

《原著》

Metabolism of ^{14}C -Octanoic Acid in Human Subjects^{1,2}

Haruo Uzawa,³ George D. Michaels,⁴ Peter D. S. Wood⁵ & Lawrence W. Kinsell⁶

As previously reported (1), ingestion of medium chain triglycerides (MCT) in place of calorically equivalent amounts of fats containing long chain fatty acids, under conditions of isocaloric intake, is associated with significant and maintained hyperglycemia. The present report represents an initial approach to elucidation of the mechanism of this effect.

MATERIALS AND METHODS

Sodium octanoate- $1\text{-}^{14}\text{C}$ was obtained from Volk Radiochemical Company, Burbank, California. The free acid was purified by thin-layer chromatography; ^{14}C -labeled triglycerides were absent from the purified material. The developing system consisted of petroleum ether-diethyl ether-glacial acetic acid (70/30/1, v/v/v). Subsequently, the isolated material was removed from the plate and eluted from the silicic acid. Preparative gas-liquid chromatography was carried out and indicated a radiopurity of 98%.

For oral administration, the isotopic material was dissolved in an aqueous solution containing approximately 0.05 ml of unlabeled octanoic acid as carrier. Sodium octanoate- $1\text{-}^{14}\text{C}$ was administered in a dose of approximately 1 microcurie per kilogram of body weight.

Two of the patients studied (RTAB and LCLA) were maintained on quantitatively constant dietary intakes on the metabolic ward prior to administration of the isotopic material. Patient RCOT consumed a mixed diet of uncertain composition. The labeled sodium octanoate was administered after an overnight

fast, and no food or liquid other than water was ingested during a 24-hour period following administration of the isotope. Five studies were carried out on three subjects. The clinical characteristics of the subjects and the composition of the maintenance diets are described subsequently.

Plasma lipids were extracted and washed by the Folch procedure (2). The upper Folch aqueous phase was carefully transferred to a counting vial and brought to pH 8 with 0.1N NaOH. After evaporation on a steam bath, the residue was acidified with 0.5ml glacial acetic acid and 9.5ml scintillation fluid was added.

The lower Folch chloroform phase was evaporated to dryness and then dissolved in scintillation fluid. Isotopic activity in the aqueous phase and chloroform phase, respectively, was then determined, using a Packard Tri-Carb Liquid Scintillation Spectrometer. Internal quench correction was performed in the pre-

1 From the Institute for Metabolic Research, Highland General Hospital, Oakland, California, U.S.A.

2 Supported in part by National Institute of Health Grant No.HE 00955.

3 Second Department of Internal Medicine, Kyushu University Faculty of Medicine, Fukuoka City, Japan, formerly Research Fellow, Institute for Metabolic Research.

4 Deceased. Formerly Senior Research Biochemist, Institute for Metabolic Research.

5 Department of Biochemistry, Stanford University, Palo Alto, California, U.S.A., formerly Senior Research Biochemist, Institute for Metabolic Research.

6 Deceased. Formerly Director, Institute for Metabolic Research

九州大学医学部第二内科 (主任: 勝木司馬之助教授)

受付: 昭和45年2月27日

別刷請求先: 福岡市 九州大学医学部 第二内科

鶴沢春生 (〒 812)

sence of acetic acid. Distribution of radioactivity in the different lipid classes in the chloroform phase was subsequently determined following separation by appropriate Florisil column chromatography (3). Preliminary studies using octanoic acid-1-¹⁴C indicated that only minimal losses of this relatively volatile acid occurred during the above procedures.

Isotopic concentrations in the various samples were subsequently corrected to a common baseline using the coefficient:

$$\frac{\text{Body weight (kg)} \times 2.22 \times 10^6}{\text{Administered dose (dpm)}}$$

Plasma total glycerides were determined by the method of Michaels (3). To establish the presence or absence of radioactivity in plasma ketones, acetoacetate and β -hydroxybutyrate were converted to acetone by methods previously described (4). The final carbon tetrachloride solution containing the 2, 4-dinitrophenylhydrazone of acetone was evaporated to dryness, dissolved in 10 ml of scintillation fluid and counted.

In order to determine the distribution of radioactivity in specific triglyceride fatty acids, the plasma glyceride fraction was hydrolyzed, methylated with diazomethane and subjected to preparative gasliquid chromatography. Diethyleneglycol succinate polyester was used as the stationary phase in an Aerograph Model A-700 chromatograph; helium was used as carrier gas with a flow rate of 300 ml per minute under a pressure of 40 p.s.i. Each fatty acid was trapped as it was eluted from the GLC column into a glass U-tube which was immersed in methanol and dry ice. After collection, the contents of the tube were dissolved in n-hexane and made up to 10 ml. The fatty acid composition of each fraction was confirmed by gas-liquid chromatography using a Barber Coleman Model No. 10 chromatograph with an ionization detector (⁹⁰Sr).

Isotopic activity in each sample was determined as described above.

In patient RTAB, carbon dioxide in the expired air was collected in 750-1000 ml of 1.5 N NaOH solution in a bubbler system during the following periods: 5-25 minutes, 45-60, 105-120, 165-180, 225-

240, 285-300, 345-360, 405-420, 465-480, 705-720 and 1425-1440 minutes after administration of the isotope. Using a microbubbler system, the CO₂ from 5 ml of the sodium hydroxide solution was expelled into 2 ml of methanol containing choline as an organic base. Aliquots were counted in vials containing 15 ml of scintillation fluid. Cumulative radioactivity during the 8-hour period was calculated by measuring the area under the activity-time curve. Total CO₂ was measured by the Van Slyke procedure (5).

Clinical Status of Patients Studied

Patient RTAB, a 61-year-old non-insulin-dependent diabetic white male, had suffered a cerebral vascular accident twelve years ago and recently had manifested some evidence of coronary insufficiency. Physical and laboratory examination revealed no marked abnormalities except generalized arteriosclerosis and mild diabetes. The diabetes was well controlled by dietary management alone. Dietary intake is shown in Figure 1.

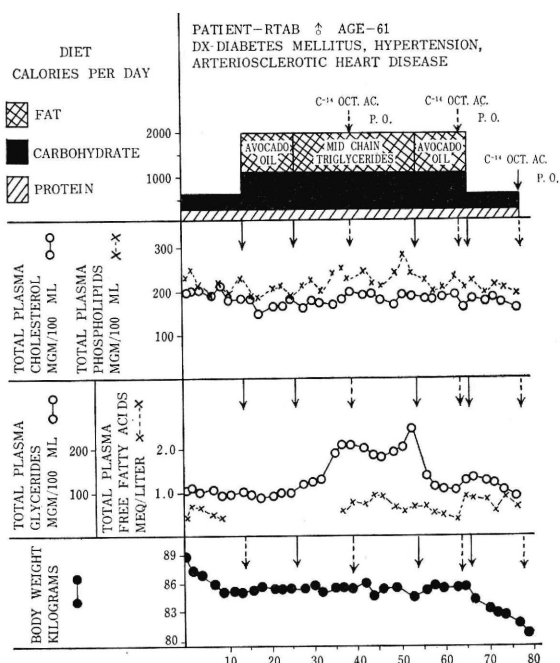


Fig. 1. Dietary intake, plasma lipid levels and body weight of patient RTAB during studies with oral sodium octanoate-1-¹⁴C.

Patient RCOT, a 41-year-old obese Negro male, had hyperglyceridemia, moderate hyperglycemia (without hyperketonemia) and essential hypertension (ca 180/90). Previous studies had demonstrated adequate control of elevated lipids and glucose by moderate calorie limitation. Prior to the period of study, he allegedly consumed an 800 calorie mixed diet. There is serious question as to his actual intake.

Patient LCLA was a 61-year-old Negro female with mild hypothyroidism, adequately controlled with desiccated thyroid. Immediately preceding and during the period of study, she was maintained on a 2000 calorie formula diet containing 45% of calories as fat (peanut oil 50% and butterfat 50%).

RESULTS

The studies were designed to answer certain specific questions which included :

- 1) The appearance and fate of water-soluble radioactivity in the plasma following ingestion of the labeled octanoate; and the identification of such water-soluble material as far as methodology would permit.
- 2) The pattern of chloroform-soluble radioactivity in plasma following the ingestion of labeled octanoate, including the identification and quantitation of lipid classes and specific fatty acids in which radioactivity was found to be present.

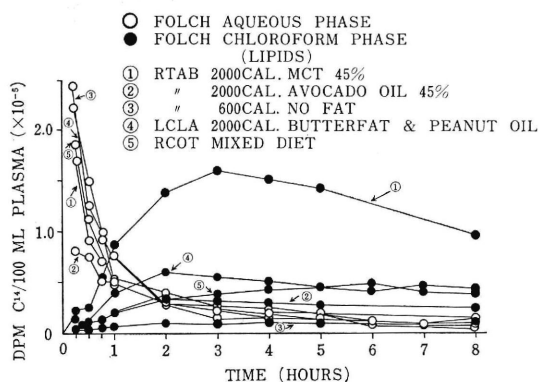


Fig. 2. 1 μ c per body weight (Kg) sodium octanoate-1- 14 C was given orally at zero time during five studies in three subjects. Much greater incorporation of 14 C into the Folch chloroform phase was present in circulating plasma on a MCT diet as compared to other dietary conditions.

3) Specific activity and actual amounts of expired CO_2 following the ingestion of labeled octanoate.

4) Evaluation of the effects of specific diets upon the preceding in one of the subjects studied.

Radioactivity in the Folch Aqueous and Chloroform Phases, Respectively

As shown in Figure 2, considerable radioactivity was present in the Folch aqueous phase 15 minutes after ingestion of the isotope. This decreased rapidly, and after two hours was present in only small quantities in all subjects. The extremely variable concentrations present during the period 15-60 minutes post-ingestion in the individual studies presumably mirror differential absorption rates and initial hepatic metabolism.

Activity in the chloroform phase rose gradually and reached a peak in two to six hours. Except in patient RTAB, during the intake of a formula containing 45% of calories as medium chain triglycerides, plasma chloroform-soluble 14 C concentration was of a relatively low order of magnitude throughout the

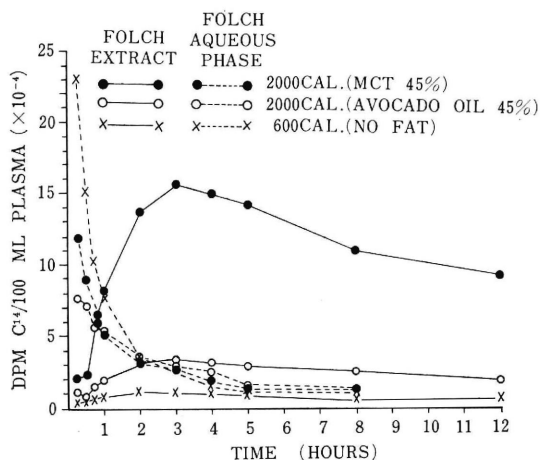


Fig. 3. 1 μ c per body weight (Kg) sodium octanoate-1- 14 C was given at zero time in three studies on one patient (RTAB). Greater incorporation of 14 C into the Folch chloroform phase from plasma was present on a MCT diet than on a calorically equivalent avocado oil diet or low calorie diet. Much more radioactivity was present in the Folch aqueous phase from plasma on a low calorie diet than was the case on other isocaloric diets.

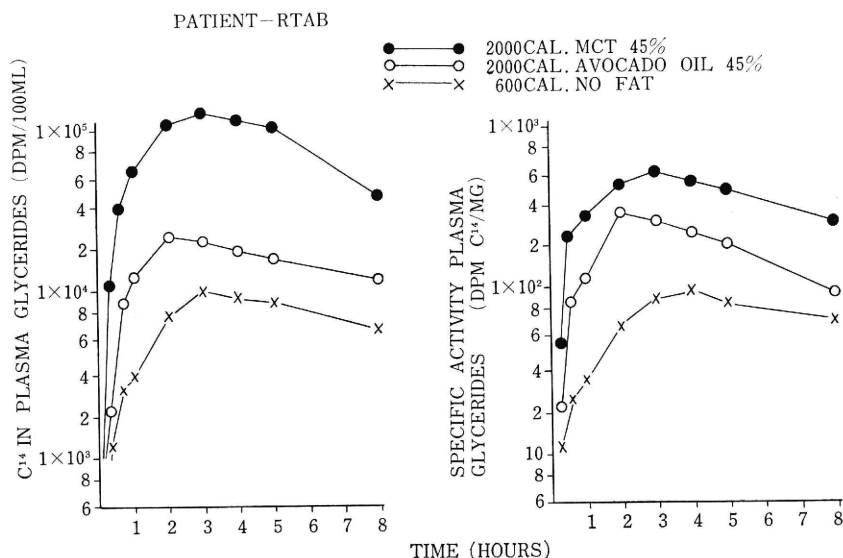


Fig. 4. 1 μ c per body weight (Kg) sodium octanoate-1- 14 C was given orally at zero time during three studies on different diets in a single subject. Much greater incorporation of C^{14} into plasma glycerides was present on a MCT diet as compared to other dietary conditions.

period of study. The lowest concentration was noted under conditions of low calorie intake.

In Figure 3 are shown in more detail the actual concentrations of isotope per 100 ml of plasma in the Folch aqueous and chloroform phases, respectively, in patient RTAB when 14 C-octanoic acid was given orally during the course of each of three dietary programs, namely, 2000 calories (isocaloric) with 45% of total calories as MCT*; 2000 calories with 45% of total calories as avocado oil**; and a 600 calorie fat-free diet.

From Figure 3 it is apparent that the greatest concentration of the isotope in the chloroform phase occurred during the intake of MCT and in the aqueous phase during the low calorie fat-free dietary regimen.

Plasma Glycerides

Incorporation of 14 C into the plasma glycerides of one subject is shown in Figure 4. It is apparent that

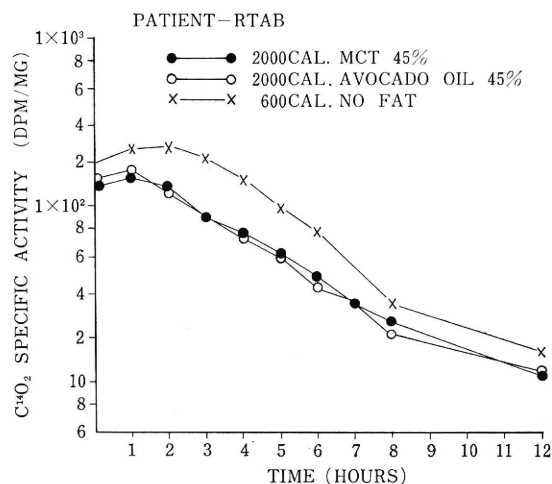


Fig. 5. 1 μ c per body weight (Kg) sodium octanoate-1- 14 C was given orally at zero time in three studies on patient RTAB. Rate of loss of 14 C in expired air on a MCT diet is the same as on a calorically equivalent avocado oil diet. On a low calorie diet, much greater loss of $^{14}CO_2$ was observed. Per cent administered dose expired within the 8-hour study period on 2000 cal MCT was 45%; and on the 2000 cal. avocado oil was 45%.

* Fatty acid composition : C8, 75%; C10, 24%; C14, 1%.

** Fatty acid composition : C18 : 1, 75%; C18 : 2, 10 %; saturates, 15%.

with regard to concentration per unit volume, as well as specific activity, by far the greatest amount of label was present in plasma glycerides during the intake of medium chain triglycerides and the least amount during the 600 calorie fat-free regimen.

Excretion of $^{14}\text{CO}_2$

In Figure 5 are shown the patterns of $^{14}\text{CO}_2$ excretion (as specific activity) on the three dietary program. Essentially the same degree of oxidation occurred during the intake of MCT and avocado oil, but there was a significantly greater contribution of the isotopic material to the total oxidative pool during the period of low calorie intake.

Water-Soluble ^{14}C Activity

Because of the rapid appearance of water-soluble ^{14}C in the plasma, there was some question as to absorption of octanoic acid from the stomach. Accordingly, labeled sodium octanoate was introduced through a tube, the tip of which was in the duodenum. Appearance of activity in the Folch aqueous phase was essentially identical under the two conditions, indicating that most of the octanoic acid is absorbed from the small intestine, or that absorption from stomach and intestine goes on at an essentially equal rate.

None of the radioactivity present in the aqueous phase was demonstrable in the plasma ketones. Therefore, the material present in the aqueous phase presumably is octanoic acid *per se* or fatty acids having a chain length shorter than 8 carbons.

Nature of the Labeled Lipids in the Folch Chloroform Phase

When labeled free octanoic acid was placed in a counting vial and heated on a steam bath for an extended period, all of the material volatilized, as evidenced by lack of any remaining radioactivity.

Following saponification of the lipids present in the Folch chloroform phase and subsequent extraction with petroleum ether, the material was heated for an extended period on a steam bath. All of the radioactivity present originally in the Folch chloroform phase remained after heating, suggesting that the radioactivity was present entirely as long chain fatty acids.

Sixty to eighty percent of isotopic activity present in the chloroform phase of plasma 1 to 5 hours after ingestion of the labeled octanoate was found in the glyceride fraction. The remainder was present almost entirely in cholesterol esters. Negligible amounts of activity were present in the phospholipid and free fatty acid fractions. Preparative gasliquid chromatography of methyl esters prepared from the labeled glyceride fatty acids demonstrated that essentially all of the activity was present in palmitic, stearic and oleic acids (Figure 6). Maximal specific activity occurred in stearic acid, but the greatest amount of activity was present in palmitic acid.

DISCUSSION

From several reports in the literature, it appears to

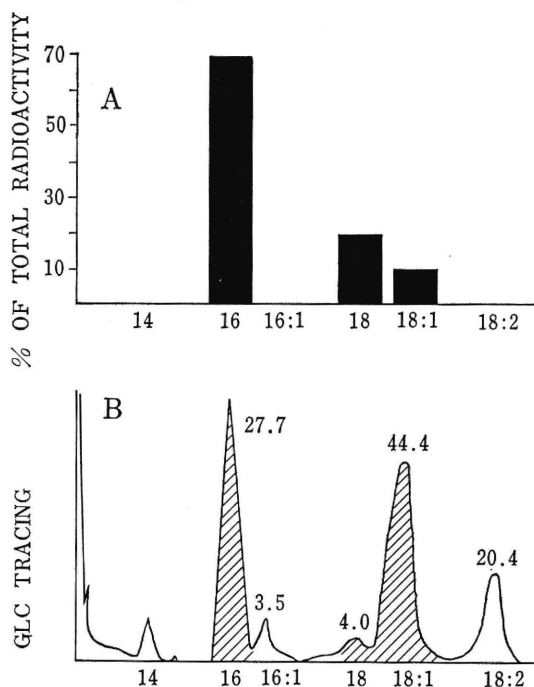


Fig. 6. Composition and ^{14}C content of individual fatty acids of plasma triglycerides of a subject 3-5 hours after oral sodium octanoate- $1\text{-}^{14}\text{C}$. A. Per cent of total glyceride ^{14}C present in individual fatty acids. B. Gas-liquid chromatographic tracing of methyl esters from triglycerides. Figures close to peaks indicate the percentage of the component concerned in the total acid mixture. Shaded peaks contained ^{14}C . Horizontal axis figures refer to specific fatty acids: 16, palmitic; 18, stearic; 18:1, oleic.

be well established that ingested C 8 : 0 and C 10 : 0 fatty acids go directly to the liver via the portal circulation rather than appearing as chylomicronous fat in the thoracic duct (6). Under isocaloric conditions where such facts constitute the major portion of the dietary fat, fasting hyperglyceridemia results (1). It seems not improbable that a cause and effect relationship exists between these two sets of observations.

The present study leaves many questions unanswered but does provide some pertinent information which may be summarized as follows :

1) Octanoic acid is rapidly absorbed from the small intestine. Some of the absorbed acid appears promptly as water-soluble material (presumably octanoic acid) in the circulating plasma. This material disappears rapidly. The initial concentration is to some degree dependent upon the basic nutritional status of the individual. The water-soluble fraction is not acetoacetic acid, betahydroxybutyric acid or acetone.

2) Over a period of several hours following the ingestion of the material, carboxyl carbon is incorporated into long chain fatty acids which appear in significant concentration in the circulating glycerides. Again, the nutritional status of the individual is of importance in determining the quantitative aspects of this series of reactions. Specifically, an isocaloric diet containing large amounts of MCT is associated with maximal concentration of labeled long chain fatty acids in plasma glycerides, whereas a low calorie regimen produces the opposite effect. This appears to indicate that synthesis of long chain from medium chain fatty acids, with subsequent esterification, lipoprotein formation, and secretion into the plasma, is accelerated by previous ingestion of large amounts of MCT.

3) As judged from studies in one patient, oxidation of MCT (or at least of the carboxyl carbon from C 8 : 0) is accelerated under conditions of low calorie, fat-free intake. No difference is observed in oxidation pattern when the maintenance fat is derived from long chain or medium chain triglycerides (at a level of 45 per cent of total calories) so long as the diet is isocaloric.

4) The carboxyl carbon of C 8 : 0 is incorporated to a significant degree into long chain fatty acids (C 16 : 0, C 18 : 0, 18 : 1) which subsequently appear in the circulating glycerides. It is not clear as yet whether C 8 : 0 is broken down to acetate and subsequently resynthesized into long chain fatty acids, or whether some other type of explanation applies.

SUMMARY

Octanoic acid is absorbed rapidly from the small intestine. Some appears promptly in the plasma. A significant amount is incorporated into long chain fatty acids and appears in the circulating glycerides. The latter process is accelerated by previous intake of an isocaloric diet in which all of the fat is derived from MCT. Oxidation of ingested octanoic acid is accelerated when the previous diet has been hypocaloric. Under these conditions, minimal incorporation of label from ingested octanoic acid is found in plasma glycerides.

REFERENCES

1. Uzawa, H., G. Schlierf, S. Chirman, G. Michaels, P. Wood and L. W. Kinsell. Hyperglyceridemia resulting from intake of medium chain triglycerides. *Am. J. Clin. Nutr.* 15 : 365, 1964.
2. Folch, J., M. Lees and G. H. Sloane-Stanley. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226 : 497, 1957.
3. Michaels, G. A Method for the determination of plasma glycerides and free fatty acids. *Metabolism* 11 : 833, 1962.
4. Michaels, G., S. Margen, G. Liebert and L. W. Kinsell. Studies in fat metabolism. I. The colorimetric determination of ketone bodies in biological fluids. *J. Clin. Invest.* 30 : 1483, 1951.
5. Van Slyke, D. D., and J. Plazin. *Micrometric Analyses*. Baltimore : The Williams and Wilkins Co., 1961.
6. Playoust, M. R., and K. J. Isselbacher studies on the intestinal absorption and intramucosal lipolysis of a medium chain triglyceride. *J. Clin. Invest.* 43 : 878, 1964.