Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides

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Abstract Although medium chain triglyceride (MCT) is less calorically dense than long chain triglyceride (LCT), it produces a greater thermic effect following ingestion. We hypothesized that the previously observed high rate of thermogenesis produced by MCT overfeeding was due to hepatic de novo synthesis of long chain fatty acids (LCFA) from the excess medium chain fatty acids (MCFA). To study this, we compared the effects of overfeeding MCT- and LCT-containing diets on blood lipid profiles. Ten in-patient, nonobese males were overfed (150% of estimated energy requirements) two formula diets for 6 days each, in a randomized crossover design. Diets differed only in the composition of the fat and contained either 40% of energy as MCT or LCT (soybean oil). The major differences between diets in the resulting pattern of blood lipids were: *1*) a reduction in fasting serum total cholesterol concentrations with the LCT, but not the MCT diet; and *2*) a threefold increase in fasting serum triglyceride concentrations with MCT, but not LCT, diet. Moreover, 10% of the fasting triglyceride fatty acids were medium chain and 40% were 16:0 with the MCT diet. This compared to 1% and 20% for medium chain and 16:0, respectively, with the LCT diet. In addition, there were increases in 16:1, 18:0, and 18:1 in the triglycerides during MCT feeding. The changes in fatty acids in triglycerides with MCT feeding are consistent with the hypothesis that excess dietary MCT cause a significant increase in the hepatic synthesis of these fatty acids from MCFA through de novo synthesis and/or chain elongation and desaturation. These processes could account for the higher rate of postprandial thermogenesis with MCT as compared to LCT.—**Hill, J. O., J. C. Peters, L. L. Swift, D. Yang, T. Sharp, N. Abumrad, and H. L. Greene.** Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides. *J. Lipid Res.* 1990. **31:** 407-416.

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Medium chain triglycerides (MCT; containing fatty acids with 6 to 12 carbon atoms) were first introduced as a well-absorbed, calorically dense nutrient used to treat patients having impaired absorption of traditional long-chain triglycerides. MCTs have also been promoted for other uses, such as in enteral and parenteral nutritional support and appetite control (1, 2). In spite of increasing interest in the use of MCT supplements for improving energy absorption in gastrointestinal disease, many questions remain concerning the nutritional advantages of such supplements. For example, MCT are calorically less dense, providing only 8.2 kcal/g of metabolizable energy as compared to an average of 9.0 kcal/g provided by long chain triglycerides (LCT) (1, 2). In addition, MCT feeding has been reported to increase thermogenesis to a greater extent than LCT, resulting in reduced efficiency of utilization (3-8). The mechanisms of increased thermogenesis associated with MCT consumption and the conditions under which it occurs should be evaluated further.

We recently completed a study (8) with nonobese males in which the effect on energy balance of 6 days of overfeeding diets containing fat as either MCT or soybean oil (LCT) was examined. Compared to the soybean oil diet, the MCT diet produced a significantly higher thermic effect of food (TEF). TEF increased by 50% after 6 days of the MCT diet, but was unchanged by 6 days of the LCT diet. As part of this study, the influence of type of dietary fat on blood lipids was also measured. This report summarizes the effects of the two dietary lipids on blood lipid profiles. The results are interpreted in the context of understanding the cause of the increased energy expenditure reported with MCT overfeeding, and understanding the dose- and/or time-dependent nature of the response to MCT feeding.

Abbreviations: MCT, medium chain triglycerides; LCT, long chain triglycerides; LCFA, long chain fatty acids; MCFA, medium chain fatty acids; TEF, thermic effect of food; HDL, high density lipoproteins; NEFA, nonesterified fatty acids; PUFA, polyunsaturated fatty acids; VLDL, very low density lipoproteins; LDL, low density lipoproteins.

MATERIALS AND METHODS

Subjects

Ten nonobese (± 10% of ideal body weight) males volunteered for this study which was approved by the Vanderbilt Committee for the Protection of Human Subjects. **Table 1** shows the characteristics of the subjects.

Procedure

All subjects were studied in the Vanderbilt Clinical Research Center (CRC) for 6 days on each diet with 1 week ad libitum eating at home between the two in-patient periods. The study was double-blind and the order of diet administration was randomized so that half of the subjects received the MCT diet first and half the LCT diet.

The in-patient periods were identical for each subject with the exception that the composition of the diet differed. On the morning of day 1 blood was taken after an overnight fast for measurement of total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and free fatty acids. The subjects then received a test meal (1000 kcal) of the same composition as the experimental diet they were to receive that period. Blood was taken at approximately hourly intervals for 6 h after the meal for determination of free fatty acid concentrations. The subjects consumed the experimental diet throughout the remainder of the 6-day period. On the morning of day 6 a fasting plasma sample was taken for measurement of lipids as on the morning of day 1. The subjects then received another 1000 kcal test meal of the same composition as the meal on day 1. Measurements of total cholesterol, triglycerides, and free fatty acids were made approximately hourly for 6 h after the meal.

The composition of the free fatty acids was measured in all subjects on days 1, 6, and 7. The fatty acid composition of plasma triglycerides, cholesteryl esters, and phospholipids was measured in four out of the ten subjects on day 6 of each diet period.

TABLE 1. Characteristics of subjects

Subject	Age	Height	Weight	Energy Ingested
	yr	*cm*	*kg*	*kcal/day*
1	30	159.4	68.1	3816
2	32	182.9	90.5	4200
3	28	176.8	72.3	3578
4	35	181.0	89.7	4486
5	44	167.6	73.0	3125
6	35	190.5	102.8	3730
7	32	166.0	72.5	3780
8	25	176.0	80.0	3730
9	22	185.4	82.1	4334
10	29	172.7	62.7	3071
Mean	31	175.8	79.4	3785
SEM	2	3.0	3.8	148

TABLE 2. Fatty acid composition of experimental diets

Fatty Acid	MCT Diet	LCT Diet
	%	
8:0	61	
10:0	32	
16:0	2	11
18:0		6
18:1	5	32
18:2		51

Diets

Subjects received all food during in-patient periods as a liquid formula diet, prepared by the CRC dietitians. The formulas were constructed from components of the Nutrisource Modular System (Sandoz Nutrition Corporation, Minneapolis, MN). The macronutrient composition of the diets was 15% protein (Nutrisource protein), 45% carbohydrate (Nutrisource carbohydrate), and 40% fat (either Nutrisource lipid-long chain triglycerides in the form of soybean oil or Nutrisource lipid-medium chain triglycerides). Diets were flavored with noncaloric flavorings to suit the tastes of the subjects. **Table 2** shows the fatty acid composition of the dietary lipid.

Each subject received each diet in a quantity estimated to provide 150% of maintenance energy requirement. Energy requirements were calculated as 1.4 × measured resting metabolic rate (measured by a ventilated hood indirect calorimetry system). This has proved to be a reasonable estimate of energy requirements of hospitalized subjects (9).

Subjects consumed their daily allotment of formula in four equal-sized meals. The exceptions were on days 1 and 6 when the subject received a 1000 kcal test meal in the morning and consumed the remainder of the liquid formula between late afternoon and 8:00 PM.

Analytical measures

Serum cholesterol and triglyceride concentrations were measured with an automatic analyzer (Du Pont Co., Wilmington, DE), using standard enzymatic techniques. The coefficient of variation for cholesterol was 3.6% and the coefficient of variation of triglyceride was 3.1%. HDL cholesterol was measured similarly after removal of VLDL and LDL from samples by precipitation with phosphotungstate (10). The coefficient of variation for this assay was 2.3%. Nonesterified free fatty acids were extracted from plasma with hexane, methanol, and methylene chloride, derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA, Supelco, Bellefonte, PA), and measured by gas-liquid chromatography (11, 12). Glucose was measured using an AutoAnalyzer (Beckman, Fullerton, CA) and insulin was measured using a double-label antibody technique (13).

We randomly selected four of the ten subjects to measure the fatty acid composition of plasma triglycerides, cholesteryl esters, and phospholipids at three time points on day 6 of each diet period. Plasma (0.5 ml) was extracted by the method of Folch, Lees, and Sloane Stanley (14). Individual lipids classes were separated by thin-layer chromatography on Silica Gel 60 plates (EM Laboratories, Elmsford, NY). Lipid extracts were dried under nitrogen at 37°C and applied to the plates. Plates were developed in petroleum ether–ethyl ether–acetic acid 80:20:1, and the lipid classes were visualized with Rhodamine B (0.05% in 95% ethanol). Lipid classes were identified by their R_f values compared with standards (Non-polar lipid Mix A, Supelco).

Cholesteryl ester and triglyceride spots were scraped from the plates and the lipids were eluted from the silica gel scrapings as described previously (15). The eluates were evaporated to dryness and methylated at 100°C in sealed tubes using BF_3–methanol (16). The phospholipid fraction was scraped from the plates and the constituent fatty acids were methylated using BF_3–methanol without elution of phospholipid from the silica gel. The samples were cooled on ice and fatty acid methyl esters were extracted with hexane.

Gas–liquid chromatography

Fatty acid methyl esters were analyzed in the hexane extracts using a Hewlett Packard 5890A gas chromatograph equipped with 30 m × 0.53 mm SPB-5 column (1.5 μm film thickness) (Supelco), flame ionization detector, and 3393A integrator. Nitrogen was used as a carrier gas at 20 ml/min, and the injector and detector temperatures were 225° and 250°C, respectively. The oven temperature was programmed as follows: 80°C (0.5 min); (20°C/min)→220°C (0.5 min); 15°C/min→280°C (3.0 min). Fatty acid methyl esters from triglycerides were also analyzed using a 6 ft × 2 mm i.d. glass column packed with 10% SP2330 on 100/120 Chromosorb W (Supelco). The oven temperature was programmed from 180° to 220°C at 2°C/min. Fatty acid methyl esters were identified by comparison of retention times to those of known standards.

Statistical analysis

Results were analyzed using Student's *t*-test for nonindependent samples (17).

RESULTS

Even though subjects were overfed, there was not a significant increase in body weight during either diet period.

Serum cholesterol

Feeding the LCT-containing formula for 6 days induced a significant reduction in fasting total cholesterol concentrations whereas consumption of the MCT-containing formula resulted in no change (**Fig. 1A**). By contrast, HDL cholesterol values were not significantly altered by either diet after 6 days (Fig. 1B). On day 6, total cholesterol had not been altered by the the ingestion of either meal (Fig. 1C).

Serum triglycerides

The LCT diet did not produce any changes in total fasting triglyceride concentrations from day 1 to day 6, whereas the MCT diet produced a significant threefold increase in fasting triglyceride from day 1 to day 6 of the study (**Fig. 2A**). The LCT test meal consumed on day 6

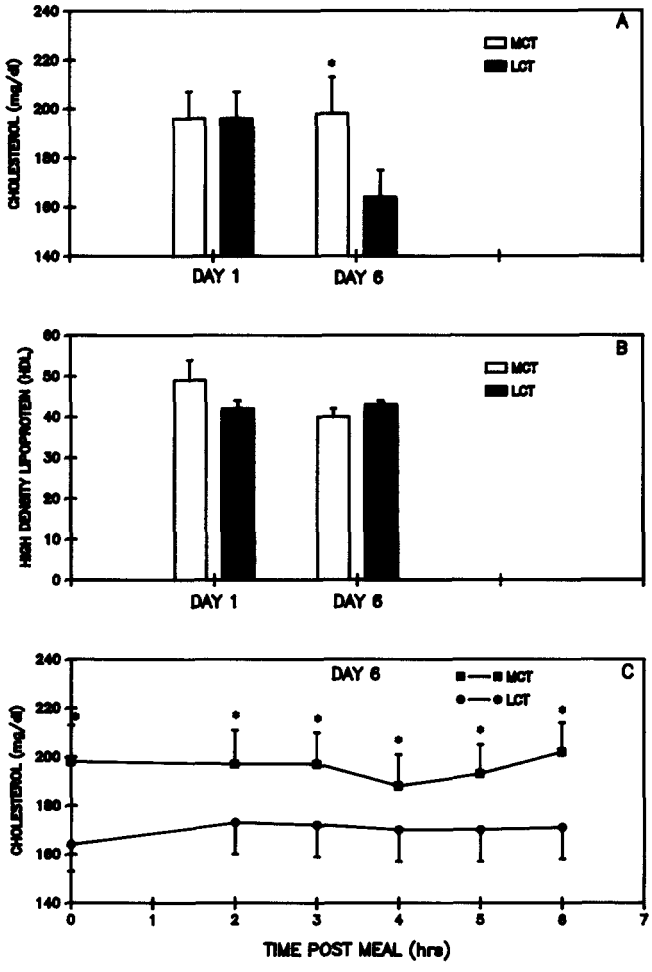

Fig. 1. Fasting concentrations of total plasma cholesterol are shown in A and fasting concentrations of plasma HDL are shown in B. Panel C shows concentrations of total plasma cholesterol before and after each test meal on day 6. The meals were consumed after the time 0 sample was taken. An asterisk (*) signifies a significant ($P<0.05$) difference between diets.

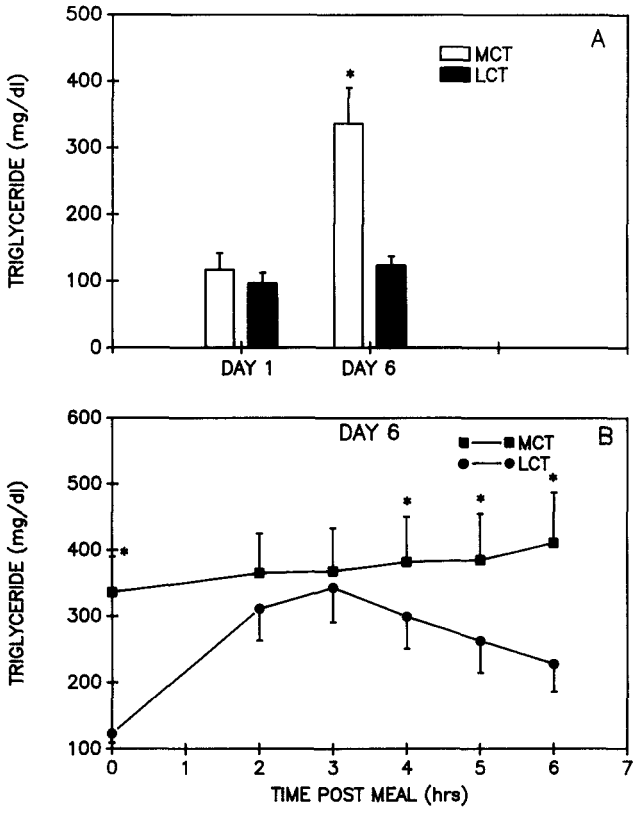

Fig. 2. Fasting concentrations of total plasma triglycerides are shown in A. Panel B shows total plasma triglyceride concentrations before and after the test meals on day 6. The meals were consumed after the time 0 sample was taken. An asterisk (*) signifies a significant ($P<0.05$) difference between diets.

produced the expected increase in triglyceride concentrations but the MCT test meal on day 6 did not result in any change in triglyceride concentrations (Fig. 2B).

The fatty acid composition of the triglyceride on day 6 is shown in **Table 3**. The samples at 2 and 3 h after feeding were not different in composition from fasting samples for either diet. However, with the MCT diet, about 10% of the triglyceride fatty acids were of medium chain length (8:0–12:0). By contrast, less than 1% of the triglyceride fatty acids were of medium chain length (in this case only 12:0 was present) with the LCT diet. The triglycerides from the subjects fed the MCT diet also contained relatively more 14:0, 16:0, and 16:1 and less 18:1–18:2 and 20:4 than were present during LCT feeding. Analysis of triglyceride fatty acids on the packed column revealed that 18:1 was increased (32.1% vs 30.4%) while 18:2 was markedly decreased (8.3% vs 35.6%) in MCT as compared to LCT feeding. Considering that fasting triglycerides were 2.5-fold higher when subjects were on MCT compared to when they consumed LCT diets, these data suggest that the absolute amounts of all triglyceride fatty acids except 18:2 and 20:4 were increased with MCT feeding. That is, in addition to an increase in triglyceride MCFAs (which were essentially absent from triglycerides during LCT feeding), 14:0 increased approximately 6-fold, 16:0 and 16:1 increased approximately 4-fold, 18:0 increased approximately 3-fold, and 18:1 increased approximately 2.5-fold compared with LCT feeding. These estimated increases assume an average molecular weight for the triglycerides of 804 (molecular weight of tripalmitin) during MCT feeding and 890 (molecular weight of triolein) during LCT feeding.

The fatty acid compositions of phospholipids and cholesteryl esters from subjects on both diets are shown in **Table 4** and **Table 5**. The differences in fatty acid patterns of these lipid classes were not as pronounced as seen in the triglycerides. In general, MCT feeding led to relatively more 16:0 in phospholipids and cholesteryl esters and less 18:0 and 18:1–18:2 compared to LCT feeding.

Serum free fatty acids

Blood concentrations of nonesterified fatty acids (NEFA) are shown in **Fig. 3** for each diet. Fasting concentrations of total NEFA did not change with the LCT diet, but were significantly ($P<0.05$) reduced by 6 days of the MCT diet

TABLE 3. Fatty acid composition of triglycerides on day 6 of each diet period in four subjects

Fatty Acid	LCT Diet			MCT Diet		
	Fasting	2 H	3 H	Fasting	2 H	3 H
	percent of total triglyceride fatty acids					
8:0	ND	ND	ND	2.3 ± 1.0	2.4 ± 0.5	2.8 ± 0.6
10:0	ND	ND	ND	5.5 ± 1.7	5.6 ± 1.0	6.1 ± 1.2
12:0	0.6 ± 0.6	1.3 ± 0.1	0.8 ± 0.4	2.3 ± 0.2	2.6 ± 0.3	2.0 ± 0.3
14:0	1.8 ± 0.6	1.7 ± 0.2	1.6 ± 0.2	5.0 ± 0.6	4.8 ± 0.6	4.7 ± 0.5
16:0	22.7 ± 2.7	19.3 ± 1.8	19.4 ± 1.8	35.9 ± 2.9	35.3 ± 2.8	34.9 ± 2.6
16:1	3.6 ± 0.2	2.2 ± 0.2	1.5 ± 0.5	6.4 ± 0.3	6.3 ± 0.3	6.0 ± 0.2
18:0	4.6 ± 1.3	4.2 ± 0.3	4.0 ± 0.1	5.4 ± 0.4	5.8 ± 0.6	6.4 ± 0.7
18:1,2	62.2 ± 4.9	66.2 ± 2.1	69.6 ± 1.8	36.8 ± 5.6	36.2 ± 5.0	36.6 ± 4.7
20:4	4.0 ± 0.6	3.0 ± 0.3	2.6 ± 0.4	0.6 ± 0.6	1.2 ± 0.7	0.6 ± 0.6

Fasting, 2-h, and 3-h values are before and after the test meal on day 6. ND, nondetectable levels.

TABLE 4. Fatty acid composition of phospholipids on day 6 of each diet period in four subjects

Fatty Acid	LCT Diet			MCT Diet		
	Fasting	2 H	3 H	Fasting	2 H	3 H
	percent of total phospholipid fatty acids					
8:0	ND	ND	ND	ND	ND	ND
10:0	ND	ND	ND	ND	ND	ND
12:0	ND	ND	ND	ND	ND	ND
14:0	ND	ND	ND	ND	ND	ND
16:0	26.0 ± 0.8	25.4 ± 0.9	25.3 ± 0.3	31.4 ± 0.6	31.2 ± 0.8	30.0 ± 0.7
16:1	ND	ND	ND	ND	ND	ND
18:0	16.9 ± 0.4	15.2 ± 1.7	17.2 ± 0.6	14.8 ± 0.5	14.5 ± 0.4	13.9 ± 0.6
18:1,2	33.9 ± 0.3	35.5 ± 1.4	35.6 ± 0.3	31.0 ± 0.5	30.8 ± 0.8	30.7 ± 0.7
20:4	18.6 ± 0.6	19.0 ± 0.9	17.6 ± 0.4	18.3 ± 0.3	18.3 ± 1.5	18.9 ± 1.9
22:6	4.6 ± 0.2	4.9 ± 0.4	4.4 ± 0.2	4.4 ± 0.3	5.2 ± 0.3	5.7 ± 0.9

Fasting, 2-h, and 3-h values are before and after the test meal on day 6. ND, nondetectable levels.

(time 0 on both panels of Fig. 3). After the meal on day 1, NEFA concentrations remained relatively constant for 6 h when the meal contained LCT, but declined progressively to reach statistical significance ($P<0.05$) by 6 h after the MCT meal (Fig. 3A). By contrast, on day 6 NEFA concentrations increased progressively to reach statistical significance ($P<0.05$) after the LCT meal, whereas NEFA concentrations remained relatively constant after MCT meal (Fig. 3B).

The decline in total fasting NEFA that occurred with MCT feeding during the 6-day period (time 0 point of Fig. 3A) was due primarily to a decrease in C18–C20 fatty acids as shown in **Fig. 4A** inasmuch as MCFA actually increased slightly during this period from 10.0 μg/ml (10% of total fasting NEFA) before the meal test on day 1 to 17.0 μg/ml (26% of the total fasting NEFA) on day 6.

After consumption of the meal on day 1, LCFA declined substantially and MCFA increased slightly (Fig. 4A), accounting for the decline in total NEFA previously shown in Fig. 3A. On a percentage basis, MCFA increased from 10% to between 30 and 50% of total NEFA after ingestion of the MCT-containing meal on day 1. After ingestion of the meal on day 6 (Fig. 4B) there were slight decreases in LCFA and increases in MCFA so that the percentage of MCFA increased from 26% to between 30 and 50% of the total NEFA.

Fig. 5 presents the changes in plasma composition of NEFAs after the LCT diet on days 1 and 6. There was no significant change in the fasting concentrations of any fatty acids after 6 days of the LCT diet; MCFA represented approximately 7% of the total NEFA over this period (time 0 on Figs. 5A and 5B). The test meal on day 1 produced no significant change in the concentrations of the different fatty acids with MCFA comprising less than 10% of the total NEFA following the meal (Fig. 5A). After the test meal consumption on day 6 (Fig. 5B), the increase in NEFA was due to increased concentrations of LCFA, specifically C18–C20 fatty acids. Neither MCFA nor C14–C16 fatty acids changed during the meal test on day 6.

TABLE 5. Fatty acid composition of cholesteryl esters on day 6 of each diet period in four subjects

Fatty Acid	LCT Diet			MCT Diet		
	Fasting	2 H	3 H	Fasting	2 H	3 H
	percent of total cholesteryl ester fatty acids					
8:0	ND	ND	ND	ND	ND	ND
10:0	ND	ND	ND	ND	ND	ND
12:0	ND	ND	ND	ND	ND	ND
14:0	0.8 ± 0.5	ND	ND	1.8 ± 0.6	1.8 ± 0.6	1.2 ± 0.7
16:0	11.0 ± 0.5	10.8 ± 0.3	10.9 ± 0.3	14.9 ± 0.5	15.5 ± 0.9	14.4 ± 0.4
16:1	1.9 ± 0.2	2.3 ± 0.6	2.5 ± 0.3	9.1 ± 0.6	9.3 ± 1.4	8.1 ± 1.3
18:0	1.2 ± 0.1	1.0 ± 0.3	1.0 ± 0.3	0.6 ± 0.6	ND	0.9 ± 0.6
18:1,2	77.0 ± 0.6	77.0 ± 0.7	76.5 ± 0.4	64.2 ± 1.4	63.9 ± 1.1	64.5 ± 0.9
20:4	8.1 ± 0.7	8.9 ± 0.7	9.4 ± 0.1	9.4 ± 1.7	9.5 ± 1.6	10.3 ± 1.2
22:6	4.6 ± 0.2	4.9 ± 0.4	4.4 ± 0.2	4.4 ± 0.3	5.2 ± 0.3	5.7 ± 0.9

Fasting, 2-h, and 3-h values are before and after the test meal on day 6. ND, nondetectable levels.

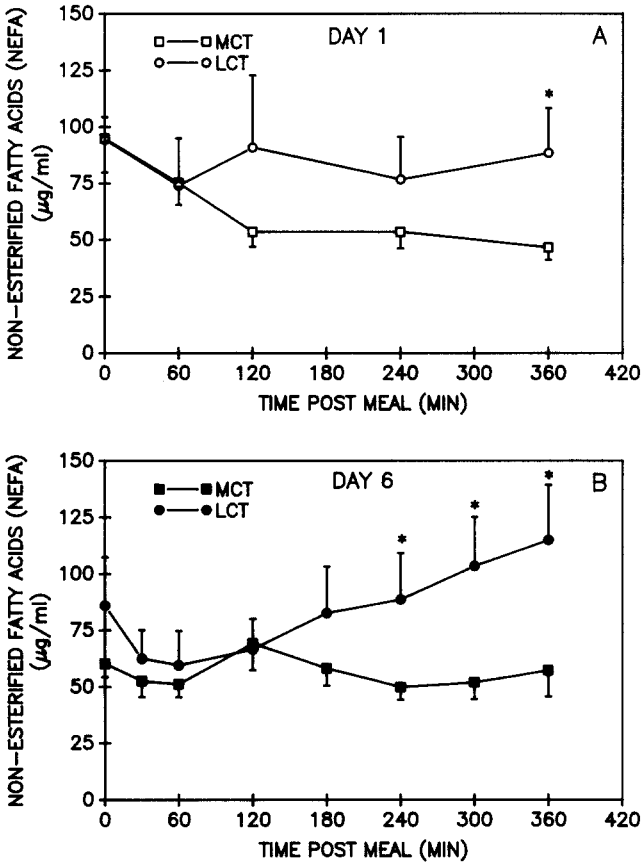

Fig. 3. Total plasma concentrations of nonesterified fatty acids are shown before and after the test meals on day 1 (A) and day 6 (B). The meals were consumed after the time 0 samples were taken. An asterisk (*) signifies a significant ($P<0.05$) difference between diets.

Insulin and glucose

There were no significant differences in either insulin or glucose concentrations as measured in subjects fasted overnight before the test meal on day 6, although insulin values tended to be higher during MCT feeding. Meal ingestion produced a rise in both insulin and glucose on both diets. The peak values for insulin and glucose occurred at 30 min after the meal for both diets. Insulin concentrations were not different between diets at any time point, and the total area under the 6-h curve was not significantly different (754 ± 163 μU/ml for MCT vs 610 ± 79 μU/ml for LCT, NS). Although glucose concentration did not differ significantly between diets at any time point, the total area under the 6-h curve was slightly (but not significantly) greater for LCT than for MCT (596 ± 11 mg/dl vs 561 ± 10 mg/dl).

DISCUSSION

The pattern of blood lipids seen during the MCT diet differed from that seen during the LCT diet and also differed from reports in the literature of the effects of lesser amounts of MCT on blood lipid metabolism. Our results are consistent with the hypothesis that ingestion of large amounts of MCT in a diet providing calories in excess of maintenance can promote substantial synthesis of LCFA by the liver. This synthesis of LCFA from acetate or from chain elongation of C8 and C10 fatty acids is energetically expensive, and could account for the unexpectedly large thermic effect of diets containing a large proportion of fat-calories as MCT (8).

The significant reduction in plasma total cholesterol with the LCT diet is consistent with numerous studies that have shown that diets rich in PUFAs lower cholesterol concentrations (18, 19). Although this response was not unexpected, three points are noteworthy. First, the cholesterol-lowering effect occurred rapidly (within 6 days), and second it occurred at a high level of total fat and calorie intake. Third, given that the P/S ratio of the LCT diet was 3.0 and that the diet was cholesterol-free, one might have expected a greater cholesterol lowering than observed. However, the excessive total caloric intake and the provision of a significant amount of simple carbohydrate in the formula diet may have mitigated the effect of the high P/S ratio.

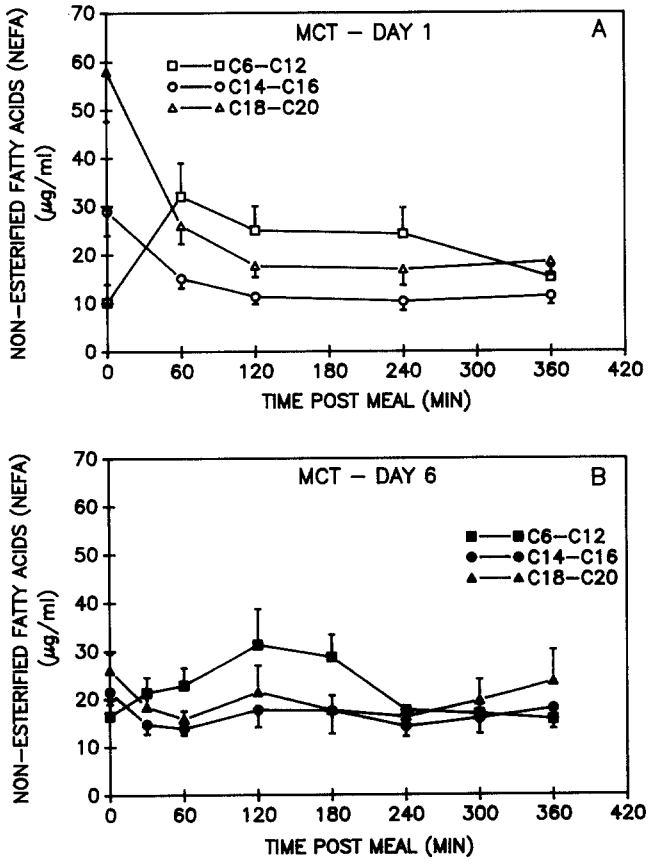

Fig. 4. The composition of nonesterified fatty acids is shown before and after the MCT meals on day 1 (A) and day 6 (B).

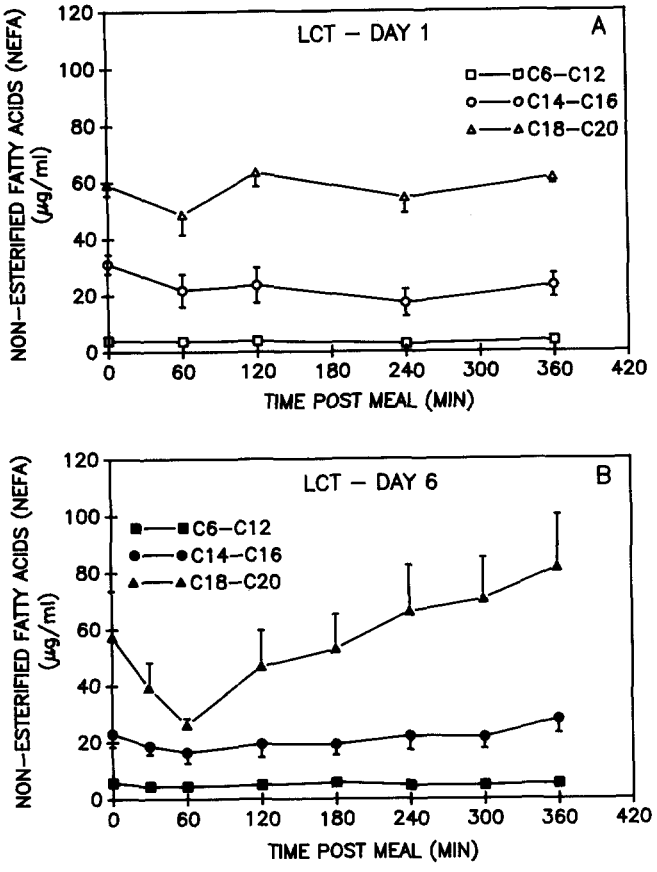

Fig. 5. The composition of nonesterified fatty acids is shown before and after the LCT meals on day 1 (A) and day 6 (B).

Although MCT feeding did not alter total cholesterol levels, it is misleading to conclude that the MCT diet had no effect on cholesterol metabolism. For example, fasting plasma triglycerides were increased by nearly threefold after 6 days of the MCT diet. If one assumes that the bulk of fasting plasma triglyceride is present in VLDL, then according to Friedewald's equation (20), VLDL cholesterol concentrations by the end of the 6-day feeding period can be estimated to have been 67 mg/dl, a level substantially above the baseline value of 23 mg/dl and above the 25 mg/dl level seen after 6 days on the LCT diet. Furthermore, using the same equation, LDL cholesterol concentrations can be estimated to have been 91 mg/dl after 1 week of MCT feeding, a drop of 33 mg/dl compared to baseline and a level that was 10 mg/dl below that of subjects fed the LCT diet. It is possible, however, that Friedewald's equation is inappropriate for estimation of LDL cholesterol during MCT feeding. If, for example, the cholesterol to triglyceride ratio of the VLDL produced during MCT treatment was decreased, we would have overestimated VLDL cholesterol. Consequently, we would have overestimated any decline in LDL cholesterol that may have occurred. Since we did not measure LDL cholesterol directly in this study, it is not possible to distinguish between these possibilities. However, it is interesting that in a follow-up study (Swift, L. L., J. O. Hill, and J. C. Peters, unpublished data) in which MCT was fed to subjects at a maintenance level of energy intake (i.e., not overfed), plasma triglycerides were elevated without any change in the cholesterol to triglyceride ratio in VLDL as compared to that seen during maintenance feeding of LCT.

Although we did not measure LDL cholesterol directly in this study and thus cannot definitively conclude that MCT caused LDL to decline, it is noteworthy that other investigators have seen effects of MCT on LDL metabolism in animals. Woollett, Spady, and Dietschy (21) recently compared the effects of MCT, hydrogenated coconut oil, and chow, either with or without added dietary cholesterol, on cholesterol metabolism in hamsters. They found that feeding MCT either with or without added dietary cholesterol consistently increased receptor-dependent LDL transport in both liver and other tissues. Despite an increase in LDL production rate in hamsters fed the MCT diet (presumably due to increased VLDL production), the increased clearance capacity prevented a large increase in plasma LDL cholesterol when compared to animals fed the low-fat chow control diet. These observations in hamsters raise the possibility that similar changes in LDL clearance capacity may occur in humans during MCT feeding, mechanism that could provide the basis for a reduction in circulating LDL cholesterol concentration.

HDL cholesterol concentrations were not significantly altered by either diet, but tended to be slightly lowered by MCT feeding. A switch from the typical diets eaten by Americans to a diet high in long-chain saturated fatty acids generally would not be expected to cause large increases in VLDL cholesterol and triglycerides or to produce a decrease in LDL levels (22). Therefore, the influence of the MCT diet on cholesterol metabolism is probably an effect of the shorter chain length of its constituent fatty acids and not due to its high degree of saturation.

Fasting NEFA levels were unchanged by 6 days of LCT feeding and the pattern of changes after the LCT meal was similar on days 1 and 6. There was a slight drop in NEFA during the first hour after each LCT meal, followed by a gradual increase in NEFA. The lack of a larger drop in total NEFA after the LCT meals may seem surprising in view of the usually dramatic suppression of 12-h fasting NEFA levels after a pure carbohydrate load. Although our test meal contained a sizeable amount of carbohydrate (112 g), it also contained a large load of fat (44 g). It is possible that endogenous release of fatty acids from adipose tissue was suppressed by the mixed meal, but that a significant amount of NEFA entered the plasma pool during the hours after the meal due to intravascular hydrolysis of diet-derived triglycerides and incomplete clearance of the released fatty acids. The data in Fig. 5

showing the responses of NEFA of different chain lengths support this idea. NEFA with 14–16 carbon atoms (mostly C16) indeed declined after meal ingestion and did not return to starting values until 6 h later, indicative of the expected response to a meal containing carbohydrate. The response of these fatty acids to the meal can be used as a marker of endogenous fatty acid release since they made up more than 30% of the NEFA pool after the 12-h fast, but were present in the diet at only 11% of the total fatty acids. In contrast to the response of the C14–16 fatty acids, C18–20 fatty acids fell below the time zero level only at the 60-min time point and actually exceeded the fasting level after 4 h. Since fatty acids of C18–20 chain length comprised 85% of the dietary fatty acids, the increase in this fraction of total NEFA 60 min after the meal suggests that they are of dietary origin. In support of this notion, Heimberg, Dunn, and Wilcox (23) have reported that even after a single fatty meal, dietary fatty acids appear in the plasma NEFA pool. These fatty acids originate from intravascular hydrolysis of diet-derived chylomicron and VLDL triglycerides. They also reported differences in NEFA response between safflower oil (LCT) and coconut oil (MCT) that were similar to the differences we found between LCT and MCT.

The postprandial drop in NEFA after the MCT meal on day 1 was the composite effect of a large drop in the C14–C20 fatty acid fractions, coupled with a rise in the C6–C12 fatty acids (mostly C8 and C10). The drop in the longer chain fatty acids reflects the expected meal-induced suppression of endogenous fat mobilization (since these fatty acids were absent from the meal). The increase in the medium chain fatty acids was most likely due to incomplete clearance by the liver of incoming diet-derived MCFA (24). This is probable because: *1*) incorporation of medium chain fatty acids into chylomicron and VLDL triglyceride is reported to be low (1) and these lipoproteins would thus not be the source of significant medium chain fatty acids, and *2*) the plasma response was essentially the same on day 6 as on day 1, but on day 1 the medium chain fatty acids would most likely not have been of adipose origin, since that was the first exposure to MCT. Other workers (25) have found similar proportions of MCFA in the plasma NEFA following an MCT load.

Six days of MCT feeding significantly lowered fasting NEFA levels. This difference could be explained by the slightly higher fasting insulin levels seen with MCT feeding in this study (40 ± 12 vs 22 ± 6 μU/ml; NS), and reported by others (26, 27). Six days of MCT feeding lowered fasting levels of C14–16 and C18–20 fatty acids, probably reflecting suppression of endogenous fat mobilization during excess calorie ingestion. These fatty acids were further reduced after the meal on day 6, presumably as a result of meal-induced insulin secretion. Six days of MCT feeding increased fasting C6–12 fatty acid levels, perhaps indicating some release of MCFA that had been stored in depot fat during the preceding MCT feeding. After the meal on day 6, the free MCFA increased to the same extent as on day 1, although at a slower rate. This difference in time course may reflect more efficient liver clearance and metabolism of MCFA on day 6 compared to day 1, or relatively more incorporation of MCFA into chylomicron triglyceride and less transport of MCFA in the free acid form.

The precise mechanism of the threefold elevation in triglycerides observed with MCT feeding is not known. However, a consideration of the pathways of MCT metabolism yields a possible explanation. Most of the C8 and C10 fatty acids released by hydrolysis of MCT in the gut are not reesterified by the enterocyte to triglyceride, but enter the portal vein as NEFA bound to albumin. The MCFA travel to the liver where they bypass the rate-controlling step in long-chain fatty acid oxidation, carnitine acyl transferase, and are rapidly and extensively oxidized to 2-carbon fragments (acetyl-CoA). The acetyl-CoA thus formed can be further degraded to CO_2 and H_2O via the Krebs cycle, can be converted into ketone bodies, or can served as substrate for de novo fatty acid synthesis. We found that increased fatty acid oxidation and ketone body formation did occur with MCT feeding, but that cannot alone explain the increased energy expenditure (8). Therefore, we believe substantial hepatic fatty acid synthesis occurred as well.

Previous studies have suggested that hepatic fatty acid synthesis can be altered by MCT diets. Kritchevsky and Tepper (28) reported enhanced rates of de novo fatty acid synthesis from acetate by livers of rats fed MCT diets for 7 days. Crozier (29) has also reported increased rates of lipogenesis in livers of rats chronically fed MCT diets. Leveille, Pardini, and Tillotson (30), demonstrated that chain lengthening activity as measured by conversion of [1-^{14}C]palmitate to C18 fatty acids was greater in livers of rats fed MCT compared to rats fed LCT. They also reported that fatty acid desaturation as measured by conversion of [2-^{14}C]stearate to oleate was also enhanced by an MCT diet. Thus, MCT diets, especially when fed in excess of caloric needs, might lead to increased de novo fatty acids synthesis and enhanced fatty acid elongation activity by the liver. This, in turn, would be expected to increase hepatic triglyceride production and very low density lipoprotein secretion, and could account for the elevated fasting plasma triglyceride concentrations seen in subjects fed the MCT diet. In support of the idea that increased de novo fatty acid synthesis occurred during excess MCT feeding was the finding that the relative percentage of 16:0 in fasting triglycerides of subjects during MCT feeding was almost twice that of subjects fed the LCT diet, even though the LCT diet provided five times more 16:0 than did the MCT diet. In fact, 16:0 was elevated in all blood lipid classes from subjects during the MCT diet feeding compared with the pattern seen during

LCT feeding. However, 16:1 was increased in triglycerides and cholesteryl esters and 18:0 and 18:1 were increased in triglycerides of MCT-fed subjects. This would suggest that MCT may also stimulate chain elongation and desaturation as reported by Leveille et al. (30). Our data are consistent with increased de novo fatty acid synthesis as well as increased chain elongation and desaturation with excess MCT feeding. These energetically costly activities could account for a substantial portion of the increased energy expenditure reported with excess MCT feeding (8).

There is a similarity between the changes in blood lipids produced by MCT feeding and those reported to occur with feeding high carbohydrate diets. The initial (1–3 weeks) response of normal healthy individuals to switching from a normal fat diet (35–40% of calories from fat) to one low in fat and high in carbohydrate is an increase in fasting plasma total triglycerides, a decrease in HDL, and little or no change in total cholesterol (31). This response is dose-related but after several weeks or months the blood lipid pattern returns to the pattern seen prior to dietary manipulation, presumably as a result of metabolic adaptation. Excessive carbohydrate intake would be expected to fuel enhanced glycogen storage and de novo fatty acid synthesis from the acetate supplied by enhanced glycolytic flux. The changes observed with MCT feeding are similar and not characteristic of high saturated fat feeding (at least not comparable to long chain saturated fat). It is tempting to speculate that the changes in blood lipids seen during MCT feeding share a common mechanism with those occurring during high carbohydrate ingestion. Whether or not the hypertriglyceridemia resulting from MCT ingestion disappears with time on the diet, as is the case with long-term high-carbohydrate intake (31), and whether the responses to MCT and carbohydrate are indeed the same awaits further investigation.

In conclusion, this study has shown that 6 days of MCT overfeeding leads to marked alterations in lipid metabolism characterized by substantial elevations of fasting triglyceride concentrations accompanied by decreases in plasma LDL and HDL cholesterol concentrations. Furthermore the triglyceride fraction was enriched in 16:0 compared to triglycerides from subjects fed the LCT diet. Overfeeding soybean oil, on the other hand, led to decreases in total cholesterol and LDL cholesterol, with no change in triglyceride or HDL cholesterol concentrations. These results are consistent with the idea that excess MCFA intake leads to increased hepatic fatty acid synthesis and triglyceride secretion. Increased fatty acid synthesis either from acetate or from chain elongation of C8 and C10 fatty acids is energetically costly and would result in a lesser efficiency of storage of ingested MCT-derived energy as depot lipid compared to deposition of energy from LCT. This scheme is consistent with our previous finding that MCT overfeeding results in a greater increase in postprandial energy expenditure than LCT overfeeding. In fact, the observed increase in postprandial thermogenesis agrees well with the theoretical costs of hepatic de novo lipogenesis (8). The inefficient storage of diet-derived MCFA relative to LCFA might suggest that MCT would be useful in helping control body weight. However, it must be noted that significant de novo lipogenesis would not be expected to occur with MCT incorporation into diets fed at restricted or maintenance levels, and under such conditions, MCT and LCT might produce similar themorgenic responses. Yost and Eckel (32) reported that an 800 kcal/day diet containing 24% of calories as MCT did not lead to greater weight loss than an isocaloric diet containing LCT. Finally, although the subjects in the present study were overfed, the differences in blood lipid responses to MCT and LCT, and the overall changes in blood lipid profiles seen relative to baseline values, were more importantly determined by the type of fat fed rather than the amount of fat consumed.

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REFERENCES

1. Bach, A. C., and V. K. Babayan. 1972. Medium-chain triglycerides: an update. *Am. J. Clin. Nutr.* **36:** 950–962.
2. Wiley, J. H., and G. A. Leveille. 1973. Metabolic consequences of dietary medium-chain triglycerides in the rat. *J. Nutr.* **103:** 829–835.
3. Geliebter, A., N. Torbay, E. F. Bracco, S. A. Hashim, and T. B. Van Itallie. 1983. Overfeeding with medium-chain triglyceride diet results in diminished deposition of fat. *Am. J. Clin. Nutr.* **37:** 1–4.
4. Baba, N., E. F. Bracco, and S. A. Hashim. 1982. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. *Am. J. Clin. Nutr.* **35:** 678–682.
5. Seaton, T. B., S. L. Welle, M. K. Warenko, and R. G. Campbell. 1986. Thermic effect of medium-chain and long-chain triglycerides in man. *Am. J. Clin. Nutr.* **44:** 630–634.
6. Contaldo, F., L. Scalfi, A. Coltorti, C. Mazzacano, L. A. Reed, A. Laviano, and M. Mancini. 1985. Thermogenic effect of dietary medium chain triglycerides in man. *In* Diabetes, Obesity and Hyperlipidemias. G. Crepaldi et al., editors. Elsevier Science Publishers, New York. 209–216.
7. Flatt, J. P., E. Ravussin, K. J. Acheson, and E. Jequier. 1985. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *J. Clin. Invest.* **76:** 1019–1024.

8. Hill, J. O., J. C. Peters, D. Yang, T. Sharp, M. Kaler, N. N. Abumrad, and H. L. Greene. 1989. Thermogenesis in man during overfeeding with medium chain triglycerides *Metabolism.* **38:** 641–648.
9. Hill, J. O., M. DiGirolamo, and S. B. Heymsfield. 1985. A new approach for studying the thermic response to dietary fuels. *Am. J. Clin. Nutr.* **42:** 1290–1298.
10. Burnstein, M., H. R. Scholnick, and R. Morfin. 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11:** 583–595.
11. Hockel, M., A. Holzer, P. Brockerhoff, and G. H. Rathgen. 1980. A microliter method for the gas chromatographic determination of long-chain non-esterified fatty acids in human serum or plasma. *J. Chromatogr.* **221:** 205–214.
12. Kukis, A., J. J. Myher, L. Marai, and K. Geher. 1976. Estimation of plasma free fatty acids as the trimethylsilyl (TMS) esters. *Anal. Biochem.* **70:** 302–312.
13. Morgan, C. R., and J. Larner. 1963. Immunoassay of insulin: two antibody system: plasma insulin levels of normal, subdiabetic and diabetic rats. *Diabetes,* **12:** 115–126.
14. Folch, J., M. Lees, and G. H. S. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animals tissues. *J. Biol. Chem.* **226:** 497–509.
15. Skipski, V. P., and M. Barclay. 1969. Thin-layer chromatography of lipids. *Methods Enzymol.* **14:** 530–598.
16. Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. *J. Lipid Res.* **5:** 600–608.
17. Winer, B. J. 1971. Statistical Principles in Experimental Design. McGraw-Hill, New York.
18. Goodnight, S. H., W. S. Harris, W. E. Connor, and D. R. Illingworth. 1982. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. *Arteriosclerosis.* **2:** 87–113.
19. Connor, W. E., and S. L. Connor. 1982. The dietary treatment of hyperlipidemia. Rationale. technique, efficacy. *Med. Clin. N. Am.* **66:** 485–518.
20. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
21. Woollett, L. A., D. K. Spady, and J. M. Dietschy. 1989. Mechanisms by which saturated triacylglycerols elevate the plasma low density lipoprotein-cholesterol concentrations in hamsters: differential effects of fatty acid chain length. *J. Clin. Invest.* **84:** 119–128.
22. Schonfeld, G., W. Patsch, L. L. Rudel, C. Nelson, M. Epstein, and R. E. Olson. 1982. Effects of dietary cholesterol and fatty acids on plasma lipoproteins. *J. Clin. Invest.* **69:** 1072–1080.
23. Heimberg, M., G. D. Dunn, and H. G. Wilcox. 1974. The derivation of plasma-free fatty acids from dietary neutral fat in man. *J. Lab. Clin. Med.* **83:** 393–402.
24. Tamir, I., D. B. Grant, A. S. Fosbrooke, M. M. Segall, and J. K. Lloyd. 1968. Effects of a single oral load of medium-chain triglyceride on serum lipid and insulin levels in man. *J. Lipid Res.* **9:** 661–666.
25. Uzawa, H., G. Schlierf, S. Chirman, G. Michaels, P. Wood, and L. W. Kinsell. 1964. Hyperglyceridemia resulting from intake of medium chain triglycerides. *Am. J. Clin. Nutr.* **15:** 365–369.
26. Pi-Sunyer, F. X., S. A. Hashim, and T. B. Van Itallie. 1969. Insulin and ketone responses to ingestion of medium- and long-chain triglycerides in man. *Diabetes.* **18:** 96–100.
27. Tantibhedhyangkul, P., S. A. Hashim, and T. B. Van Itallie. 1967. Effects of ingestion of long-chain and medium-chain triglycerides on glucose tolerance in man. *Diabetes.* **16:** 796–799.
28. Kritchevsky, D., and S. A. Tepper. 1965. Influence of medium-chain triglycerides (MCT) on cholesterol metabolism in rats. *J. Nutr.* **86:** 67–72.
29. Crozier, G. L. 1988. Medium-chain triglyceride feeding over the long term: the metabolic fate of [^{14}C]octanoate and [^{14}C]oleate in isolated rat hepatocytes. *J. Nutr.* **118:** 297–304.
30. Leveille, G. A., R. S. Pardini, and J. A. Tillotson. 1967. Influence of medium-chain triglycerides on lipid metabolism in the rat. *Lipids.* **2:** 287–294.
31. Jackson, R. L., M. T. Yates, C. A. McNerney, and M. L. Kashyap. 1987. Diet and HDL metabolism: high carbohydrate vs high fat diets. *Adv. Exp. Med. Biol.* **210:** 165–172.
32. Yost, T. J., and R. H. Eckel. 1989. Metabolic effects of medium chain triglyceride substitution during hypocaloric feeding in obese women. *Am. J. Clin. Nutr.* **49:** 326–330.