Differences Between the Metabolism of Linoleic and Palmitic Acid: Utilization for Cholesterol Synthesis and Oxidation to Respiratory CO_2[1]

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ABSTRACT

Measurements were made of the incorporation of intragastrically administered 1-C^{14}-labeled linoleic and palmitic acid carbon into the total body cholesterol of the intact rat and of the rat's ability to oxidize these two labeled acids to respiratory CO_2. As compared with palmitic acid, the intact rat and isolated rat tissues exhibit a greater ability to metabolize linoleic acid. This is evidenced by a greater utilization of linoleic acid carbon for synthesis of total body cholesterol and also by a preference for the oxidation of linoleic acid. The greater incorporation of linoleic acid carbon into cholesterol appears to reflect the preferential oxidation of linoleic acid by the liver, a main site of fatty acid oxidation and cholesterol biosynthesis. This preference of the rat to utilize linoleic acid carbon for the synthesis of cholesterol may help to explain the well documented observation that the plasma cholesterol of the rat increases as the linoleic acid content of the diet increases.

INTRODUCTION

In recent years there has been much interest in differences between the metabolism of essential and nonessential fatty acids and particularly in relationships between the fatty acids and the metabolism of cholesterol. Considerable attention has been devoted to relationships between the composition of the dietary fatty acids and the cholesterol content of the plasma (1) and also the composition of the plasma cholesterol esters (1,2). Investigations of this area have revealed that in the rat plasma cholesterol levels increase as the linoleic acid content of the diet increases (1); whereas, in man the circulatory levels of cholesterol decrease in response to an increase in the polyunsaturated fatty acid intake (3). The reasons for these changes are largely unexplained. Since large amounts of acetyl CoA are formed during catabolism of fatty acids, it is conceivable that these substances contribute a significant portion of the total carbon required for the cholesterol biosynthesis and that differences between the degree to which fatty acids furnish carbon for cholesterol synthesis may partially explain the relationships between intake of a particular type of fatty acid and changes in the levels of body cholesterol. In an effort to investigate this possibility and to further study differences between the metabolism of essential and nonessential fatty acids, the present study measures the incorporation of labeled carbon from intragastrically administered 1-^{14}C-linoleic acid, an essential fatty acid, and 1-^{14}C-palmitic acid, a nonessential fatty acid, into the total body cholesterol of the intact rat. In addition, simultaneous measurements were also made on the extent of oxidation of these two labeled fatty acids to respiratory CO_2 in order to provide an index of the rates at which these two fatty acids were generating acetyl-CoA. A preliminary report on these experiments has been presented (4).

EXPERIMENTAL PROCEDURES

Three month old male rats of the Wistar strain, maintained on Purina rat chow, were fed by stomach tube, 1 ml of USP olive oil to which was added 12 μc of either 1-^{14}C-palmitic or 1-^{14}C-linoleic acid, having a specific activity of 1 μc/mg. Since the palmitic acid and linoleic acid content of USP olive oil is essentially identical (5), the specific activity of the 1-^{14}C-palmitate and 1-^{14}C-linoleate absorbed from the intestines should also be essentially identical. At various time intervals after administration of the labeled fatty acids, respiratory CO_2 was collected for a few hours (6) and the animals then killed. Rats were given access to their regular diet during the time period between administration of the labeled fatty acids and collection of respiratory CO_2.

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TABLE I

Per Cent of the Total Absorbed 1-^{14}C-Palmitate and Linoleate Carbon Found Incorporated Into the Total Cholesterol of the Entire Rat, 6, 29 and 77 hr After Feeding 1-^{14}C-Palmitate or Linoleate[a]

Hours Killed	Number of rats	^{14}C-Cholesterol (^{14}C-Palmitate-fed rats)	Number of rats	^{14}C-Cholesterol (^{14}C-Linoleate-fed rats)
6	3	0.266 ± 0.024	3	1.252 ± 0.342
29	4	0.231 ± 0.030	4	0.804 ± 0.222
77	4	0.249 ± 0.067	4	0.800 ± 0.122

[a] Each value is expressed as the arithmetic mean ± 1 SD. The statistical significance of the differences between the incorporation of 1-^{14}C-palmitate and linoleate at each time studied is less than 0.01 (as measured by the Student's "t" test).

Unabsorbed lipid was collected by washing the entire gastrointestinal tract with acidic-aqueous-ethanol followed by petroleum ether and combining these washings with the excreted fecal matter. The unabsorbed lipid fraction and the entire rat were than saponified separately in alcoholic KOH and the fatty acids and colesterol extracted, essentially as described by Van Bruggen et al. (7). The isolated fatty acids were oxidized to CO_2 by wet combustion (8) and converted to baruim carbonate; the cholesterol converted to the digitonide (9), and the respiratory CO_2 precipitated as baruim carbonate. All ^{14}C-containing samples were plated to infinite thickness and counted with the Nuclear Model C110B detector. The total activity of all radioactive samples were calculated by multiplying the specific activity as baruim carbonate multiplied by the amount of carbon in the sample. The amounts of the ^{14}C fatty acids absorbed from the intestines were calculated by subtracting the total ^{14}C recovered in the fecal plus intestinal contents from the total ^{14}C fed. About 60% of both the fed 1-^{14}C-palmitate and linoleate was absorbed by the sixth hour after feeding; about 80% of each was absorbed by the twenty ninth and seventy-seventh hours. The percentage of absorbed ^{14}C fatty acid carbon incorporated into cholesterol in the rat was calculated directly by dividing the total activity of the cholesterol isolated from the whole rat by the total activity of the absorbed ^{14}C-labeled fatty acids multiplied by 100. The percentage of endogenous ^{14}C-labeled fatty acid carbon undergoing conversion to respiratory CO_2 per hour during various time intervals after feeding the labeled fatty acids was calculated as follows: the average-hourly total activity of the respiratory $^{14}CO_2$ collected during the 1 or 5 hr time intervals was divided by the total ^{14}C fatty acid activity recovered from the entire rat, minus intestinal contents, immediately after completion of the respiratory CO_2 collection. This value was then multiplied by 100.

Inasmuch as conversion of ^{14}C-labeled fatty acid carbon to cholesterol and respiratory CO_2 in some instances was measured several days after feeding of the labeled fatty acids, the question arose as to whether the endogenous ^{14}C-labeled fatty acids at such time were still essentially the same as the fed ^{14}C-labeled fatty acids. By chromatographic separation of the endogenous fatty acids into major classes (1), followed by ^{14}C assay (11), it was shown that the ^{14}C-fatty acids derived from the administered labeled palmitate and linoleate were present in the rat predominantly as saturated and diunsaturated fatty acid respectively even 7 days after the fatty acids were administered.

TABLE II

Per Cent of the Total Absorbed 1-^{14}C-Palmitate and Linoleate Carbon Found Incorporated Into the Total Cholesterol of Various Tissue Fractions 6 hr After Feeding These ^{14}C-Labeled Fatty Acids[a]

Fatty acid fed	Number of rats	Carcass[b]	Liver	Gut[c]	Skin[d]	Organs[e]
1-^{14}C-Pal.	3	0.058±0.015	0.085±0.020	0.055±0.025	0.050±0.027	0.017±0.004
1-^{14}C-Lin.	3	0.325±0.147	0.472±0.161	0.208±0.050	0.132±0.071	0.108±0.046

[a] Each value is expressed as the arithmetic mean ± 1 SD.
[b] Carcass refers to the tissue fraction remaining after removal of the liver, gut, skin and organs.
[c] Gut refers to the gastrointestinal tract from the stomach to the anus inclusive.
[d] Skin refers to the entire hide, ie., subcutaneous fat, skin and hair.
[e] Organs refers to the heart, spleen, pancreas, kidneys, adrenals and reproductive system.

TABLE III

Per Cent of the Total Endogenous ^{14}C-Labeled Fatty Acid Carbon Oxidized to Respiratory $^{14}CO_2$ Per Hour by the Intact Rat During Various Time Intervals After Feeding 1-^{14}C-Palmitate or Linoleate[a]

Hours after feeding	Number of rats	$^{14}CO_2$ (^{14}C-Palmitate-fed rats)	Number of rats	$^{14}CO_2$ (^{14}C-Linoleate-fed rats)
5--6	4	10.88 ± 2.99	4	20.94 ± 7.16
24--29	5	2.11 ± 0.51	5	2.43 ± 0.59
48--53	3	0.94 ± 0.10	3	0.79 ± 0.22
72--77	4	0.75 ± 0.49	4	0.80 ± 0.20
168--173	1	0.59	1	0.67

[a] Each value is expressed as the arithmetic mean ± 1 SD. The difference between the oxidation of 1-^{14}C-palmitate and linoleate during the 5 to 6 hr time interval is significant to the 0.05 level (as measured by the Student's "t" test). The differences at the other time intervals are not statistically significant.

RESULTS

The results shown in Table I indicate that at 6, 29 and 77 hr after feeding of the labeled fatty acids, a significantly greater fraction of the absorbed ^{14}C-linoleate than ^{14}C-palmitate carbon was recovered as ^{14}C-cholesterol in the entire rat. For example, after one or three days, about 0.8% of the absorbed ^{14}C-linoleate carbon was present as cholesterol compared to about 0.2% of the absorbed ^{14}C-palmitate. In several additional experiments it was demonstrated that this preferential incorporation of administered linoleate carbon into cholesterol in the entire rat was occurring in all of a large variety of tissues examined following the feeding of the labeled fatty acids (Table II).

The observed differences between the fraction of absorbed linoleate and palmitate carbon converted to cholesterol appears to be the result of events that occurred soon after the labeled fatty acids were absorbed, since as shown in Table I, the concentration of ^{14}C in the cholesterol of the rats fed either labeled palmitate or linoleate remained essentially constant between one and three days after administration of the fatty acids.

The explanation for the preferential incorporation of absorbed linoleate carbon into cholesterol appears related to the observation that there was an accompanying preferential oxidation of ^{14}C-labeled linoleate compared to ^{14}C-labeled palmitate shortly after administration of these fatty acids. This is shown in both Tables III and IV. Between the fifth and sixth hours after feeding the labeled fatty acids, approximately twice as much endogenous ^{14}C-fatty acid carbon was oxidized to CO_2 per hour by the intact, 1-^{14}C-linoleate-fed rats than by the intact, 1-^{14}C-palmitate-fed rats (Table III). In addition, when removed from the rats 6 hr after feeding and incubated in vitro, both the liver and skeletal muscle of these animals showed a preference for oxidation of endogenous linoleate (Table IV). As further equilibration of the labeled fatty acids with the body

TABLE IV

Per Cent of the Endogenous Tissue ^{14}C-Labeled Fatty Acid Carbon Oxidized to Respiratory $^{14}CO_2$ Per Gram of Tissue (Wet Weight)[a,b]

Hours after feeding	Number of rats	$^{14}CO_2$ (^{14}C-Palmitate-fed rats)		Number of rats	$^{14}CO_2$ (^{14}C-Linoleate-fed rats)	
		Liver	Muscle		Liver	Muscle
6	4	1.43 ± 0.41	8.67 ± 2.50	4	2.82 ± 0.23	16.61 ± 3.63
29	2	1.07 ± 0.07	2.29 ± 0.10	2	1.13 ± 0.01	2.83 ± 0.03
77	4	1.24 ± 0.08	1.62 ± 0.56	4	0.98 ± 0.06	2.22 ± 0.83

[a] At 6, 29 and 77 hr after feeding the ^{14}C-labeled fatty acid 1 g samples of liver and skeletal muscle were removed, sliced (Stadie tissue slicer) and incubated in 20 ml of Krebs phosphate buffer (pH = 7.4) for 2 hr at 37 C.

[b] Each value is the mean ± 1 SD. Significantly more endogenous ^{14}C from 1-^{14}C-linoleate than 1-^{14}C-palmitate was oxidized to $^{14}CO_2$ by both liver and muscle at the sixth hour, P (t) $<$ 0.02(as measured by the Student's "t" test).

lipid occurred over a period of a day or more, the fraction of endogenous ^{14}C-fatty acid carbon oxidized to CO_2 per hour in the linoleate-fed rat was similar to that observed in the palmitate-fed rat (Tables III and IV).

DISCUSSION

Since the liver is a primary site of oxidation of fatty acids recently absorbed from the intestines and also a main site of cholesterol biosynthesis, the observed preferential utilization of dietary 1-^{14}C-linoleate carbon for cholesterol biosynthesis by the intact rat could largely represent a preferential catabolism of 1-^{14}C-linoleate by the liver. Indeed, the data indicate that soon after ingestion of 1-^{14}C-linoleic and palmitic acids the liver was oxidizing significantly more 1-^{14}C-linoleic acid carbon than 1-^{14}C-palmitic acid carbon. The greater oxidation of the fed 1-^{14}C-linoleate than 1-^{14}C palmitate by the liver perhaps could reflect a smaller pool of hepatic linoleic acid available to dilute the newly absorbed ^{14}C-linoleate than hepatic palmitic acid to dilute the ^{14}C-palmitate. It is also possible that the enzymes of the β oxidation pathway exhibit a preference for linoleic over palmitic acid as substrate for oxidation.

Irrespective of the mechanism involved, as judged by ability to oxidize linoleic and palmitic acids and to utilize the carbon of these acids as substrate for cholesterol synthesis, the rat exhibits a greater ability to catabolize linoleic than palmitic acid. The greater incorporation of linoleic acid carbon into cholesterol most probably reflects the preferential oxidation of linoleic acid by the intact rat and by the rat liver in particular. Although utilization for cholesterol biosynthesis is a quantitatively minor pathway of fatty acid metabolism, the fact that animals ingest considerable amounts of fatty acids and that cholesterol represents a small fraction of the total body carbon makes it likely that a large fraction of the carbon required for total cholesterol synthesis is derived from fatty acid carbon. Thus, the observed preference of the intact rat to utilize linoleic acid carbon for the synthesis of cholesterol may help to explain the observation of Klein (1) and others (12) that the plasma cholesterol of the rat increases as the linoleic acid content of the diet increases. By comparison, the decrease in plasma cholesterol of humans in response to increased intake of linoleic acid may relate to the observation of Fredrickson and Gordon (13) that the human does not catabolize linoleic acid as readily as palmitic and oleic acids.

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