EARLY EFFECTS OF FASTING AND OF CARBOHYDRATE INGESTION ON LIPIDS AND LIPOPROTEINS OF SERUM IN MAN

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It is generally assumed that plasma lipoproteins function as carriers in the transport of fatty acids to and from the liver, fat depots, and peripheral tissues, but the roles of the various lipoprotein classes in this regard are not established. A previous study showed that the concentration of triglycerides in serum was lower several hours after the ingestion of a meal containing no fat than after an overnight fast (1). In the present study large quantities of glucose were fed to fasting young adults; the resulting alterations in various lipid constituents of ultracentrifugally separated lipoprotein classes were compared with changes observed during comparable periods of continued fasting. These comparisons showed that the concentrations of all major lipid constituents of the very low density lipoproteins of serum fall significantly after the ingestion of glucose. This finding suggests that very low density lipoproteins have a significant role in the transport of fatty acids derived from endogenous sources.

METHODS

Experimental subjects and procedure

Seven adults who had no known systemic disease were studied during 2 periods, each preceded by a 12 to 15-hour fast (water allowed *ad libitum*).

Period I. The subjects continued to fast for an additional 11 hours, during which serial blood samples were withdrawn.

Period II. The same subjects fasted for 3 additional hours, after which they were fed 100 grams of glucose as lemonade at hourly intervals for 4 hours. Two or three hours thereafter they were allowed to eat a fat-free supper. Serial blood samples were withdrawn at intervals during the test and again the next morning before breakfast (*cf.* Figure 1). In three of the subjects period I preceded period II. In the other four subjects the periods were reversed.

Analytical methods

These have been described in the preceding paper (1). All serum samples were extracted in chloroform-methanol, 2:1, v/v. Serum total protein concentrations were determined on all samples by the biuret method (2), and lipoprotein lipid and protein concentrations were corrected to a constant total protein concentration to minimize changes resulting from fluctuations in the concentration of plasma proteins.

RESULTS

Changes in total lipid concentration in Fraction I + II lipoproteins ($S_f > 10$) in the seven subjects are shown in Figure 1. Mean values are given in Table I. As shown in Table I, the concentration of total lipids fell slightly during the first 3 hours of the test. With continued fasting, the changes were variable, but resulted in no significant change in mean values. When glucose was administered, providing 1600 calories over a period of 4 hours, total lipid concentration fell consistently, irrespective of the initial concentration, and was lowest 8 hours after glucose was first administered. The following morning, after another 12 to 15 hours of fasting, the levels had risen consistently in the five subjects tested.

In four of the subjects (Table II) detailed lipoprotein analyses were carried out during both periods. In the fasting state there were no consistent changes in any fraction, except for a slight increase in Fraction IV (high density lipoprotein) phospholipids. After ingestion of glucose, all lipid constituents in Fraction I + II were reduced. In general, the decrease was most marked in triglycerides, slightly less in phospholipids, and least in total cholesterol. In Fraction III (S_f 0-10 lipoproteins) there was a slight fall in the mean values for all constituents. In every instance Fraction IV phospholipid concentration fell 5 and 8 hours after ingestion of glucose; no comparable changes occurred in cholesterol or protein concentration.

DISCUSSION

Rubin and Aladjem (3), using the analytical centrifuge, studied lipoprotein concentrations in six healthy subjects during 96 hours of fasting.

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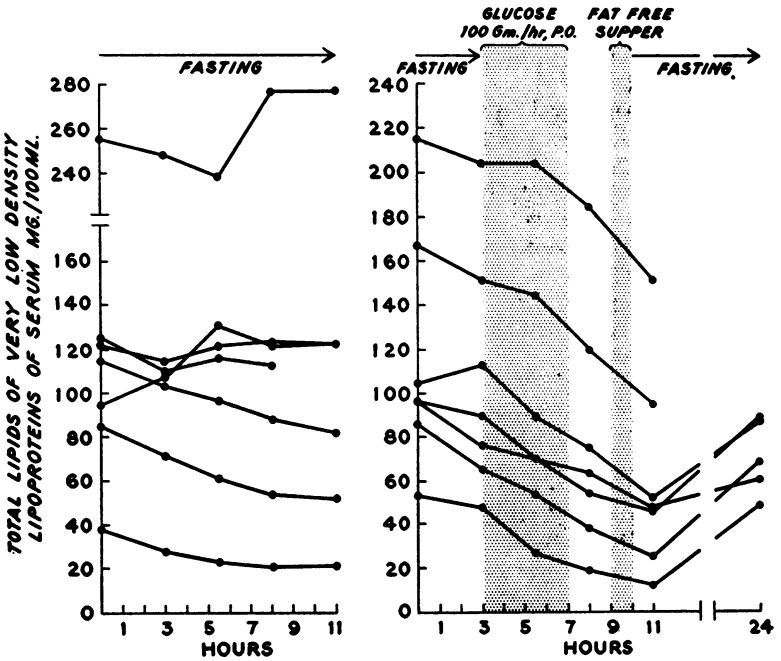

FIG. 1. ALTERATIONS IN VERY LOW DENSITY (FRACTION I + II) LIPOPROTEIN TOTAL LIPID CONCENTRATIONS IN HEALTHY ADULTS DURING FASTING AND AFTER INGESTION OF GLUCOSE

All tests begun after preliminary 12 to 15-hour fast.

In accord with the present data, their results showed that the concentration of S_f 12-400 (Fraction I + II) lipoproteins was variable during the first 24 hours of fasting and that the concentrations of S_f 0-12 (Fraction III) and high density (Fraction IV) lipoproteins did not change significantly. After 96 hours the concentrations of S_f 12-400 and 0-12 lipoproteins had increased significantly,

TABLE I

MEAN VALUES FOR TOTAL LIPID IN FRACTION I + II LIPOPROTEINS AFTER FASTING AND GLUCOSE INGESTION IN SEVEN HEALTHY ADULTS

Hour of Test	0	3	5-1/2	8	11
Fasting:					
Mean	119	112	113	114	113
Difference from 3-hour value			+1	+2	+1
Standard error*			5	6	8
P			0.9 > P > 0.8	0.8 > P > 0.7	0.8 > P > 0.7
Glucose:					
Mean	117	107	93	79	61
Difference from 3-hour value			-14	-28	-46
Standard error*			4	2	4
P			0.02 > P > 0.01	< 0.01	< 0.01

* $\left(\frac{\Sigma(d-\bar{d})^2}{n(n-1)}\right)^{1/2}$

TABLE II

CONCENTRATIONS OF SERUM LIPOPROTEIN CONSTITUENTS AFTER FASTING AND GLUCOSE INGESTION IN HEALTHY ADULTS

Hour of Test		Fasting					Glucose					
		0	3	5-1/2	8	11	0	3	5-1/2	8	11	24
Fraction I + II												
Total Cholesterol	RH	16	18	20	21	22	17	15	12	11	8	11
	LN	27	23	28	25	--	23	22	21	18	13	19
	CD	66	66	61	69	73	52	50	50	46	45	--
	MG	18	16	14	14	12	17	14	13	10	8	16
	Mean:	32	31	31	32	--	27	25	24	21	19	--
Phospholipids	RH	22	25	29	25	30	21	20	16	13	10	13
	LN	30	28	32	28	--	26	24	22	18	13	22
	CD	54	53	56	62	62	49	46	44	38	32	--
	MG	26	23	21	20	17	23	19	16	13	9	22
	Mean:	33	32	35	34	--	30	27	25	21	16	--
Glycerides	RH	53	58	69	62	57	52	48	37	34	26	31
	LN	54	51	61	49	--	46	44	34	22	17	37
	CD	89	89	101	126	117	97	91	88	71	45	--
	MG	64	57	51	46	41	50	43	33	26	22	50
	Mean:	65	64	71	71	--	61	57	48	38	28	--
Fraction III												
Total Cholesterol	RH	109	111	111	106	109	135	133	133	131	129	128
	LN	90	92	97	100	---	98	93	92	95	93	94
	CD	117	112	108	108	109	116	117	117	115	112	--
	MG	86	87	95	96	89	92	90	94	89	88	91
	Mean:	100	100	103	100	--	110	108	109	108	106	--
Phospholipids	RH	75	80	78	74	76	92	92	92	87	90	93
	LN	68	71	75	76	--	72	66	67	66	66	70
	CD	84	81	78	82	79	80	79	77	76	76	--
	MG	64	64	66	69	68	64	63	64	62	60	63
	Mean:	73	74	74	75	--	77	75	75	73	73	--
Protein	RH	63	63	63	57	60	75	73	73	71	69	72
	LN	58	58	60	60	--	52	54	48	52	51	53
	CD	78	75	68	72	69	73	71	69	70	71	--
	MG	54	53	56	55	57	53	53	53	52	51	54
	Mean:	63	62	62	61	--	63	63	61	61	61	--
Fraction IV												
Total Cholesterol	RH	41	38	37	39	39	41	38	39	41	40	37
	LN	36	36	37	37	--	40	40	40	43	41	43
	CD	42	41	40	38	38	40	37	36	36	37	--
	MG	44	44	45	44	45	48	47	47	47	45	46
	Mean:	41	40	40	40	--	42	41	41	42	41	42
Phospholipids	RH	71	70	71	76	79	76	70	68	67	65	67
	LN	66	69	75	76	--	75	75	74	72	70	81
	CD	82	80	81	81	81	74	72	67	67	63	--
	MG	81	83	85	86	86	80	79	79	74	71	84
	Mean:	75	76	78	80	82	76	74	72	70	67	77
Protein	RH	156	151	158	162	170	176	---	---	176	172	172
	LN	174	171	172	170	---	164	169	164	163	170	177
	CD	164	163	164	164	168	154	153	151	150	151	---
	MG	185	203	203	201	191	188	184	190	188	181	197
	Mean:	170	172	174	174	---	173	---	---	168	169	---

whereas the concentration of the high density lipoproteins had remained unchanged. They then administered 1 gram of sucrose per kgm. of body weight; no significant changes occurred in the next 3 hours, but normal values were found 24 hours later after the subjects had resumed their usual diet. The failure of Rubin and Aladjem to observe a prompt decrease in the concentration of S_f 12-400 lipoproteins after ingestion of sucrose may have resulted from the relatively small quan-

tities of carbohydrate they administered. In the present study more than enough glucose was supplied over a period of 4 hours to satisfy the subjects' caloric requirements, and it is likely that they were utilizing carbohydrate as the major source of energy for at least 6 hours. The next morning they undoubtedly were deriving considerable energy from fat combustion, and the concentration of Fraction I + II lipoproteins rose significantly, although no fat had been eaten in the interim.

The results presented demonstrate the considerable lability of very low density lipoproteins independent of chylomicron transport, and suggest that their concentration, like that of ketone bodies and unesterified fatty acids (4, 5), may depend on the availability of carbohydrate for energy production. The marked increase in the concentration of this lipoprotein fraction in diabetic acidosis (6) and its rapid fall with treatment, along with the decrease in ketone bodies and unesterified fatty acids (7), support this concept.

Others have noted the lability of the very low density lipoproteins. Chandler, Lawry, Potee, and Mann (8) found that the diurnal variability in the concentration of S_f 20-100 but not of S_f 12-20 lipoproteins exceeded any variation that could be attributed to technical error, but observed no diurnal trends. This is not surprising, for the opposing effects of fat and carbohydrate ingestion would tend to be neutralized after a mixed meal. On the other hand, Boyd (9), in a study of subjects on mixed diets supplying 2000 to 2500 calories per day, observed a diurnal variation in the concentration of neutral fat which reached a nadir at 5 P.M. In this case, the effect of carbohydrate on very low density lipoproteins probably outweighed the effect of fat. Critical data concerning the importance of very low density lipoproteins in the transport of fatty acids derived from endogenous sources remain to be obtained. Available data regarding fatty acid transport pertain only to whole serum. Harper, Neal, and Hlavacek (10) observed in dogs that the turnover of the nonphospholipid fatty acid fraction of plasma (triglycerides, cholesterol esters, and unesterified fatty acids) was considerably more rapid than that of the phospholipid fatty acid fraction. These observations have been confirmed in man by Lipsky, McGuire, Bondy, and Man (11). Recent studies indicate that unesterified fatty acids may account for a significant portion of fatty acid transport during fasting (5) and during removal of chylomicra from the plasma (12). In chylomicra, triglycerides are the chief vehicle for fatty acid transport (13), but their role in the transport of endogenous fatty acids remains to be clarified. Since the lipid of Fraction I + II lipoproteins consists mainly of triglycerides, it appears likely that the lability of this fraction is related to their turnover.

The prolonged effects of fasting and of ingesting fat-free foods must be differentiated from those occurring during the first 24 hours. Kartin, Man, Winkler, and Peters (14), in a study of the effects of prolonged fasting on serum lipid concentrations in human subjects, found that free and esterified cholesterol and phospholipid concentrations rose consistently and that neutral fats showed variable changes. In their series the effects of fasting were complicated by concomitant water deprivation, and the extent of hemoconcentration in their subjects is not known. In contrast to Rubin and Aladjem's report that very low density lipoproteins were increased after 72 hours of fasting (3), this study did not imply an elevation of this fraction during more prolonged fasting. The changes in serum lipid concentrations (as well as ketosis) observed by Kartin and his colleagues could be prevented by the ingestion of 100 grams of carbohydrate daily.

Prolonged ingestion of fat-free diets results in rapid fall of cholesterol and phospholipid concentrations within one week (15–17), even in the absence of weight loss, but may not appreciably change fasting serum triglyceride concentrations. It is apparent that lipoproteins other than those of very low density must be involved in these changes. On the other hand, when an excess of calories in the form of carbohydrates and proteins is supplied over a period of several days, S_f 12-400 lipoprotein concentrations rise strikingly (18). This suggests that transport of newly synthesized fatty acids to depots may take place via very low density lipoproteins.

The physiologic roles of Fraction III and IV lipoproteins are not clarified by the present study. A close relation between Fraction I + II and III is suggested by the fact that a) their protein moieties have the same N terminal amino acid residue (19), and b) the concentrations of these fractions tend to vary inversely in certain pathologic states, such as idiopathic hyperlipemia (20) and the nephrotic syndrome (21). The protein moiety of

Fraction IV has a different N terminal amino acid residue (19) and therefore cannot be a precursor or product of the other fractions. The fall in phospholipid concentration in Fraction IV after ingestion of glucose and the slight rise observed during fasting suggest a possible role for this fraction in fatty acid transport as well.

SUMMARY

1. The concentrations of lipid and protein constituents of ultracentrifugally separated serum lipoprotein fractions were studied in seven healthy adults during short periods of fasting and after ingestion of a large quantity of glucose. To minimize the effects of chylomicronemia, a 12 to 15-hour interval of fasting preceded each period.

2. The total lipid concentration in very low density ($S_f > 10$) lipoproteins was variable during fasting, but fell consistently after ingestion of 400 grams of glucose over a 4-hour period. The levels 5 and 8 hours after starting glucose feeding were significantly lower than those after comparable periods of fasting. These changes resulted from reductions in all lipid constituents of this lipoprotein fraction, but the mean per cent fall was greatest in triglycerides, less in phospholipids, and least in cholesterol.

3. No significant changes occurred in the concentrations of lipid constituents or protein in the major low density (S_f 0-10) lipoproteins during either of the periods of study.

4. The concentrations of cholesterol and protein in high density lipoproteins did not change during the periods of study, but the concentration of phospholipids rose slightly during continued fasting and fell during the period following ingestion of glucose.

5. The possible implications of these findings in relation to lipid transport in the blood are discussed.

REFERENCES

1. Havel, R. J., Early effects of fat ingestion on lipids and lipoproteins of serum in man. J. Clin. Invest., 1957, **36**, 848.
2. Gornall, A. G., Bardawill, C. J., and David, M. M., Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 1949, **177**, 751.
3. Rubin, L., and Aladjem, F., Serum lipoprotein changes during fasting in man. Am. J. Physiol., 1954, **178**, 263.
4. Dole, V. P., A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest., 1956, **35**, 150.
5. Gordon, R. S., Jr., and Cherkes, A., Unesterified fatty acid in human blood plasma. J. Clin. Invest., 1956, **35**, 206.
6. Tuller, E. F., Mann, G. V., Schertenleib, F., Roehrig, C. B., and Root, H. F., The effects of diabetic acidosis and coma upon the serum lipoproteins and cholesterol. Diabetes, 1954, **3**, 279.
7. Laurell, S., Plasma free fatty acids in diabetic acidosis and starvation. Scandinav. J. Clin. & Lab. Invest., 1956, **8**, 81.
8. Chandler, H. L., Lawry, E. Y., Potee, K. G., and Mann, G. V., Spontaneous and induced variations in serum lipoproteins. Circulation, 1953, **8**, 723.
9. Boyd, E. M., Diurnal variations in plasma lipids. J. Biol. Chem., 1935, **110**, 61.
10. Harper, P. V., Jr., Neal, W. B., Jr., and Hlavacek, G. R., Lipid synthesis and transport in the dog. Metabolism, 1953, **2**, 69.
11. Lipsky, S. R., McGuire, J. S., Jr., Bondy, P. K., and Man, E. B., The rates of synthesis and the transport of plasma fatty acid fractions in man. J. Clin. Invest., 1955, **34**, 1760.
12. Havel, R. J., and Fredrickson, D. S., The metabolism of chylomicra. I. The removal of palmitic acid-1-C^{14} labeled chylomicra from dog plasma. J. Clin. Invest., 1956, **35**, 1025.
13. Bloom, B., Chaikoff, I. L., Reinhardt, W. O., and Dauben, W. G., Participation of phospholipides in lymphatic transport of absorbed fatty acids. J. Biol. Chem., 1951, **189**, 261.
14. Kartin, B. L., Man, E. B., Winkler, A. W., and Peters, J. P., Blood ketones and serum lipids in starvation and water deprivation. J. Clin. Invest., 1944, **23**, 824.
15. Starke, H., Effect of the rice diet on the serum cholesterol fractions of 154 patients with hypertensive vascular disease. Am. J. Med., 1950, **9**, 494.
16. Watkin, D. M., Froeb, H. F., Hatch, F. T., and Gutman, A. B., Effects of diet in essential hypertension. II. Results with unmodified Kempner rice diet in fifty hospitalized patients. Am. J. Med., 1950, **9**, 441.
17. Mellinkoff, S. M., Machella, T. E., and Reinhold, J. G., The effect of a fat-free diet in causing low serum cholesterol. Am. J. M. Sc., 1950, **220**, 203.
18. Walker, W. J., Lawry, E. Y., Love, D. E., Mann, G. V., Levine, S. A., and Stare, F. J., Effect of weight reduction and caloric balance on serum lipoprotein and cholesterol levels. Am. J. Med., 1953, **14**, 654.
19. Brown, R. K., Clark, B., Davis, R., and van Vunakis, H., Chemical studies of the protein portion of human lipoproteins. Biochemical Problems of Lipids. Proc. Third International Conference, Butterworths Scientific Publications, London. In press.
20. Havel, R. J., and Gordon, R. S., Jr., An enzymatic defect associated with altered lipoprotein metabolism in an individual with idiopathic hyperlipemia. Circulation, 1955, **12**, 485.
21. Havel, R. J., Unpublished data.