Effects of a single oral load of medium-chain triglyceride on serum lipid and insulin levels in man

I. TAMIR, D. B. GRANT, AUDREY S. FOSBROOKE, M. M. SEGALL, and JUNE K. LLOYD

Institute of Child Health, University of London, London, England

ABSTRACT Analysis of serum free fatty acids by gas–liquid chromatography showed high proportions (27–57%) of octanoic acid for up to 4 hr after the ingestion of a single oral load of medium-chain triglyceride (approximately 1 g/kg body weight) in four volunteers.

The effects of a medium-chain triglyceride load on the concentrations of plasma free long-chain fatty acids, plasma glucose, serum insulin, and serum triglyceride were observed and compared with the effects of a glucose load. A rapid fall in the free long-chain fatty acids followed both loads but only a small rise in serum insulin was observed after medium-chain triglyceride. The fall in free long-chain fatty acids following ingestion of medium-chain triglyceride cannot therefore be caused mainly by the release of insulin and may be due to a direct action on adipose tissue.

No medium-chain fatty acids were detected in the serum triglyceride after ingestion of medium-chain triglyceride, but there was a small but significant increase in the percentage of hexadecenoic acid in this fraction.

KEY WORDS medium-chain triglyceride · octanoic acid · free fatty acids · insulin · glucose · hexadecenoic acid · man

THE INTRADUODENAL INFUSION of octanoic acid (8:0) together with glucose in man has been shown by Linscheer, Slone, and Chalmers (1) to produce higher serum insulin levels than occur after glucose alone, although the rise in serum glucose was not affected by the addition of octanoic acid. The reduction of serum FFA in this experiment did not appear to be affected by the inclusion of octanoic acid, but Jenkins (2) has pointed out that when the levels of endogenous FFA are calculated the concentration of these fatty acids (LCFA) does fall to a greater extent after the infusion of glucose plus octanoic acid than after glucose alone. The intravenous administration of sodium octanoate has been shown to result in a significant reduction in glucose concentration (3, 4).

These effects of octanoic acid on serum FFA and glucose concentrations have been attributed mainly to the stimulation of insulin secretion caused by the rise in serum ketone bodies, which results from the hepatic metabolism of medium-chain fatty acids (MCFA) (1, 2, 4, 5). Octanoic acid has also been shown to directly stimulate insulin output from the isolated rat pancreas (6). As trioctanoin is absorbed into the portal blood as octanoic acid (7), the effects of giving the triglyceride orally should be comparable with those of giving the free acid. Unlike the latter, trioctanoin can be tolerated after oral administration and may be given in the form of medium-chain triglycerides (MCT) without the addition of glucose. The effects of octanoic acid absorption can therefore be studied independently of glucose, with which it can then be compared.

We report here studies of the effect of a single oral load of MCT on the concentrations of plasma LCFA, plasma glucose, serum insulin, and serum triglyceride in man, and also on the fatty acid composition of the serum FFA and triglyceride. In each subject we have compared the results with those produced by an oral load of glucose and, in addition, in some cases, with the effect of an oral load of glycerol.

Abbreviations: GLC, gas–liquid chromatography; FFA, free fatty acids; LCFA, free long-chain fatty acids (carbon chain length 12 and greater); MCFA, free medium-chain fatty acids (carbon chain length 8 and 10): MCT, medium-chain triglyceride. Fatty acids are designated by chain length:number of double bonds.

MATERIALS AND METHODS

The investigation was carried out on four volunteers: three healthy males aged 30–35 yr and a 12 yr old boy (J.D.) with carbohydrate-induced hypertriglyceridemia, who at the time of the study had been successfully treated with a restricted carbohydrate diet (25% calories from carbohydrate) for 6 months and had a normal oral glucose tolerance test, serum triglyceride level, and lipoprotein pattern.

The subjects fasted overnight, and were recumbent for 30 min before and throughout the test. MCT (fatty acid composition 8:0, 71%; 10:0, 25%; 12:0, 3%), approximately 1 g/kg body weight, was taken as a 50% w/v emulsion in water flavored with black coffee. Glucose, 100 g, was taken as a 25% solution in water. In the three adults glycerol in quantities equivalent to the amount present in the MCT load was given as a 10% w/v solution in water.

Venous blood was taken before and 15, 30, 45, 60, 90, 120, and 150 min after the oral load.

Heparinized blood for plasma glucose and FFA estimations was cooled immediately in ice and the plasma was separated within 30 min.

Analytical Methods

Plasma glucose was estimated by a glucose oxidase method (8); plasma FFA, by the titration method of Dole and Meinertz (9); serum insulin, by a modification (10) of the immunoassay procedure of Morgan and Lazarow (11); serum triglyceride, by infrared absorptiometry (12); serum cholesterol by a Liebermann–Burchard method (13).

The fatty acid composition of serum FFA and triglyceride was determined by the following procedure. Serum lipids were extracted overnight with ethanol–ether 3:1 at room temperature and separated by thin-layer chromatography on Rhodamine-impregnated Silica Gel G (Merck) in hexane–diethyl ether–acetic acid 80:20:1. The FFA and triglyceride spots were identified under ultraviolet light and scraped directly into methanolysis tubes. Equivalent areas of gel from tracks on which no lipid material had been loaded were taken for use as blanks. 1 ml of freshly prepared methylating reagent (concentrated sulphuric acid 2%, benzene 0.2%, dry methanol to 100 ml) was added and the closed tubes were heated at 60°C for 16 hr. The methyl esters were extracted by adding 0.5 ml of hexane and 0.5 ml of water and mixing vigorously for 1 min. The hexane phase was used for GLC *without* prior concentration to avoid loss of the volatile methyl esters of octanoic and decanoic acids. GLC was carried out on a 5 ft polyethylene glycol adipate column equipped with a flame ionization detector (Pye model 104). Two isothermal runs were made for each sample; at 90°C for separation of the C_8–C_{14} fatty acids, and at 170°C for separation of the C_{14}–C_{20} fatty acids. The peak area was obtained by multiplying the peak height by the width at half the height. The blank areas of gel gave no peaks on GLC. Quantitative results with fatty acid standards (KD, Applied Science Laboratories, and 189-3, Sigma Chemical Co.) agreed with the stated composition data with a relative error less than 4% for the major components (>10% of total mixture) and less than 9% for the minor components (<10% of total mixture).

RESULTS

Free Fatty Acids

Table 1 shows the fatty acid composition of serum FFA following ingestion of MCT in three subjects. Octanoic acid was not detected in the fasting state, but appeared in each subject by 30 min and persisted for the duration of the test (150 min), reaching concentrations of 27–57% of the total fatty acids. In two individuals in whom estimations were continued to 240 min, octanoic acid was still detected in serum FFA (39.3 and 6.5%). Decanoic

TABLE 1 FATTY ACID COMPOSITION OF SERUM FFA FOLLOWING ORAL MCT IN THREE SUBJECTS

Fatty Acid	Minutes after Ingestion																	
	0			30			60			90			120			150		
	IT	MS	EJ	IT	MS	EJ	IT	MS	EJ	IT	MS	EJ	IT	MS	EJ	IT	MS	EJ
	g/100 g of total fatty acids																	
8:0	0	0	0	49.5	7.6	11.5	52.5	22.1	27.6	57.4	24.2	36.3	32.7	16.9	36.3	32.0	27.3	35.7
10:0	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1.8	<1	<1	2.3	<1	<1	1.3
12:0	<1	<1	2.0	<1	<1	<1	<1	<1	1.6	<1	<1	<1	<1	<1	<1	<1	<1	<1
14:0	4.5	3.5	3.8	1.6	2.6	2.1	4.0	4.1	2.4	2.2	2.7	2.5	2.5	2.2	2.2	2.6	2.9	1.6
16:0	29.5	30.3	38.3	14.4	29.3	32.3	14.5	27.6	24.0	12.0	26.7	20.8	18.7	37.3	17.6	26.5	37.1	24.0
16:1	3.6	4.0	5.2	1.6	3.6	2.0	2.0	3.7	2.0	3.2	3.5	2.0	4.1	3.3	1.5	2.7	2.3	3.1
18:0	14.7	12.3	12.6	6.6	10.7	23.6	5.5	11.9	10.8	4.3	12.6	10.8	5.8	10.8	11.6	8.2	7.9	8.6
18:1	32.1	33.5	27.2	17.4	34.4	16.6	13.2	22.1	18.8	12.3	22.2	18.8	23.2	18.2	20.5	14.6	14.9	17.2
18:2	15.6	14.7	10.7	8.8	11.6	9.8	8.1	8.5	5.9	8.5	8.1	5.9	12.9	11.7	7.9	13.0	7.4	6.6

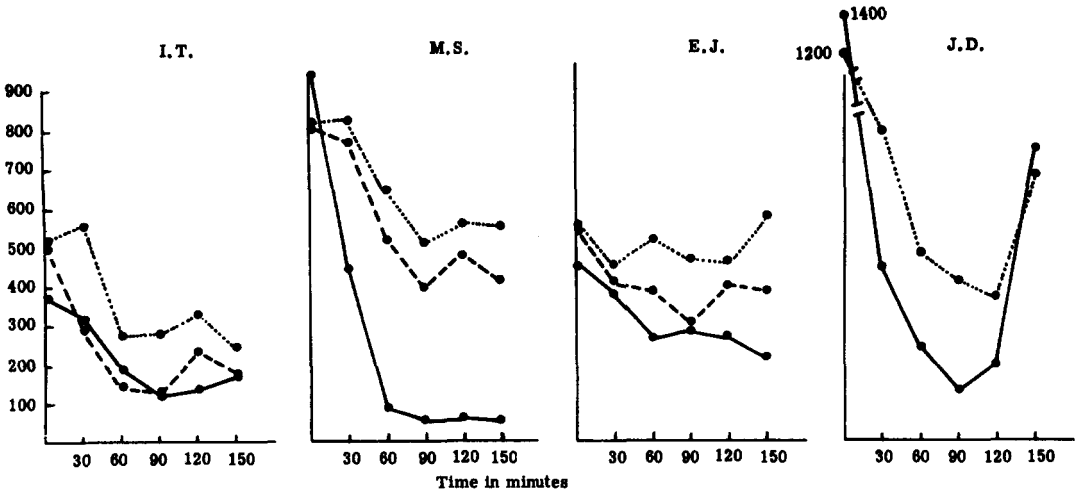

FIG. 1. Effect of MCT and glucose on plasma free fatty acid concentrations (shown on ordinate in μeq/liter). ●···●, FFA after MCT (estimated by titration). ●- - -●, LCFA after MCT (calculated after subtraction of octanoic acid). ●—●, FFA after glucose (estimated by titration. After glucose ingestion, all FFA is LCFA).

acid was usually present in concentrations of less than 1% and on no occasion exceeded 2.3%.

Estimations of FFA after ingestion of MCT will therefore include octanoic acid in addition to endogenous LCFA and allowance must be made for this in following the changes in LCFA after MCT ingestion. In the titration method for plasma FFA, however, octanoic acid is only partially extracted and we have obtained a recovery of 46.5% ± 4.3 (mean ± SD) of octanoic acid added to plasma over a concentration range of 200–4,000 μeq/liter. We have therefore calculated the concentrations of plasma LCFA after MCT ingestion from the following formula:

$$\text{LCFA} = (100 - a)/[(0.465 \times 2a) + (100 - a)] \times b,$$

where a = percentage of 8:0 as estimated by GLC and b = FFA in μeq/liter as estimated by titration. The derivation of the formula is as follows.

Given that the FFA titration estimates only 46.5% of the octanoic acid present in the plasma and that the LCFA is completely estimated, then for plasma in which octanoic acid is the principal (>97.7%) MCFA present (Table 1) the FFA titer may be considered to derive from the contributions of 8:0 (molecular weight 144) and of 12:0 to 18:2 (LCFA, average molecular weight 275, taking C_{16} fatty acids as $^1/_3$ and C_{18} fatty acids as $^2/_3$ of the total LCFA).

To obtain the molar ratio of 8:0 to LCFA from the GLC data it is necessary to multiply the weight of 8:0 by the ratio of the molecular weights (275/144), which for the purpose of this calculation we have taken to be 2. Let a = percentage by weight of 8:0 estimated by GLC; then the ratio 8:0/LCFA (by weight) = $a/(100 - a)$ and the *molar* ratio 8:0 /LCFA = $2a/(100 - a)$. However, since only 46.5% of 8:0 is recovered, the molar ratio obtained in the titration is $0.465 \times 2a/(100 - a)$. Total moles titrated (8:0 plus LCFA) = $(0.465 \times 2a) + (100 - a)$ and if b is the amount of FFA in μeq/liter as determined by titration, then LCFA in μeq/liter is given by the formula

$$(100 - a)/[(0.465 \times 2a) + (100 - a)] \times b$$

Fig. 1 shows the calculated levels of plasma LCFA in three subjects after MCT, compared with the levels of plasma LCFA after glucose. A fall occurred in each subject after ingestion of MCT as after glucose. In two subjects (I.T. and E.J.) the fall after MCT was of similar magnitude to that after glucose, although in the third (M.S.) the effect of glucose was much greater. In the fourth subject (J.D.) GLC estimation of octanoic acid was not made and the exact reduction in plasma LCFA is therefore unknown. Nevertheless, a considerable fall in plasma FFA as measured by titration was observed after MCT. The fall in plasma LCFA was presumably even greater and would have more nearly approached the fall after glucose.

No consistent changes were observed in plasma FFA after oral glycerol in three subjects or after coffee alone in one subject (Table 2).

Glucose and Insulin

The levels of plasma glucose and serum insulin after MCT, glucose, and glycerol are given in Table 3. Although a small rise in serum insulin occurred after ingestion of MCT (7–18 μU/ml above fasting values) this was considerably less than that observed after glucose (80–111 μU/ml above fasting values). No significant rise in serum insulin was observed after glycerol. The maximum fall in plasma glucose after MCT was 13–23 mg/100 ml.

TABLE 2 PLASMA FFA CONCENTRATIONS* (ESTIMATED BY TITRATION) FOLLOWING ORAL MCT, GLUCOSE, AND GLYCEROL

Subject and Test Load	Minutes after Ingestion					
	0	30	60	90	120	150
	μeq/liter					
I.T. MCT	500	550	270	280	330	240
Glucose	370	310	180	120	130	170
Glycerol	630	500	520	590	600	600
Coffee	395		325	500		
M.S. MCT	800	825	640	500	560	550
Glucose	940	435	70	<50	<50	<50
Glycerol	425	860	680	750	560	730
E.J. MCT	540	450	510	460	450	570
Glucose	450	370	260	280	260	200
Glycerol	380	360	250	450	440	390
J.D. MCT	1200	790	470	400	360	670
Glucose	1400	440	230	120	190	740

* SD of FFA estimation over the range 250–1500 μeq/liter = 25.

TABLE 3 PLASMA GLUCOSE AND SERUM INSULIN CONCENTRATIONS AFTER ORAL MCT, GLUCOSE, AND GLYCEROL

Subject and Test Load	Minutes after Ingestion															
	0		15		30		45		60		90		120		150	
	G	I	G	I	G	I	G	I	G	I	G	I	G	I	G	I
I.T. MCT	98	4	—	21	78	22	—	14	86	19	89	5	78	6	78	13
Glucose	98	5	—	—	164	98	—	—	98	42	106	84	88	23	64	9
Glycerol	92	3	—	3	100	3	—	2	96	3	98	4	97	3	101	1
M.S. MCT	98	8	—	8	98	15	—	—	93	11	82	9	82	10	82	8
Glucose	87	—	—	—	159	—	—	—	170	—	132	—	136	—	111	—
Glycerol	89	9	—	7	94	8	—	7	108	7	103	8	99	7	95	6
E.J. MCT	126	25	—	20	129	23	—	—	128	43	117	30	113	38	113	36
Glucose	115	19	—	—	208	120	—	—	180	130	113	42	107	58	109	32
Glycerol	104	5	—	7	106	8	—	6	108	12	104	5	108	5	101	8
J.D. MCT	98	2	—	—	88	9	—	—	98	5	88	4	87	3	75	—
Glucose	103	4	—	—	161	60	—	—	198	81	149	84	122	58	90	15

G, glucose (mg/100 ml); I, insulin (μU/ml).

Triglyceride

Serum triglyceride concentrations did not rise significantly after oral MCT, glucose, or glycerol (Table 4). Analysis of the fatty acid composition of the serum triglyceride showed that in all subjects there was a small but significant rise in the percentage of hexadecenoic acid (16:1) 60–90 min after MCT, with a return towards basal values by 150 min. In two of the four subjects similar changes were seen after ingestion of glucose (Table 5). No consistent changes in the other fatty acids were observed. No octanoic or decanoic acids were detected in the serum triglyceride after MCT.

DISCUSSION

Our studies show that the absorption of MCT is associated with a fall in the concentration of plasma LCFA, which is equal in some cases to that produced by an oral load of glucose of similar calorific value. The small rise in serum insulin after ingestion of MCT compared with the large increase after glucose makes it unlikely that stimulation of insulin secretion (whether by a direct action of octanoic acid or via the production of ketone bodies) plays a major part in the fall of LCFA. Indeed in the subject J.D., who appeared to have the greatest fall in LCFA after MCT, the increase in serum insulin was only 7 μU/ml. The possibility that a rise in insulin might occur earlier after MCT than after glucose was excluded by showing that at 30 min the levels were higher than at 15 min. Jenkins (2) has suggested that blood ketones may have a direct inhibitory effect on the release of FFA from the adipose cell. We did not measure plasma ketone levels, but Bergen, Hashim, and Van Itallie (14) found only a mild degree of hyperketonemia

TABLE 4 SERUM TRIGLYCERIDE CONCENTRATIONS* FOLLOWING ORAL MCT, GLUCOSE, AND GLYCEROL

Subject and Test Load	Minutes after Ingestion					
	0	30	60	90	120	150
	mg/100 ml					
I.T. Glucose	90	84	101	79	77	75
MCT	83	84	88	78	77	70
Glycerol	92	100	96	99	97	101
M.S. Glucose	96	93	97	93	92	95
MCT	115	108	127	125	120	119
Glycerol	89	94	108	103	99	95
E.J. Glucose	91	91	79	101	102	104
MCT	95	95	95	90	100	92
Glycerol	104	106	103	104	108	101
J.D. Glucose	99	91	96	91	92	95
MCT	82	84	82	87	81	83

* SD of triglyceride estimation over the range 50–150 mg/100 ml = 4.

TABLE 5 HEXADECENOIC ACID CONCENTRATION* FOLLOWING ORAL MCT AND GLUCOSE

Time	I.T.		M.S.		E.J.		J.D.	
	MCT	Glucose	MCT	Glucose	MCT	Glucose	MCT	Glucose
min	*g/100 g of total fatty acid*							
Fasting	3.8	3.3	5.4	5.6	5.5	5.2	3.8	4.0
30	3.8	3.8	7.4	7.9	7.0	5.7	4.6	4.6
60	4.0	3.4	8.6	8.1	7.1	5.2	4.8	7.0
90	5.6	3.4	6.8	7.7	5.6	5.4	6.7	5.0
120	3.8	4.0	6.8	8.1	6.2	5.3	5.9	4.8
150	3.9	3.4	6.6	7.7	5.5	4.9	5.0	4.2

* SD of the method = 0.26, around a mean of 5.1.

after a larger oral load of MCT (100 g) and we think it unlikely that the fall in LCFA in our subjects could be due solely to the action of ketones on the adipose cell. We did not observe any consistent changes in LCFA after our oral glycerol loads and conclude that the effect of MCT on LCFA was not due to an effect of the glycerol component of MCT. We have shown that high concentrations of octanoic acid persist in the plasma for several hours after MCT, and it is therefore possible that octanoic acid itself exerts an inhibitory effect on the release of FFA from adipose tissue.

Our results illustrate the limitations of the titration method in measuring plasma FFA levels when octanoic acid is present. In at least two of our subjects the fall in LCFA after ingestion of MCT would have been appreciably underestimated, and in one subject (E.J.) missed altogether if allowance had not been made for the extracted octanoic acid in interpreting the FFA titer. Greenberger, Ruppert, and Tzagournis (15) have reported no significant change in plasma FFA levels estimated by titration following a smaller oral MCT load (30 ml of MCT oil). However, inspection of their data shows that five of their ten normal subjects had a decrease in the estimated level of FFA of 143–254 μeq/liter. From our observations on the persistence of octanoic acid in the plasma and its partial extraction in the titration method, we infer that octanoic acid contributed to the plasma FFA titer in their subjects and that the fall in LCFA was probably greater and present in more of their subjects than is apparent from their uncorrected FFA data.

A rise in the proportion of hexadecenoic acid in serum triglyceride after a single oral MCT load does not appear to have been reported before, although we have observed similar rises in children receiving MCT diets over long periods (unpublished observations).

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REFERENCES

1. Linscheer, W. G., D. Slone, and T. C. Chalmers. 1967. *Lancet.* **i:** 593.
2. Jenkins, D. J. A. 1967. *Lancet.* **i:** 847.
3. Werk, E. E., H. T. McPherson, L. W. Hamrick, Jr., J.

D. Myers, and F. L. Engel. 1955. *J. Clin. Invest.* **34:** 1256.
4. Sanbar, S. S., G. Helenyi, Jr., N. Forbath, and J. R. Evans. 1965. *Metabolism.* **14:** 1311.
5. Fajans, S. S., J. C. Floyd, Jr., R. F. Knopf, and J. W. Conn. 1964. *J. Clin. Invest.* **43:** 2003.
6. Sanbar, S. S., and J. M. Martin. 1967. *Metabolism.* **16:** 482.
7. Hashim, S. A., S. S. Bergen, Jr., K. Krell, and T. B. Van Itallie. 1964. *J. Clin. Invest.* **43:** 1238.
8. Blood Sugar: Colorimetric Method. 1964. C. F. Boehringe and Soehne, Mannheim, Germany.
9. Dole, V. P., and H. Meinertz, 1960. *J. Biol. Chem.* **235:** 2595.
10. Grant, D. B., 1967. *Arch. Dis. Child.* **42:** 375.
11. Morgan, C. R., and A. Lazarow. 1963. *Diabetes.* **12:** 115.
12. Freeman, N. K. 1964. *J. Lipid Res.* **5:** 236.
13. Brown, W. D. 1961. *Aust. J. Exptl. Biol.* **39:** 223.
14. Bergen, S. S., Jr., S. A. Hashim, and T. B. Van Itallie. 1966. *Diabetes.* **15:** 723.
15. Greenberger, N. J., R. D. Ruppert, and M. Tzagournis. 1967. *Ann. Int. Med.* **66:** 727.