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Effect of ingested fat on fatty acid composition of serum lipoproteins

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SUMMARY

The fatty acid composition of several serum lipoprotein fractions of a human subject was studied before and after the ingestion of a fat meal composed of corn oil. It was found that the fatty acids of the chylomicrons, low density lipoproteins ($d < 1.019$), and of the $d > 1.21$ fraction tend to resemble the fatty acids of the recently ingested fat. It was established that this resemblance was not the result of exchange *in vitro* from chylomicrons to other fractions. Similar changes in composition of the other lipoprotein fractions were also observed, but these were small in magnitude and not outside the range of experimental error.

This investigation was undertaken to determine the effect of recently ingested fat on the fatty acid composition of several serum lipoprotein fractions. It had been previously demonstrated that following the ingestion of different fats, the fatty acid composition of chylomicrons of both rat and man resembles the composition of the fat fed (1). This finding has been confirmed in human chyle (2), in which it also appeared that the fatty acid composition of other lipoproteins underwent similar, but less definite, changes. A second purpose of the present study was to determine what part exchange of fatty acids between chylomicrons and various lipoproteins *in vitro* might play in these observations.

METHODS

The subject was a healthy 46-year-old male technician. No attempt was made to control his usual diet, except that he was requested to eat a relatively low fat meal the evening before the experiment. A blood sample was taken, in the fasting state, at about 9 A.M. He then drank 100 g of corn oil emulsified in skim milk. He was bled again 6 hours later. The blood was permitted to clot and the serum was removed. The fasting serum sample was clear; the second sample was very lipemic. The same experiment was repeated on the same subject 15 days later.

Corn oil was selected for these experiments because of its relatively high concentration of linoleic acid compared to that of most serum lipids. Since oleic acid was found in substantial concentration in each of the lipoproteins, an increase in the linoleic to oleic ratio of these two acids, rather than the total fatty acid composition, is presented.

The chylomicrons were separated from the serum by layering 7 ml of the latter under a phosphate-citrate buffer (pH 7.2, 0.05 M, density 1.006) in lusteroid tubes with a total volume of about 13.5 ml. Four tubes of each serum sample were so prepared. They were centrifuged for 30 minutes at 20,000 rpm at room temperature in the 40 rotor of the Spinco preparative ultracentrifuge. The chylomicrons were thereby concentrated in the top few milliliters of the tube, from which they were removed after slicing the tube. The chylomicrons were washed by centrifuging three times through the buffer under conditions similar to the original isolation, except that no attempt was made to layer them. In the case of the fasting sample, the chylomicrons from the original four tubes were progressively pooled, so that the final wash was accomplished in one tube only.

In order to diminish the possibility that some chylomicrons might remain in the serum, the infranatant material from the first centrifugation was again layered under buffer, centrifuged under the same conditions, and the tops (slightly lactescent in the case of the second sample) were discarded.

The serum, thus twice cleared of chylomicrons, was then fractionated by repeated centrifugations at increasing densities by the method of Havel *et al.* (3). The fractions were extracted in chloroform-methanol and the extracts washed with slightly acid water. Suitable portions of the chloroform phase were evaporated, and methyl esters of the fatty acids were prepared by incubation of the dried lipid with 1-ml aliquots of 98% methanol and 2% sulfuric acid for 16 hours at 65° in sealed test tubes. Water (5 ml) was then added to each tube and the lipids were extracted into petroleum ether. The petroleum ether extract was carefully blown to dryness with a stream of nitrogen; the lipids were taken up in isooctane; and aliquots of these solutions were analyzed by gas-liquid chromatography. The columns used were 6-foot glass columns filled with Chromosorb W coated with a quantity of ethylene glycol adipate polyester approximately 10% of the weight of the Chromosorb. The columns were run at 200° and the gas flow was 100 ml/minute. A modified argon ionization detector (4), which had been calibrated for quantitative accuracy within a specific range of vapor concentration, was used, and the size of the aliquots analyzed was experimentally adjusted so that no component of the mixture gave a vapor concentration during chromatography which exceeded that range. Quantification was accomplished by measuring the areas under the curves.

RESULTS

The results are presented in Table 1, in which the ratio between the concentrations of linoleic and oleic acids are given for the several fractions. It is apparent from the fasting samples that this ratio increased in all fractions during the interval between the two experiments. Six hours following the ingestion of the corn oil, in both experiments, there was an increase in this ratio in all fractions. The increase was greatest in the chylomicrons. The ratio approximately doubled in the fraction of density <1.019, and in the fraction of density >1.21. The changes in the other fractions were smaller.

TABLE 1. RATIO OF LINOLEIC TO OLEIC ACIDS IN SEVERAL SERUM LIPOPROTEIN FRACTIONS BEFORE AND AFTER FEEDING CORN OIL*

	Experiment 1		Experiment 2	
	Before	After	Before	After
Chylomicrons	0.37	1.81	0.48	1.82
1.019 top	0.44	0.95	0.55	1.04
1.063 top	1.08	1.32	1.33	1.44
1.21 top	1.01	1.13	1.24	1.59
1.21 bottom	0.49	1.41	0.77	1.33

* This ratio in the corn oil fatty acids was 2.28.

To assess the reliability of the methods, and to ascertain if an appreciable exchange of fatty acids between chylomicrons and other lipoproteins occurred, the fasting serum in the first experiment, after twice centrifuging off the chylomicrons, was treated in the following way: Five milliliters of serum was placed in each of three test tubes; 1.5 ml of buffer was added to the first; and 1.5 ml of washed chylomicrons from the postprandial sample was added to each of the other two tubes. In tube 2 the chylomicrons were added at the end of the incubation, and in tube 3 they were added at the beginning of the incubation. Incubation took place at room temperature for 2 hours. Lipid (8.2 mg) was added as chylomicrons to 5 ml of serum containing 22.5 mg of lipid. It should be realized that the lipid content of human serum chylomicrons is about 90% triglyceride, whereas in fasting serum the triglycerides constitute only about 15% of the total lipid.

The contents of these three tubes were then fractionated and analyzed exactly as described above. The results are shown in Table 2. The greatest differences occur between tubes 1 and 2, i.e., between the control without added chylomicrons and the zero-time control. In most fractions the zero-time sample actually shows a lower linoleic to oleic ratio than the control. We believe that these differences demonstrate the range of experimental error. A comparison of tube 3 with either tube 1 or tube 2 demonstrates that no significant exchange of fatty acids from chylomicrons to other lipoproteins occurs *in vitro*.

TABLE 2. RATIO OF LINOLEIC TO OLEIC ACIDS IN SEVERAL SERUM LIPOPROTEIN FRACTIONS AFTER INCUBATING FASTING SERUM WITH CORN-OIL CHYLOMICRONS

	Serum and Buffer	Serum and Chylomicrons	
	Tube 1	Tube 2	Tube 3
	2 hours	*zero time*	*2 hours*
Chylomicrons		1.75	1.79
1.019 top	0.44	0.39	0.47
1.063 top	1.08	0.78	1.16
1.21 top	1.01	0.82	0.96
1.21 bottom	0.49	0.57	0.60

The approximate doubling of the linoleic to oleic ratio in the 1.019 top and 1.21 bottom fractions, as seen in Table 1, appears to be greater than one might expect from experimental error.

DISCUSSION

We have no explanation for the increased linoleic to oleic ratio in the fractions of fasting serum in the interval between experiments. One might conclude that such observations without rigid prior control of diet have little significance.

The fatty acids in the 1.21 bottom fraction represent primarily the free fatty acids (FFA) of the serum, although from the data of Phillips (5), it would appear that about 300 μEq/liter of fatty acids in this fraction are present as phosphatides, primarily lysolecithin. It was reported by Dole (6) that the chemical composition of the FFA of the serum could not be significantly altered by short-term changes in the type of fat fed. We accepted these data and in a previous paper (1) interpreted them as indicating that the FFA were in such rapid exchange with the depot fats that chemical equilibrium with the latter would be a constant feature. It has been shown by several groups of workers (7) that the half life of intravenously injected radioactively labeled FFA is a matter of only 1 or 2 minutes. It has also been shown that the feeding of a fatty meal results in only slight increases in levels of FFA. But the data presented here, although limited in extent, indicate that the composition of the FFA of the serum can presumably be influenced by the composition of fat recently ingested.

The doubling in the ratio linoleic to oleic in the 1.019 top fraction after feeding corn oil (Table 1) could be explained in several ways. Some fat may be absorbed from the intestine in the form of these very low density lipoproteins. Chylomicrons certainly vary greatly in size, and presumably include a spectrum just as do the low density lipoproteins. The problems of defining, and indeed of separating, these spectra have not yet been resolved. It can be pointed out in this regard, however, that the 1.019 top fractions in these two experiments showed only minimal lactescence. In experiment 1 the amount of total lipid in these fractions was determined chemically. The postprandial sample contained less than a 10% increment over the fasting sample.

Another possible explanation is that the chylomicrons are degraded *in vivo*, by the enzymatic removal of triglyceride, into low density lipoproteins. This opinion has been expressed by Lindgren *et al.* (8). We tend to favor the view that these very low density lipoproteins have a relatively short half life (a matter of hours), and that their fatty acid composition reflects that of the fat recently transported to the liver, where they presumably arise (9).

The increase in linoleic to oleic ratio in the other lipoprotein fractions (1.063 top and 1.21 top) after feeding corn oil, although not significant, is consistent. It should be recalled, in this respect, that only a small percentage of their constituent lipid is triglyceride. The increased ratio may represent binding of FFA (10).

REFERENCES

1. Bragdon, J. H., and A. Karmen. *J. Lipid Research* **1:** 167, 1960.
2. Kayden, H. J., A. Karmen, A. E. Dumont, and J. H. Bragdon. *J. Clin. Invest.* **39:** 1001, 1960.
3. Havel, R. J., H. A. Eder and J. H. Bragdon. *J. Clin. Invest.* **34:** 1345, 1955.
4. Lovelock, J. E. *J. Chromatog.* **1:** 34, 1958.
5. Phillips, G. B. *Proc. Soc. Exptl. Biol. Med.* **100:** 19, 1959.
6. Dole, V. P., A. T. James, J. P. W. Webb, M. A. Rizack, and M. F. Sturman. *J. Clin. Invest.* **38:** 1544, 1959.
7. Fredrickson, D. S., and R. S. Gordon, Jr. *Physiol. Revs.* **38:** 585, 1958.
8. F. T. Lindgren, N. K. Freeman, A. V. Nichols, and J. W. Gofman. In *The Blood Lipids and the Clearing Factor*, 3rd International Conference on Biochemical Problems of Lipids, Brussels, 1956, p. 224.
9. Radding, C. M., and D. Steinberg. *J. Clin. Invest.* **39:** 1560, 1960.
10. Shafrir, E. *J. Clin. Invest.* **37:** 1775, 1958.