Metabolism of parenterally administered fat emulsions in the rat: studies of fatty acid oxidation with 1-^{13}C- and 8-^{13}C-labelled triolein

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To reassess the hypothesis that fatty acid catabolism occurs to completion via β-oxidation, male Sprague–Dawley rats receiving continuous total parenteral nutrition (TPN) including 43% energy as fat were infused with [1-^{13}C]- or [8-^{13}C]triolein. Expired CO_2 was collected continuously for 4 h and its $^{13}C:^{12}C$ ratio determined by isotope–ratio mass spectrometry. Bicarbonate retention was also assessed over 4 h by infusion of $NaH^{14}CO_3$ and measurement of the expired $^{14}CO_2$. A possible loss of label from [8-^{13}C]oleic acid from the citric acid cycle via labelled acetyl-CoA without oxidation to CO_2 was assessed by infusing further animals with acetate labelled with ^{14}C either at C atoms 1 or 2 and determination of its conversion to expired $^{14}CO_2$. At isotopic steady state, 63·2 (SE 1·6)% (*n* 8) of the infused [1-^{14}C]acetate and 46·0 (SE 1·2)% (*n* 8) of [2-^{14}C]acetate was recovered as expired $^{14}CO_2$. After correction for bicarbonate retention and non-oxidative isotope loss, 37·3 (SE 1·2)% (*n* 20) of the [1-^{13}C]triolein was found to have been oxidized, whereas 32·6 (SE 1·0)% (*n* 20) of the [8-^{13}C]triolein was oxidized ($P \leq 0.01$). The lower oxidation of the C atom at position 8 of oleic acid than that at position 1 indicates incomplete oxidative breakdown of the fatty acid after entering β-oxidation.

^{13}C-labelled triolein: β-Oxidation: Total parenteral nutrition

Parenteral fat is an integral part of nutritional support, providing essential fatty acids and high amounts of energy in a low volume of fluid (Silberman, 1986). Generally, it is assumed that the portion of the infused fat that is not stored will be completely degraded by mitochondrial β-oxidation to CO_2 and water, thereby producing heat and ATP. The nature and efficacy of β-oxidation have generally been assessed by employing ^{14}C-labelled triacylglycerols (Geyer *et al.* 1948; Lerner *et al.* 1949; Eckart *et al.* 1973; Nordenström *et al.* 1982; Chen, 1984; Bender *et al.* 1985; Johnson *et al.* 1990), or in more recent studies, by means of ^{13}C-labelled triolein (Paust *et al.* 1984, 1989; Brösicke *et al.* 1985; Park *et al.* 1986; Adolph *et al.* 1989) or trioctanoin (Watkins *et al.* 1977; Wolfram & Metges, 1988; Sulkers *et al.* 1989). In human beings, the degree of oxidation of long-chain triacylglycerols and medium-chain triacylglycerols varies between 11 and 30% and 31 and 52% respectively (Weinman *et al.* 1950, 1951; Eckart *et al.* 1973; Watkins *et al.* 1977; Allsop *et al.* 1978; Nordenström *et al.* 1982; Wolfram & Metges, 1988; Paust *et al.* 1989; Sulkers *et al.* 1989; Thompson *et al.* 1989). In rats, however, the extent of oxidation is markedly higher: 36–67% for long-chain triacylglycerols and 70–90% for medium-chain triacylglycerols (Geyer *et al.* 1948; Lerner *et al.* 1949; Chen, 1984; Paust *et al.* 1984; Bender *et al.* 1985; Brösicke *et al.* 1985; Park *et al.* 1986; Adolph *et al.* 1989; Johnson *et al.* 1990).

Most studies of fatty acid catabolism using isotopes are performed with fatty acids labelled at the first C atom (carboxyl-C), i.e. there is a tacit assumption that β-oxidation of fatty acids goes to completion so that the release of the first C atom as CO_2 indicates oxidation of all of the C atoms (Weinman *et al.* 1950). The validity of this assumption has never been definitely proven. Early work with tripalmitin ^{14}C-labelled in positions 1, 6 or 11 supported the hypothesis of complete β-oxidation (Wein-

Abbreviations: TPN, total parenteral nutrition; V_{CO_2}, total CO_2 production.

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man *et al.* 1950). However, results of a subsequent study with tristearin ^{14}C-labelled in positions 1 or 6 did not confirm this hypothesis (Weinman *et al.* 1951).

In the present study we administered 1-^{13}C- and 8-^{13}C-labelled triolein to rats during total parenteral nutrition (TPN). One hypothesis is that fatty acids labelled on an even-numbered C atom yield acetyl-CoA labelled in C2, which may partly be shunted to fates other than oxidation to CO_2 in the citric acid cycle. To test this, we infused, separately, [1-^{14}C]- and [2-^{14}C]acetate (i.e. the direct precursors of acetyl-CoA) so that any difference in the recoveries of the differently labelled acetate molecules as expired $^{14}CO_2$ would indicate diversion of the second C atom in acetyl-CoA derived from [8-^{13}C]triolein, e.g. to gluconeogenesis or other fates.

Materials and methods

Animals and total parenteral nutrition

Male Sprague–Dawley rats, weighing 220–240 g, were adapted for 5 d in metabolic cages in a light- and temperature-controlled room. They consumed *ad libitum* a commercial standard rat chow (Altromin®, Altromin, Lage, Germany) and tap water.

A central catheter was inserted into the external jugular vein and placed in the right side of the heart after the animals were anaesthetized by an intraperitoneal injection of Inoketam® 100 ml/l (Virbac GmbH, Bad Oldesloe, Germany) and Rompun® 20 ml/l (Bayer, Leverkusen, Germany). The end of the catheter was passed subcutaneously to be exteriorized in the dorsal midscapular region by means of a trocar. It was protected by a metal harness, thus allowing continuous parenteral nutrition through the catheter while enabling the animals to move about freely.

After a 3 d recovery period on oral feeding, the animals received continuous TPN with (g/kg per d) : N (Traumasteril 10 %®) 2, glucose (Glucosteril 50 %®) 30, and fat (Lipovenös 20 %®) 12. All solutions were provided by Fresenius AG (Bad Homburg, Germany). The TPN solution was supplemented with vitamins (Multibionta®, E. Merck, Darmstadt, Germany) and trace elements (Tracitrans®, Fresenius AG) and was infused by means of a syringe pump (Fresenius AG) at a rate of 2·60 ml/h. Total energy supply was 1175 kJ/kg per d.

Carbon dioxide trapping system

After 24 h on TPN, the animals were placed in a gas-tight metabolic cage of approximately 2·5 litres. A current of CO_2-free air was drawn through the cage at a rate of 15 (SE 1) litres/h. Expired CO_2 was collected by aspiration for consecutive periods of 10 min through CO_2-traps arranged in two parallel series, each containing 10 ml 1 M-NaOH allowing CO_2 to be absorbed quantitatively as carbonate. A constant light vacuum of 7–10 mmHg prevented loss of expired CO_2 from the system.

Bicarbonate retention

A portion of the labelled CO_2 produced during the oxidation of a C-labelled substrate is retained in the body; the extent of this bicarbonate retention was determined as recommended by Thompson *et al.* (1989). Briefly, the animals were infused with $NaH^{14}CO_3$ (200 µl/kg per h of a 1·2 mM solution; specific activity: 307 Mbq/mmol; Sigma, St. Louis, MO, USA) during ongoing TPN over 4 h, and the expired CO_2 was trapped at 10 min intervals. Duplicate 1 ml portions of these samples were added to 4 ml liquid scintillation cocktail (Rotiszint® eco plus, Roth, Karlsruhe, Germany), and the radioactivity was quantified in a liquid scintillation counter (Beckman LS 1801, Beckman, Irvine, CA, USA).

At isotopic steady state, the percentage of the infused [^{14}C]bicarbonate retained was calculated by dividing the rate of $^{14}CO_2$ excretion (disintegrations/min per min) by the rate of radioactivity infusion.

[^{13}C]oleic acid oxidation

At 48 h after the start of TPN, rats in two equal groups (n 20) randomly received a combined bolus of $NaH^{13}CO_3$ (0·06–0·42 µmol/kg) and [1-^{13}C]- or [8-^{13}C]triolein (2–20 µmol/kg) and immediately afterwards, a constant infusion of a tracer amount of either [1-^{13}C]- or [8-^{13}C]triolein (16–24 µmol/kg per h) over 4 h. Tracer priming of the bicarbonate pool decreases the time until attainment of a new steady state of the ^{13}C-enrichment in expired CO_2 (Allsop *et al.* 1978). [^{13}C]bicarbonate and ^{13}C-labelled triolein emulsions were provided by Fresenius AG.

Between 60 and 30 min before and throughout each ^{13}C-infusion experiment, expired CO_2 was collected by absorbing it into NaOH for consecutive periods of 10 min. The total CO_2 production (V_{CO_2}) was determined titrimetrically from the amount of CO_2 trapped during each sampling period.

Isotope-ratio mass spectrometry

Duplicate portions of each trapped CO_2 sample were injected into evacuated 20 ml Vacutainers® (Becton Dickinson, Heidelberg, Germany), and the CO_2 released by injection of 1 ml 15 M-H_3PO_4. The ^{13}C-enrichment of each CO_2 sample was determined by means of a Finnigan MAT 251 Delta E dual-inlet multi-collector isotope-ratio mass spectrometer. The operation and performance of this automated system have been described in detail by Scrimgeour & Rennie (1988).

The percentage recovery of the infused label was calculated from the expired $^{13}CO_2$ according to a modification of the formula given by Schoeller *et al.* (1980):

$$\% \text{ recovery of infused label} = \frac{E \times V_{CO_2}}{F_I \times P \times n \times {}^{14}CO_{2\,exp}},$$

where E is the mean ^{13}C enrichment in the CO_2 samples taken at isotopic steady state in atom % excess. The enrichment is the difference between the mean ^{13}C content

in the breath CO_2 samples taken before isotope infusion (baseline ^{13}C expiration) and the samples taken at isotopic steady state in atom % ^{13}C; V_{CO_2} is the CO_2 production during isotopic steady state (mmol/kg per h); F_I is the infusion rate of [^{13}C]triolein (μmol/kg per h); P is the isotopic purity of [^{13}C]triolein (= 99 %); n is the number of ^{13}C atoms per [^{13}C]triolein molecule (= 3); $^{14}CO_{2\,exp}$ is the recovery of infused $H^{14}CO_3$ as breath $^{14}CO_2$.

^{14}C-labelled acetate infusion

In a separate experiment, sixteen rats in two equal groups randomly received a constant infusion of either [1-^{14}C]- or [2-^{14}C]acetate (8·7 μl/kg per h of a 3·8 mM aqueous solution; specific activity: 1961 and 2079 Mbq/mmol respectively; Amersham Buchler GmbH, Braunschweig, Germany) over 4 h admixed to the TPN solution. At isotopic steady state, the rate of $^{14}CO_2$ exhalation (% recovery) was determined for both groups as described earlier.

The ratio of the recoveries of the differently labelled acetate molecules as expired $^{14}CO_2$ was used to correct the possible loss of label derived from [8-^{13}C]triolein:

$$\%\ \text{oxidation of [8-}^{13}\text{C]triolein} = \%\ \text{recovery of infused label} \times \frac{\text{recovery of [1-}^{14}\text{C]acetate as breath } ^{14}CO_2}{\text{recovery of [2-}^{14}\text{C]acetate as breath } ^{14}CO_2}.$$

Definition of steady state

We operationally define a quasi-steady state by considering the end period of each infusion experiment to be 45 min. All data sets of this period were averaged for calculation.

Statistical analyses

Differences between groups were tested for significance by performing a Wilcoxon two-sample test, using the software package Statistical Analysis System SAS® (Statistical Analysis Systems, 1988) running on an IBM personal computer. The results are expressed as means with their standard errors.

Results

The mean recovery of infused [^{14}C]bicarbonate as $^{14}CO_2$ at isotopic steady state was 90·5 (SE 1·0)% (Fig. 1). The interindividual variation was between 82 and 100 % (n 23).

During infusion of [1-^{14}C]- and [2-^{14}C]acetate, isotopic steady state of $^{14}CO_2$ was attempted at 63·2 (SE 1·6)% (n 8) of the infused [1-^{14}C]acetate radioactivity and at 46·0 (SE 1·2)% of the infused [2-^{14}C]acetate radioactivity (n 8) (Figs. 2 and 3).

At isotopic steady state, mean recovery of [8-^{13}C]triolein was 23·8 (SE 0·7)% (n 20) whereas that of [1-^{13}C]triolein was 37·3 (SE 1·2)% (n 20) (Fig. 4). The difference was significant ($P \leq 0{\cdot}001$).

The recovery of infused [1-^{13}C]triolein as expired $^{13}CO_2$ was corrected for bicarbonate retention to determine the

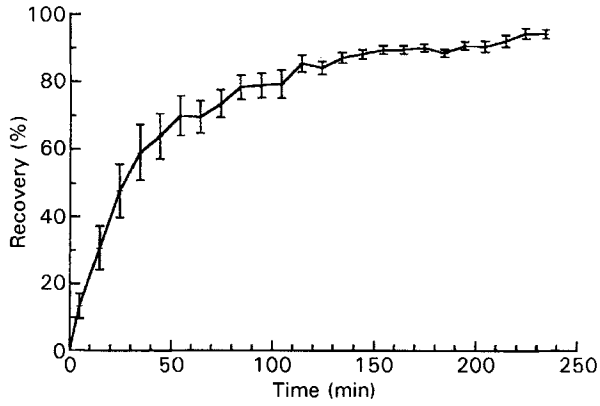

Fig. 1. Percentage recovery of ^{14}C as $^{14}CO_2$ in expired air from rats infused with $NaH^{14}CO_3$. The expired $^{14}CO_2$ was trapped over successive periods of 10 min and quantified in a liquid scintillation counter. Values are means for 23 rats, with standard deviations represented by vertical bars.

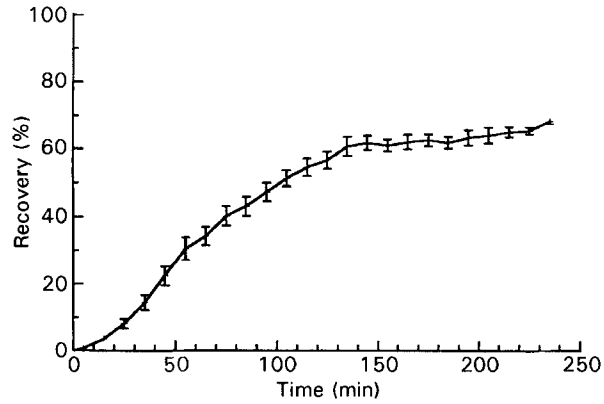

Fig. 2. Percentage recovery of ^{14}C as $^{14}CO_2$ in expired air from rats infused with [1-^{14}C]acetate. The expired $^{14}CO_2$ was trapped over successive periods of 10 min and quantified in a liquid scintillation counter. Values are means for eight rats, with standard deviations represented by vertical bars.

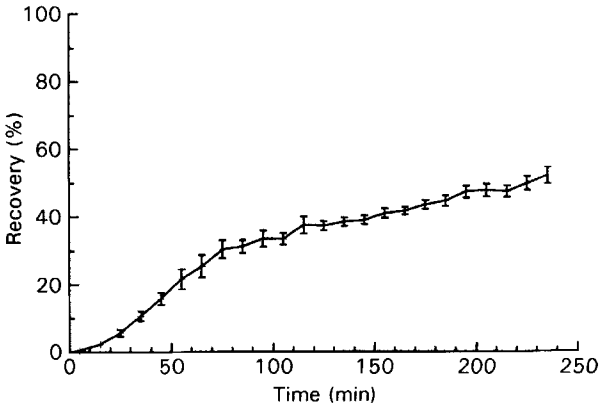

Fig. 3. Percentage recovery of ^{14}C as $^{14}CO_2$ in expired air from rats infused with [2-^{14}C]acetate. The expired $^{14}CO_2$ was trapped over successive periods of 10 min and quantified in a liquid scintillation counter. Values are means for eight rats, with standard deviations represented by vertical bars.

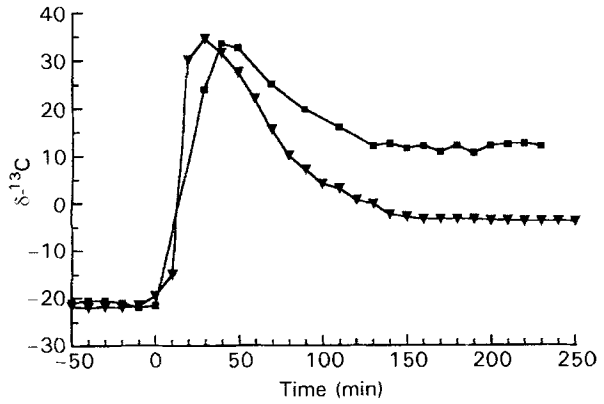

Fig. 4. Example of the pattern of exhalation of $^{13}CO_2$ by rats during the infusion of [1-^{13}C]triolein (–■–) or [8-^{13}C]triolein (–▼–). After priming with [^{13}C]bicarbonate and the respective labelled emulsion, the enrichment of the exhaled CO_2 with ^{13}C rose exponentially. After about 180 min, a new steady-state enrichment was attained. The difference between baseline ^{13}C-enrichment of the exhaled CO_2 and steady-state enrichment during ^{13}C infusion was used to calculate the percentage recovery of the infused label.

size of the oxidized fraction. To quantify the oxidized fraction of [8-^{13}C]triolein, it is necessary to consider not only bicarbonate retention, but also the isotope loss from the second C atom in acetyl-CoA. Thus, the percentage oxidation of [8-^{13}C]triolein was calculated by multiplying the percentage recovery by the ratio mean recovery of [1-^{14}C]- : [2-^{14}C]acetate as breath $^{14}CO_2$ (= 1·37, see pp. 382–383). The resulting value, 32·6 (SE 1·0)%, was still 13% lower ($P \leq 0{\cdot}01$) than the equivalent value for oxidation of [1-^{13}C]triolein, 37·3 (SE 1·2)% (Table 1).

Table 1. Fractional oxidation of infused [1-^{13}C]- and [8-^{13}C]triolein during isotope steady state (% of infused dose) in rats

(Values are corrected for bicarbonate retention and non-oxidative isotope loss)

Animal no.	Fractional oxidation of [1-^{13}C]triolein	Animal no.	Fractional oxidation of [8-^{13}C]triolein
1	34·7	21	28·6
2	38·1	22	29·9
3	39·8	23	34·8
4	42·8	24	41·0
5	37·9	25	26·0
6	42·4	26	38·3
7	36·8	27	32·5
8	31·1	28	33·9
9	34·0	29	39·2
10	39·5	30	32·2
11	38·9	31	32·5
12	34·3	32	32·4
13	40·5	33	28·7
14	53·6	34	35·8
15	28·6	35	22·8
16	33·0	36	34·0
17	39·3	37	36·2
18	33·1	38	32·9
19	34·0	39	31·4
20	36·9	40	28·5
Mean	37·3	Mean	32·6
SEM	1·2	SEM	2·0

Discussion

The easiest definition of a 'true steady state' is to take a period near the end of the study that is used to calculate the plateau enrichment and to do a linear regression of those points against time, computing the slope. Then the slope value for all of the animals is averaged together and a *t* test is used to determine whether the mean value is significantly different from zero or not. If it is not, then the 'group' is in steady state. In our study we define a 'quasi-steady state' by considering the last 45 min of each infusion experiment. Thus, the data are 'relative' and much of the error of not being in a true steady state is diminished because of the assumption that all data will be affected. This is probably reasonable for comparing the bicarbonate and acetate results with the triolein because the oleate has to pass through the acetate and bicarbonate pools on its way to be oxidized.

In our experiments with rats, we observed a 13% difference in percentage oxidation of intravenously supplied triolein labelled with ^{13}C either at position 1 or 8 of the constituent oleic acid chains. This finding means that knowledge of the location of the label within a fatty acid chain is crucial for interpreting the results of isotope tracing of fatty acid oxidation. The observation of a 13% lower oxidation of the label in position 8 of oleic acid than that in position 1 indicates an incomplete β-oxidative breakdown of this fatty acid.

These results are in contrast to those of experiments using carboxyl-labelled fatty acids which are based on the assumption of complete breakdown of the fatty acid after initiation of β-oxidation. This hypothesis is based on studies first carried out by Weinman *et al.* (1950): they injected tripalmitin, labelled with ^{14}C at the C atoms 1, 6 or 11 of palmitic acid, intravenously into rats. They concluded that the location of the label did not influence significantly the amounts of $^{14}CO_2$ expired. Nevertheless, 1 year later they interpreted the observation of a 20% difference in the recoveries of [1-^{14}C]- *v.* [6-^{14}C]tristearin at $^{14}CO_2$ as representing incomplete β-oxidation (Weinman *et al.* 1951).

Armstrong *et al.* (1961) infused tripalmitin ^{14}C-labelled at chain-C atoms 1 or 6 into dogs and found that the amount of label recovered in breath was 31% lower for [6-^{14}C]- than for [1-^{14}C]tripalmitin, providing additional support for incomplete β-oxidation. After these early studies, the question of whether or not β-oxidation was complete received little attention. The almost exclusive use of carboxyl-labelled triacylglycerols or fatty acids for the determination of lipid oxidation has left the question hanging. Our observations suggest that the reported values of fatty acid oxidation (Geyer *et al.* 1948; Lerner *et al.* 1949; Eckart *et al.* 1973; Watkins *et al.* 1977; Nordenström *et al.* 1982; Chen, 1984; Paust *et al.* 1984, 1989; Bender *et al.* 1985; Brösicke *et al.* 1985; Park *et al.* 1986; Silberman, 1986; Wolfram & Metges, 1988; Adolph *et al.* 1989; Sulkers *et al.* 1989; Johnson *et al.* 1990) would have to be reduced by approximately 13% (the relative difference between the percentage oxidation of [8-^{13}C]- and [1-^{13}C]triolein).

The question arises whether or not the fed state influences the rate of lipid oxidation. In theory, feeding

carbohydrate should cause glucose oxidation to increase markedly. Therefore, the fed state may alter relative oxidation *v.* other fates. Indeed, preliminary studies investigating the percentage recovery of [1-^{14}C]- and [2-^{14}C]acetate as exhaled $^{14}CO_2$ in fasted and TPN-fed rats exhibited higher $^{14}CO_2$ values in the fasted (72·6 (SE 2·4) and 55·8 (SE 3·2) respectively) than in the fed state (65·53 (SE 3·8) and 49·2 (SE 3·6) respectively). Nevertheless the 1-^{14}C:2-^{14}C ratio remained unaffected (D Scharff, W Bäurle and P Fürst, unpublished results). It is unlikely that once the fatty acid is broken down to the small acetate units, the C is not oxidized.

Some other aspects, pertinent to the quantification of substrate oxidation by means of ^{13}C-labelled compounds, are worthy of discussion. First, not all of the labelled CO_2 being released as final product of the oxidative breakdown of a labelled substrate is actually expired. Some of the CO_2 is retained in the bicarbonate pools of the body (Wolfe, 1984; Irving *et al.* 1985). We determined, using a constant infusion of NaH$^{14}CO_3$, that 90·5 % of the metabolically produced CO_2 is excreted, as observed by most other workers using such techniques (Morris & Simpson-Morgan, 1963; Vazquez *et al.* 1986; Yagi & Walser, 1990). This value for bicarbonate retention is within the same range as those assessed by intraperitoneal or intravenous bolus administration of [^{14}C]bicarbonate in rats, i.e. from 90 to 98 % (Gould *et al.* 1949; Greenberg & Winnick, 1949; Shipley *et al.* 1959). Two studies reported substantially lower recoveries of 76 % and 50 % (Brooks & Donovan, 1983; Moldawer *et al.* 1983). The discrepancy is probably caused by differences in experimental conditions. The animals used in the latter studies were, for example, in the fasting state, which enhances the retention of bicarbonate and the rate of CO_2 refixation compared with the fed state (Yang *et al.* 1983).

Second, the ^{13}C-enrichment of expired CO_2 must be steady before the isotope administration. Because the natural variation of the ^{13}C-enrichment of the diet influences the natural ^{13}C-enrichment of the exhaled CO_2 (Schoeller *et al.* 1984), TPN was commenced 2 d before the stable isotope infusion period. This early changeover from oral feeding to intravenous nutrition was designed to adjust the animals to TPN and to achieve a constant ^{13}C-enrichment in breath CO_2 ('^{13}C-baseline') for each animal before the isotope administration.

The isotope steady state in expired breath was reached after a mean infusion time of 170 min, which is surprisingly rapid considering the complex metabolism of the infused [^{13}C]lipid particles before intracellular oxidation.

The most important aspect is the possible loss of label from fatty acids isotopically labelled at an even-numbered C atom (like [8-^{13}C]oleic acid) into non-oxidative pathways: β-oxidation of [8-^{13}C]oleic acid generates an acetyl-CoA molecule labelled with ^{13}C in the methyl group (C 2), whereas β-oxidation of [1-^{13}C]oleic acid yields acetyl-CoA labelled at its carbonyl C (C 1). When the resulting acetyl-CoA passes through the citric acid cycle, the C 1 is quantitatively cleaved as $^{13}CO_2$, whereas the methyl C of acetyl-CoA may be directed partly to other metabolic pathways (e.g. formation of phosphoenolpyruvate from the citric acid cycle intermediate oxaloacetate).

Such a different mode of C handling, as identified by Weinman *et al.* (1957), may result in underestimation of the oxidation of both [8-^{13}C]oleic acid and of the second C atom of the acetyl-CoA molecule generated from [8-^{13}C]oleic acid during β-oxidation.

For acetyl-CoA labelled in its C atoms 1 or 2, the loss of isotope in the citric acid cycle was recently demonstrated by Wolfe & Jahoor (1990) who showed that the recovery of infused [1-^{13}C]acetate as expired CO_2 was higher than the recovery of [2-^{13}C]- or [2-^{14}C]acetate.

In the present study the extent of loss of ^{13}C from the second C in acetyl-CoA, which is generated from [8-^{13}C]oleic acid, was quantified by comparing the rates of conversion of infused acetate (as precursor to acetyl-CoA), labelled either at C 1 or 2, to labelled expiratory CO_2. The correction to account for the difference in the recoveries of [2-^{14}C] *v.* [1-^{14}C]acetate as breath $^{14}CO_2$ resulted in a true percentage oxidation of 32·6 % for [8-^{13}C]triolein. Even after correction for the partial loss of the [8-^{13}C]triolein label through the citric acid cycle using [1-^{14}C]- and [2-^{14}C]acetate, we still found a significantly lower recovery of the [8-^{13}C]triolein relative to the [1-^{13}C]triolein. This difference indicates an incomplete breakdown of oleic acid after onset of β-oxidation which might be caused by partial oxidation and subsequent chain elongation and storage of newly formed fatty acids or triacylglycerol.

We conclude from our experiments that the isotope loss only partly explains the observed differences in ^{13}C-recovery of the differently labelled triacylglycerols in breath CO_2. This conclusion is in contrast to that of Wolfe & Jahoor (1990) who stated that the observed isotope loss would completely account for the difference in label recovery in breath between odd- and even-numbered fatty acids.

A small number of studies has been performed up to now using triolein labelled with ^{13}C at the C 8 of the oleic acid chains. Metges *et al.* (1992) administered [1-^{13}C]- and [8-^{13}C]triolein to human volunteers as an enteral bolus. They could not find a significant difference between the mean recoveries of [1-^{13}C] and [8-^{13}C]triolein in breath CO_2. However, when Adolph *et al.* (1991*a*,*b*) infused the labelled emulsions during TPN in ventilated traumatized patients using the steady-state technique, they found that the mean percentage oxidation of [8-^{13}C]triolein was 68 % of that of [1-^{13}C]triolein, the difference being significant ($P \leq 0{\cdot}005$). Neither group determined the bicarbonate retention of their respective study subjects under identical experimental conditions. Instead, they used a standard factor which does not necessarily reflect the retention of bicarbonate during the specific experimental and physiological conditions of their respective studies. Neither did they consider the sequestration of the label derived from [8-^{13}C]triolein in the citric acid cycle, leading to erroneous low values of oxidation of [8-^{13}C]triolein. Applying the correction factor derived from our acetate infusion experiments to the data of Adolph *et al.* (1991*a*,*b*), the ratio label recovery from [8-^{13}C]- : that from [1-^{13}C]triolein would be 0·93, which means that the relative difference between the percentage oxidation of [8-^{13}C]triolein and [1-^{13}C]triolein would be 7 %.

Conclusions

(1) Our observation of a higher percentage oxidation of [1-^{13}C]triolein compared with [8-^{13}C]triolein reflects an incomplete oxidative breakdown of oleic acid after onset of β-oxidation. Thus, our results do not support the hypothesis of a complete β-oxidation. Whether alternative metabolic pathways of fatty acids like chain elongation after partial oxidation with subsequent enhanced storage are of physiological or clinical relevance remains to be evaluated.

(2) The use of carboxyl-([1-^{13}C]-)labelled triacylglycerols or fatty acids results in the calculation of erroneously high values of fatty acid oxidation. Furthermore, the use of triacylglycerols or fatty acids labelled at an even-numbered C atom (like [8-^{13}C]triolein) results in the calculation of erroneously low values of fatty acid oxidation, if the isotope flux from acetyl-CoA in the course of the citric acid cycle is not considered.

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