acids with those of the simultaneous serum samples.

*Methods and materials.* Synovial fluid aspirations and simultaneous serum collections were performed on 10 patients with classical or definite rheumatoid arthritis, 7 patients with degenerative joint disease, and 5 patients with gouty arthritis. To insure the existence of equilibrium between the plasma and the synovial fluid lipid, all patients were fasted for 6 hours or more at the time of arthrocentesis. An aliquot of each synovial fluid was used for a white cell count, differential analysis, mucin content and microscopic examination for crystals. The remainder of the synovial fluid was centrifuged at 2,000 RPM for 20 minutes and the supernatant fraction from which the clot had been separated was stored at −20°C until further analysis. Parallel serum samples were similarly stored.

Lipid extraction was done on the aliquots of synovial fluid and serum according to the method of Folch(7) and fatty acid analyses were performed on the Folch extract. After evaporating the extract to dryness under a stream of oxygen-free nitrogen, the residue was methylated using the method of Metcalfe and Schmitz(8). The fatty acids were analyzed as methyl esters by gas-liquid chromatography using an F-M Model 400 apparatus with a hydrogen flame ionization detector. The column used was glass (6 feet, 0.5 inch outside diameter) packed with 6% DEGS (diethylene glycol succinate) coated on 80/100 mesh Diatoport S (obtained from F & M Scientific Corp., Avondale, Pa.) at a column temperature of 180°C. Helium was used as the carrier gas at the flow rate of 60 ml/min. Fatty acids were identified by comparison of their retention times with those of commercially available pure standards (Applied Science Labs., State College, Pa.) and National Heart Institute Fatty Acid Standards were used to establish the linearity of response. The stated composition data of N.H.I. Fatty Acid Standards could be reproduced with a relative error less than 4.0% for major components and less than 8.4% for minor components. The areas under peaks were determined by multiplying peak height by width at half height as suggested by the

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TABLE I. Fatty Acid Composition of Synovial Fluid and Matching Serum. (Values expressed as mean peak area in sq cm ± standard deviation.)

| Diagnosis | Rheumatoid arthritis (10)* | | Degenerative joint disease (7)* | | Gout (5)* | |
|---|---|---|---|---|---|---|
| Fatty acid | Synovial fluid | Serum | Synovial fluid | Serum | Synovial fluid | Serum |
| 14:0 | .8 ± .3 | 2.2 ± .9 | .9 ± .2 | 2.1 ± .5 | 1.0 ± .5 | 1.8 ± .9 |
| 16:0 | 15.2 ± 5.1 | 47.4 ± 17.7 | 16.1 ± 5.3 | 54.0 ± 6.9 | 27.1 ± 11.4 | 65.4 ± 33.1 |
| 16:1 | 2.7 ± 1.1 | 8.9 ± 3.4 | 3.2 ± 1.5 | 9.5 ± 3.1 | 4.4 ± 2.9 | 9.1 ± 4.3 |
| 18:0 | 5.1 ± 1.4 | 14.8 ± 6.4 | 5.6 ± 1.8 | 18.5 ± 4.3 | 8.4 ± 3.1 | 19.6 ± 10.5 |
| 18:1 | 18.6 ± 7.5 | 57.2 ± 18.0 | 19.3 ± 7.6 | 67.5 ± 10.6 | 34.3 ± 15.6 | 80.1 ± 38.6 |
| 18:2 | 16.5 ± 7.9 | 48.3 ± 24.6 | 18.5 ± 8.2 | 50.8 ± 16.0 | 28.4 ± 15.9 | 60.6 ± 39.6 |
| 20:4 | 6.0 ± 1.5 | 15.6 ± 6.3 | 7.9 ± 4.8 | 17.8 ± 7.2 | 9.9 ± 4.0 | 20.1 ± 13.5 |
| Total fatty acid | 65.6 ±20.7 | 193.9 ± 69.2 | 71.5 ±24.1 | 222.0 ± 33.1 | 113.5 ± 45.0 | 257.4 ±122.5 |

* No. of different samples analyzed indicated in parentheses.

TABLE II. Relative Fatty Acid Composition of Synovial Fluid and Matching Serum. (Values expressed as % of total fatty acid esters.)

| Diagnosis | Rheumatoid arthritis (10) | | Degenerative joint disease (7) | | Gout (5) | |
|---|---|---|---|---|---|---|
| Fatty acid | Synovial fluid | Serum | Synovial fluid | Serum | Synovial fluid | Serum |
| 14:0 | 1.2 | 1.1 | 1.3 | .9 | .9 | .7 |
| 16:0 | 23.4 | 24.4 | 22.5 | 24.5 | 23.8 | 25.4 |
| 16:1 | 4.2 | 4.6 | 4.5 | 4.3 | 3.9 | 3.5 |
| 18:0 | 7.8 | 7.6 | 7.8 | 8.4 | 7.4 | 7.6 |
| 18:1 | 28.7 | 29.4 | 26.9 | 30.6 | 30.2 | 31.4 |
| 18:2 | 25.4 | 24.8 | 25.8 | 23.1 | 25.1 | 23.5 |
| 20:4 | 9.2 | 8.0 | 11.1 | 8.1 | 8.7 | 7.8 |

Advisory Committee, Lipid Standard Program, National Heart Institute(9).

*Results.* Routine synovial fluid analyses were as expected(10) in each type of synovial fluid. The average white cell count for the synovial fluids from patients with rheumatoid arthritis was 21,690 ± 11,991 cells per cu mm (75% polymorphonuclear leukocytes and 25% monocytes), whereas it was 590 ± 363 cells per cu mm (4% polymorphonuclear leukocytes and 96% monocytes) for those from patients with degenerative joint disease and 11,500 ± 9,383 cells per cu mm (68% polymorphonuclear leukocytes and 32% monocytes) for the gouty effusions. Furthermore, urate crystals were demonstrated in all the gouty synovial fluids. No cholesterol crystals were seen in any of the synovial fluids.

The fatty acid composition of synovial fluids from patients with 3 different articular diseases and matching serums is shown in Tables I, II and III. There was no qualitative difference between synovial fluid and its matching serum and all the fatty acids which are normally present in serum were also found to be present in synovial fluid irrespective of the disease category. Quantitatively, the total fatty acids present in the synovial fluids of patients with rheumatoid arthritis and degenerative joint disease were approximately a third of the amount present in the matching sera, whereas this ratio was roughly one-half in the cases of gouty arthritis. However, the synovial fluid fatty acid composition in terms of the ratio of each fatty acid to the total fatty acids was essentially the same as that of the matching serum. Thus, both in synovial fluid and in serum, palmitic (16:0), oleic (18:1) and linoleic (18:2) acids constituted

TABLE III. Ratio of Serum Fatty Acids to Synovial Fluid Fatty Acids.

| | Mean | S.D. | Range |
|---|---|---|---|
| Rheumatoid arthritis | 3.1 | .95 | 1.4-4.7 |
| Degenerative joint disease | 3.5 | 1.23 | 2.1-5.6 |
| Gout | 2.3 | .87 | 1.6-3.8 |

roughly 80% of the total fatty acids. Myristic (14:0), palmitoleic (16:1), stearic (18:0) and arachidonic (20:4) acids were present in smaller quantities.

The quantity of the total fatty acids present in the synovial fluid was highest in the cases of gouty arthritis when compared to rheumatoid and degenerative joint disease fluids. Although statistical analyses indicated that the difference between gouty and rheumatoid fluid was significant ($P<0.05$) a fatty acid analysis clearly could not differentiate one from another because of the lack of specificity of this finding. The correlation between synovial fluid white cell count and its fatty acid concentration was poor. A scattergram was drawn and the correlation coefficient was determined for the various synovial fluid white blood cell levels and fatty acid concentration. The result ($r = -0.16$) indicated that no significant ($p>0.1$) correlation existed between the two. Finally, difference between the synovial fluid fatty acid concentration and that in the serum was highly significant ($p<0.001$), and the former value was invariably lower.

*Discussion.* Since an initial fatty acid profile of a chylous effusion in a patient with rheumatoid arthritis suggested that gross differences in the fatty acid profile might exist between synovial fluid and serum, the present study was carried out preliminary to the more definitive fractionation of synovial fluid lipid to its component parts (phospholipids, triglycerides, free fatty acids, cholesterol, and cholesterol esters) and determination of the fatty acid composition of each moiety. In the current investigation, the total fatty acid profile (containing both the free and ester-linkage fatty acids) of synovial fluid and its matching serum was determined by gas chromatography after hydrolysis and methylation of a total lipid extract of a sample. Our data indicate that the fatty acid composition of synovial fluid is similar to that of serum (*i.e.*, the same fatty acids were present in the same relative amount) and that it is little influenced by the underlying articular disease. In both synovial fluid and serum, 3 fatty acids, palmitic, oleic and linoleic acids, accounted for nearly 80% of the total fatty acids. A similar study of a chylous rheumatoid effusion by one of us(2) demonstrated that there were no qualitative alterations between comparable serum and chylous synovial fluid samples but that relatively more stearic acid and less palmitoleic acid were found in the chylous synovial effusion than in the matching sera. This apparent alteration in the relative fatty acid composition in the chylous synovial fluid as compared to serum was not found to exist in our current samples which included no chylous effusions. On a quantitative basis the synovial fluid fatty acid concentration was found to be significantly lower than that of serum. The gouty synovial fluid contained approximately one-half of the serum concentration, whereas that of synovial fluid of rheumatoid arthritis and degenerative joint disease was about one-third of the serum concentration.

Due to the lack of similar studies in the literature, our results cannot be compared with others. However, Pietropaolo *et al*(11) examined the fatty acid composition of exudate, transudate and edema and obtained results not dissimilar from the present data. They reported that the fatty acid concentration was highest in exudate which contained 31% of the serum concentration and was lower in transudate and edema which contained 22% and 15% of the serum concentration, respectively; that the predominant fatty acids were palmitic, oleic, and linoleic acids; and finally that despite some individual variations in the concentration of the minor component, the relative composition of the major fatty acids in pathologic body fluids generally paralleled that of serum. Bole(5) also observed the similarity of synovial fluid lipids to those of serum in his study of rheumatoid joint fluid lipids.

Although gouty synovial fluids tend to have higher fatty acid content than rheumatoid synovial fluids, the significance of this observation is not clear, for there are considerable individual variations in the fatty acid concentration and the correlation with the synovial fluid white cell count is poor. Certainly, the analysis of total fatty acid composition of synovial fluid would not be a rewarding procedure as a diagnostic aid in differentiating

an inflammatory synovial fluid (*i.e.*, rheumatoid arthritis and gout) from a non-inflammatory synovial fluid (*i.e.*, degenerative joint disease). Chung *et al*(4) also found it impossible to differentiate degenerative joint disease synovial fluid from that of rheumatoid arthritis by lipid analysis, as did Bole(5) who felt that the contribution to the total lipid by pleocytosis in synovial fluid was minimal. This apparent lack of correlation between the white cell count and lipid concentration of synovial fluid is of some interest since Buchanan(12) and Marks, Gellhorn, and Kidson(13) have established that leukocytes can synthesize lipids. It may be that, as suggested by isotopic data in the chylous fluid(2), the synovial membrane routinely contributes significantly to local lipid content. This suggestion is also supported by electron micrographs taken in our laboratory of a variety of synovial membranes which not uncommonly demonstrate lipids in the synovial lining cells.

*Summary.* Total fatty acid analyses of synovial fluids from patients with rheumatoid arthritis, gout, and degenerative joint disease and matching sera were carried out by gas chromatography. The relative fatty acid composition of synovial fluid was similar to that in serum. Palmitic, oleic and linoleic acids constituted the major components and myristic, palmitoleic, stearic and arachidonic acids were the minor components. The synovial fluid fatty acid concentration was roughly one-half to one-third of that of the matching serum. However, correlation between the synovial fluid fatty acid concentration and its white cell count was poor and total fatty acid analyses were not helpful in differentiating an inflammatory synovial fluid from a non-inflammatory fluid.

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## Formalinized Tanned Cell Hemagglutination Test for Demonstration Of Autoantibodies in Myasthenia Gravis.* (31408)

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Several immunological methods have been employed to demonstrate muscle antibodies in the sera of patients suffering from myasthenia gravis. These include direct immunofluorescent (IF) staining(1,2,3), complement IF staining(1,2), indirect IF staining(2,4), complement fixation(2,6) and tanned cell agglutination, using fresh cells(5,7). The last technique appears to be the most sensitive in our hands. However, the preparation of fresh tanned cells coated with muscle antigen is somewhat cumbersome and the product is unstable. An improved method has now been devised utilizing more stable formalinized cells. One aim of this report is to compare the results obtained with fresh and

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