

The fatty acid composition of chylomicrons of chyle and serum following the ingestion of different oils

J. H. BRAGDON and A. KARMEN

Section on Metabolism and the Laboratory of Technical Development, National Heart Institute, National Institutes of Health, Bethesda 14, Maryland

[Received for publication November 12, 1959]

SUMMARY

Chylomicrons were isolated from the thoracic duct chyle and from the blood serum of rats, and from the serum of human subjects following the ingestion of different natural oils. The fatty acid composition of the chylomicrons was compared with that of the dietary fat by gas chromatography and was found to be very similar.

Dole *et al.* (1) have recently reported a remarkable constancy of fatty acid composition of chylomicrons separated from human plasma during the period of absorption of fats differing greatly in fatty acid composition. They speculated as to possible mechanisms involved. Either the fatty acids were chemically altered during absorption through the intestinal wall or there was rapid recycling between chylomicron fatty acids and tissue fatty acids. The purposes of this paper are to report the analyses of chyle and serum chylomicrons following the feeding of different fats to rats, and to attempt to confirm Dole's findings in human blood chylomicrons.

METHODS

Polyethylene cannulae were inserted in the thoracic ducts of three rats. The following day the animals were fed 1 ml. of coconut oil, olive oil, and cod liver oil, respectively. The chyle was collected and the chylomicrons isolated and washed as previously described (2). In brief, the chyle was layered under 0.15 M NaCl and centrifuged at $100,000 \times g$ for 30 minutes. The butterlike material at the top of the tube was re-emulsified in 0.15 M NaCl and recentrifuged. This process of washing was repeated. In order to get rat serum chylomicrons, groups of four fasting rats were fed each of the three oils. They were bled from the aorta 3 hours later; the blood from each group was pooled, and the serum was treated essentially as the chyle.

Two young women, who had spent several months in the Clinical Center as volunteer controls and who had been on a mixed diet (limited to 35 g. of fat per

day) were fed a formula breakfast in which the sole source of fat was either coconut oil or corn oil. The dose was 2 g. per kg. of body weight. They were bled fasting and 6 hours postprandially. Their serum samples were treated differently from the rat serum samples. Layering and washing took place in saline containing 0.01 M phosphate buffer at pH 7.4 and 0.05 per cent of the disodium salt of ethylene diamine tetraacetic acid. Centrifugation was carried out for 30 minutes at only $26,000 \times g$ and the chylomicrons were subjected to three washings in addition to the original separation.

Samples of the fats fed the rats, of the formula diets fed the human subjects, and of the chylomicrons were extracted in chloroform-methanol as previously described (2). Aliquots of the chloroform phase were evaporated to dryness at 20°C under nitrogen. Methyl esters were prepared by the transesterification procedure of Stoffel *et al.* (3), which involves refluxing the lipids with methanol, through which dry HCl gas has been passed until the HCl concentration is 5 per cent (w/w) in the methanol. The methyl esters were then extracted into redistilled hexane, and concentrated by evaporation under nitrogen. Aliquots were analyzed by gas chromatography.

The column used was a glass column, 8 feet long, 5 mm. I.D., filled with chromosorb W, siliconized with dimethyl dichlorosilane according to the method of Horning *et al.* (4), on which 15 per cent (w/w) of a polyester of adipate diethylene glycol had been coated. Analyses were performed at 200°C with a gas flow of 100 ml. per minute. Detection devices used in this study included a hot wire katharometer (model K5,

TABLE 1. THE PERCENTAGE FATTY ACID COMPOSITION OF 3 OILS COMPARED WITH THE COMPOSITION OF CHYLOMICRONS SEPARATED FROM CHYLE AND SERUM OF RATS FOLLOWING INGESTION OF THE OILS

Fatty acids *	12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:0 †	20:1 †	22:1 †
Olive oil		0.0	16	1.8	2.1	67	13			
Chyle chylomicrons		0.0	16	2.2	2.9	63	15			
Serum chylomicrons		0.0	17	2.3	2.0	64	15			
Cod liver oil		6.2	18	16	3.3	32	1.1	2.0	10	12
Chyle chylomicrons		6.5	21	17	4.1	34	4.6	1.0	8.1	4.5
Serum chylomicrons		5.1	21	15	4.6	33	6.3	1.5	6.7	6.7
Coconut oil ‡	71	17	5.7	0.0	1.9	3.2	1.0			
Chyle chylomicrons	56	20	8.2	0.0	2.1	8.2	4.3			
Serum chylomicrons	37	16	17	1.2	3.9	16	8.6			

* The first number refers to the number of carbon atoms; the second number refers to the number of double bonds.

† The fatty acids labeled 20:0, 20:1, and 22:1 are designated as such from retention volume data on adipate-diethylene glycol polyester gas chromatography columns. Their identity has not been confirmed by chemical determinations or by the use of other columns. They may represent more highly unsaturated esters of shorter chain length.

‡ The acids shorter than 12:0 have been ignored in computing the composition.

Burell and Co.) and an argon-ionization detector similar to that developed by Lovelock (5), employing a 300 μ c. radium foil. Voltage and amplifier settings were kept constant throughout the analyses.

At least two analyses of each sample were performed. The volume of the aliquots analyzed was varied in order to present long- and short-chain esters of the mixture to the detector cells in approximately equal concentrations, thus permitting more accurate quantification. Acids present in trace amounts have been ignored.

RESULTS

The fatty acid composition of the three fats that were fed to the rats and of the chylomicrons obtained both from the chyle and from the serum are presented in Table 1. In the case of olive oil and cod liver oil feeding, the composition of the chylomicrons, whether from chyle or serum, exhibits a remarkable similarity to the dietary fat. The only significant difference in this regard occurs in the chylomicrons after cod liver oil feeding. This oil contains very little linoleic (18:2) acid, and it appears that some of this acid was added from nondietary sources in the intestinal mucosa. In the rat fed coconut oil, which has a very high content of lauric (12:0) acid, there is a significant decrease in the concentration of this acid in the chyle chylomicrons. This is not surprising in view of the findings of Bloom *et al.* (6) that only 15 to 55 per cent of fed lauric acid is absorbed via the lymph in rats; the re-

mainder of this relatively short-chained acid is presumably absorbed via the portal system, as less than 5 per cent of the dose could be recovered in feces and intestinal contents. Shorter acids are absorbed entirely through the portal system and for this reason have been ignored in computing the composition of the

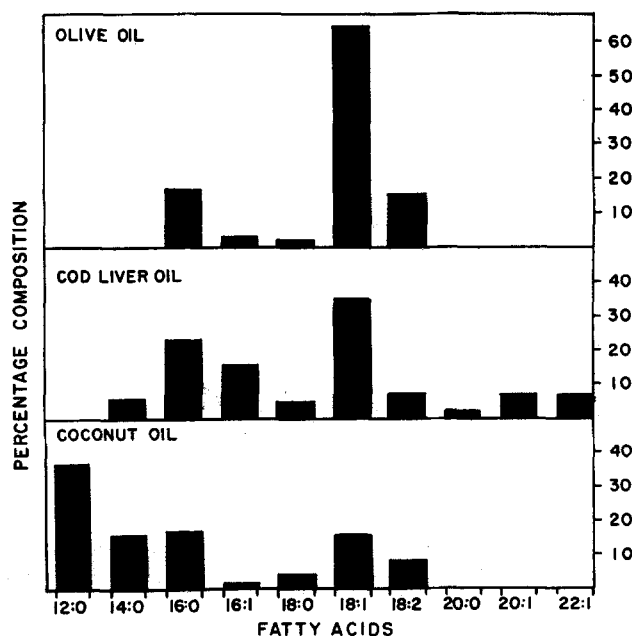


FIG. 1. The percentage fatty acid composition of rat serum chylomicrons following the ingestion of 3 different oils. The fatty acids are identified as follows: the first figure refers to the number of carbon atoms; the second figure refers to the number of double bonds.

TABLE 2. THE PERCENTAGE FATTY ACID COMPOSITION OF 2 OILS COMPARED WITH THE COMPOSITION OF HUMAN SERUM CHYLOMICRONS 6 HOURS FOLLOWING INGESTION OF THE OILS

Fatty acids	12:0	14:0	16:0	16:1	18:0	18:1	18:2
Corn oil	0.0	0.5	17	0.0	1.2	25	57
Chylomicrons	0.7	0.7	16	1.2	2.4	28	51
Coconut oil *	73	19	6	0.0	2.0	0.0	0.0
Chylomicrons	24	23	27	1.2	5.0	16	3.2

* The source of this oil differed from that fed the rats.

coconut oil. Comparing the composition of the chyle chylomicrons with the composition of the coconut oil, it is apparent that oleic (18 : 1) and linoleic (18 : 2) acids have been added from nondietary sources. This is the only case in which the serum chylomicrons differ significantly from the chyle chylomicrons. There is a further reduction in the short-chain acids with a relative increase in those of 16 and 18 carbon atoms. The chemical composition of the serum chylomicrons is shown graphically in Figure 1.

In the two human subjects one can compare only the serum chylomicrons with the dietary fat, and these data are presented in Table 2. When corn oil, which contains long-chain acids exclusively, was fed, the

fatty acid composition of the serum chylomicrons was almost identical to that of the corn oil. When coconut oil, with a preponderance of short-chain acids, was fed, the result was similar to that observed in the rat, and presumably for the same reason: that most of the lauric acid (12 : 0) was absorbed via the portal system. The composition of the serum chylomicrons is shown graphically in Figure 2.

DISCUSSION

Dole *et al.* (1) have reviewed the older literature, with its conflicting data, on the effect of dietary fat on the composition of chylomicrons. In most cases whole lymph, rather than chylomicrons only, was analyzed.

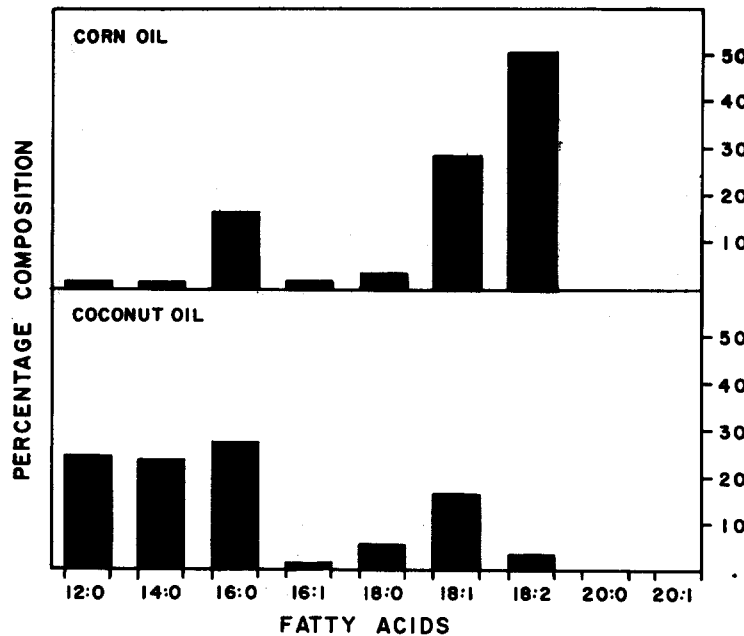


FIG. 2. The percentage fatty acid composition of human serum chylomicrons following the ingestion of 2 different oils. The fatty acids are identified as follows: the first figure refers to the number of carbon atoms; the second figure refers to the number of double bonds.

It is well known that whole lymph contains lipids other than those immediately absorbed from the intestine. Dole and his co-workers, although they did find significant differences in the fatty acid composition of serum chylomicrons following the feeding of coconut oil or corn oil, stressed their relative similarity and emphasized the differences between the composition of the chylomicrons and the composition of the dietary fat. The findings presented here differ from those of Dole, and show a similarity in composition between chylomicrons and the dietary fat. This discrepancy can perhaps be explained by the difference in methods used for the separation of the chylomicrons. Dole and his co-workers layered human serum under physiological saline and centrifuged at $100,000 \times g$ for 30 minutes. They then analyzed the material in the top of the tube. This gravitational field is sufficient to cause many low density lipoproteins, as well as chylomicrons, to rise to the top of the tube. Furthermore, they did not wash their chylomicron preparations, which were presumably contaminated with fatty acids not of immediate dietary origin. The human chylomicrons analyzed here, on the other hand, were removed from the top of the tube after centrifuging for 30 minutes at only $26,000 \times g$ and they were washed three times to free them of contaminating lipoproteins. It is true that the rat chylomicrons described here were isolated under centrifugal conditions identical to those of Dole, but rat serum contains very low concentrations of low density lipoproteins, only a small fraction of those often encountered in human serum, and again the chylomicrons were washed.

Ten ml. of the fasting human serum yielded so few chylomicrons that analysis was impossible. The usual sample of fatty acid esters placed on the column was in the neighborhood of $30 \mu\text{g}$.

In the rat fed cod liver oil, linoleic acid appeared to have been added to the chyle chylomicrons from non-dietary sources. In the coconut oil-fed rat both oleic

and linoleic appear to have been added. It is possible that bile, pancreatic juice, and digested epithelial cells are the source of these acids.

The only significant change in the composition of chylomicrons occurring after they have entered the blood stream appears in the case of coconut oil-fed rats, in which lauric (12:0) and myristic (14:0) acids appear to have been selectively removed, with the result that the long-chain acids appear relatively more abundant. No explanation is offered for this observation.

The fact that the fatty acid composition of serum chylomicrons closely resembles that of the chyle chylomicrons indicates that very little, if any, exchange or recycling occurs between chylomicron fatty acids and tissue fatty acids. At least this appears true for the longer-chained acids, a fact which tends to validate recent work from this laboratory on the metabolism of chylomicrons (7, 8). Such is not true for the free fatty acids of the blood, which, as shown by Dole *et al.* (1), can be only slightly influenced in composition by short-term changes in the diet, and which presumably rapidly reach chemical equilibrium with adipose tissue (9).

REFERENCES

1. Dole, V. P., A. T. James, J. P. W. Webb, M. A. Rizaack, and M. F. Sturman. *J. Clin. Invest.* **38**: 1544, 1959.
2. Bragdon, J. H. *J. Lab. Clin. Med.* **52**: 564, 1958.
3. Stoffel, W., F. Chu and E. H. Ahrens, Jr. *Anal. Chem.* **31**: 307, 1959.
4. Horning, E. C., E. A. Moscatelli and C. C. Sweeley. *Chem. & Ind. London*, 751 (1959).
5. Lovelock, J. E. *J. Chromatog.* **1**: 35, 1958.
6. Bloom, B., I. L. Chaikoff and W. O. Reinhardt. *Am. J. Physiol.* **166**: 4451, 1951.
7. Bragdon, J. H., and R. S. Gordon, Jr. *J. Clin. Invest.* **37**: 574, 1958.
8. Fredrickson, D. S., D. L. McColester and K. Ono. *J. Clin. Invest.* **37**: 1333, 1958.
9. Fredrickson, D. S., and R. S. Gordon, Jr. *Physiol. Revs.* **38**: 585, 1958.