

Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat

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1. The oxidation rates of lauric, myristic, palmitic, stearic, oleic, α -linolenic, linoleic, γ -linolenic, dihomo- γ -linolenic and arachidonic acids were studied by use of a radioisotope tracer technique in weanling rats at rest in a metabolism chamber over 24 h.
2. Of the saturated fatty acids, lauric acid (12:0) was the most efficient energy substrate: the longer the chain length of the saturated fatty acids, the slower the rate of oxidation.
3. Oleic acid (18:1) was oxidized at a remarkably fast rate, similar to that of lauric acid.
4. Of the ω 6 essential fatty acids studied, linoleic acid (18:2 ω 6) was oxidized at a faster rate than any of its metabolites, with arachidonic acid (20:4 ω 6) being oxidized at the slowest rate.
5. The rate of oxidation of γ -linolenic acid (18:3 ω 3) was almost as fast as that of lauric and oleic acids.

Triglycerides serve as a major component of energy intake in the human diet in western countries (Rizek *et al.* 1983). However, the composition of the triglyceride fatty acids vary considerably depending on the nature of foods eaten. Studies of the comparative metabolism of fatty acids in a whole-body system indicated that the cellular uptake and oxidation of long-chain fatty acids varied with the degree of unsaturation (Mead *et al.* 1956; Coats, 1964; Cenedella & Allen, 1969). Ockner *et al.* (1972) showed that there were differences in the intestinal absorption of saturated and unsaturated fatty acids and differences were also noted in their level of incorporation into chylomicrons (McDonald *et al.* 1980). These findings contradict a common assumption that dietary fats are all oxidized at the same rate.

Whole-body oxidation rates in animals given radiolabelled fatty acids have yielded inconsistent findings, since laboratories have used different models and procedures and have studied different fatty acids. The present study was therefore carried out to investigate the difference in rates of whole-body oxidation of medium-chain fatty acids and essential polyunsaturated fatty acids in one model system. Using radioactively labelled substrates, the oxidation rates of lauric, myristic, palmitic, stearic, oleic, linoleic, γ -linolenic, dihomo- γ -linolenic, arachidonic and α -linolenic acids were determined in weaned rats by measuring the extent of labelling in expired $^{14}\text{CO}_2$ over 24 h.

METHODS AND MATERIALS

Animals

For the early part of the experiment, Sprague-Dawley female rats of the CFY strain were bred in the Nuffield Laboratory and, after weaning at 21 d, were used for the metabolism experiment. However, at a later date weanling rats of the same genetic strain as those bred in the Laboratory were obtained from Benton and Kingmon (Yorkshire), since it proved to be more convenient.

The rats were kept under controlled conditions with a 12 h light–12 h dark cycle (06.00–18.00 hours light), a temperature range of 19–23° and relative humidity of about 55%. All rats were allowed free access to food (Special Dietary Services, London) and tap water at all times. Rats used for the metabolism experiments weighed between 60 and 80 g and were 23–26 d old.

All experiments within a series were performed at the same time of day to avoid any diurnal variations. Animals were kept in the metabolism chamber for a period of 24 h and maintained in the previously described conditions with free access to food and water.

Radioactively labelled fatty acids

[1-¹⁴C]lauric acid (26 mCi/mmol), [1-¹⁴C]palmitic acid (58 mCi/mmol), [1-¹⁴C]stearic acid (60 mCi/mmol), [1-¹⁴C]oleic acid (57 mCi/mmol), [1-¹⁴C]linoleic acid (57 mCi/mmol), [1-¹⁴C] α -linolenic acid (50 mCi/mmol) and [1-¹⁴C]arachidonic acid (50 mCi/mmol) were purchased from Amersham International plc, Amersham, Bucks. [1-¹⁴C]myristic acid (54.5 mCi/mmol) was obtained from Fluorochem Ltd, Gosport, and [1-¹⁴C] γ -linolenic acid (55 mCi/mmol) and [1-¹⁴C]dihomo- γ -linolenic acid (55 mCi/mmol) were obtained from Roche Product Ltd, Herts. Radiolabelled fatty acid (5–6 μ Ci) was introduced into a vial containing 0.2 ml olive oil, and the solvent evaporated under a stream of nitrogen. The oil and isotope mixture was then administered orally to the rat through a Jackson catheter (Arnold, Veterinary Product Ltd, Reading).

Preparation of the metabolism chamber

After oral dosing, the rat was immediately placed in the metabolism chamber (Metabowl; Jencon Ltd, Berks). The principle of the Metabowl is that the animal is maintained in a sealed glass chamber, in which urine and faeces are collected separately. A current of dry, carbon-dioxide-free air is pumped through the metabolism chamber by a Hyflo Technical Model C pump (Scientific Suppliers, London), carrying with it the expired CO₂ from the rat. The exhaled ¹⁴CO₂ from the rat was dried by passing it through concentrated sulphuric acid and trapping it in two collection columns containing a mixture of methoxyethanolamine and ethanolamine (2:1, v/v); 500 ml in the first column and 300 ml in the second column.

In vivo recovery of ¹⁴CO₂

The oxidation of fatty acids was determined by administration of radioactively labelled fatty acids to weanling rats and measuring the expired ¹⁴CO₂ over a 24 h period. From the first collection column, 10-ml portions of trapping agent were removed at hourly intervals for the first 7 h and at 17, 19, 21 and 24 h thereafter. At 24 h, 10 ml were also removed from the second column to account for any overflow of ¹⁴CO₂. From each sample, four 2-ml portions were removed and added to a vial containing 10 ml scintillation fluid (0.81 g Scintimix 2, PPO (250 ml/l), dimethyl POPOP (50 ml/l)), dissolved in 180 ml of a mixture of toluene–methoxyethanolamine–ethanolamine (100:70:10, by vol.) and then counted. The counting efficiency, which was normally about 85%, was determined by addition of an internal standard ([1-¹⁴C]hexadecane) and also by external standard channel ratio mode using a liquid scintillation counter (Model SL30; Intertechnique). In all experiments, radioactive counts in the second collection column were found to be similar to background counts. A total of eight rats was used to study the oxidation of each fatty acid. Four rats for the early 1–7 h and four rats for the late 17, 19, 21 and 24 h. However, with lauric acid six rats were used for the early hours and three rats for the late hours. With palmitic acid, six and four rats were used for the early and late hours respectively and with linoleic acid the corresponding numbers of rats were five and four.

An additional study was also carried out to compare the rate of oxidation of [1-¹⁴C]linoleic acid after oral and intraperitoneal administration over a 5 d period.

Analytical procedures

Lipids were extracted using the method of Folch *et al.* (1957). The tissues were homogenized in chloroform–methanol (2:1, v/v) containing 10 mg butylated hydroxytoluene/l as an

antioxidant, flushed with N₂ and left to extract overnight at 4°. The homogenate was filtered through Whatman no. 1 filter paper and the residue washed with a further 10 ml chloroform-methanol (2:1, v/v). The filtrate was transferred to a separation funnel and to it was added twenty times its volume of saline (8.5 g sodium chloride/l); it was then shaken and left overnight at 4°. The lower organic phase was transferred to a round-bottom flask and evaporated in a Rotavap-R (Buchi) under reduced pressure at 37°. The lipid extract was then redissolved in 20 ml chloroform for the liver, carcass and faeces and in 10 ml chloroform for the plasma. The lipid extracts were flushed with N₂ and stored at 4°.

Radioactivity in tissues, plasma, faeces and urine

To determine the radioactivity retained in the tissues and plasma after 24 h, the rat was anaesthetized under chloroform and 3–5 ml blood were withdrawn by cardiac puncture into a syringe containing heparin to prevent coagulation. The blood was then centrifuged at 1500 g for 10 min and plasma removed for lipid extraction. When the animal was dead the whole liver was removed, washed in ice-cold saline, blotted dry and weighed. The remainder of the carcass was homogenized in a grinding machine from which 10 g of the minced carcass were removed and extracted as described previously. The radioactivity in the faeces was also extracted as previously described. The radioactivity in the lipid extracts was determined by removing two 2-ml portions from the chloroform extract for the liver, faeces and carcass and two 1-ml portions for the plasma sample, placing in a scintillation vial, drying under N₂, then adding 10 ml scintillation fluid (Unisolve 1) before counting. For the urine samples, four 2-ml portions were added to scintillation vials containing 10 ml scintillation fluid and the samples counted.

Recovery of radioactivity from the whole rat

Six rats were divided into two groups. Three were injected with 5 µCi [¹⁴C]lauric acid in 0.2 ml olive oil intraperitoneally and the other three received the same dose of [¹⁴C]linoleic acid intraperitoneally. After 10 min the rats were killed and the whole animal homogenized in a grinding machine, from which 10 g were removed and lipids extracted as described previously. Radioactivity in the lipid phase and the aqueous phase after partition was measured by scintillation counting.

Statistical analysis

One-way analysis of variance was performed in all sets of results; where this showed a treatment effect, unpaired *t* tests were performed comparing the different fatty acid groups.

RESULTS

The oxidation of labelled saturated fatty acids

The oxidation of labelled fatty acid was determined from the respired ¹⁴CO₂ from rats. The results shown in Fig. 1 are given as the cumulative rate of expired ¹⁴CO₂ recovered as a function of time and expressed as a percentage of the administered dose.

Of the saturated fatty acids the order of oxidation after 24 h was: [¹⁴C]lauric acid > [¹⁴C]myristic acid > [¹⁴C]palmitic acid > [¹⁴C]stearic acid. Both [¹⁴C]lauric acid and [¹⁴C]myristic acid were oxidized at a significantly faster rate than [¹⁴C]palmitic acid and [¹⁴C]stearic acid (*P* < 0.01). [¹⁴C]palmitic acid and [¹⁴C]stearic acid were oxidized at the same rate until 21 h; a small rise was noted at 24 h which may indicate another phase in oxidation. However, it should be pointed out that the results for the late intermediate times of 17, 19 and 21 h were obtained from a separate experiment, using the same strain of rats, and then combined with the early 1–7 h and 24 h results.

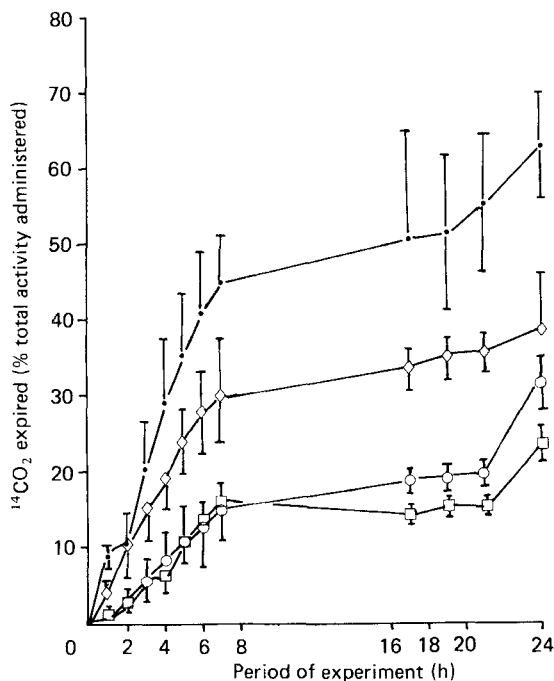


Fig. 1. The in vivo oxidation of saturated fatty acids in rats over a 24 h period. Each animal received an oral dose of 5–6 μCi ^{14}C -labelled fatty acid in 0.2 ml olive oil, was put into a metabolism chamber and the expired $^{14}\text{CO}_2$ collected (for details of procedure, see p. 384). Points are mean values, with their standard errors represented by vertical bars, for the radioactivity recovered in CO_2 from [^{14}C]lauric acid (●), [^{14}C]myristic acid (◇), [^{14}C]palmitic acid (○) and [^{14}C]stearic acid (□).

The oxidation of labelled unsaturated fatty acids

The results showed that with the $\omega 6$ fatty acid family, increasing desaturation and chain elongation resulted in a gradual reduction in the rate of fatty acid oxidation (Fig. 2). This differential rate of oxidation was in the order: [^{14}C]linoleic acid > [^{14}C] γ -linolenic acid > [^{14}C]dihomo- γ -linolenic acid > [^{14}C]arachidonic acid.

The results also showed that [^{14}C] α -linolenic acid and [^{14}C]oleic acid were both preferentially oxidized ($P < 0.01$) when compared with [^{14}C]linoleic acid. All the C_{18} fatty acids were oxidized in preference to C_{20} polyunsaturated derivatives of linoleic acid ($P < 0.01$). The rates of oxidation of 18:3 $\omega 6$, 20:3 $\omega 6$ and 20:4 $\omega 6$ were 27, 14 and 14% respectively of that observed for 18:2 $\omega 6$. At 7 h [^{14}C]oleic acid, [^{14}C] α -linolenic acid and [^{14}C]linoleic acid were oxidized to a significantly greater extent ($P < 0.01$) than [^{14}C] γ -linolenic acid, [^{14}C]dihomo- γ -linolenic acid and [^{14}C]arachidonic acid.

In comparing the oxidation of saturated and unsaturated fatty acids, [^{14}C]linoleic acid [^{14}C] α -linolenic acid and [^{14}C]oleic acid were oxidized at a significantly faster rate than [^{14}C]stearic acid and [^{14}C]palmitic acid at 24 h ($P < 0.01$). No significant differences could be found between [^{14}C] γ -linolenic acid and [^{14}C]palmitic acid oxidation. At 7 h the rate of oxidation showed a similar pattern, where the C_{18} fatty acids were preferentially oxidized ($P < 0.01$) compared with [^{14}C]stearic acid, [^{14}C]palmitic acid and the long-chain polyunsaturated [^{14}C]dihomo- γ -linolenic acid and [^{14}C]arachidonic acid.

Table 1 shows the rates of oxidation of labelled fatty acid during a 5 d period after oral ingestion and intraperitoneal injection. The results show a faster rate of oxidation during

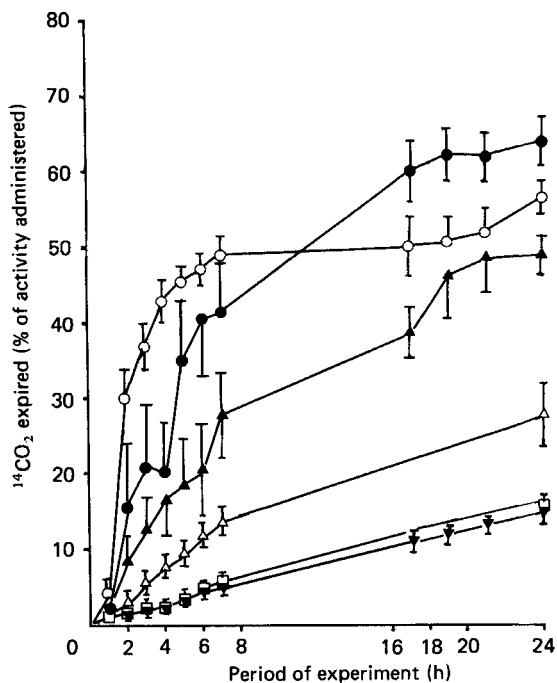


Fig. 2. The *in vivo* oxidation of unsaturated fatty acids in rats over a 24 h period. Each animal received an oral dose of 5–6 μCi ^{14}C -labelled fatty acid in 0.2 ml olive oil, was put into a metabolism chamber and the expired $^{14}\text{CO}_2$ collected (for details of procedure, see p. 384). Points are mean values, with their standard errors represented by vertical bars, for the radioactivity recovered in CO_2 from [^{14}C] α -linolenic acid (\bullet), [^{14}C]oleic acid (\circ), [^{14}C]linoleic acid (\blacktriangle), [^{14}C] γ -linolenic acid (\triangle), [^{14}C]dihomo- γ -linolenic acid (\square) and [^{14}C]arachidonic acid (\blacktriangledown).

Table 1. $^{14}\text{CO}_2$ expired (% of radioactivity administered) by rats after intraperitoneal and oral administration of ^{14}C -labelled linoleic acid
(Values are means with their standard errors for two animals)

| Period after dose (h) | [^{14}C]linoleic acid | | | |
|-----------------------|----------------------------------|-----|-----------------|------|
| | Oral | | Intraperitoneal | |
| | Mean | SEM | Mean | SEM |
| 1 | 2.9 | 1.4 | 12.9 | 4.0 |
| 2 | 10.4 | 4.1 | 25.0 | 6.9 |
| 3 | 17.0 | 5.7 | 31.0 | 8.7 |
| 4 | 23.9 | 6.3 | 35.5 | 8.2 |
| 5 | 31.9 | 4.9 | 39.2 | 8.2 |
| 6 | 36.8 | 3.5 | 41.5 | 7.9 |
| 24 | 56.8 | 2.4 | 54.9 | 8.9 |
| 48 | 60.7 | 1.6 | 62.3 | 6.5 |
| 72 | 62.3 | 1.3 | 67.6 | 10.9 |
| 96 | 71.3 | 0.0 | 67.6 | 0.0 |
| 120 | 69.9 | 1.5 | 73.5 | 3.9 |

For details of procedure, see p. 384.

Table 2. *Distribution of ^{14}C activity (% of radioactivity administered) in rat liver, plasma, erythrocytes, carcass, urine, faeces and carbon dioxide 24 h after oral administration of labelled saturated fatty acids*
(Values are means with their standard errors for the number of animals shown)

| | ^{14}C -labelled fatty acids | | | | | | | | | | | |
|------------------------------|---------------------------------------|-----|--------------------|------|--------------------|---|-------------------|-----|----|------|-----|---|
| | Lauric (12:0) | | Myristic (14:0) | | Palmitic (16:0) | | Stearic (18:0) | | | | | |
| | Mean | SEM | n | Mean | SEM | n | Mean | SEM | n | Mean | SEM | n |
| Liver | 0.3 | 0.0 | 10 | 0.6 | 0.2 | 8 | 3.2 | 0.3 | 8 | 4.8 | 0.0 | 8 |
| Plasma | 0.02 | 0.0 | 10 | 0.04 | 0.0 | 8 | 0.1 | 0.0 | 10 | 0.4 | 0.3 | 8 |
| Erythrocytes | 0.02 | 0.0 | 10 | 0.03 | 0.0 | 8 | 3.0 | 0.0 | 10 | 0.1 | 0.0 | 8 |
| Carcass* | 6.1 | 1.4 | 10 | 17.0 | 2.2 | 8 | 22.0 | 1.7 | 10 | 26.0 | 1.9 | 8 |
| Urine | 2.4 | 0.5 | 10 | 1.2 | 0.2 | 8 | 1.8 | 0.2 | 10 | 1.1 | 0.2 | 8 |
| Faeces | 1.1 | 0.5 | 10 | 0.4 | 0.1 | 8 | 0.9 | 0.4 | 10 | 2.8 | 3.9 | 8 |
| CO_2 (%) | 63.1 | 7.5 | 10 | 40.0 | 7.3 | 8 | 32.0 | 3.2 | 10 | 25.0 | 2.8 | 8 |
| Total activity recovered (%) | 73.1 | | | 59.3 | | | 63.0 | | | 60.2 | | |

* The activity recovered from the carcass was from the whole animal (minus the liver and blood).

Table 3. Distribution of ¹⁴C activity (% of radioactivity administered) in rat liver, plasma, erythrocytes, carcass, urine, faeces and carbon dioxide 24 h after oral administration of labelled unsaturated fatty acids
(Values are means with their standard errors for the number of animals shown)

| | ¹⁴ C-labelled fatty acids | | | | | | | | | | | |
|------------------------------|--------------------------------------|-----|----|-------------------|-----|---|----------------------|-----|---|----------------------|-----|---|
| | Oleic (18:1) | | | Linoleic (18:2ω6) | | | α-Linolenic (18:3ω3) | | | Arachidonic (20:4ω6) | | |
| | Mean | SEM | n | Mean | SEM | n | Mean | SEM | n | Mean | SEM | n |
| Liver | 1.3 | 0.3 | 13 | 2.4 | 0.4 | 9 | 2.1 | 0.3 | 8 | 19.0 | 1.6 | 7 |
| Plasma | 0.1 | 0.0 | 13 | 0.2 | 0.0 | 9 | 0.03 | 0.0 | 8 | 0.9 | 0.3 | 7 |
| Erythrocytes | 0.03 | 0.0 | 13 | 0.1 | 0.0 | 9 | 0.03 | 0.0 | 8 | 0.2 | 0.0 | 7 |
| Carcass* | 23.0 | 1.8 | 13 | 27.0 | 3.5 | 9 | 16.0 | 1.9 | 8 | 46.0 | 2.6 | 7 |
| Urine | 2.6 | 0.4 | 13 | 1.1 | 0.3 | 9 | 2.4 | 0.1 | 8 | 0.8 | 0.1 | 7 |
| Faeces | 0.4 | 0.1 | 13 | 0.6 | 0.2 | 9 | 1.4 | 0.2 | 8 | 0.6 | 0.1 | 7 |
| CO ₂ | 57.0 | 2.1 | 8 | 48.0 | 2.8 | 9 | 64.0 | 2.9 | 8 | 14.0 | 1.5 | 7 |
| Total activity recovered (%) | 84.4 | | | 79.4 | | | 86.0 | | | 81.5 | | |

* The activity recovered from the carcass was from the whole animal (minus the liver and blood).

the first 6 h after intraperitoneal administration compared with oral dosing. However, after 6 h the oxidation rate was similar for both groups.

Distribution of ^{14}C radioactivity

Tables 2 and 3 summarize the distribution of labelled saturated and unsaturated fatty acids in the rat liver, carcass, plasma and erythrocytes, and their excretion in urine and faeces, after 24 h. The recovery of labelled fatty acids reflected their oxidation rates, with the least oxidized of the fatty acids having the greatest retention in the carcass. Thus, the degree of retention for the saturated fatty acids was in the order: [^{14}C]lauric acid < [^{14}C]myristic acid < [^{14}C]palmitic acid < [^{14}C]stearic acid and for the unsaturated fatty acids, [^{14}C] α -linolenic acid < [^{14}C]oleic acid < [^{14}C]linoleic acid < [^{14}C]arachidonic acid. The oxidation rates for γ -linolenic acid and dihomo- γ -linolenic acid were studied previously and the activity in the carcass was not measured.

The amounts of labelled fatty acids recovered in the liver were much smaller, ranging from 0.2 to 4.0% of the total activity administered. The exception again was [^{14}C]arachidonic acid, 19.5% of which was recovered. As in the carcass, these values reflected the rate of oxidation of the fatty acids to expired CO_2 , i.e. the faster the rate of oxidation, the lower the retention.

The amount of radioactivity recovered in the urine and faeces was relatively low, about 1–3% of the administered dose, indicating good absorption of the labelled fatty acids from the rat intestine. In plasma the radioactivity was very low, about 0.1% of the administered dose. This indicated rapid uptake of labelled fatty acids by the liver and adipose tissue, where they are readily esterified into triglycerides and phospholipids.

Recovery of radioactivity from the whole rat

Of the administered dose of labelled fatty acid, the mean total radioactivity recovered from three animals was 67.7% for the [^{14}C]lauric acid-treated group and 78.6% for the [^{14}C]linoleic acid-treated group; this gave factors of 1.5 and 1.3 respectively (i.e. $100/67.7$ and $100/78.6$). Thus, when 1.5 is multiplied by the total activity recovered for the individual fatty acids shown in Table 1, approximately 100% of the activity can be accounted for as the saturated fatty acids. Similarly, when 1.3 is multiplied by the total activity recovered for the individual fatty acids in Table 2, approximately 100% of the activity can thus be accounted for as the unsaturated fatty acids.

As lipid-soluble materials were extracted, the radioactivity that was not accounted for may be associated with non-lipid materials, such as proteins and carbohydrates which were not recovered in the lipid extract.

DISCUSSION

Oxidation of saturated fatty acids

Greenberg *et al.* (1965) have shown that oxidation of short-chain fatty acids by the liver occurs more rapidly than oxidation of long-chain fatty acids. The fatty acids of medium-chain triglycerides (MCT) released by the action of intramucosal lipase are transported directly to the liver via the portal-venous system for oxidation (Bloom *et al.* 1951). The results from the present study show that lauric acid and myristic acid were preferentially oxidized over palmitic acid and stearic acid. These findings suggest that MCT fatty acids such as lauric and myristic acids may be taken up directly into the liver via the portal system and subsequently oxidized. By contrast, palmitic and stearic acids are transported to the liver via the lymphatic system (Coots, 1965) and therefore a longer period of time elapses before they become available for oxidation. Whereas lauric acid and myristic acid serve as

the principal fuels of respiration for working tissues, in the postabsorptive state, palmitic and stearic acids may be utilized directly or stored in tissue triglycerides and phospholipids for later use.

Weinman *et al.* (1950), studying the oxidation of palmitic and stearic acids, showed that regardless of whether the ^{14}C was introduced into the carboxyl carbon end or the 6th or the 11th C atoms of the palmitic acid molecule, there were no differences in the percentage of ^{14}C recovered in the expired CO_2 . These results suggest that once palmitic acid enters the catabolic pathway the entire C chain is converted to CO_2 at about the same rate. However, this was not the case for stearic acid. Weinman *et al.* (1951) found that the ^{14}C recovered in expired CO_2 24 h after administration of $[6\text{-}^{14}\text{C}]$ tristearin was about 20% less than that for $[1\text{-}^{14}\text{C}]$ tristearin. These findings indicate that the carboxyl-C of stearic acid was converted to CO_2 more rapidly than the 6th C.

Oxidation of oleic acid

An important finding in the present work was the remarkably high oxidation rate for oleic acid. The preferential oxidation of oleic acid over longer-chain fatty acids in mice (Mead *et al.* 1956) and rats (Cenedella & Allen, 1969) has also been reported. Evidence obtained from perfused-heart studies (Willebrands, 1964) showed a greater uptake rate for oleic acid compared with linoleic acid and an even greater uptake rate when compared with stearic acid. Jones *et al.* (1985) provided evidence in human subjects which showed the preferential oxidation of oleic acid compared with linoleic acid. Oleic acid is preferentially incorporated into triglycerides which are a ready source of energy and this probably explains the relatively high oxidation rate for this fatty acid. This high rate of oxidation is of special interest since oleic acid is the major fatty acid found in human milk (Crawford *et al.* 1985), ranging from 30 to 40% of total fatty acids, compared with 25% for palmitic acid in milks of all the species so far studied (Crawford *et al.* 1976).

These results suggest that oleic acid has a dual role in milk. It serves as a major energy source as well as being utilized in cell structure for growth and development. Oleic acid is one of the principal fatty acids in brain phosphoglycerides (Rao & Rao, 1973). The human brain develops at a rapid rate, from 450 to 1200 g, during the first year of life. This weight gain is largely due to an expansion of the cell volume and myelination associated with development of interconnections between cells. Both oleic acid and its metabolic product, nervonic acid (24:1), are major fatty acids in mature myelin.

Oxidation of unsaturated fatty acids

Another important finding from the present work was the fast oxidation rate observed for α -linolenic acid, which was similar to that of lauric acid and thus may also serve as an energy source. This finding is consistent with the compositional findings for various tissues in different species, for example the concentration of α -linolenic acid is seldom significant in choline phosphoglycerides, even in species such as the zebra (Crawford *et al.* 1976) which eat grass and have high levels of α -linolenic acid in adipose tissue. Evidence provided by Bandyopadhyay *et al.* (1982) showed that the liver of carnivorous catfish (*Heteropneustes fossilis*) oxidized α -linolenic acid preferentially over linoleic acid, and that the liberated $[1\text{-}^{14}\text{C}]$ acetyl-CoA was incorporated into saturated fatty acids such as myristic and palmitic acids. This suggests that during in vivo oxidation of labelled α -linolenic acid to expired $^{14}\text{CO}_2$, the label may not be derived from the original fatty acid but could also come from fatty acids elongated or synthesized *de novo*. Therefore, in the present study, the complete oxidation of the administered fatty acid to CO_2 may also include those fatty acids that subsequently incorporate the labelled acetyl-CoA into their structure, undergo chain elongation and desaturation, and eventually become oxidized to CO_2 .

By contrast the low oxidation rate of C₂₀ fatty acids, like dihomo- γ -linolenic acid and arachidonic acid, suggests that they share a common pathway, which could be quite different from that of the C₁₈ poly-unsaturated fatty acids. In the few reports on the oxidation of arachidonic acid, it was found to have a very low rate of oxidation and was incorporated mainly into phospholipids (Coots, 1965). Coniglio *et al.* (1964) found that fat-deficient rats oxidized only 7% in 6 h compared with 10% in control animals. The C₂₀ fatty acids are oxidized via the extramitochondrial peroxisomal pathway (Lazarow & de Duve, 1976), which functions mainly by chain shortening to C₁₈ fatty acids which in turn enter the mitochondria for subsequent further oxidation. The reduced rate of oxidation of C₂₀ fatty acids also stems from their greater and more selective incorporation into phospholipids, which in turn are incorporated into permanent cell structures more readily than triglycerides (Sinclair, 1974).

The rapid oxidation rate of α -linolenic acid described here might offer an explanation for a consistent and puzzling difference in the balance between parent fatty acid and long-chain products of the tissue phosphoglycerides. In the tissues of land mammals, the choline phosphoglycerides of plasma, liver and muscle tend to have relatively high concentrations of linoleic acid and lower amounts of arachidonic acid; the opposite is the case for α -linolenic acid and its derivatives. For example, human plasma lecithin contains (mmol/mol) about 220 linoleic acid and 80 arachidonic acid but only 7 α -linolenic acid associated with 80 docosahexaenoic acid. These contrasts in the relation between parent and derived fatty acids in the ω 6 and ω 3 series is found even in the phosphoglycerides of herbivorous mammals (Crawford *et al.* 1976). It is already known that α -linolenic acid is desaturated faster than linoleic acid (Mead, 1971; Brenner, 1981) and that the long-chain derivatives of α -linolenic acid are incorporated into the cell membrane phosphoglycerides much faster than α -linolenic acid itself (Sinclair, 1974). Consequently, the combination of the fast oxidation rate and faster desaturation of α -linolenic acid could explain the low proportion of parent fatty acid to their long-chain product which is almost universally found in structural lipids in the ω 3 but not in the ω 6 fatty acid series.

The present results emphasize that fatty acids are by no means utilized on an equal basis for oxidation. Fatty acids such as lauric acid and myristic acid which occur in MCT are a potential energy source. However, the observation that α -linolenic acid and oleic acid were oxidized at equally fast rates provides us with a new view of the relative significance of the different fatty acids for biological use. For example, oleic acid is not only an efficient energy source but, unlike the fatty acids in MCT, it is also of potential use in structural lipids in cells during growth and tissue repair. The clinical situations in which MCT have been used are almost always associated with a high demand for cell repair or growth. Given this, olive oil might be a more appropriate form of treatment than MCT because oleic acid and the other fatty acids of olive oil can serve the dual purpose of providing energy and serve as fatty acids for cell-structure lipids (e.g. palmitic, stearic, oleic and linoleic acids). The MCT are mainly C₁₀, C₁₂ C₁₄ compounds which are good energy substrates but poor precursors for cell membrane lipids.

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