Dietary Linoleic, α-Linolenic and Oleic Acids Are Oxidized at Similar Rates in Rats Fed a Diet Containing These Acids in Equal Proportions

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The objective of this study was to examine whether whole body oxidation rates of dietary linoleic, α-linolenic and oleic acids differ when the acids are provided in identical quantities. Male rats were fed for 10 wk a 15% fat (w/w) diet containing equal amounts of linoleic, α-linolenic and oleic acids (22.7, 23.0 and 23.2% of total fatty acids, respectively). At week 10, after overnight fasting, rats were intragastrically administered 20 μCi of either [1-^{14}C]-labelled linoleic, α-linolenic or oleic acid in a 200-μL bolus of oil containing equal quantities of each fatty acid. The appearance of $^{14}CO_2$ in expired air was then monitored hourly for 12 h for each animal. A preliminary study had shown that growth and food consumption patterns in animals consuming the oil containing equal quantities of each of the fatty acids paralleled the patterns of animals that were self-selecting among separate diets, each of which contained one of the component oils. The appearance of ^{14}C, expressed as percent dose administered, peaked at 2–3 h post-dose for ^{14}C-labelled linoleic (5.28 ± 0.37%/h), α-linolenic (6.92 ± 0.51%/h) and oleic (5.98 ± 0.44%/h) acids. Statistically these values were not significantly different. Cumulative $^{14}CO_2$ excretion rates over 12 h were also similar for linoleic (27.2 ± 0.9%), α-linolenic (26.8 ± 1.2%) and oleic (25.9 ± 1.2%) acids. The results suggest that the rat's capacity to oxidize 18-carbon unsaturated fatty acids is not affected by fatty acid unsaturation when these fatty acids are provided at equal dietary levels.

***Lipids* 29, 491–495 (1994).**

A number of previous studies have indicated that disposal of dietary fat for energy rather than storage depends on the long-chain fatty acid composition of the fat (1–11). Respiratory gas exchange data obtained in animal experiments suggested that greater peroxisomal oxidation (1) and oxygen consumption (2) occurs with consumption of fats rich in polyunsaturated fatty acids (PUFA) compared with fats containing monounsaturated (MUFA) or saturated fatty acids (SAFA). Similarly, energy intake/balance experiments have generally shown that consumption of n-3 PUFA results in lesser weight and energy gains when compared with fats containing other fatty acids (3–5).

Discrimination between various fatty acids in the oxidation process has also been suggested from animal and human experiments with tracer-labelled fatty acids (6–11). In rats fed normal chow diets and given ^{14}C-labelled long-chain fatty acids orally, differences between oxidation rates for α-linolenic acid, oleic acid and linoleic acid were seen over the first 8 h of a 24 h study, with α-linolenic acid being converted to carbon dioxide most rapidly, followed by oleic acid and linoleic acid (6). Arachidonic and γ-linolenic acid exhibited the lowest rates of oxidation. Other studies with labelled fatty acids suggested a similar trend of PUFA toward oxidation rather than retention within body pools (7–10). In humans, expired $^{13}CO_2$ from labelled oleic acid after oral administration appeared more rapidly than from labelled linoleic or stearic acids (11). The general consensus that emerged from these studies is that the blend of fatty acids consumed, which depends on the type of dietary fat or oil selected, can modify an individual's susceptibility to long-term body weight gain.

However, limitations exist in the interpretation of the results from previous experiments when one compares tracer appearance rates in expired CO_2 derived from labelled dietary fatty acids. A major difficulty has often been the vastly different quantity of specific fatty acids consumed. For instance, tracer fatty acids were typically added directly to dietary olive oil (6), or lard and corn oil followed by fasting (7), or were transesterified directly with soybean (8), olive (9) or corn oil (10). Also, tracer fatty acids were added directly to chow diets. Such diets contain, of course, widely varying pools of specific fatty acids. Thus, the quantities of tracee (i.e., the unlabelled fatty acid pool with which the tracer fatty acid became associated varied between experiments and fatty acids tested. Differences in the tracer/tracee ratio imply that it may be erroneous to conclude preferential fatty acid oxidation on the basis of a greater proportion of label excreted, if the tracer was mixed with a fatty acid pool of different size. Moreover, endogenous pools of specific fatty acids which are targeted for oxidation will likely be depleted or expanded in response to the exogenous fatty acid blend delivered by feeding the diet. For these reasons, interpretation of results from many of the earlier tracer studies has remained difficult. Various approaches aimed at controlling the tracer/tracee ratio for specific fatty acids at the time of label administration have previously been used (12); however, animals previously have not been prefed a diet with which internal body pool sizes could be standardized.

To permit the study of the oxidation capacity for specific dietary fatty acids under identical conditions, an approach was chosen here in which equivalent quantities of three important dietary fatty acids, differing in unsaturation, were provided during both the precedent dietary and the comparison phases. The objective was to test the hypothesis that when provided in equal amounts, the capacity of the rat to oxidize dietary linoleic (18:2n-6), α-linolenic (18:3n-3) and oleic acids (18:1n-9) is similar and independent of the degree of unsaturation.

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Abbreviations: FAME, fatty acid methyl esters; GLC, gas–liquid chromatography; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids.

MATERIALS AND METHODS

Animals and diets. Thirty male Sprague Dawley rats (80–100 g; Harlan Sprague Dawley, Indianapolis, IN) were initially fed rodent Purina Laboratory Chow (Purina Mills, St. Louis, MO) and water *ad libitum*. Animals were housed at constant temperature (20°C) and humidity (54–56%) at a 12-h light–dark cycle throughout the experiment. After one week, rats were individually housed in steel cages and switched to an elemental diet containing 15% fat blended to contain equal proportions of linoleic, α-linolenic and oleic acids as triglycerides for ten weeks (Table 1). All oils were obtained from local sources. The equal proportion oil diet was prepared by blending sunflower, flax and olive oil, high in linoleic, α-linolenic and oleic acids, respectively, at a ratio of 1.11:3.00:0.92 (w/w/w). Within individual oils, weight ratios of linoleic, α-linoleic and oleic acids for sunflower, flax and olive oil were 0.456:0.007:0.153, 0.179:0.379:0.180, and 0.116:0.008:0.479, respectively. Overall fatty acid composition of the oil provided was 18:2n-6, 22.69%; 18:3n-3, 23.24%; 18:1n-9, 23.16%; 18:0, 2.48%; and 16:0, 5.88%. Butylated hydroxytoluene was added at a level of 0.02%, and diets were prepared every two to three days. Oils were stored at –5°C. The fatty acid ratio in the final diet was determined by gas–liquid chromatography (GLC). All procedures were approved by the Animal Care Experimentation Committee of the University of British Columbia (Vancouver, British Columbia, Canada).

Fatty acid oxidation. After ten weeks on the blended-oil diet, rats were randomized into three groups on the basis of weight. Food was removed eight hours prior to the experiment. Animals in each group were administered 20 μCi each of either [1-^{14}C]linoleic, α-[1-^{14}C]linolenic or [1-^{14}C]oleic acid (50–60 mCi/mmol; Amersham, Oakville, Ontario, Canada) mixed with 200 μL of the blended oil and given intragastrically *via* an oral catheter. Immediately thereafter animals were placed in a metabolic chamber, and $^{14}CO_2$ was collected for 10 min every hour for 12 h. Carbon dioxide from the metabolic chamber was collected by passing the air from the chamber through a sulfuric acid trap for drying, followed by two collection columns containing a mixture of methoxyethanolamine and ethanolamine (2:1, vol/vol) (6). Samples from the mixture were removed after each collection period and added to scintillation fluid. Radioactivity was assayed using a scintillation counter (Isocap; Nuclear Chicago, Des Plaines, IL).

The trapping efficiency of the methoxyethanolamine/ethanolamine mixture used to collect the CO_2 exhausted from the metabolic chamber was examined in a pilot study. It was determined that the saturation point of the mixture occurred at least 3 min after the 10-min collection period, thus ensuring $^{14}CO_2$ collection without losses.

Gas chromatography. The fatty acid composition of individual oils and of the blended oil diet was determined using a gas–liquid chromatograph (model 5890, Hewlett Packard, Palo Alto, CA) equipped with a flame-ionization detector and a 30 m × 0.2 μm i.d. SP-2330 (10%) capillary column using helium as carrier gas (1 mL/min). Fat was extracted from homogenized aliquots of the blended oil diet using chloroform/methanol (2:1, vol/vol), and the extracts were saponified with KOH and then transesterified with boron trifluoride/methanol (13). Hexane was added, and the mixture was shaken to partition the fatty acid methyl esters (FAME) into the hexane phase. The resulting FAME were injected into the GLC column at 160°C. After 8 min, the column temperature was increased at 2°C/min for 30 min then held constant for an additional 20 min. Chromatographic peaks were identified by comparison of retention data with those of authentic FAME standards (Supelco, Bellefonte, PA).

TABLE 1

Composition of Diet Containing Equal Levels of Linoleic, α-Linolenic and Oleic Acids

Nutrient	Content (g/100 g diet)
Casein	18.00
Sucrose	62.77
Fixed ratio oil[a]	15.00
DL-Methionine	0.23
Mineral mixture[b]	4.00
Vitamin mixture[c]	—[c]

[a]Oil contained equal proportions of linoleic, α-linolenic and oleic acids by combining sunflower, flax and olive oil in a ratio of 1.11:3:0.92, respectively.

[b]Contained calcium carbonate, 38.14%; copper sulfate · $5H_2O$, 0.048%; ferrous sulfate · $7H_2O$, 2.70%; magnesium sulfate · $7H_2O$, 5.73%, manganese sulfate · H_2O, 0.45%; potassium phosphate monobasic (KH_2PO_4), 38.90%; potassium iodide, 0.079%; sodium chloride, 13.93%; zinc chloride, 0.026%; and cobalt chloride · $6H_2O$, 0.002%.

[c]Contained (in g/kg of diet): vitamin A (50,000 IU/g), 0.040; vitamin D (85,000 IU/g), 0.0026; vitamin E (25,000 IU/g), 0.44; choline chloride, 1.65; menadione, 0.049; *p*-aminobenzoic acid, 0.111; nicotinic acid, 0.10; riboflavin, 0.022; pyridoxine hydrochloride, 0.022; thiamin hydrochloride, 0.022; calcium pantothenate, 0.066; biotin, 0.0004; folic acid, 0.002; inositol, 0.111; and vitamin B_{12}, 30 mg.

RESULTS

An initial study was carried out to test the palatability of the blended oil diet compared to diets containing the individual composite oils. Groups of rats (n = 5) consumed either the 15% (w/w) blended oil diet (Table 1) or chose between three food cups containing diets identical in macronutrient content with fats added either as sunflower, flax or olive oil. Figure 1 depicts the growth curves of groups fed both the blended oil and the three optional diets over a 22-d period. Body weight gains were similar across the groups, indicating good acceptability of the blended oil diet by the rat. Food consumption of animals on diets containing the blended oil was also similar to that of animals on diets containing the individual composite oils.

The body weights of the groups given ^{14}C-labelled linoleic (508 ± 22 g, mean ± SEM), α-linolenic (528 ± 23 g) and oleic (518 ± 26 g) acids were similar. Recovery of $^{14}CO_2$ over the 12-h interval post-dose is shown in Figure 2. ^{14}C appeared in expired air within in the first hour of collection with a rapid increase reaching a peak at two to three hours for all three ^{14}C-labelled fatty acids examined. Peak excretion rates for [1-^{14}C]linoleic (5.28 ±

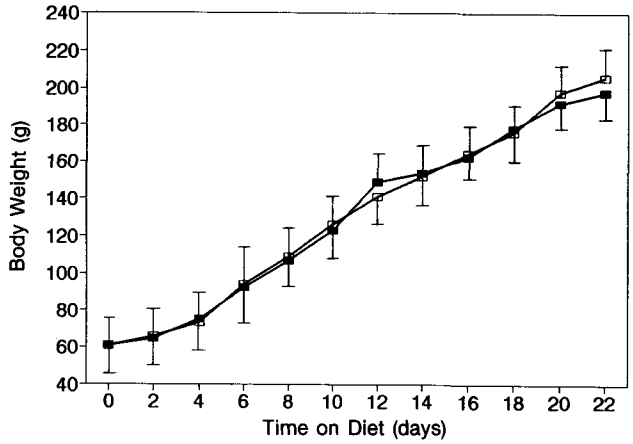

FIG. 1. Body weights of rats given either (*ad libitum*) a choice between three food cups containing identical diets with fat added either as sunflower, flax or olive oil (solid squares) or the blended oil 15% (w/w) fat diet (open squares) over 22 d; data are means ± SEM.

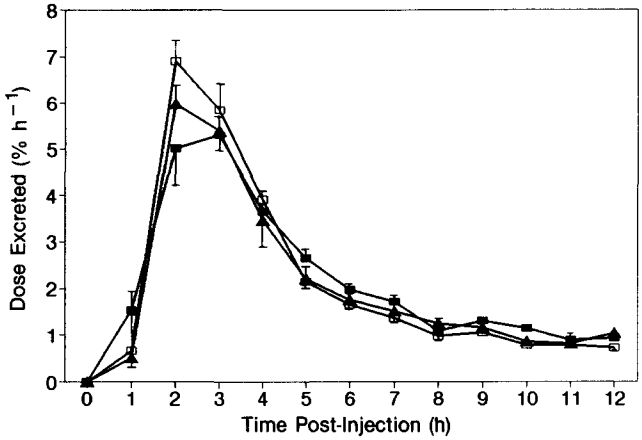

FIG. 3. Cumulative ^{14}C recovery as $^{14}CO_2$ over the 12-h interval post-dose after administration of 1-^{14}C-labelled linoleic (solid squares), α-linolenic (open squares) and oleic (solid triangles) acids; data are means ± SEM.

0.37%/h), α-[1-^{14}C]linolenic (6.92 ± 0.51%/h) and [1-^{14}C]-oleic (5.98 ± 0.44%/h) acids were not significantly different. There were no statistically significant differences in the oxidation rate at two or three hours. Each peak was followed by a gradual decline in excretion rate of label until 12 h; then a levelling off occurred at an excretion rate of about 1%/h.

Cumulative elimination rates for ^{14}C from rats given the labelled fatty acids are shown in Figure 3. There were no differences in cumulative excretion of label among [1-^{14}C]linoleic (27.2 ± 0.9%), α-[1-^{14}C]linolenic (26.8 ± 1.2%) and [1-^{14}C]oleic (25.9 ± 1.2%) acids over the 12-h duration, nor did any differences become significant when data were corrected for body weight.

DISCUSSION

The purpose of this study was to examine whether fatty acid oxidation rates would differ when both dietary and endogenous pool sizes were controlled. In previous studies, pool size often varied when fat oxidation was compared (6–10). The present work demonstrates that when the dietary pool size of the fatty acid examined is maintained at a consistent ratio prior to and during the oxidation period, ^{14}C elimination representative of oxidation rates is virtually identical for the fatty acids tested. These findings contrast results of a number of earlier studies with animals and humans that suggested that differences exist in whole body excretion of labelled fatty acids, but in these previous studies the labelled fatty acids were either provided in tracee pools of varying size, or the pretreatment diet was not standardized.

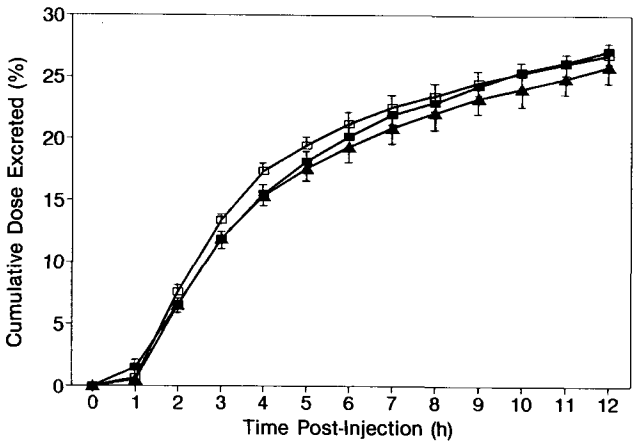

FIG. 2. ^{14}C recovery as $^{14}CO_2$ over the 12-h interval post-dose after administration of 1-^{14}C-labelled linoleic (solid squares), α-linolenic (open squares), or oleic (solid triangles) acid; data are means ± SEM.

In mice given ^{14}C-labelled oleic, linoleic and stearic acids transesterified with linoleic acid-rich corn oil (10), 50% of the administered label in oleic acid was recovered in expired CO_2 at 10-h post-administration, compared with 31 and 19% for linoleic acid and stearic acid, respectively. The comparative oxidation of oleic, palmitic and stearic acids was studied in rats (8) intubated with ^{14}C-labelled fatty acids transesterified with soybean triglyceride. Stearic acid ^{14}C appeared in expired air more slowly allowing 57% recovery after 51 h, compared to 66% from oleic acid. More rapid oxidation of linoleic as compared to stearic acid had been reported in rats fed each ^{14}C-labelled fatty acid mixed with olive oil (9). Similar studies in rats fed diets containing 15% of calories as triolein, trilinolein or tripalmitin, labelled with the respective ^{14}C-labelled fatty acid, had shown that 64, 52 and 51% of the dietary oleate, linoleate and palmitate, respectively, was oxidized after 72 h (14). Overall, these findings suggested differences in oxidation between MUFA, PUFA and SAFA; however, pool sizes of unlabelled (tracee) fatty acids likely varied widely between the fatty acids that were compared.

A similar oxidation pattern had been observed in humans (11). Subjects fed ^{13}C-labelled fatty acids mixed with a breakfast meal, exhibited greater 9-h cumulative $^{13}CO_2$ excretion in expired air from absorbed oleate (15.1%) than from absorbed linoleate (10.2%) or stearate (2.9%) (11). Data from fat malabsorption studies had shown that

orally ingested [^{13}C]triolein was converted to $^{13}CO_2$ more rapidly than [^{13}C]palmitic acid when given under the same conditions (15). Six hours after administration to healthy subjects, of each labelled fatty acid mixed in 0.7 g/kg of lipomul, 11.3 and 6.6% of label from triolein and palmitic acid, respectively, had appeared in breath. Other work has shown that triolein was oxidized more rapidly than tripalmitin when these ^{14}C-labelled fats were administered to healthy humans (16). However, when a comparison was made between whole body oxidation of orally fed ^{14}C-labelled oleic, linoleic and α-linolenic acids in healthy individuals after 24 h, no significant differences in $^{14}CO_2$ recoveries were observed (17). As with animal studies, the tracer/tracee ratio of specific fatty acids varied within the fat bolus fed. Thus, the findings of tracer studies carried out both in animals and in humans indicate that the shift of long-chain fatty acids toward oxidation, rather than storage, varies depending on the degree of unsaturation. However, without controlling fatty acid pool size, interpretation of the results of these studies remains difficult.

In other approaches aimed at examining whether fatty acid oxidation is related to structure, the tracer was directly introduced into the bloodstream, bypassing digestive and absorptive processes. When comparing whole body fatty acid oxidation, direct injection can compensate for potential variations in gastrointestinal absorption and dietary pool size between the various fatty acids tested. In rats maintained on formula diets and injected with ^{14}C-labelled linoleic, oleic and stearic acids, the oxidation rate as determined from cumulative $^{14}CO_2$ production was found to be highest for linoleic acid and lowest for stearic acid (18). The same group later reported increased oxidation of linoleic acid over palmitic acid (19), postulating that production of a highly metabolizable gluconeogenic propionyl-CoA from linoleic acid during oxidation explained the elevated rate of oxidation of linoleic acid. The oxidation rates of infused albumin bound [^{14}C]palmitic and [^{14}C]oleic acids were similar in dogs (20). Conversely, in humans, oxidation rates of injected ^{14}C-labelled palmitic, oleic and linoleic acids were 9.4, 10.0 and 6.3% of the dose administered, respectively, after one hour (7).

Although direct injection of tracer bypasses digestive processes, data interpretation in studies using injection when comparing dietary fatty acid oxidation can be difficult as the amount of injected label appearing in CO_2 represents the fraction of the plasma pool oxidized, not the absolute amount. Net or absolute oxidation measurement depends on the plasma pool size as well as the fractional turnover rate. Markedly smaller plasma pools of linoleic acid in comparison with palmitic acid have been reported (21). Thus, whether the appearance rate of a tracer directly corresponds to net fatty acid oxidation remains to be determined.

By contrast, other investigators have noted no difference in fatty acid oxidation rates. Awad *et al.* (22) found no difference in energy deposition between rats fed PUFA vs. MUFA/SAFA containing diets at 20% of energy for four weeks. Similarly, no difference was observed in humans in $^{14}CO_2$ excretion after oral administration of ^{14}C-labelled palmitic, stearic, oleic, linoleic and α-linolenic acids in healthy individuals after 24 h (17). Toorop *et al.* (12) controlled both the specific activity and net quantity of dietary linoleate and palmitate given to rats and found that, expressed relative to fatty acid absorbed, the oxidation rates of each of the fatty acids were similar. In other studies, such as those of Leyton *et al.* (6), enhanced conversion of α-[^{14}C]linolenic acid to $^{14}CO_2$, when compared to other fatty acids, would be expected given the very small pool size of linolenic acid within the olive oil carrier. In this case, although the proportion of α-[^{14}C]linolenic acid oxidized from the carrier oil was large, net oxidation would have been minimal.

During movement between gut and site of oxidation, labelled fatty acids must cross several transport and metabolite pools, the size of which will be at least partially dependent on prior dietary intake. Given two different labelled fatty acids entering this network of pools from the gut at equal rates, the fatty acid traversing a series of larger pools before arriving at the site where it is oxidized will become more diluted when compared to a fatty acid crossing smaller pools. This dilution will result in a diminished rate of labelled CO_2 expiration. Our findings suggest that a pretreatment diet containing equal fatty acid pools may correct for differences in oxidation due to variability across these internal fatty acid pools. When the labelled fatty acid is then delivered in an oil of equal fatty acid pools, each fatty acid behaves in a similar manner from the absorptive to the oxidative stages of metabolism. Previous work has suggested that absorption efficiencies of different MUFA and PUFA are similar in rats (23,24) and in humans (25), although transport from gut to liver may vary depending on chain length (26). Thus, it cannot be ruled out that absorption and oxidation kinetics varied between the fatty acids tested in a self-compensating manner. However, the similarity in the shape of the curves across the three fatty acids tested suggests similar kinetics.

In summary, the present data suggest that the capacity to oxidize fatty acids varying in degree of saturation is similar when rats are both prefed equal quantities of each fatty acid over a prolonged period and are administered the fatty acid at equivalent tracer/tracee ratios in a fat bolus. The results suggest that differences previously observed in fatty acid oxidation were due to the particular blend of fatty acids provided in the diet and not to the capacity of the organism to oxidize different fatty acids at different rates.

ACKNOWLEDGMENTS

This research was supported by a grant from the Natural Sciences and Engineering Research Council (Canada). The author is grateful to Franco Ciolli, Zbysek Masin and Brian Toy for technical assistance.

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[Received October 4, 1993, and in final revised form May 17, 1994; Revision accepted May 18, 1994]