# Polyunsaturated Fatty Acids in the Lipids of the Atherosclerotic Femoral Artery

## Changes after Corn Oil Supplementation of the Diet

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THE effect of a diet with polyunsaturated fat on lowering the serum lipid levels has been amply demonstrated by many investigators.[1–3] Whether these lowered serum lipid levels have caused any improvement of the diseased patient is only rarely reported. This, of course, is due to the difficulty of registering such an improvement (if any) and the long time of treatment required before such a supposed improvement can be objectively observed. Kinsell and his collaborators were among the first to observe the lipid-lowering effect of vegetable oils rich in polyunsaturated fatty acids, and they now recorded a long period of observations of their patients. Kinsell et al.[4] reported a subjective and objective regression of atherosclerotic symptoms in a few of their patients who were treated with polyunsaturated fat. However, it is too early to draw any conclusions concerning the benefit of treating atherosclerosis by lowering serum lipid levels through the use of polyunsaturated fat.

There is a possibility of determining whether polyunsaturated fat in the diet has not only a lowering effect on the serum lipids but also an influence on the lipid composition of the atherosclerotic artery lesions. This is accomplished by analysing the lipid distribution in the artery wall before and after the introduction of polyunsaturated fat into the diet. In the present study, the lipid distribution and the pattern of the polyunsaturated fatty acids in the intimal layer of the femoral artery have been analysed in patients treated by surgical reconstructive methods for occlusive atherosclerotic lesions. In some of the cases the effect of introduction of polyunsaturated fat in the diet on the lipids in the serum and in the artery was also studied.

### MATERIAL

The material consists of ten patients (nine men and one woman) with typical histories of intermittent claudication. The mean age was sixty-four years, with the range of fifty-five to seventy-three years. Occlusive atherosclerotic changes of the common iliac artery and/or the femoral artery were diagnosed by angiography. In five patients a pathologic electrocardiogram was obtained at rest. In none of them were there signs of diabetes, hypothyroidism or the nephrotic syndrome. The woman had hypertension. All the patients were on an ordinary diet and the mean serum cholesterol value (two or more determinations in each patient) was 303 mg. per 100 ml., with a range of 230 to 425 mg. per 100 ml.

Four patients were instructed to replace the "visible" fat in their ordinary diet by the same amount of unsaturated fat in the form of special unsaturated margarines or corn oil. The daily consumption was roughly 50 gm. of polyunsaturated fatty acids corresponding to about 50 per cent of the fat intake. The periods on unsaturated fat before the operation for the four patients were, respectively: half a month (R. S.), two months (S. E. O.), five and eight months (G. S.) and five months (G. B.). Two patients (G. S. and G. B.) were operated upon also before the change of diet, one patient (G. S.) twice.

In the patients on the unsaturated diet the serum

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TABLE I

The Absolute and Relative Amounts of Lipids Extracted from the Intima of Femoral Artery in Patients on an Ordinary Diet

| Lipid | No. of Patients | mg. per 100 gm. Wet Weight | | No. of Patients | Per cent of Sum of Lipids | |
|---|---|---|---|---|---|---|
| | | Mean | Standard Deviation | | Mean | Standard Deviation |
| Cholesterol | 7 | 1176 | 170 | 7 | 52.5 | 4.1 |
| Glycerides | 7 | 337 | 103 | 7 | 16.3 | 4.7 |
| Phospholipids | 8 | 698 | 357 | 7 | 31.3 | 6.0 |
| Sum of lipids | 7 | 2226 | 969 | .. | ... | ... |

NOTE: Glycerides were calculated as triglycerides with fatty acids of the mean molecular weight of 282. Phospholipids were obtained by multiplying the phosphorus value by 25.

TABLE II

The Percentage Fatty Acid Distribution in the Different Lipid Fractions Extracted from the Intima of the Femoral Artery in Eight Patients on an Ordinary Diet and from the Serum of Sixteen Patients with Essential Hypercholesterolemia[10] on an Ordinary Diet

| Lipid Fraction | Cholesterol Esters (mean ± standard deviation) | | Glycerides plus Unesterified Fatty Acids (mean ± standard deviation) | | Phospholipids (mean ± standard deviation) | |
|---|---|---|---|---|---|---|
| | Femoral Artery | Serum | Femoral Artery | Serum | Femoral Artery | Serum |
| Dienes | 35.0 ± 4.6 | 38.5 ± 4.2 | 7.2 ± 1.8 | 8.1 ± 3.1 | 5.1 ± 1.5 | 13.4 ± 3.6 |
| Trienes | 2.7 ± 0.2 | 2.9 ± 0.7 | 2.2 ± 1.5 | 2.1 ± 1.0 | 2.0 ± 1.4 | 3.9 ± 1.4 |
| Tetraenes | 5.3 ± 0.7 | 5.1 ± 1.0 | 2.0 ± 0.8 | 1.5 ± 0.4 | 5.2 ± 1.6 | 5.7 ± 1.3 |
| Pentaenes | 2.2 ± 1.1 | 2.2 ± 0.8 | 1.8 ± 1.3 | 1.1 ± 0.7 | 1.4 ± 0.6 | 2.5 ± 1.0 |
| Hexaenes | 1.9 ± 1.4 | 2.0 ± 1.0 | 1.5 ± 2.0 | 1.3 ± 1.6 | 0.8 ± 0.7 | 2.5 ± 1.4 |

cholesterol levels were determined approximately twice a month. In one patient (R. S.) the decrease in the serum cholesterol level was insignificant, in all the others a decrease of about 40 mg. per 100 ml. was obtained. In two patients (G. S. and G. B.) the polyunsaturated fatty acids of the serum lipids were also determined simultaneously with the operation.

### *Sampling Method*

The operation technic described by Dos Santos[5] and Reboul and Laubry[6] was used. The artery was liberated from the surrounding tissue and a longitudinal incision was made into the medial layer of the artery. The part of the artery towards the center from the incision was then dissected free by following the medial layer around the whole circumference of the wall of the vessel. The preparation thus obtained consisted of the intimal layer and a part of the medial layer, leaving a smooth central wall in the artery.[7]

The remains of the media on the liberated inner part of the artery were macroscopically visible as a chagrination. These remains were carefully dissected away. However, they adhered to the medial side of the atherosclerotic lesions of the intima and thus could not be removed completely. After this the remaining tube was opened. All parts completely occluded by plaques or by thrombotic material were removed to ensure that only those parts of the vessel wall which had been in contact with the blood stream were taken for analysis. The remaining tissue consisted of atherosclerotic plaques, mostly of advanced degree of development with ulcerations and calcifications, and between them the intimal layer, thin and translucent. On the peripheral wall of the intima and the plaques were small fragments of the medial layer adhering to the atherosclerotic lesions.

### *Chemical Methods*

The artery preparation and the serum were ex-

TABLE III

The Percentage Fatty Acid Distribution in the Different Lipid Fractions Extracted from the Intima of the Femoral Artery in Patient R. S. after One-Half Month and Patient S. E. O. after Two Months on a Diet Rich in Unsaturated Fat

| Lipid Fraction | Cholesterol Esters | | Glycerides plus Unesterified Fatty Acids | | Phospholipids | |
|---|---|---|---|---|---|---|
| | R. S. | S. E. O. | R. S. | S. E. O. | R. S. | S. E. O. |
| Dienes | 31.1 | 35.0 | 13.1 | 8.9 | 2.0 | 0 |
| Trienes | 3.4 | 2.9 | 6.3 | 4.0 | 1.1 | 1.0 |
| Tetraenes | 7.2 | 5.3 | 4.1 | 3.6 | 3.1 | 1.1 |
| Pentaenes | 2.3 | 2.0 | 2.2 | 1.1 | 0.6 | 0.4 |
| Hexaenes | 3.0 | 1.3 | 1.5 | 2.0 | 1.0 | 0.9 |

tracted according to the method of Folch et al.[8] the vessel preparation after homogenization with a Bühler homogenizer. The extraction of the artery was tested by homogenizing the extracted material in a Potter-Elvehjem apparatus in chloroform: methanol, 2:1. The new extraction gave negligible amounts of additional lipids.

Chromatography was then used according to the method of Hirsch and Ahrens[9] to separate the lipids, silicic acid into cholesterol esters, free cholesterol plus glycerides plus unesterified fatty acids and phospholipids. The completeness of separation and recovery was ascertained as described earlier.[10]

The cholesterol ester fatty acids and the glyceride fatty acids plus unesterified fatty acids were saponified and the fatty acids extracted by petroleum ether from the acidified solution.[10] The phospholipid fatty acids were obtained via re-esterification to methyl esters according to the method described by Hallgren et al.[11]

The fatty acids from the different fractions were determined titrimetrically, and the polyunsaturated fatty acids by the alkali isomerization technic described by Holman and Hayes.[12] Cholesterol was determined according to Cramér and Isaksson,[13] glyceride-glycerol according to Carlson and Wadström[14] and lipid phosphorus according to Svanborg and Svennerholm.[15]

## RESULTS

The lipid distribution in the artery preparations taken from patients on an ordinary diet is listed in Table I.

In order to obtain a rough idea of the composition of the phospholipids in the vessel walls the molar quotient, phospholipid fatty acids: lipid phosphorus, was calculated. It was found to be 1.05 with a standard deviation 0.21. The same quotient in phospholipids from the serum of patients with essential hypercholesterolemia and atherosclerotic manifestations was found to be 1.49.

The fatty acid composition of the different lipids in the artery walls of patients on an ordinary diet is given in Table II.

The serum polyunsaturated fatty acids were not determined in the six patients who had remained on the ordinary diet and were operated upon. For comparison between the fatty acids in the artery wall and in serum, also listed in Table II are the serum values from a group of patients with essential hypercholesterolemia with a high frequency of atherosclerotic manifestations. These figures are taken from an earlier study.[10]

It may be seen that the fatty acid composition of the cholesterol esters and of the fraction containing glycerides plus unesterified fatty acids in the artery wall in the patients analysed in this work shows a considerable similarity to the fatty acid composition of those in the serum from a comparable group of patients. However, the dienes are somewhat lower in the artery lipid fractions mentioned. In the phospholipid fatty acids there are clearly lower values for dienes. This also holds true for the other individual polyunsaturated fatty acids in the phospholipid fraction although it is less pronounced.

Table III presents the results of the analyses of the arterial lipids in the two patients who had been on an unsaturated fat diet one-half and

TABLE IV

The Percentage Fatty Acid Distribution in the Different Lipid Fractions Extracted from the Intima of the Femoral Artery in Patient G. B. on an Ordinary Diet (O) and After Five Months on a Diet Containing Unsaturated Fat (U)

| Lipid Fraction | Cholesterol Esters | | Glycerides plus Unesterified Fatty Acids | | Phospholipids | |
|---|---|---|---|---|---|---|
| | O | U | O | U | O | U |
| Dienes | 29.8 | 38.5 | 9.5 | 15.0 | 4.9 | 4.3 |
| Trienes | 3.0 | 4.4 | 2.6 | 2.0 | 1.9 | 1.8 |
| Tetraenes | 4.3 | 5.3 | 1.2 | 1.0 | 4.9 | 4.3 |
| Pentaenes | 1.6 | 2.4 | 2.7 | 0.5 | 1.2 | 1.0 |
| Hexaenes | 0.9 | 2.5 | 5.5 | 0 | 0.7 | 1.0 |

TABLE V

The Percentage Fatty Acid Distribution in the Different Lipid Fractions Extracted from the Intima of the Femoral Artery in Patient G. S. Two Preparations Taken on an Ordinary Diet ($O_1$ and $O_2$), One After Five Months ($U_1$) and One After Eight Months ($U_2$) on a Diet Containing Unsaturated Fat

| Lipid Fraction | Cholesterol Esters | | | | Glycerides plus Unesterified Fatty Acids | | | | Phospholipids | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $O_1$ | $O_2$ | $U_1$ | $U_2$ | $O_1$ | $O_2$ | $U_1$ | $U_2$ | $O_1$ | $O_2$ | $U_1$ | $U_2$ |
| Dienes | 38.1 | 35.9 | 44.5 | 42.5 | 6.0 | 7.0 | 16.8 | 10.8 | 5.3 | 5.0 | 3.8 | 3.7 |
| Trienes | 2.9 | 2.0 | 3.9 | 5.5 | 0 | 1.0 | 3.5 | 1.6 | 1.5 | 1.4 | 1.2 | 1.4 |
| Tetraenes | 5.4 | 5.2 | 7.6 | 8.0 | 2.0 | 1.2 | 2.3 | 1.7 | 4.4 | 4.2 | 2.2 | 3.4 |
| Pentaenes | 1.7 | 1.3 | 2.1 | 2.2 | 0.4 | 0.2 | 1.1 | 0.6 | 0.9 | 1.1 | 0.5 | 0.6 |
| Hexaenes | 1.3 | 1.0 | 1.2 | 1.3 | 0 | 0 | 1.1 | 0.9 | 0.7 | 1.2 | 0.5 | 0.4 |

two months, respectively, before the operation was performed.

When the figures in Table III are compared with the corresponding figures for patients on an ordinary diet (Table II) there seem to be some differences in the fatty acid composition. Thus the glyceride plus unesterified fatty acids seem to contain a greater proportion of polyunsaturated fatty acids. Several of the percentage figures in the fraction in question are above the range of the figures for the patients on the oridinary diet. The significance of these findings is open to discussion, however. These two patients were on the diet rich in unsaturated fat for only a limited time. In two other patients (G. B. and G. S.) on the unsaturated fat diet, however, it was possible not only to analyse the artery preparations while they were on the ordinary diet and then after a longer period on the diet rich in polyunsaturated fat, but also to follow the fatty acid distribution in the different serum lipids. The results of the analyses of the artery fatty acids are given in Table IV and V. The sum of polyunsaturated fatty acids and the monoenes plus saturated fatty acids in percentage values in the different lipid fractions in serum and in the artery wall for these patients are given in Figures 1 and 2.

In Tables IV and V may be seen how the degree of polyunsaturation rises in the cholesterol esters and in the fraction containing glycerides plus unesterified fatty acids with the diet rich in unsaturated fat. This rise is most pronounced in the dienes, but it can also be found in the other polyene fatty acids. If anything, the percentage content of polyunsaturated fatty acids in the intima phospholipids decreases.

In Figures 1 and 2 it can be seen that the degree of polyunsaturation in the lipids from the artery preparation and from serum rises in

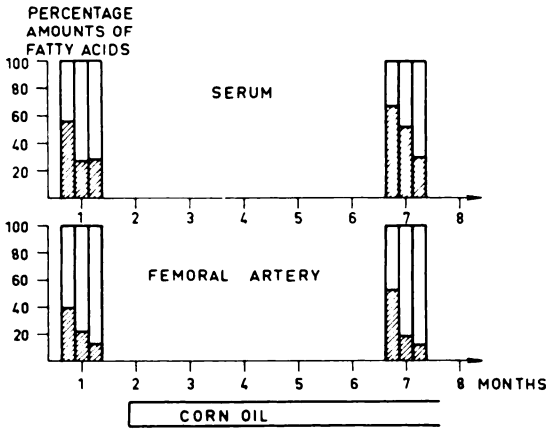


FIG. 1. Patient G. B. The percentage distribution of polyunsaturated fatty acids (shaded) and monoenoic plus saturated fatty acids (unshaded) of the serum and femoral artery lipid fractions before and after unsaturated fat supplementation of the diet Bars from left to right: cholesterol esters, glycerides plus unesterified fatty acids, phospholipids.

the cholesterol esters and in the glycerides plus unesterified fatty acids, but in the phospholipids only in the serum from one of the patients (G. S.). The changes in the serum were considerably more pronounced than in the arteries.

## COMMENTS

When analysing the fatty acid composition of the lipids in the arterial walls several factors should be taken into consideration if the values given by different authors are to be compared. Böttcher et al.[16,17] have pointed out the variation of the proportions of the fatty acids in different stages of atherosclerosis, the percentage amounts of polyunsaturated fatty acids rose with increasing severity of the atherosclerotic changes. The opinions as to whether the fatty acid composition varies in different arteries are divergent. Böttcher et al.[17] found different percentage amounts of the individual fatty acids in atherosclerotic aortas and in coronary arteries; Wright and associates,[18] however, found no pronounced difference between the two arteries mentioned, but they analysed a pool of coronary arteries only. The results of analyses of fatty acids in atherosclerotic plaques seem to be different as compared with those in the remaining aortic wall. Wright et al.[18] found no difference, but Swell and co-workers[19] found that the media contained considerably more linoleic acid than the plaques. Böttcher et al.[16,17] included a considerable part of the media in their preparation from aorta, but found that the dilution of the intimal lipids by lipids from the media was of small magnitude and could be neglected. The serum lipid levels and the possibility of the blood streams coming into contact with the atheromatous lesions seem to be other factors that might cause differences

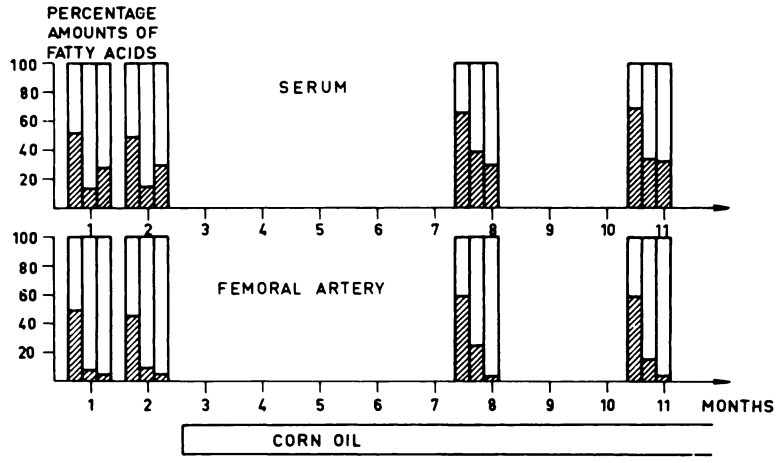


FIG. 2. Patient G. S. The percentage distribution of polyunsaturated fatty acids and monoenoic plus saturated fatty acids of the serum and femoral artery lipid fractions before and after unsaturated fat supplementation of the diet. Symbols as in Figure 1.

between the results of different workers, as the lipids in serum and the atheromas seem to be exchangeable. Differences in the methods used must also be taken into consideration. It seems necessary for these circumstances to be defined if comparisons between different authors are to be meaningful.

In the present work, the preparations analysed seem to be comparable with those of Böttcher et al.[16,17] except in regard to the vessels analysed. The degree of atherosclerosis in the present work, as determined by macroscopical inspection, is comparable to stages II and III in the works of Böttcher and his group. The composition of the lipids extracted from the artery wall supports this assumption. The fatty acid composition of the different lipid fractions is in quite good agreement with those found in stages II and III of atherosclerosis by Böttcher et al. They also agree fairly well with those found by Tuna, Reckers and Frantz[20] who analysed plaques from aorta. The preparations of these authors also seem to be comparable with the ones analysed in the present work.

When comparing the fatty acid composition of the femoral artery wall and the serum from a material of patients who, from the points of view of clinical picture and serum lipid levels, were similar to the material of patients analysed in the present work, some striking points are to be observed. The fatty acid composition of the cholesterol esters and of the fraction containing glycerides plus unesterified fatty acids shows considerable similarities in the two places. There is a somewhat lower percentage value of the dienes in the artery, but the difference is not statistically significant.

Another striking feature is the discrepancy between the phospholipid fatty acids. The vessel lipids contain a much smaller amount of dienes, and a somewhat lower amount of the other polyunsaturated fatty acids. This is probably due to different compositions of the individual types of phospholipids in the serum and in the artery wall. The lower molar quotient, fatty acids:lipid phosphorus, in the vessel phospholipids indicates a higher concentration of phospholipids with one fatty acid per molecule, e.g., sphingomyelin, in the intima than in the serum. The sphingomyelin fatty acids in arteries are mainly saturated or monounsaturated $C_{24}$ acids, as in sphingomyelins from other sources.[17] This is probably the explanation of the lower proportion of polyunsaturated fatty acids in the artery phospholipids.

When unsaturated fat is introduced into the diet, the fatty acid composition of the artery wall is altered. In the cholesterol esters and in the glycerides plus unesterified fatty acids, a rise in the proportion of the polyunsaturated fatty acids is produced. In the phospholipids, however, no significant change could be observed: there is, if anything, a slight decrease. The rise in the proportion of polyunsaturated fatty acids can be observed in serum, the change being most pronounced in glycerides plus unesterified fatty acids, then in the cholesterol esters and least in the phospholipid fraction.[10,21] The changes in the fatty acid composition of the artery lipids seem to require a much longer time to develop than in the serum lipids, as judged from the fact that after two months on an unsaturated fat diet, the alterations were not observable with any certainty. The poor response to unsaturated fat in the diet of the phospholipid fatty acids in the artery lipids might be explained by the demonstration by Zilversmit and McCandless[23] that atheroma phospholipids are largely produced *in situ*.

The possibility of producing changes in the fatty acid composition of artery lipids by a change of the dietary fat has also recently been indicated in a report by Farquhar et al.[22] These investigators also found the most pronounced degree of change in the glycerides and the cholesterol esters. The changes required a period of time considerably longer than that required for the same phenomenon in the serum lipids.

The diet-induced changes in the fatty acid composition of the lipids in the artery wall seem to follow the changes in the serum lipids, but develop more slowly. This indicates an exchange between the lipids or between the fatty acids in both places. Whether the change in the lipid composition of the artery signifies any change of importance in the size and extent of the atheromas is impossible to deduce. More

work on the experimental animal is needed on this point.

## SUMMARY

The composition of the fatty acids in the different lipids in the atherosclerotic femoral artery was determined. The femoral artery specimens were obtained when the patients were treated surgically for atherosclerotic disease.

On an ordinary diet the fatty acid composition of the artery lipids was almost similar to the composition in serum, in regard to cholesterol esters and glycerides plus unesterified fatty acids. There was a considerable difference, in the phospholipids with lower values for the polyunsaturated fatty acids in the artery walls. This discrepancy is probably due to a higher concentration of sphingomyelin in the artery walls than in serum, as judged from the molar quotient, phospholipid fatty acids: lipid phosphorus.

When polyunsaturated fatty acids were supplied in the diet, the degree of polyunsaturation of the serum lipids rose markedly. These changes were followed by the same phenomenon in the artery wall, observed in the cholesterol esters and in the glycerides plus unesterified fatty acids, but here a considerably longer period of time was required for the changes to be observable. This indicates an exchange between the lipids or the fatty acids in serum and in the artery wall.

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