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The Metabolic Fate of Dietary 1-[^{14}C]Linoleate and 1-[^{14}C]Palmitate in Meal-Fed Rats[1] (40440)

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Dietary polyunsaturated fatty acids have been shown to be more effective than saturated fatty acids in reducing the activity of lipogenic enzymes and the rates of fatty acid synthesis in rat liver (1–8). However, the mechanism whereby dietary polyunsaturated fatty acids exert their greater inhibitory effect has not been elucidated.

The relative uptake of circulating polyunsaturated fatty acids by the liver has been reported to be greater than the uptake of saturated fatty acids (9). Consequently, more dietary fatty acids might reach the liver when rats are fed polyunsaturated fatty acids than when saturated fatty acids are consumed. Polyunsaturated fatty acids have also been reported to be oxidized to respiratory CO_2 at a faster rate than saturated fatty acids (10–13). Long chain acyl CoA derivatives, intermediates in fatty acid oxidation, inhibit fatty acid synthesis (14–17).

The objectives of the present study were: (a) to determine the relative rates of oxidation to respiratory $^{14}CO_2$ of dietary 1-[^{14}C]linoleate and 1-[^{14}C]palmitate, (b) to examine the net accumulation of these fatty acids in liver and epididymal fat pads and (c) to determine the distribution of these fatty acids in various lipid classes in the liver of rats fed a single meal containing the labelled fatty acids. To relate the results of the present experiments with previous studies on dietary fatty acids and fatty acid synthesis we elected to utilize the same general experimental design as utilized in investigations on the influence of dietary linoleate and palmitate on rates of fatty acid synthesis and on activities of associated enzymes in rats (6–8).

Materials and methods. Male Sprague-Dawley rats[2] were housed individually in stainless steel cages and had free access to water. Temperature in the room was 22 ± 1° and the lights were on from 7 AM to 7 PM. Rats had access to food from 0900 to 1100 hr daily. The composition of the fat-free basal diet has been presented (7). The dietary protocol for all five experiments is presented in Table I.

In experiments I and II rats were fed a single 45 minute meal containing labelled fatty acids (phase 3 of dietary protocol) and were then placed in metabolic chambers to collect respired $^{14}CO_2$. Each rat received 10 g of the fat-free basal diet and 300 mg methyl linoleate or 700 mg methyl palmitate.[3] The rats were fed only 10 g of the fat-free diet to ensure that the entire amount fed would be consumed. More palmitate than linoleate was added to the diet in an attempt to equalize the amount of the two fatty acids absorbed (7). Tracer doses of 1-[^{14}C]linoleate and 1-[^{14}C]palmitate[4] were thoroughly mixed with the unlabelled fatty acids immediately prior to feeding. Thus, the specific activity (dpm ^{14}C/mg fatty acid) of the dietary fatty acid was known. Rats in experiment I were fed 10 g of the fat-free basal diet 24 hr after feeding the diet containing the labelled fatty acids and were killed 4 hr later. Rats in experiment II were killed either 8 or 24 hr after consuming the single meal containing the labelled fatty acids.

In experiment III rats were fed a single meal containing the labelled fatty acids without the fat-free basal diet. The fatty acids (300 mg methyl linoleate or 700 mg methyl palmitate) were mixed with an equal weight of cellulose and fed. Respired $^{14}CO_2$ was collected for 8 hr.

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[2] Spartan Research Animals, Haslett, Michigan.

[3] 99% purity, Sigma Chemical Company, St. Louis, Missouri.

[4] >97% radiochemical purity, Amersham/Searle Corporation, Arlington Heights, Illinois.

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TABLE I. DIETARY PROTOCOL.

Experiment	Phase 3[a]
I	Meal-fed 10g fat-free diet plus [^{14}C]-fatty acid; meal-fed 10g fat-free diet 24 hr later
II	Meal-fed 10g fat-free diet plus [^{14}C]-fatty acids
III	Meal-fed [^{14}C]fatty acids
IV	Meal-fed 15g fat-free diet plus unlabelled fatty acids for 6 meals; meal-fed 10g fat-free diet plus [^{14}C]fatty acids for 1 meal
V	Meal-fed 10g fat-free diet plus [^{14}C]-fatty acids

[a] All rats were meal-fed the basal fat-free diet (7) supplemented with 2% corn oil for 10–14 days (phase 1). The rats were then meal-fed the fat-free basal diet for an additional 6 days (phase 2).

Consumption of the fat-free diet for 6 days immediately prior to the meal containing the labelled fatty acids might influence the metabolism of the labelled fatty acids. Consequently, in experiment IV rats were fed six meals, in which 450 mg methyl linoleate or 1050 mg methyl palmitate were added to 15 g of the fat-free diet. Tracer doses of 1-[^{14}C]-linoleate and 1-[^{14}C]palmitate were added to the seventh meal which contained 300 mg methyl linoleate or 700 mg methyl palmitate and 10 g of the fat-free diet. Respired $^{14}CO_2$ was collected for 24 hr.

The last experiment was conducted to determine the distribution of the labelled fatty acids in various lipid classes in the liver of the rats. Rats were fed a single meal (10 g fat-free diet plus 300 mg methyl linoleate or 700 mg methyl palmitate) containing the labelled fatty acids. The animals were killed 24 hr later.

The amount of dietary fatty acid absorbed was determined in each experiment. When animals were killed gastrointestinal contents were removed. Lipids were extracted from the gastrointestinal contents and from the feces; radioactivity in the lipid was then quantitated. Based on the specific activity of the fatty acids fed and on the recovery of radioactivity in the gastrointestinal contents and in the feces, the amount of each fatty acid absorbed was determined.

Respired $^{14}CO_2$ was collected by placing individual rats in metabolic chambers. Room air was passed through a soda lime chamber, through the metabolic chamber and then into a series of traps containing 0.5 *N* NaOH to collect the respired CO_2. Aliquots of the NaOH solution were removed at timed intervals to quantitate radioactivity in a liquid scintillation counter.

The accumulation of [^{14}C]fatty acids in liver, in epididymal fat pads and in the remaining carcass was estimated. Tissues were homogenized in an equal weight of water. Aliquots of the homogenate were saponified. Nonsaponifiable lipids were extracted with petroleum ether. The solution was then acidified and the fatty acids were extracted with petroleum ether (7). The amount of radioactivity in the fatty acids was then quantitated in a liquid scintillation counter.

Liver lipids were extracted with chloroform-methanol (2:1) and separated by thin layer chromatography on silica gel G plates. The solvents were petroleum ether, diethylether and glacial acetic acid (8–20–1). The plates were scraped and the radioactivity in each lipid class was determined by liquid scintillation counting.

Significant treatment effects were determined by the student's *t* test (18).

Results. The fraction of the absorbed dietary fatty acids oxidized to respiratory $^{14}CO_2$ was determined. Within 24–28 hr after feeding a single high-carbohydrate meal containing labelled fatty acids, 23–27% of the radioactivity was recovered in the respired CO_2 (Tables II and III). Both dietary linoleate and dietary palmitate were oxidized to a similar extent. When the rats received seven meals containing the fatty acids (only the last meal contained labelled fatty acids) comparable results were obtained (Table IV). Thus, the oxidation of absorbed dietary linoleate and palmitate occurred at similar rates regardless of whether the six preceding meals contained fatty acids or were fat-free.

The time sequence of $^{14}CO_2$ expiration following consumption of the labelled fatty acids is described in Fig. 1 and Table III. During the first 8–10 hr after consuming the high-carbohydrate meal the oxidation of the dietary fatty acids occurred at a low rate. The rate of absorbed dietary fatty acid converted to respired $^{14}CO_2$ progressively increased from the 10th to the 14th-hr after the meal and then remained elevated until the rats were fed a fat-free diet 24 hr later (Fig. 1). At this time the rate of $^{14}CO_2$ expiration

decreased rapidly. Initially, oxidation of 1-[^{14}C]palmitate occurred at a faster rate than did oxidation of 1-[^{14}C]linoleate; however, between 14 and 24 hr after the meal this observation was reversed (Fig. 1). Consequently, during the entire 24-hr observation period the cumulative oxidation of the absorbed dietary fatty acids was not influenced by the composition of the fatty acids fed.

TABLE II. EFFECTS OF ONE MEAL OF A FAT-FREE DIET CONTAINING [^{14}C]LINOLEATE OR [^{14}C]PALMITATE ON RESPIRED $^{14}CO_2$ AND ON THE ACCUMULATION OF ^{14}C-FATTY ACIDS IN LIVER AND EPIDIDYMAL ADIPOSE TISSUE OF RATS. (Exp. I—28 hr).[a]

Parameter	Dietary fatty acid	
	3% $C_{18:2}$	7% $C_{16:0}$
[^{14}C]fatty acid absorbed, mg	289 ± 1	358 ± 23
[^{14}C]fatty acid oxidation, %[b]	27.1 ± 1.3	23.3 ± 2.1
[^{14}C]fatty acid in liver, %[b]	4.8 ± 0.3	2.7 ± 0.2[d]
[^{14}C]fatty acid in fat pads, %[b]	3.7 ± 0.3	2.7 ± 0.3
Total ^{14}C recovery, %[c]	86 ± 3	72 ± 4[d]

[a] Mean ± SEM for seven rats weighing 231 ± 5 g; livers weighed 12.8 ± 0.3 g and fat pads weighed 2.6 ± 0.2 g. Rats had consumed 16 ± 1 g of the fat-free diet per day for 6 days prior to receiving a single meal containing the [^{14}C]labelled fatty acids.

[b] Expressed as a percent of the [^{14}C]fatty acid absorbed.

[c] The sum of total respired ^{14}C and carcass (including liver and epididymal fat pads) [^{14}C]fatty acids divided by total ^{14}C absorbed.

[d] Significantly different from value obtained when rats consumed 3% $C_{18:2}$ ($P < 0.05$).

Concomitant consumption of the high-carbohydrate diet might alter the relative rates of linoleate and palmitate oxidation. Thus, rats were also fed the fatty acids with cellulose as a carrier (Table III-experiment III). As expected, the rate of oxidation of the absorbed fatty acids was severalfold higher than that observed when the fatty acids were consumed with the high-carbohydrate meal. There were no differences in the rate of oxidation between the two fatty acids fed.

TABLE IV. EFFECTS OF 7 MEALS OF A FAT-FREE DIET CONTAINING METHYLLINOLEATE OR METHYL PALMITATE WITH THE LAST MEAL CONTAINING [^{14}C]FATTY ACIDS ON RESPIRED $^{14}CO_2$ AND ON ACCUMULATION OF [^{14}C]FATTY ACIDS IN LIVER AND EPIDIDYMAL ADIPOSE TISSUE OF RATS. (Exp. IV-24 hr)[a].

Parameter	Dietary fatty acid	
	3% $C_{18:2}$	7% $C_{16:0}$
[^{14}C]fatty acid absorbed, mg	280 ± 3	333 ± 16[d]
[^{14}C]fatty acid oxidized, %[b]	24.7 ± 0.8	23.1 ± 2.0
[^{14}C]fatty acid in liver, %[b]	4.9 ± 0.5	2.8 ± 0.3[d]
[^{14}C]fatty acid in fat pads, %[b]	4.6 ± 0.3	3.6 ± 0.4
Total ^{14}C recovery, %[c]	93 ± 2	89 ± 3

[a] Mean ± SEM for 10 rats weighing 265 ± 3 g; livers weighed 13.2 ± 0.3 g and fat pads weighed 3.5 ± 0.2 g. Rats had been meal-fed a fat-free diet for 6 days prior to the experiment.

[b] Expressed as a percent of the [^{14}C]fatty acid absorbed.

[c] See Table II.

[d] Significantly different from value obtained when rats were fed 3% $C_{18:2}$ ($P < 0.05$).

TABLE III. EFFECTS OF CONSUMING [^{14}C]LINOLEATE OR [^{14}C]PALMITATE WITH OR WITHOUT THE FAT-FREE BASAL DIET ON RESPIRED $^{24}CO_2$ AND ON ACCUMULATION OF [^{14}C]FATTY ACIDS IN LIVER AND EPIDIDYMAL ADIPOSE TISSUE OF RATS.[a]

Parameter	Exp. II-Fed fat-free basal diet plus fatty acids				Exp. III-fed fatty acids only	
	8 hr		24 hr		8 hr	
	3% $C_{18:2}$	7% $C_{16:0}$	3% $C_{18:2}$	7% $C_{16:0}$	300 mg $C_{18:2}$	700 mg $C_{16:0}$
[^{14}C]fatty acid absorbed, mg	176 ± 12	211 ± 14[d]	276 ± 4	282 ± 10	212 ± 13	378 ± 23[d]
[^{14}C]fatty acid oxidized, %[b]	3.9 ± 0.7	7.0 ± 1.1[d]	26.1 ± 1.8	25.6 ± 1.4	34.8 ± 3.2	34.8 ± 1.7
[^{14}C]fatty acid in liver, %[b]	6.7 ± 2.1	7.0 ± 1.4	5.3 ± 0.3	3.0 ± 0.3[d]	4.7 ± 0.4	3.2 ± 0.3[d]
[^{14}C]fatty acid in fat pads, %[b]	3.9 ± 0.8	4.3 ± 0.8	5.1 ± 0.4	2.2 ± 0.3[d]	ND	ND
Total ^{14}C recovery, %[c]	87 ± 7	80 ± 5	91 ± 3	82 ± 5	ND	ND

[a] Mean ± SEM for six rats weighing 244 ± 3 g (Exp. II) or eight rats weighing 221 ± 4 g (Exp. III). All rats had consumed 17 g of a fat-free basal diet daily for 6 days prior to the experiment. In exp. II rats were fed the fat-free basal diet plus the labelled fatty acids whereas in exp. III the rats consumed the labelled fatty acids which had been mixed with a small amount of cellulose. ND = not determined.

[b] Expressed as percent of the ^{14}C fatty acids absorbed.

[c] See Table II.

[d] Significantly different from value obtained when rats consumed $C_{18:2}$ ($P < 0.05$).

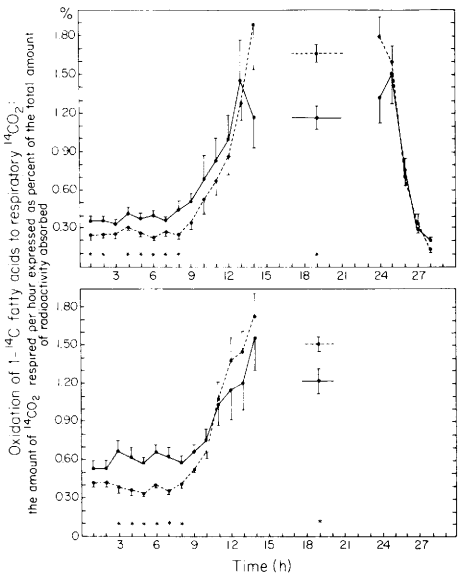

FIG. 1. Time sequence of fatty acid oxidation to respired $^{14}CO_2$ in the rat. The top panel represents data from experiment I and the lower panel represents data from experiment IV. Oxidation of linoleic acid is indicated by the dashed line and oxidation of palmitic acid is indicated by the solid line. Samples were obtained hourly for the first 14 hr. The total respired $^{14}CO_2$ was collected between the 14th and 24th hours; the average rate of $^{14}CO_2$ expired per hour during this period is presented. Each point represents the mean ± SEM for 7 or 10 rats. The asterisks indicate significant differences between the rate of linoleate and palmitate oxidation ($P < 0.05$).

Accumulation of radioactivity in fatty acids in liver and epididymal fat pads was determined. Increased retention of [^{14}C]fatty acids occurred in livers of rats fed 1-[^{14}C]linoleate rather than 1-[^{14}C]palmitate (Tables II–V). One exception occurred; after 8 hours similar amounts of radioactivity accumulated in livers of rats fed linoleate and palmitate (Table III-experiment II). Epididymal fat pads retained similar amounts of radioactivity in fatty acids regardless of the fatty acid fed (Tables II to IV). Again, one exception occurred; after 24 hr rats fed 1-[^{14}C]linoleate retained more radioactivity in their fat pads than did rats fed 1-[^{14}C]palmitate (Table III–experiment II).

A greater percentage of the radioactivity was recovered in hepatic phospholipids when 1-[^{14}C]linoleate was fed than when 1-[^{14}C]palmitate was consumed (Table V). Recovery of radioactivity in hepatic triglycerides was not influenced by the source of fatty acid fed; whereas an increased fraction of the radioactivity was recovered in cholesterol and nonesterified fatty acids when 1-[^{14}C]palmitate was fed.

TABLE V. EFFECT OF ONE MEAL OF A FAT-FREE DIET CONTAINING [^{14}C]PALMITATE ON DISTRIBUTION OF [^{14}C]FATTY ACIDS IN VARIOUS LIPID CLASSES IN RAT LIVER. (Exp. V-24 hr)[a].

Parameter	Dietary fatty acid	
	3% $C_{18:2}$	7% $C_{16:0}$
[^{14}C]fatty acid absorbed, mg	279 ± 2	317 ± 14
[^{14}C]fatty acid in liver, %[b]	4.9 ± 0.3	2.9 ± 0.2[d]
^{14}C in lipid classes, %[c]		
Phospholipids	78.5 ± 1.2	60.8 ± 2.6[d]
Free cholesterol	2.2 ± 0.2	6.9 ± 0.8[d]
Cholesterol esters	2.1 ± 0.2	6.9 ± 1.1[d]
Nonesterified fatty acids	1.7 ± 0.2	7.8 ± 1.2[d]
Triglycerides	14.9 ± 1.2	16.8 ± 0.7
Recovery of ^{14}C from TLC plate, %	92 ± 4	92 ± 6

[a] Mean ± SEM for eight rats weighing 199 ± 5 g. Rats consumed 15 g of a fat-free basal diet per day for 6 days prior to receiving a single meal containing [^{14}C]labelled fatty acids.

[b] Expressed as percent of the [^{14}C]fatty acid absorbed.

[c] Percent of total activity recovered from the TLC plate in each lipid class.

[d] Significantly different from value obtained when rats consumed 3% $C_{18:2}$ ($P < 0.05$).

Discussion. The results of the present study demonstrate that absorbed dietary linoleate and palmitate are oxidized to respiratory $^{14}CO_2$ with equal efficiency in meal-fed rats consuming a high-carbohydrate diet containing approximately 8% of absorbed energy from fatty acids. In other experiments tracer doses of radioactive fatty acids were mixed with olive oil, which contained approximately similar amounts of both fatty acids under study, and then fed to rats (12). In these studies 1-[^{14}C]linoleate was oxidized to respired $^{14}CO_2$ faster than was 1-[^{14}C]palmitate during the first several hours after feeding, but these differences were not apparent later. It is possible that the labelled fatty acids were absorbed before the triglyceride fatty acids and that the initial results reflect the dilution of the newly absorbed [^{14}C]linoleate in a smaller body pool of linoleate than the dilution of [^{14}C]palmitate in the body pool of palmitate. In the present report a similar experiment (rats consumed fat in the absence of other nutrients) was conducted except that

the dietary triglycerides were replaced by methyl esters of the fatty acids. Under these conditions the same amount of respired $^{14}CO_2$ was recovered when 1-[^{14}C]palmitate was fed as when 1-[^{14}C]linoleate was consumed (Table III).

Tracer doses of radioactive fatty acids have also been injected into rats to obtain estimates of rates of fatty acid oxidation (11, 13). More respired $^{14}CO_2$ was collected when the unsaturated fatty acids were injected; therefore, it was concluded that polyunsaturated fatty acids were oxidized at a faster rate than saturated fatty acids. Differential dilution of the label by the endogenous pools of the fatty acids makes interpretation of these results difficult. Nestel and Barter (19) have shown that the fractional turnover of linoleic acid was approximately 35% faster than the fractional turnover of palmitic acid in humans, but the pool of linoleate was considerably smaller than the pool of palmitate. Consequently, the total turnover (umoles per minute) of the unsaturated fatty acid was actually 38% less than the turnover rate of the saturated fatty acid.

The rats retained more label in hepatic fatty acids when the polyunsaturated fatty acid was fed than when the saturated fatty acid was fed. These results are in agreement with experiments which have demonstrated that the liver has a greater affinity for linoleate than for palmitate (9). In agreement with the present results, others (20, 21) have also shown a greater incorporation of linoleate than of palmitate into hepatic phospholipids. Musch and coworkers (5) have suggested that polyunsaturated fatty acids might exert a specific action on lipogenic enzymes by altering membrane lipid properties.

Summary. The relative rates of oxidation to respiratory $^{14}CO_2$ of dietary 1-[^{14}C]linoleate and 1-[^{14}C]palmitate in rats fed a single meal containing the labelled fatty acids were investigated. The oxidation of the dietary linoleate and palmitate, expressed as a fraction of the radioactivity absorbed, occurred at similar rates. Significantly more [^{14}C]linoleate than [^{14}C]palmitate accumulated in the livers of the rats and accumulation of label in hepatic phospholipids was greater when [^{14}C]linoleate was fed than when [^{14}C]palmitate was fed.

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