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# ANALYSIS OF HUMAN SERUM TRIGLYCERIDES BY HIGH-TEMPERATURE GAS CHROMATOGRAPHY

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SUMMARY

The usefulness of high-temperature gas chromatography has been evaluated as a technique to monitor changes in the composition of intact triglycerides of human serum lipoprotein fraction after ingestion of triglycerides containing fatty acids of widely differing chain lengths (*i.e.* coconut, corn and rapeseed oils).

The technique proved to be useful in this type of metabolic experiment, since the appearance of specific molecular weight triglycerides with random or non-random distributions of fatty acids in serum lipoproteins could be detected after ingestion of the oils. With simple fatty acid analysis, only changes in the proportions of fatty acids in triglycerides can be measured.

## INTRODUCTION

Gas chromatography (GC) has been widely used to investigate the effects of dietary fats on the fatty acid composition of serum triglycerides. The analysis of fatty acid composition alone is, however, of limited use since it yields little information about the molecular composition of the dietary and serum triglycerides.

High-temperature GC is a sensitive and rapid technique whereby intact lipids can be separated without prior hydrolysis to their constituent fatty acids (see KUKSIS[1] for review). The method separates triglycerides according to their total carbon number (*i.e.* molecular weight) without distinguishing molecules on the basis of double bond number or position on the glycerol backbone. This technique enables more information to be obtained about the molecular weight and degree of intermolecular specificity or randomness of the fatty acid distribution of glycerides than fatty acid analysis alone does.

Except for a few studies[2–4], the method has not been widely used to study lipid metabolism. KUKSIS *et al.*[5,6] have reported that blood glycerides can be separated by high-temperature GC. The experiments reported here were devised to evaluate the

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usefulness of this technique in determining changes in the composition of intact triglycerides of circulating lipoproteins in man after ingestion of triglycerides with fatty acids of widely differing chain lengths. For simplicity, serum has been separated into two fractions (density ($d$) $\leqslant$ 1.00 and density ($d$) $>$ 1.00), since it was not our intention to carry out a detailed survey of changes in the triglyceride composition of individual serum lipoprotein classes.

## MATERIALS AND METHODS

Samples of refined deodorized coconut oil and refined rapeseed oils were gifts from Loders and Nucoline Ltd., Cairn Mills, Silvertown, London E. 16, Great Britain. Corn oil was purchased from domestic suppliers.

Thick-layer chromatography[7] followed by iodine staining showed that the coconut and rapeseed oils were >99% triglyceride. Though the corn oil contained some diglyceride and free fatty acids, high temperature GC (see below) showed it to be >94% triglyceride. Tables II and III show the fatty acid and triglyceride compositions of the oils used in these experiments. The fatty acid composition of the coconut oil agrees with published data[8,9], as does the triglyceride composition[4,9]. The fatty acid composition of corn oil is similar to that quoted by HILDITCH AND WILLIAMS[10]. Only the elution pattern of corn oil triglycerides on GC, but not the molar composition, has been published previously[11]. Both fatty acid and triglyceride compositions of the rapeseed oil used agree with published data[12,13].

A fasting (12 h) male, age 37, was given 40 g of coconut, corn or rapeseed oil. The same samples of these oils were used throughout. Except for the coconut oil, which was spread on toasted bread, the oils were ingested with orange juice. The subject of these experiments was one of the authors (R.D.).

Venous blood (5–10 ml) was drawn after fasting and 3–3.5 h after ingestion of oil. Directly after drawing the blood, serum was prepared, layered under water and subjected to centrifugation at 10,000 $g$ for 10 min. Lipoproteins of density $\leqslant$1.00 concentrated in the upper layer and lipoproteins of density >1.00 concentrated in the lower layer. The former were washed by gentle emulsification in water and recentrifugation at 10,000 $g$ for 10 min. Lipids were extracted from both lipoprotein fractions and washed free from non-lipid contaminants[14]. In each case, lipid classes were separated by thick-layer chromatography[7]. The developing solvent was diethyl ether–light petroleum (b.p. 40–60°) (15:85) and the tank was flushed with nitrogen at the start of development. Using a triolein marker, the triglyceride area was scraped from the plate, eluted with chloroform, evaporated to a small volume and immediately analysed by GC for intact lipid structure (see below). In a few cases, samples were stored dry and under nitrogen prior to saponification, methylation and fatty acid analysis.

The apparatus and methods used to analyse intact triglycerides by high-temperature GC have been described[7,15]. A column (1 ft. 9 in.) of 10% SE-30 was used as stationary phase with a column load of approx. 20–40 $\mu$g lipid. The temperature programme used to analyse rapeseed oil terminated at 345°. Methyl esters of fatty acids from triglycerides were analysed on an aged column of 10% diethyleneglycol adipate/3% phosphoric acid[7]. Molar correction factors[15] were applied to all GC results.

The average fatty acid carbon number for fatty acid methyl esters and for

triglycerides was calculated as described previously[7]. Triglyceride carbon numbers refer to the total number of acyl carbon atoms in the molecule.

*Assessment of specificity or randomness of triglycerides*

The intermolecular specificity or randomness of the fatty acid distribution in triglycerides was evaluated by comparing the molar percentage of each triglyceride species found experimentally ($F$) with the calculated[16] molar percentage of each triglyceride species assuming a random ($R$) distribution of fatty acids. In the simplest case where experimental errors in fatty acid and triglyceride analyses are ignored, ($F - R$) represents the absolute degree of specificity inherent in the triglyceride structure *i.e.* the moles % of the triglyceride species which has a non-random distribution of fatty acids. For a completely random intermolecular distribution of fatty acids, ($F - R$) = 0.

In practice, each fatty acid and triglyceride analysis by GC has inherent experimental errors. The errors involved in determining the fatty acid composition will be reflected in the calculated values for $R$, and values for $F$ will reflect in addition the errors involved in the triglyceride analysis. Hence, values for $F$ and $R$ will each have their own errors. This is illustrated in Table I, which shows the most specific triglyceride structures which could, in theory,be obtained with the fatty acid composition of the triglycerides of fasting serum lipoproteins, $d > 1.00$, (see Table II). It can be seen that the overall moles % specificity of triglyceride structures is related to the sum of either the positive or the negative values for ($F - R$). Since it is difficult to assign an error to this overall moles % specificity when experimental errors for component fatty acid and/or triglyceride compositions are not of the same order, average values for these overall errors have been used.

TABLE I

CALCULATION OF OVERALL MOLE % SPECIFICITY

The fatty acid composition used in this calculation is that of the triglycerides of fasting serum lipoproteins of $d > 1.00$ (see Table II) *i.e.* $C_{12} = 0.2 \pm 0.1$ moles %; $C_{14} = 2.2 \pm 0.1$ moles %; $C_{16} = 41.5 \pm 1.4$ moles %; $C_{18} = 56.2 \pm 0.5$ moles %. However, the $F^*$-values are theoretical, and have been calculated assuming a complete specificity for monoacyl triglyceride distribution (*i.e.* $C_{12} + C_{12} + C_{12}$ etc.). The $R$-values are calculated assuming a random distribution of fatty acids.

| *Triglyceride (carbon No.)* | *$F^*$-values* | *$(R)$-values* | *$(F^* - R)$-values* |
|---|---|---|---|
| | *Moles %* | *Moles %* | *Moles % specificity* |
| 36 | 0.2 ± 0.1 | — | 0.2 ± 0.1 |
| 38 | — | — | — |
| 40 | — | — | — |
| 42 | 2.2 ± 0.1 | — | 2.2 ± 0.1 |
| 44 | — | 0.2 ± 0.1 | — 0.2 ± 0.1 |
| 46 | — | 1.5 ± 0.5 | — 1.5 ± 0.5 |
| 48 | 41.5 ± 1.4 | 10.4 ± 0.7 | 31.1 ± 2.1 |
| 50 | — | 31.1 ± 0.3 | —31.1 ± 0.3 |
| 52 | — | 39.2 ± 0.7 | —39.2 ± 0.7 |
| 54 | 56.2 ± 0.5 | 17.7 ± 0.2 | 38.5 ± 0.7 |
| | Overall moles % specificity | | = 72.0 ± (av.) 2.3 |

## TABLE II

FATTY ACID COMPOSITION (MOLES %) OF INGESTED TRIGLYCERIDES AND OF SERUM LIPOPROTEIN TRIGLYCERIDES AFTER FASTING AND INGESTION OF COCONUT, CORN AND RAPESEED OILS

| *Fatty acid (No. of carbon atoms: No. of double bonds)* | *Fatty acid (moles %)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *After fasting* | *Coconut oil*[b] | *After ingestion of coconut oil* | | *Corn oil*[b] | *After ingestion of corn oil* | | *Rapeseed oil*[b] | *After ingestion of rapeseed oil* | |
| | *Lipoproteins* | | *Lipoproteins* | | | *Lipoproteins* | | | *Lipoproteins* | |
| | $d > 1.00$[a] | | $d \leqslant 1.00$[c] | $d > 1.00$[a] | | $d \leqslant 1.00$[d] | $d > 1.00$[b] | | $d \leqslant 1.00$[d] | $d > 1.00$[b] |
| 8:0 | — | 9.7 ± 0.3 | — | — | — | — | — | — | — | — |
| 10:0 | — | 6.8 ± 0.1 | — | — | — | — | — | — | — | — |
| 12:0 | 0.2 ± 0.1 | 52.9 ± 1.1 | 8.5 ± 1.2 | 1.8 ± 0.2 | — | — | — | 0.6 ± 0.2 | — | — |
| 14:0 | 2.2 ± 0.1 | 14.6 ± 0.7 | 16.6 ± 2.3 | 7.3 ± 2.0 | — | 2.0 ± 0.2 | 2.5 ± 0.1 | 0.3 ± 0.1 | 1.5 ± 0.3 | 2.0 ± 0.2 |
| 16:0 | 34.1 ± 1.0 | 7.4 ± 0.0 | 41.6 ± 1.8 | 45.5 ± 6.7 | 14.0 ± 0.2 | 27.9 ± 1.0 | 34.8 ± 2.0 | 4.5 ± 0.4 | 23.8 ± 0.7 | 26.7 ± 0.7 |
| 16:1 | 7.3 ± 0.4 | — | 16.6 ± 0.2 | 14.1 ± 0.9 | — | 7.0 ± 0.5 | 9.2 ± 0.3 | 0.4 ± 0.1 | 2.9 ± 0.6 | 3.9 ± 0.8 |
| 18:0 | 7.4 ± 0.1 | 3.0 ± 0.1 | 6.6 ± 0.0 | 6.3 ± 0.4 | 2.3 ± 0.1 | 6.1 ± 0.4 | 7.3 ± 0.2 | 1.1 ± 0.1 | 3.1 ± 0.6 | 3.1 ± 0.6 |
| 18:1 | 38.8 ± 0.2 | 4.8 ± 0.1 | 10.1 ± 0.2 | 22.6 ± 10.4 | 33.0 ± 0.4 | 33.1 ± 2.5 | 31.0 ± 1.5 | 13.1 ± 0.6 | 30.7 ± 2.5 | 41.3 ± 2.5 |
| 18:2 | 10.0 ± 0.2 | 1.0 ± 0.1 | — | 2.4 ± 2.4 | 50.7 ± 0.2 | 22.1 ± 0.1 | 13.9 ± 0.5 | 15.0 ± 0.2 | 15.6 ± 0.5 | 9.5 ± 0.1 |
| 18:3 | — | — | — | — | — | 1.8 ± 0.2 | 1.5 ± 1.0 | 8.8 ± 0.8 | 3.6 ± 0.5 | 3.0 ± 0.4 |
| 20:1 | — | — | — | — | — | — | — | 6.7 ± 0.0 | 3.2 ± 0.5 | 1.7 ± 0.2 |
| 22:1 | — | — | — | — | — | — | — | 49.7 ± 2.2 | 15.6 ± 0.5 | 8.8 ± 0.5 |
| Average fatty acid carbon number | 17.1 | 12.6 | 15.6 | 16.4 | 17.7 | 17.2 | 17.0 | 19.9 | 18.1 | 17.7 |

[a] Average of 2 different samples.
[b] Average of 2 determinations on the same sample.
[c] Mean of 4 different samples ± S.E.
[d] Single analysis on one sample with estimated maximum variation.

## RESULTS AND DISCUSSION

### *Fasting samples*

A number of samples of fasting serum lipoproteins with $d > 1.00$ had similar triglyceride fatty acid compositions to those quoted in Table II, but only these two samples contained sufficient lipid to determine their intact triglyceride compositions. Overall, 15.6 ± (av.)2.6 moles % of the triglycerides had specific distributions of fatty acids (Table III). No lipoproteins of $d \leqslant 1.00$ were detected in any sample of fasting serum.

### *The effects of ingested oils*

There was considerable variation in the concentrations of the two serum lipoprotein fractions obtained after ingestion of the same quantity of oil. We report in Tables II and III those experiments where a sufficient concentration of both serum lipoprotein fractions was obtained (*i.e.* at least 0.6 mg lipid per fraction) to enable their patterns of fatty acids and triglycerides to be determined. In each case, the effect of the ingested triglyceride on the fatty acid pattern of the serum lipoprotein triglycerides is described, followed by the effect on the intact triglyceride composition.

### *Coconut oil*

When coconut oil was ingested, $C_{8:0}$ and $C_{10:0}$ fatty acids were not detected in either serum lipoprotein fraction (Table II), indicating their absorption by the portal route[17,18]. The proportions of $C_{12:0}$ and $C_{14:0}$ (the major fatty acids of coconut oil) in lipoproteins $d \leqslant 1.00$ were greater than in lipoproteins $d > 1.00$, whereas fasting samples contained little of these fatty acids. These changes were strongly reflected in the average fatty acid carbon numbers quoted in Table II, and agree well with those obtained by BRAGDON AND KARMEN[19] and KAYDEN *et al.*[20] in similar studies.

Table III shows that the lower molecular weight triglycerides (carbon numbers 26–34) of the coconut oil were not detected in either serum lipoprotein fraction. Triglycerides with carbon numbers 36–40 were present in both lipoprotein fractions, but absent in fasting samples. The average fatty acid carbon numbers of the triglycerides reflect the increased proportion of these shorter-chain triglycerides in lipoproteins $d \leqslant 1.00$ compared with lipoproteins $d > 1.00$. It is interesting that the effects observed by BÉZARD AND BUGAUT[4] on the triglyceride composition of rat adipose tissue after long-term feeding of coconut oil were strikingly similar to the effects reported here on the triglyceride composition of human serum lipoproteins $d \leqslant 1.00$ after a single dose of the oil.

The ingested coconut oil had approximately the same overall fatty acid specificity (16.4 ± (av.)3.2 moles %) as the fasting serum lipoprotein triglycerides (15.6 ± (av.)2.6 moles %). Though the values for lipoproteins $d > 1.00$ were variable, the overall specificity of the triglycerides of both lipoprotein fractions had increased considerably. The greatest increase in specificity was seen in triglycerides of carbon numbers 48 and 52, which may indicate that $C_{16}$ and $C_{18}$ fatty acids tend to re-esterify partial glycerides already containing these chain-length fatty acids.

### *Corn oil*

After taking corn oil, there was both a greater proportion of $C_{18:2}$ (the major

## TABLE III

TRIGLYCERIDE COMPOSITION (MOLES %) OF INGESTED OILS AND OF SERUM LIPOPROTEIN FRACTIONS AFTER FASTING AND INGESTION OF COCONUT, CORN AND RAPESEED OILS

$F$ = values found experimentally; $R$ = values calculated assuming a random distribution of fatty acids.

| *Triglyceride (carbon No.)* | *Triglyceride (moles %)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *After fasting* | | *Coconut oil* | | *After ingestion of coconut oil* | | | | *Corn oil* | |
| | *Lipoproteins d>1.00* | | | | *Lipoproteins d<1.00* | | *Lipoproteins d>1.00* | | | |
| | $F$[a] | $(F-R)$[a] | $F$[b] | $(F-R)$[b] | $F$[c] | $(F-R)$[c] | $F$[a] | $(F-R)$[a] | $F$[b] | $(F-R)$[b] |
| 24 | — | | — | −0.1 ± 0.1 | — | | — | | — | |
| 26 | — | | 0.1 ± 0.1 | −0.1 ± 0.1 | — | | — | | — | |
| 28 | — | | 1.0 ± 0.1 | −0.7 ± 0.2 | — | | — | | — | |
| 30 | — | | 4.4 ± 0.1 | 1.9 ± 0.2 | — | | — | | — | |
| 32 | — | | 15.3 ± 0.3 | 5.6 ± 0.4 | — | | — | | — | |
| 34 | — | | 18.6 ± 0.3 | 7.7 ± 0.4 | — | | — | | — | |
| 36 | — | | 20.3 ± 0.3 | −1.0 ± 0.9 | 2.0 ± 1.4 | 1.9 ± 1.4 | 0.7 ± 0.7 | 0.7 ± 0.7 | — | |
| 38 | — | | 16.2 ± 0.3 | −1.4 ± 0.5 | 4.1 ± 2.0 | 3.7 ± 2.1 | 1.4 ± 0.6 | 1.4 ± 0.6 | — | |
| 40 | — | | 9.6 ± 0.8 | −3.2 ± 0.9 | 6.9 ± 2.3 | 4.9 ± 3.0 | 2.9 ± 0.1 | 2.8 ± 0.1 | — | |
| 42 | 0.4 ± 0.0 | 0.4 ± 0.0 | 6.2 ± 0.4 | −5.8 ± 0.5 | 10.3 ± 4.2 | 4.6 ± 5.5 | 3.9 ± 0.3 | 3.3 ± 0.5 | — | |
| 44 | 1.3 ± 0.1 | 1.1 ± 0.2 | 3.4 ± 0.3 | −2.5 ± 0.4 | 12.4 ± 1.7 | − 2.5 ± 3.5 | 5.7 ± 1.0 | 2.4 ± 1.7 | — | |
| 46 | 2.8 ± 0.2 | 1.3 ± 0.7 | 2.0 ± 0.2 | −1.0 ± 0.3 | 14.7 ± 1.6 | − 8.4 ± 3.4 | 8.6 ± 1.2 | − 2.2 ± 4.4 | — | |
| 48 | 5.7 ± 0.3 | −4.7 ± 1.1 | 1.2 ± 0.5 | −0.6 ± 0.5 | 14.8 ± 3.3 | −15.3 ± 5.5 | 12.4 ± 0.2 | −17.5 ± 7.0 | 0.6 ± 0.1 | 0.3 ± 0.1 |
| 50 | 21.8 ± 0.8 | −9.2 ± 0.5 | 0.9 ± 0.3 | 0.4 ± 0.3 | 15.0 ± 3.8 | − 3.4 ± 5.2 | 21.3 ± 0.4 | −12.7 ± 2.4 | 4.7 ± 0.1 | −0.4 ± 0.3 |
| 52 | 51.9 ± 0.1 | 12.7 ± 1.1 | 0.5 ± 0.3 | 0.3 ± 0.4 | 15.4 ± 6.6 | 10.5 ± 7.7 | 37.8 ± 2.0 | 20.6 ± 8.2 | 31.4 ± 0.5 | 0.2 ± 0.8 |
| 54 | 16.1 ± 1.3 | −1.7 ± 1.6 | 0.3 ± 0.3 | 0.2 ± 0.2 | 4.4 ± 3.4 | 3.9 ± 3.5 | 5.2 ± 0.3 | 1.6 ± 2.6 | 63.3 ± 0.5 | −0.1 ± 1.0 |
| 56 | — | | — | | — | | — | | — | |
| 58 | — | | — | | — | | — | | — | |
| 60 | — | | — | | — | | — | | — | |
| 62 | — | | — | | — | | — | | — | |
| 64 | — | | — | | — | | — | | — | |
| 66 | — | | — | | — | | — | | — | |
| e | | 15.6 ± (av.) 2.6 | | 16.4 ± (av.) 3.2 | | 29.6 ± (S.D.) 7.5 | | 32.4 ± (av.) 14.1 | | 0.5 ± (av.) 1.1 |
| f | 17.1 | | 12.3 | | 15.7 | | 16.4 | | 17.7 | |

**TABLE III** *(Continued)*

| *Triglyceride (carbon No.)* | *Triglyceride (moles %)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *After ingestion of corn oil* | | | | *Rapeseed oil* | | *After ingestion of rapeseed oil* | | | |
| | *Lipoproteins d⩽1.00* | | *Lipoproteins d>1.00* | | | | *Lipoproteins d⩽1.00* | | *Lipoproteins d>1.00* | |
| | $F^d$ | $(F-R)^d$ | $F^d$ | $(F-R)^d$ | $F^b$ | $(F-R)^b$ | $F^d$ | $(F-R)^d$ | $F^d$ | $(F-R)^b$ |
| 24 | — | | — | | — | | — | | — | |
| 26 | — | | — | | — | | — | | — | |
| 28 | — | | — | | — | | — | | — | |
| 30 | — | | — | | — | | — | | — | |
| 32 | — | | — | | — | | — | | — | |
| 34 | — | | — | | — | | — | | — | |
| 36 | — | | — | | — | | — | | — | |
| 38 | — | | — | | — | | — | | — | |
| 40 | — | | — | | — | | — | | — | |
| 42 | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.4 ± 0.2 | 0.4 ± 0.2 | — | | — | | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 44 | 1.3 ± 0.3 | 1.3 ± 0.3 | 1.1 ± 0.3 | 1.1 ± 0.3 | — | | — | | 0.4 ± 0.2 | 0.4 ± 0.2 |
| 46 | 2.5 ± 0.5 | 1.7 ± 0.6 | 2.6 ± 0.5 | 1.1 ± 0.6 | — | | — | −0.4 ± 0.1 | 1.5 ± 0.3 | 0.9 ± 0.4 |
| 48 | 5.8 ± 0.5 | −1.1 ± 1.3 | 6.3 ± 0.5 | − 5.7 ± 1.8 | 0.3 ± 0.1 | 0.0 ± 0.2 | 1.4 ± 0.3 | −1.5 ± 0.6 | 4.9 ± 0.5 | 0.0 ± 1.1 |
| 50 | 18.1 ± 0.5 | −7.4 ± 2.1 | 18.8 ± 0.5 | −14.3 ± 2.3 | 1.4 ± 0.2 | 1.9 ± 0.5 | 11.2 ± 0.5 | −0.3 ± 0.6 | 12.5 ± 0.5 | −4.7 ± 2.5 |
| 52 | 43.8 ± 1.8 | 2.1 ± 2.2 | 51.4 ± 1.5 | 13.6 ± 2.6 | 3.1 ± 0.5 | 0.4 ± 1.3 | 33.4 ± 1.0 | 10.0 ± 1.4 | 34.7 ± 1.0 | 3.6 ± 1.1 |
| 54 | 28.0 ± 1.0 | 2.9 ± 3.2 | 19.4 ± 1.0 | 3.9 ± 3.0 | 6.5 ± 0.4 | − 0.6 ± 1.1 | 24.6 ± 1.0 | 2.7 ± 1.2 | 23.2 ± 0.8 | −0.1 ± 1.9 |
| 56 | — | | — | | 9.6 ± 1.0 | 0.8 ± 2.0 | 12.7 ± 0.5 | −3.6 ± 0.7 | 9.9 ± 0.5 | −1.4 ± 0.6 |
| 58 | — | | — | | 12.8 ± 0.9 | −10.5 ± 1.5 | 5.8 ± 0.5 | −8.8 ± 0.9 | 5.9 ± 0.5 | −3.1 ± 1.5 |
| 60 | — | | — | | 19.3 ± 0.2 | 8.1 ± 0.3 | 5.7 ± 0.5 | 1.4 ± 0.7 | 3.7 ± 0.4 | 2.4 ± 0.5 |
| 62 | — | | — | | 46.2 ± 1.4 | 17.4 ± 2.9 | 5.1 ± 0.5 | 1.0 ± 0.7 | 3.1 ± 0.4 | 1.8 ± 0.6 |
| 64 | — | | — | | — | − 5.0 ± 0.5 | — | −0.3 ± 0.1 | — | −0.1 ± 0.1 |
| 66 | — | | — | | — | −12.4 ± 1.7 | — | −0.4 ± 0.0 | — | −0.1 ± 0.1 |
| e | | 8.5 ± (av.) 5.0 | | 20.0 ± (av.) 5.4 | | 28.5 ± (av.) 6.0 | | 15.3 ± (av.) 3.5 | | 9.5 ± (av.) 5.3 |
| f | 17.2 | | 17.2 | | 19.9 | | 18.0 | | 17.7 | |

[a] Average of 2 different samples.
[b] Average of 2 determinations on the same sample.
[c] Mean of 4 samples ± S.D.
[d] Single analysis on one sample with estimated maximum variation.
[e] Moles % specificity.
[f] Average fatty acid carbon No.

fatty acid of the oil) and a higher ratio of $C_{18:2}$ to $C_{18:1}$ in triglycerides of lipoproteins $d \leqslant 1.00$ than in triglycerides of lipoproteins $d > 1.00$ (Table II). These results are in agreement with previous observations[17,20–22].

The same range of triglycerides (*i.e.* with carbon numbers 42–54) was found in the serum lipoprotein fractions as in the fasting lipoproteins (Table III). However, the proportion of triglycerides with carbon number 54 (the major component of the corn oil) was considerably increased in lipoproteins $d \leqslant 1.00$ at the expense of triglycerides with carbon number 52. This change did not significantly affect the average fatty acid carbon number. There was no significant difference in the proportion of triglycerides in lipoproteins $d > 1.00$ compared with fasting samples.

The random pattern of fatty acids in the corn oil was largely retained in the serum lipoprotein triglycerides, except for triglycerides with carbon numbers 50 (*i.e.* $C_{16} + C_{16} + C_{18}$) and 52 (*i.e.* $C_{16} + C_{18} + C_{18}$) in lipoproteins $d > 1.00$.

*Rapeseed oil*

Erucic acid ($C_{22:1}$) is the major fatty acid of rapeseed oil (Table II). After taking rapeseed oil, there was nearly twice the proportion of this fatty acid in the triglycerides of lipoproteins $d \leqslant 1.00$ as in those of lipoproteins $d > 1.00$. This clearly affected the average fatty acid carbon numbers compared with fasting samples (Table II).

The triglycerides of both lipoprotein fractions had also increased average fatty acid carbon numbers, and showed a striking increase in triglycerides with carbon numbers 54–62 (the major components of rapeseed oil). Triglycerides with carbon numbers 56–62 could not be detected in fasting samples.

The rapeseed oil had 28.5 ± (av.)6.0 moles % chain length specificity overall. This was mainly accounted for by triglycerides with carbon numbers 58–66. (The theoretical random composition ($R$) includes triglycerides with carbon numbers 64 and 66, though these were not present in the oil.) Both serum lipoprotein fractions had a more random distribution of fatty acids, especially in triglycerides with carbon numbers 60 and 62, and this was reflected in the lower values for their overall moles % specificity.

## CONCLUSION

The technique of GC of intact triglycerides has proved to be useful in this type of metabolic experiment. It enables the appearance of specific molecular weight triglycerides with random or non-random distributions of fatty acids to be monitored With simple fatty acid analysis, only changes in the proportions of fatty acids in triglycerides can be measured. Since high-temperature GC provides information about the intermolecular fatty acid chain-length-specificity of glycerides, it is complementary to lipase hydrolysis which identifies intramolecular positional specificity of fatty acids esterification on the glycerol backbone.

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## REFERENCES

1 A. KUKSIS, in G. V. MARINETTI (Editor), *Lipid Chromatographic Analysis*, Marcel Dekker, New York, 1 (1967) pp. 239–337.
2 R. WATTS AND R. DILS, *Lipids*, 3 (1968) 471.
3 W. C. BREKENRIDGE L. MARAI AND A. KUKSIS, *Can. J. Biochem.*, 47 (1969) 761.
4 J. BÉZARD AND M. BUGAUT, *J. Chromatogr. Sci.*, 7 (1969) 639.
5 A. KUKSIS, L. MARAI AND D. A. GORNALL, *J. Lipid Res.*, 8 (1967) 352.
6 A. KUKSIS, O. STACHNYK AND B. J. HOLUB, *J. Lipid Res.*, 10 (1967) 660.
7 S. SMITH, R. WATTS AND R. DILS, *J. Lipid Res.*, 9 (1968) 52.
8 T. P. HILDITCH AND P. N. WILLIAMS, *The Chemical Constitution of Natural Fats*, 4th Ed., Chapman and Hall, London, 1964, pp. 341, 342.
9 A. KUKSIS, M. J. MCCARTHY AND J. M. R. BEVERIDGE, *J. Amer. Oil Chem. Soc.*, 41 (1964) 201.
10 T. P. HILDITCH AND P. N. WILLIAMS, *The Chemical Constitution of Natural Fats*, 4th Ed., Chapman and Hall, London, 1964, pp. 280–283.
11 A. KUKSIS AND M. J. MCCARTHY, *Can. J. Biochem. Physiol.*, 40 (1962) 679.
12 T. P. HILDITCH AND P. N. WILLIAMS, *The Chemical Constitution of Natural Fats*, 4th Ed., Chapman and Hall, London, 1964, pp. 298–301.
13 R. D. HARLOW, C. LITCHFIELD AND R. REISER, *Lipids*, 1 (1966) 216.
14 J. FOLCH, M. LEES AND G. H. SLOANE-STANLEY, *J. Biol. Chem.*, 226 (1957) 497.
15 R. WATTS AND R. DILS, *J. Lipid Res.*, 9 (1968) 40.
16 A. KUKSIS, M. J. MCCARTHY AND J. M. R. BEVERIDGE, *J. Amer. Oil Chem. Soc.*, 40 (1963) 530.
17 P. WOOD, K. IMAICHI, J. KNOWLES, G. MICHAELS AND K. LINSELL, *J. Lipid Res.*, 5 (1964) 225.
18 I. TAMIR, D. B. GRANT, A. S. FOSBROOK, M. M. SEGALL AND J. K. LLOYD, *J. Lipid Res.*, 9 (1968) 661.
19 J. H. BRAGDON AND A. KARMEN, *J. Lipid Res.*, 1 (1960) 167.
20 H. J. KAYDEN, A. KARMEN AND A. E. DUMONT, *J. Clin. Invest.*, 42 (1963) 1373.
21 H. J. KAYDEN, A. KARMEN, A. E. DUMONT AND J. H. BRAGDEN, *J. Clin. Invest.*, 39 (1960) 1001.
22 J. H. BRAGDON AND A. KARMEN, *J. Lipid Res.*, 2 (1961) 400.