Hepatic Metabolism of 1-^{14}C Octanoic and 1-^{14}C Palmitic Acids

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Abstract

The hepatic metabolism of 1-^{14}C octanoic acid was compared with that of 1-^{14}C palmitic acid in male rats which were fed. After intraportal injection only 1/6 to 1/18 as much octanoic acid as palmitic acid was incorporated into hepatic lipids. In contrast, octanoic acid yielded two to four times as much water-soluble product as did palmitic acid. Similar, but even more impressive, differences between the incorporation of these fatty acids into hepatic lipids were observed in liver slices incubated with ^{14}C octanoate and ^{14}C palmitate. The oxidation of octanoate to CO_2 was more than 10 times as great as that of palmitate. With both substrates, triglycerides comprised almost half the labeled lipid recovered. However octanoate yielded a higher proportion of labeled, unesterified fatty acids and a lower proportion of labeled phospholipid and monoglycerides than did palmitate. Most of the ^{14}C recovered in hepatic lipids after incubation with 1-^{14}C octanoate was found in the carboxyl groups of long-chain fatty acids, suggesting that the latter had been synthesized from 2-carbon fragments formed from the oxidation of octanoate. In contrast, only a small fraction of the palmitate was elongated.

The similarities and differences between the metabolism of octanoic and palmitic acid in liver and intestine, and the possible nutritional significance of octanoic acid are discussed.

Introduction

TRIGLYCERIDES containing medium-chain fatty acids and those containing long-chain fatty acids differ with respect to their intestinal absorption and intramucosal metabolism (1–6). Thus long-chain triglycerides (LCT) undergo intraluminal hydrolysis to monoglycerides, fatty acids, and glycerol by pancreatic lipase before absorption, are reesterified to triglyceride within the mucosa, and are then exported as chylomicra via the lymphatics. In contrast, medium-chain triglycerides (MCT), containing principally octanoic and decanoic acids, can be absorbed intact, undergo hydrolysis within the mucosa by a mucosal lipase (4–6), and are then transported to the liver via the portal vein either as a salt or as an albumin complex (1–3).

Little attention has been given to a consideration of possible differences in the metabolism of medium- and long-chain fatty acids once they reach the liver. In the present report, evidence, based on both in vivo and in vitro experiments in the rat, is presented to show that medium-chain and long-chain fatty acids differ also with respect to their metabolism in the liver.

Methods

The male Sprague-Dawley type of albino rats (Charles River Rat Farm, North Wilmington, Mass.), weighing 180–220 g and maintained on Purina Lab Chow, were used in all experiments and were not fasted prior to use.

1-^{14}C octanoic acid and 1-^{14}C palmitic acid (New England Nuclear Corporation, Boston, Mass.) were purified by thin-layer chromatography prior to use. After dilution to the appropriate specific activity with unlabeled fatty acid (Hormel Foundation, Austin, Minn.) the mixture was dissolved in a small volume of diethyl ether and homogenized in a pH 7.4 Krebs-Ringer bicarbonate buffer (1/2 calcium), containing 10% Janel fatty acid-poor bovine albumin (Gallard-Schlesinger Chemical Manufacturing Corporation, Carle Place, New York), until the ether was evaporated.

Experiments in vivo

After anesthetization of rats with Nembutal the portal vein was isolated through a midline incision and injected in 1.0 min with 1.2 μMoles (2.4 μc) of either ^{14}C octanoic or ^{14}C palmitic acid in 1.0 ml of buffer-albumin solution. A 1-ml tuberculin syringe and 23-gauge needle were used for injection, and a pledget of Gelfoam was affixed to the injection site to ensure hemostasis.

Each of the fatty acids was given to a group of five rats. Livers were removed 1,3,5,10, and 15 min after completion of the injection and were then weighed and homogenized in a Waring Blendor with four volumes of Krebs-Ringer bicarbonate buffer. Lipids were extracted by the method of Folch et al. (7). In experiments with octanoic acid, the chloroform-methanol used for extraction contained, as carrier, 2.3 mM octanoic acid and 0.3 mM trioctanoin and was adjusted to pH < 3.0, as previously recommended (4). In experiments with either fatty acid substrate, radioactivity was determined after evaporating the lipid-containing phase to dryness in a rotary evaporator at 40C and redissolving the lipid in hexane. This method yielded recoveries of 88–92% in control albumin solutions containing either ^{14}C octanoic or ^{14}C palmitic acid. Radioactivity in water-soluble products was measured in a dioxane-counting solution (8). In all cases, counting efficiency was determined by a channels ratio method (9).

Experiments in vitro

Immediately after the sacrifice of the rats by decapitation, the liver was removed and sliced with a Stadie-Riggs tissue slicer. Liver slices, weighing 0.4 ± 0.02 g, were incubated in 5 ml of pH 7.4 Krebs-Ringer bicarbonate buffer (1/2 calcium), containing 5% albumin and 3 μMoles of ^{14}C fatty acid substrate. The buffer mixture was gassed with 5% CO_2-95% oxygen for 30 min prior to the introduction of the liver slices and again just prior to affixing the serum stoppers. Incubations were carried out in 25-ml Erlenmeyer flasks, fitted with center wells, in a Dubnoff shaker at 37C.

To measure the conversion of ^{14}C octanoic or ^{14}C palmitic acid to $^{14}CO_2$, the reaction was stopped by transferring the flask to an ice bath and adding 0.2 ml of 10 N H_2SO_4 to the main compartment and 0.5 ml of hyamine hydroxide (Pilot Chemicals Inc.,

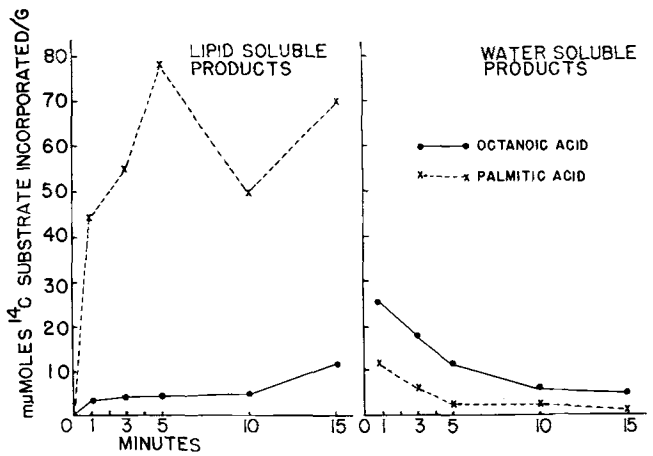

FIG. 1. Incorporation of 1-^{14}C octanoic acid and 1-^{14}C palmitic acid into lipid and water-soluble products of rat liver after intraportal injection.

Watertown, Mass.) to the center well. The flasks were shaken in ice for 30 min, after which the hyamine was transferred to 15 ml of 0.04% Liquifluor in toluene for an assay of radioactivity. Under these conditions $^{14}CO_2$ collection was quantitative, and less than 0.1% of the ^{14}C octanoate was volatilized and taken up by the hyamine. To measure conversion of the fatty acid substrate to lipid and water-soluble products, the reaction was stopped by transferring the flasks to an ice bath, after which the liver slices were removed, washed × 3 in ice-cold distilled water, homogenized with 2.0 ml of Krebs-Ringer bicarbonate buffer at 4C, extracted and assayed for radioactivity, as in the in vivo experiments. In addition, aliquots of the lipid-containing phase were subjected to a) thin-layer chromatography to separate their lipids into classes and to determine the radioactivity in each (10), and b) gas-liquid chromatography (GLC) to analyze the fatty acid composition and isotope content (11). The radioactivity of the carboxyl carbon of the fatty acids was determined, after saponification of the lipid extract, by a modification of the Schmidt reaction (12, 13).

Results

Experiments in vivo

As shown in Figure 1, far less octanoic acid than palmitic acid was incorporated into hepatic lipids

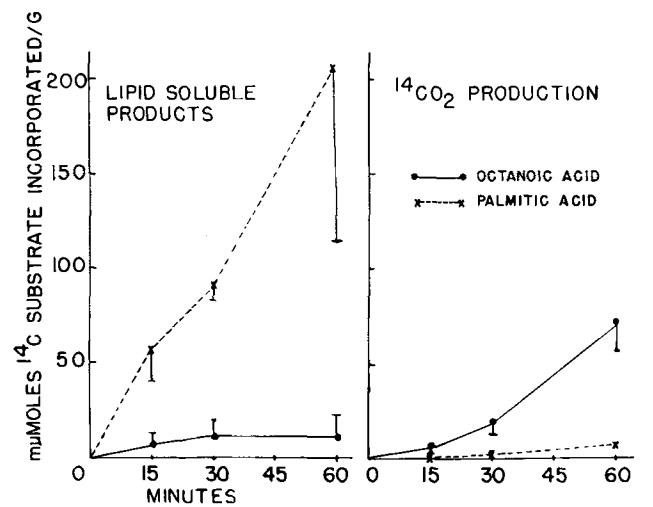

FIG. 2. Incorporation of 1-^{14}C octanoic and 1-^{14}C palmitic acid into lipid-soluble products and $^{14}CO_2$ by rat liver slices. Each point represents the mean ± 1 S.D. of three experiments.

TABLE I

Metabolism of 1-^{14}C Octanoic and 1-^{14}C Palmitic Acid to $^{14}CO_2$ and Lipid-Soluble Products by Liver Slices[1]

Substrate	$^{14}CO_2$	Lipid-soluble products
(3 μMoles)	(mμMoles ^{14}C fatty acid incorporated/g liver slice)	
Octanoic acid	213.7 ± 26.3	14.0 ± 4.3
Palmitic acid	15.0 ± 2.5	307.0 ± 15.0
p value	<0.01 (n = 3)	<0.001 (n = 6)

[1] Incubations were for 2 hr. Results expressed as mean ± SEM.

after the injection of these fatty acids into the portal vein. During the 15-min period of observation the ratio of octanoic:palmitic acid incorporation ranged between 1:6 and 1:18; the difference between the two was evident as early as 1 min after the injection. The amount of labeled lipid in the liver after injection of either fatty acid tended to increase with time, suggesting that, under the conditions of this experiment, repeated passages through the liver were required for extraction of the fatty acids from the plasma.

In contrast to its slower rate of incorporation into hepatic lipids, octanoic acid yielded two to four times more water-soluble material than palmitic acid. After injection of either fatty acid the concentration of labeled water-products in the liver was maximal at 1 min, then fell progressively (Figure 1).

Experiments in vitro

When liver slices were incubated with fatty acids, significantly less octanoic acid than palmitic acid was incorporated into hepatic lipids, as in the in vivo experiments (Figure 2). In contrast, the oxidation of octanoic acid to CO_2 by liver slices was significantly greater than that of palmitic acid. As shown in Table I, a high proportion of the labeled octanoic acid taken up by liver slices during a 2-hr incubation period was oxidized to CO_2, and very little was incorporated into lipid-soluble products. The converse was true of palmitic acid.

The lipid-soluble products formed from octanoic and palmitic acid in this experiment were characterized by separating the lipid classes by TLC (Table II). Triglycerides accounted for approximately half the radioactivity incorporated into the lipids of incubated liver slices. However the other lipid products of these two fatty acids differed in that octanoic acid yielded a higher proportion of unesterified fatty acids and a lower proportion of phospholipids and monoglycerides than did palmitic acid.

The lipid-soluble products were further characterized by GLC of the fatty acid methyl esters which were formed by transmethylation of the lipid extract. This revealed that fatty acids accounted for 92.8 ± 2.0% of the lipid radioactivity in the octanoate experiments and for 96.0 ± 0.4% in the palmitate experiment, indicating that little conversion of these

TABLE II

Lipid-Soluble Products Formed from 1-^{14}C Octanoic and 1-^{14}C Palmitic Acid by Liver Slices[1]

^{14}C Substrate (3 μMoles)	Percentage distribution of total lipid radioactivity		
	Phospholipids and monoglycerides	Unesterified fatty acids	Triglycerides
Octanoic acid (6)	10.3 ± 2.4	38.2 ± 6.9	48.3 ± 4.6
Palmitic acid (6)	31.7 ± 1.7	17.0 ± 1.8	46.3 ± 5.6
p value	<0.001	<0.05	NS

[1] Lipid-soluble products migrating with diglycerides, cholesterol, and cholesterol esters comprised 3.2% ± 1.2 of the lipid-soluble products from octanoic acid and 5.0% ± 0.3 from palmitic acid. Numbers in parenthesis refer to the number of experiments. The same six rat livers were used for these data as were employed for the lipid products data in Table I. Incubations were for 2 hr. Results expressed as mean ± SEM.

TABLE III

Distribution of ^{14}C Among Fatty Acids After Incubating Liver Slices with 1-^{14}C Octanoic or 1-^{14}C Palmitic acid[1]

^{14}C Substrate (3 μMoles)	Fatty acid product					Fatty acid label in carboxyl groups
	<16:0[2]	16:0	16:1	18:0	18:1, 18:3 20:0, 20:4	
	(% Total fatty acid label in each)					(%)
Octanoic acid	17.5 ± 6.4	42.2 ± 8.6	12.5 ± 2.2	4.8 ± 0.5	23.0 ± 5.1	80.1 ± 2.4
Palmitic acid	1.8 ± 0.8	77.4 ± 2.9	12.0 ± 1.7	4.0 ± 0.8	4.8 ± 0.7	96.8 ± 1.1
p value	<0.05	<0.01	NS	NS	<0.05	<0.001

[1] The same six rat livers were used for these data as were employed for the data in Table II. Incubations were for 2 hr. Results expressed as mean ± SEM.

[2] <16:0 refers to fatty acids the carbon chain-lengths of which are less than 16 but greater than six.

substrates to glyceride glycerol, phospholipid bases, or cholesterol had occurred. From the data in Table III it can be seen that, after 2 hr of incubation, less than 10% of the label in ^{14}C palmitic acid was found in hepatic fatty acids of longer chain-length. In contrast, most of the ^{14}C octanoic acid underwent elongation. Of particular note, less than 2% of the fatty acid radioactivity after incubation was recovered in the octanoate fraction. Moreover a large proportion of the label (23%) was found in C18 and C20 unsaturated fatty acids. Since 80% of the ^{14}C in these longer-chain fatty acids which were derived from 1-^{14}C octanoic acid was present in carboxyl groups (Table III), it is highly probable that the octanoate was oxidized to acetate prior to its incorporation into long-chain fatty acids.

Discussion

From the data presented it is evident that, compared with palmitic acid, octanoic acid is a poor substrate for lipid synthesis in the liver but is more readily catabolized to CO_2 and water-soluble products. Similar differences in the metabolism of these two fatty acids have been observed in intestinal slices (5). However their behavior in the liver differs from that in the intestine in several respects. Whereas, in the liver, the bulk of the octanoic acid that is not catabolized to CO_2 and water-soluble products is converted to long-chain fatty acids, little of the octanoic acid that enters the intestinal mucosa is elongated; most of the uncatabolized fraction was recoverable intact and unesterified. Elongation of palmitic acid occurs to a small but significant degree in the liver but almost not at all in the intestinal mucosa. In the liver, comparable fractions of fatty acids derived from octanoic and palmitic acid are incorporated into triglycerides whereas, in the intestine, there is preferential incorporation of fatty acids derived from palmitic acid but not octanoic acid.

Previous studies have shown that excluding the liver from the circulation does not significantly reduce the amount of $^{14}CO_2$ recovered in the expired air of animals given 1-^{14}C octanoic acid (20). This finding suggests that octanoic acid may be preferentially oxidized to CO_2 rather than incorporated into other lipids not only in the liver and intestine but also in other extrahepatic tissues. It is quite possible therefore that octanoic acid, in supporting growth in man (15) and animals (16,17), merely spares carbohydrate and fat by providing caloric energy.

ACKNOWLEDGMENTS

Technical assistance given by Miss Rose Marie Moquin. This study was supported by Grant AM 05966 from the National Institutes of Health.

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