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by E. Araki and T. Kitagawa

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Compositional analysis of fatty acids of
serum total lipids in healthy subjects.

by Eiji Araki and Toshiko Kitagawa*

Previous reports on the principal fatty acids of human serum have presented the results of analyses of healthy subjects and patients with various diseases.^{1,2} In recent years, moreover, the diagnostic significance of small quantities of fatty acids in serum, such as 3, 7, 11, 15-tetramethyl hexadecanoic acid in Refsum's disease,³ 14-methyl hexadecanoic acid in malignant tumors,⁴ and 5, 8, 11-eicosatrienoic acid in primary hepatocellular carcinoma⁵ have come to be recognized. In an earlier study we identified total fatty acids including polyunsaturated fatty acids through analysis by combined chemical ionization mass spectrometry - gas chromatography.⁶ The present report, however, concerns the percentage composition of fatty acid methyl esters of serum from healthy subjects, fractionated with the aid of silver nitrate-infiltrated thin-layer chromatography.

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Materials and Methods

Five litres of serum from 25 fasting adults, tested as biochemically normal, was used. The serum was filtered through Toyo No. 2 filter paper and then centrifuged at 105,000 x g for 3 hours. The supernatant was lyophilized. Immediately after drying and pulverization, fatty acid methyl esters, obtained by methylation (with 5% methanolic hydrochloric acid) or total serum lipids extracted and refined by the Folch method, were purified by thin-layer silica gel chromatography, using 10% ethyl ether in n-hexane for development. Subsequently these fatty acid methyl esters were separated, through 5% silver nitrate-infiltrated silica gel thin-layer chromatography, with solvents of 10% and 25% ethyl ether in n-hexane, into saturated, mono- and diethenoid fatty acids, and tri-, tetra-, and polyethenoid fatty acids respectively. For each fraction of unsaturated fatty acids thus obtained, hydrogenation was performed directly with palladium carbon as catalyst, and gas chromatography was then carried out. The fatty acids of each fraction were identified with the aid of the results reported in the previously-mentioned analysis through chemical ionization mass spectrometry gas chromatography,⁶ and their structures were determined by measurement of the relative areas under the peaks of the gas chromatograms, areas being calculated by the half-height with method.

Results

As shown in Table 1, a total of 27 fatty acids were identified: 8 straight-chain saturated fatty acids, 5 monoethenoid fatty acids, 5 diethenoid fatty acids, 2 triethenoid fatty acids, 4 tetraethenoid fatty acids and 3 polyethenoid fatty acids. The composition of each group of normal serum fatty acids, fractionated according to the level of unsaturation, was investigated.

Table 1. Composition of human serum fatty acids, by level of saturation or unsaturation (tr. : below 0.1%)

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① 直鎖飽和脂肪酸		⑥ Triethenoid 脂肪酸	
② 炭素類	③ 百分率	② 炭素類	③ 百分率
C ₁₂	0.1	C ₁₉	13.1
C ₁₄	2.9	C ₂₀	86.9
C ₁₅	0.5	⑦ Tetraethenoid 脂肪酸	
C ₁₆	72.2	② 炭素類	③ 百分率
C ₁₇	tr.	C ₁₆	tr.
C ₁₈	23.9	C ₁₈	3.5
C ₂₂	tr.	C ₂₀	95.4
C ₂₄	tr.	C ₂₂	1.1
④ Monoethenoid 脂肪酸		⑧ Polyethenoid 脂肪酸	
② 炭素類	③ 百分率	② 炭素類	③ 百分率
C ₁₅	1.1	C _{20:5}	22.0
C ₁₆	10.1	C _{22:5}	9.8
C ₁₇	tr.	C _{22:6}	68.2
C ₁₈	88.5		
C ₂₀	0.2		
⑤ Diethenoid 脂肪酸			
② 炭素類	③ 百分率		
C ₁₆	tr.		
C ₁₈	98.8		
C ₂₀	0.5		
C ₂₂	0.7		
C ₂₄	tr.		

Key to Table 1

1. Straight-chain saturated fatty acids
2. Carbons
3. Percentages
4. Monoethenoid fatty acids
5. Diethenoid fatty acids
6. Triethenoid fatty acids
7. Tetraethenoid fatty acids
8. Polyethenoid fatty acids

Discussion

Many of the previous studies of human serum fatty acids have dealt with the principal fatty acids from $C_{14:0}$ to $C_{20:4}$, but few have concerned highly unsaturated fatty acids above $C_{20:5}$. Among the latter, Nelson⁷ and Williams et al.⁸ have published detailed reports on human serum phospholipids and their fatty acid composition. Their findings agree with our results on $C_{22:5}$ and $C_{22:6}$, but the fatty acids they identify as $C_{22:1}$, $C_{22:4}$ and $C_{24:1}$ may be inferred, from their percentage compositions, to correspond to $C_{20:5}$ and $C_{22:5}$ ω -6 as reported in our study. In addition, among components occurring in trace quantities, $C_{15:0}$, $C_{17:0}$ and $C_{17:1}$, etc., were detected, but their structures are currently under investigation, especially for the question of the existence of branching.

To bring about reliable identification of human serum fatty acids in this manner is not only to contribute to research on fatty acid metabolism; it may also be of use in identifying fatty acids in tissues and body fluids, as serum is a standard material available at any time and in any laboratory.

Conclusion

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27 fatty acids identified in human serum lipids were fractionated according to levels of unsaturation by silver nitrate-infiltrated silica gel thin-layer chromatography, and the fatty acid composition of each fraction was indicated.

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Chemical Abstracts

vivo ultrafiltration (a 5-15 min tourniquet compression of the arm), whereas the plasma uric acid was not affected. Thus, these 2 plasma variables are probably indirectly related via a common assocn. with an unknown factor or factors other than the protein binding of urate.

87: 149443a Compositional analysis of fatty acids of serum total lipids in healthy subjects. Araki, Eiji; Kitagawa, Toshiko (Clin. Lab., Nat. Cancer Cent., Tokyo, Japan): *Igaku To Seibutsugaku* 1977, 94(1), 17-19 (Japan). Human serum total lipids were fractionated and identified with the aid of AgNO₃-infiltrated silica gel thin-layer chromatog. Serum lipids were fractionated into 8 satd. fatty acids (FA), 5 monoethenoid FA, 5 diethenoid FA, 2 triethenoid FA, 4 tetraethenoid FA, and 3 polyethenoid FA. The availability of reliable fractionation and identification techniques for serum may permit serum to be used as the std. material for analyzing fatty acids in tissues and body fluids. S. Yamashita