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Orally administered [^{14}C]DPA and [^{14}C]DHA are metabolised differently to [^{14}C]EPA in rats

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Abstract

Previous studies have revealed that C20 PUFA are significantly less oxidised to CO_2 in whole-body studies compared with SFA, MUFA and C18 PUFA. The present study determined the extent to which three long-chain PUFA, namely 20 : 5*n*-3 EPA, 22 : 5*n*-3 docosapentaenoic acid (DPA) and 22 : 6*n*-3 DHA, were catabolised to CO_2 or, conversely, incorporated into tissue lipids. Rats were administered a single oral dose of 2·5 μCi [1-^{14}C]DPA, [1-^{14}C]EPA, [1-^{14}C]DHA or [1-^{14}C]oleic acid (18 : 1*n*-9; OA), and were placed in a metabolism chamber for 6 h where exhaled $^{14}CO_2$ was trapped and counted for radioactivity. Rats were euthanised after 24 h and tissues were removed for analysis of radioactivity in tissue lipids. The results showed that DPA and DHA were catabolised to CO_2 significantly less compared with EPA and OA ($P<0·05$). The phospholipid (PL) fraction was the most labelled for all three *n*-3 PUFA compared with OA in all tissues, and there was no difference between C20 and C22 *n*-3 PUFA in the proportion of label in the PL fraction. The DHA and DPA groups showed significantly more label than the EPA group in both skeletal muscle and heart. In the brain and heart tissue, there was significantly less label in the cholesterol fraction from the C22 *n*-3 PUFA group compared with the C20 *n*-3 PUFA group. The higher incorporation of DHA and DPA into the heart and skeletal muscle, compared with EPA, suggests that these C22 *n*-3 PUFA might play an important role in these tissues.

Key words: ***n*-3 PUFA: Docosapentaenoic acid: EPA: DHA: β-Oxidation**

There is a paucity of literature regarding the metabolism of docosapentaenoic acid (DPA), an elongated metabolite of EPA and an immediate precursor of DHA, in mammals. Previously, we have shown that with short-term supplementation of *n*-3 DPA in rats, there was evidence of the retro-conversion of DPA to EPA in the liver, muscle, adipose and heart and of the metabolism of DPA to DHA in the liver[1]. Holub *et al.*[2] extended this work and demonstrated, in young rats, that DPA was predominantly retro-converted to EPA, with selective incorporation into phospholipid (PL) pools. There was a particularly high capacity for this retro-conversion in the kidney. It should be noted that DPA in foods is accompanied by other *n*-3 and *n*-6 fatty acids. It is possible that DPA competes with these fatty acids for the enzymes required for its metabolism (desaturases and elongases) and that DPA metabolism is affected by this competition which may minimise its conversion into DHA in various cell-culture models[3] and animal studies[1].

However, it is still not known what proportion of DPA is β-oxidised to form CO_2 compared with deposition of DPA in various tissues, though previous studies have shown that C20 PUFA were oxidised the least after 7 h compared with SFA, MUFA or PUFA, including linoleic acid, γ-linolenic acid and α-linolenic acid (ALA). Leyton *et al.*[4] investigated the whole-body oxidation of medium-chain and essential long-chain PUFA (LC-PUFA) in rats and reported that of the fatty acids that were studied, ALA and oleic acid (OA) had the equal highest rates of oxidation compared with other LC-PUFA such as linoleic acid, γ-linolenic acid and arachidonic acid[4]. Fu & Sinclair[5] reported that in guinea pigs, ALA was more prone to β-oxidation or excretion via the skin rather than metabolism to DHA. In a human study, Burdge *et al.*[6] investigated the rate of β-oxidation of ALA in human subjects and also its conversion to EPA, DPA and DHA. The proportion of [^{13}C]ALA recovered as $^{13}CO_2$ in breath at the baseline tracer study was 34 % over 24 h and was not altered by diets rich in ALA or LC *n*-3 PUFA. The authors have indicated that the actual extent of partitioning of labelled ALA towards oxidation may have been up to 30 % greater than the measured value[6]. As with ALA, EPA has also been reported to undergo rapid oxidation in tissues such as brain, which may explain the low levels of (ALA and) EPA in the brain, compared with DHA[7,8].

Abbreviations: ALA, α-linolenic acid; DPA, docosapentaenoic acid; LC-PUFA, long-chain PUFA; OA, oleic acid; PL, phospholipid.

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DHA has been reported to be conserved from β-oxidation[9,10] and efficiently deposited in various tissues including the liver, heart, retina and brain[1,11,12]. There have been no studies to investigate the whole-body metabolism of DPA, comparing tissue deposition with the degree to which it is oxidised to CO_2.

The aims of the present study were to (1) determine the extent to which [^{14}C]DPA is excreted as CO_2 compared with [^{14}C]EPA and [^{14}C]DHA, and (2) examine the incorporation of radioactivity into the various lipid fractions including PL, diacylglycerol, cholesterol, NEFA or TAG, compared with EPA and DHA.

Methodology

Animals and diets

A total of twenty, 3-week-old male weanling Wistar rats were randomly divided into four groups of five animals. The rats were maintained on a rat chow diet with free access to water. The total lipid content of the chow diet used was 5·7 g/100 g diet. The three main unsaturated fatty acids present in the diet were OA (29·7 %; weight percentage of total fatty acids), linoleic acid (31·2 %; weight percentage of total fatty acids) and ALA (3·4 %; weight percentage of total fatty acids). There were no *n*-3 LC-PUFA detected in the diet lipids.

Collection of CO_2 expired by the animals

Rats were pair-housed and allowed 1 week to acclimatise. The weight of the animals was recorded on the first day of the experiment. The rats were then orally administered (see below) a single dose (*n* 5 rats per isotope) of 2·5 μCi [1-^{14}C]DPA (52 mCi/mmol), [1-^{14}C]EPA (56·2 mCi/mmol), [1-^{14}C]DHA (53 mCi/mmol) or [1-^{14}C]OA (55·6 mCi/mmol) (Moravek Radiochemicals). The isotopes were supplied in the NEFA form in ethanol and evaporated under a stream of N_2 gas. The dried, labelled fatty acids were each dissolved in 10 ml OA to obtain a concentration of 2·5 μCi in 0·5 ml olive oil. This 0·5 ml olive oil containing the different isotopically labelled fatty acids was then given to the rats by inserting a feeding tube into the rat stomach via the throat and the oil was injected into the tube using a syringe. The doses were administered to all animals at the same time of the day (in the morning). After dosing, the animals were immediately placed in a metabolism chamber (Columbus Instruments) for the next 6 h, which was connected to a suction pump and a flowmeter. The rats had access to water but not food during these 6 h. A constant airflow of 1·1 litres/min per cage was maintained. The exhaled $^{14}CO_2$ was bubbled into a 50 ml solution of methoxyethylamine–ethanolamine (2:1, v/v)[4]. In an initial trial experiment, the exhaled $^{14}CO_2$ was bubbled into two bottles containing the trapping liquid to test the saturation of the trapping liquid. However, no radioactivity was detected in the second bottle. Therefore, only one bottle was used for the final experiment. The air was then passed through another flask of distilled water to trap any vapours produced. Thereafter, 1 ml of the trapping solution was removed from the bottle containing methoxyethylamine–ethanolamine every hour for the next 6 h and counted for radioactivity using scintillation fluid (Ultima Gold; PerkinElmer). The dpm of CO_2 collected were then converted into the percentage of dose. After 6 h, the animals were returned to their home cages, with free access to food and water. At 24 h after dosing, the animals were killed and tissues were collected for analysis as described below.

Tissue analysis

Total radioactivity in whole tissue. In order to calculate the total radioactivity in whole tissue, the tissue weights of heart, liver and kidney were recorded. Since the total adipose and muscle were not dissected from these rats, it was not possible to directly estimate the amount of radioactivity in the whole adipose and muscle compartments. From the literature[13,14], it was found that the weight of adipose and skeletal muscle in these animals might be expected to be of the order of 8·9 and 27 % of body weight, respectively. Therefore, these values were used to estimate these two tissue weights and derive the total incorporation into adipose and muscle.

The tissues were dissolved in Solvable™ (PerkinElmer) according to the following method[15,16]: 1 ml of plasma and the weighed amounts of tissues – 100 mg of liver and brain; 50 mg of heart, liver, adipose, kidney and muscle – were added to scintillation vials. Then, 2 ml of Solvable™ were added to each vial. The samples were incubated at 55°C for 1 h in the case of plasma and for 3 h in the case of other tissues. To reduce foaming caused by H_2O_2, 0·1 ml of 0·1 M-EDTA disodium solution was added to the samples. Then, 0·2 ml of 30 % H_2O_2 was added in 0·1 ml increments. The samples were allowed to stand for a few minutes to allow any reaction/foaming to subside. They were heated again at 55°C for 30 min for complete decolourisation. Then, 10 ml of the scintillation fluid (Ultima gold; PerkinElmer) were added to the samples. Before counting, the samples were allowed to adapt to light and temperature for 1 h at room temperature in the scintillation counter with the lid closed.

Lipid extraction from tissues

Tissues were weighed (ranging between 350 mg and 1 g), minced and tissue lipids were extracted in 3 ml chloroform–methanol (2:1, v/v) overnight at 4 °C, as described by Sinclair *et al.*[17]. The next day, the samples were allowed to reach room temperature and then filtered. To the filtrate, a volume of 0·9 % NaCl was added, which was equal to 20 % of the volume of the filtrate. The samples were vortexed and then centrifuged at 1500 rpm for 10 min to allow the layers to separate, and the lower phase was taken and stored at −20 °C before further analysis.

TLC

An aliquot of the extracted lipids was subjected to TLC using silica gel 60 G (Merck), using hexane–diethylether–acetic acid (85:15:2, by vol.) as the developing solvent. The lipids were visualised with 0·1 % 2′,7′-dichlorofluroescein indicator (Scharlau). The PL, diacylglycerol, cholesterol, NEFA and

TAG bands were identified using standards and scraped off into scintillation vials and counted using 3 ml of the scintillation fluid. There was a clear separation of the PL, diacylglycerol and cholesterol fractions; however, there was an unexpectedly poor separation of TAG and NEFA in most cases, and so these two fractions were combined before counting.

Statistical analysis

Data calculations and statistical analysis were performed using SPSS software (SPSS version 17.0; SPSS, Inc.). Datasets were first tested for normality using the Kolmogorov–Smirnov test. For datasets with a normal distribution, significant differences between the dietary groups were tested using a one-way ANOVA for each type of fatty acid. If the homogeneity of variance test was non-significant, *post hoc* comparisons were made using the least significant difference test with a significance level of $<0{\cdot}05$. If the homogeneity of variance test was significant, data were analysed using Welch test, and *post hoc* comparisons were made using Dunnett's T3 test with a level of $<0{\cdot}05$ considered as significant.

Datasets with a non-normal distribution were analysed using the non-parametric Kruskal–Wallis test, with a level of $<0{\cdot}05$ considered as significant.

Ethics approval

All experimental procedures involving animals were performed under the Australian Code of Practice for the care and use of animals for Scientific Purposes and were approved by the Deakin University Animal Welfare Committee.

Results

Body and tissue weights

The mean body weight of rats in the [^{14}C]OA, [^{14}C]EPA, [^{14}C]DPA and [^{14}C]DHA treatment groups was 72 (SEM 6), 77 (SEM 4), 71 (SEM 8) and 79 (SEM 6) g, respectively. There was no significant difference between the body weights and tissue weights of rats in any of the groups.

Incorporation of radioactivity into CO_2 released by the animals

Fig. 1 shows the amount of expired $^{14}CO_2$ recovered as a function of time, expressed as a percentage of dose administered to the rats. To quantify the total amount of $^{14}CO_2$ expired in 6 h, the area under each curve for Fig. 1 was calculated using the trapezoidal rule. Since the data did not show a normal distribution, statistical analysis was performed using the Kruskal–Wallis test. Rats in the OA group had more label ($P<0{\cdot}05$) in the collected CO_2 after 6 h compared with the EPA (by 3·3-fold), DPA (by 12·5-fold) and DHA (by 9·2-fold) groups. After 6 h, the amount of $^{14}CO_2$ collected for the DPA rats was not different from that of the DHA rats ($P=0{\cdot}251$; $\chi^2 = 1{\cdot}32$; df = 1) but significantly lower than

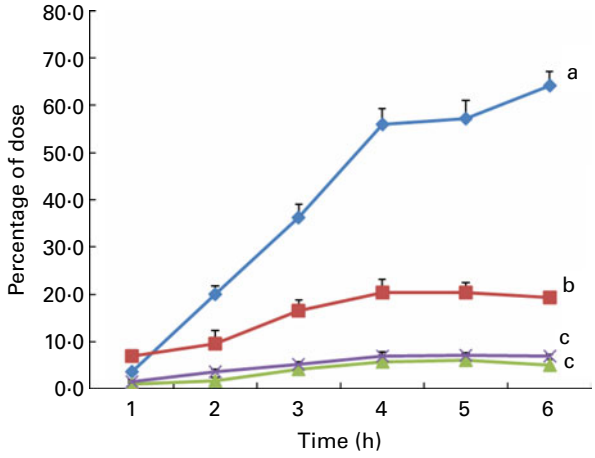

Fig. 1. Incorporation of [^{14}C]oleic acid (OA; ◆), [^{14}C]EPA (■), [^{14}C]docosapentaenoic acid (DPA; ▲) or [^{14}C]DHA (×) into CO_2 released by the animals in 6 h after supplementation with 2·5 μCi [^{14}C]OA, [^{14}C]EPA, [^{14}C]DPA or [^{14}C]DHA. Values are means (percentage of dose; *n* 5 per group), with their standard errors represented by vertical bars. Data obtained at 6 h were analysed using the non-parametric Kruskal–Wallis test, with a level of $<0{\cdot}05$ considered as significant. [a,b,c] Mean values with unlike letters were significantly different between the groups (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

that of the EPA rats ($P=0{\cdot}009$; $\chi^2 = 6{\cdot}82$; df = 1). After 6 h, the order of the expired $^{14}CO_2$ was [^{14}C]OA > [^{14}C]EPA > [^{14}C]DHA = [^{14}C]DPA.

Incorporation of radioactivity in the tissues

The incorporation of radioactivity in the liver, heart, brain, kidney, muscle and adipose after 24 h of the oral administration of [^{14}C]OA, [^{14}C]EPA, [^{14}C]DPA or [^{14}C]DHA is shown in Fig. 2 and was statistically analysed using one-way ANOVA (due to the normal distribution of the dataset) except for adipose tissue data, which was analysed using the non-parametric Kruskal–Wallis test (due to the non-normal distribution). Incorporation of radioactivity was higher in the *n*-3 LC-PUFA groups compared with the OA group in all tissues. DHA was deposited in significantly ($P<0{\cdot}05$) higher amounts compared with OA, EPA and DPA in the liver, heart, brain and kidney. In the liver, incorporation of radioactivity in the DHA group was significantly ($P<0{\cdot}05$) higher by 9-fold compared with the OA group and by approximately 1·8-fold compared with the EPA and DPA groups. In the heart, both DPA and DHA groups showed significantly ($P<0{\cdot}05$) higher label compared with the OA (by 11-fold) and EPA (by 3-fold) groups. In the brain, it was observed that the EPA, DPA and DHA groups had significantly ($P<0{\cdot}05$) higher amounts of radioactivity compared with the OA group by 3·1-, 4·2- and 6·1-fold, respectively. Similarly in the kidney, the EPA, DPA and DHA groups had significantly ($P<0{\cdot}05$) higher amounts of radioactivity compared with the OA group by 2·1-, 2·3- and 3·5-fold, respectively.

In the muscle, the DPA and DHA groups had a significantly ($P<0{\cdot}05$) higher incorporation of the label compared with the OA and EPA groups by approximately 3·1- and 2·1-fold, respectively. Although the OA group showed a higher

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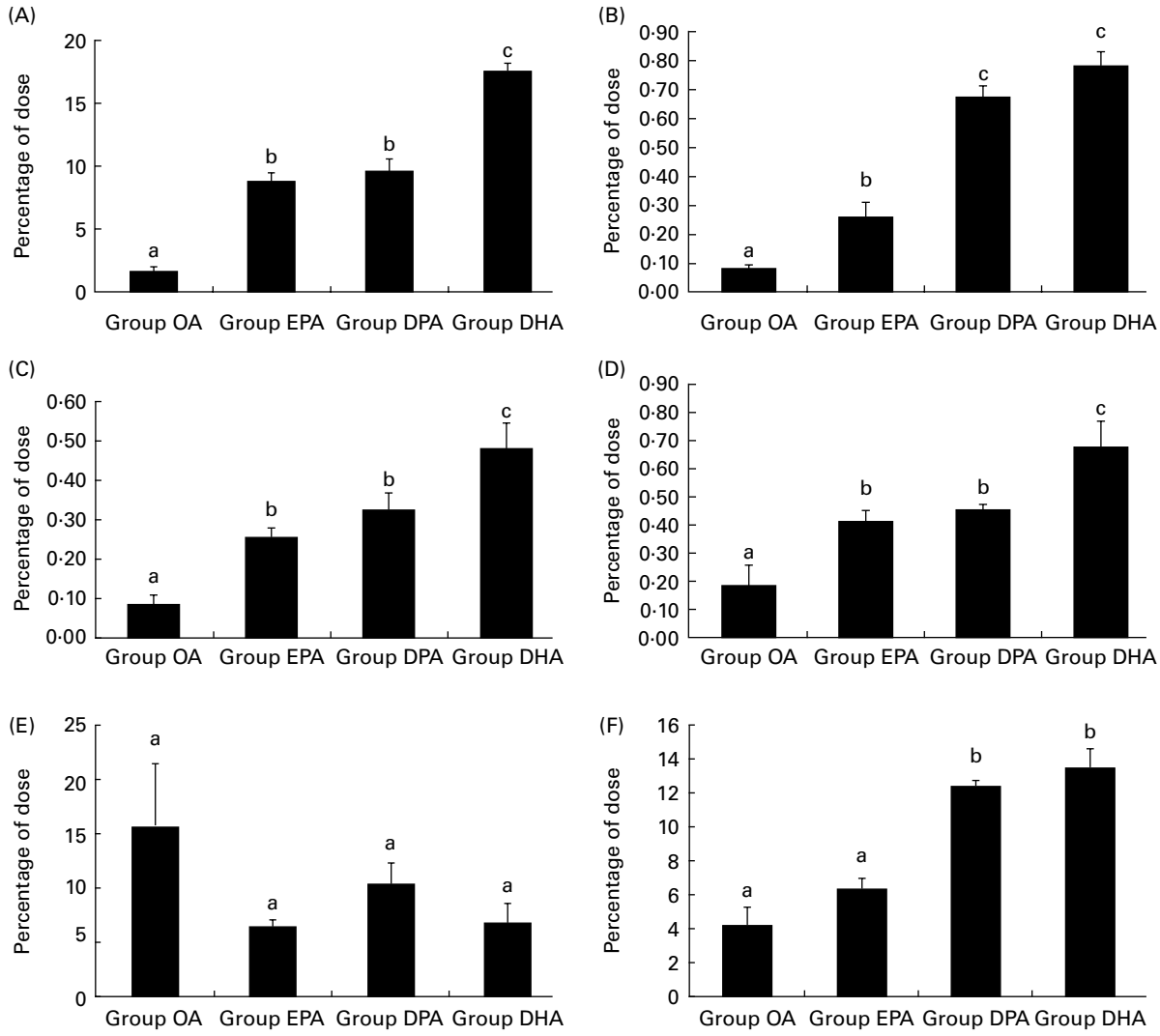

Fig. 2. ^{14}C counts in the (A) liver, (B) heart, (C) brain, (D) kidney, (E) adipose tissue and (F) skeletal muscle of animals supplemented with 2·5 μCi [^{14}C]oleic acid (OA), [^{14}C]EPA, [^{14}C]docosapentaenoic acid (DPA) or [^{14}C]DHA. Values are means (percentage of dose; *n* 5 per group), with their standard errors represented by vertical bars. Data for liver, heart, brain, kidney and muscle were analysed using one-way ANOVA (due to a normal distribution) and *post hoc* comparisons were made using the least significant difference test. Data for adipose tissue counts were analysed using the non-parametric Kruskal–Wallis test (due to a non-normal distribution), with a level of $<0·05$ considered as significant. [a,b,c] Mean values with unlike letters were significantly different between the groups. The derived values were taken from the literature ((E) adipose tissue 8·9 % body weight, skeletal muscle 27 % of body weight and total plasma volume of 4·68 ml/100 g body weight; see the Methodology section).

incorporation of the dose in the adipose tissue compared with the *n*-3 LC-PUFA groups, this difference was not statistically significant ($P>0·05$). A comparison of radioactivity detected in CO_2 (at 6 h) and tissues (at 24 h) is presented in Table 1.

Incorporation of radioactivity in various lipid fractions

Table 2 shows the radioactivity incorporated into various lipid fractions in the analysed tissues.

Phospholipid fraction

The PL fraction was clearly the most labelled for the *n*-3 LC-PUFA compared with OA in all the tissues. In all tissues except adipose, the tissue labelling in the PL fraction was between 43 and 80 %. The labelling of PL in adipose was <19 % of the label in all lipid fractions in this tissue. In the brain and liver, the label from DHA was most highly incorporated into the PL, while DPA was the second most highly incorporated. In the heart, the label from DPA was highly incorporated into the PL and was 3-fold higher than OA. The brain, heart and kidney showed the greatest differential for the *n*-3 LC-PUFA compared with OA; in the case of the brain, there was approximately twice as much label in the PL for the *n*-3 LC-PUFA (1·6-fold for EPA, 2·0-fold for DPA and 2·3-fold for DHA), compared with OA. In the case of the heart, there was approximately twice the amount of radioactivity in the PL for EPA, compared with OA. For DPA and DHA, the differential was 2·5- and 2·3-fold, respectively, compared with OA. In the case of the kidney, the differential

Table 1. Comparison of radioactivity recovered from all tissues and from the $^{14}CO_2$ expired by the rats*

(Mean values with their standard errors (percentage of dose), *n*-5 per group)

	Brain		Liver		Heart		Kidney		Muscle†		Adipose†		CO_2 (6 h)	
Group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
OA	0·1[a]	0·0	1·6[a]	0·3	0·1[a]	0·0	0·2[a]	0·1	4·2 [a]	1·0	15·6[a]	5·8	64·2[a]	3·0
EPA	0·3[b]	0·0	8·8[b]	0·7	0·3[b]	0·1	0·4[b]	0·0	6·3[a]	0·6	6·5[a]	0·6	19·3[b]	1·1
DPA	0·3[b]	0·0	9·6[b]	1·0	0·7[c]	0·0	0·5[b]	0·0	12·4[b]	0·4	10·4[a]	1·9	5·1[c]	1·1
DHA	0·5[c]	0·1	17·6[c]	0·6	0·8[c]	0·0	0·7[c]	0·1	13·5[b]	1·1	6·8[a]	1·9	7·0[c]	0·4

OA, oleic acid; DPA, docosapentaenoic acid.

[a,b,c] Mean values with unlike superscript letters within a row were significantly different between the dietary groups.

* ^{14}C counts in all tissues (24 h after dosing) and expired $^{14}CO_2$ (6 h after dosing) of animals supplemented with 2·5 μCi [^{14}C]OA, [^{14}C]EPA, [^{14}C]DPA or [^{14}C]DHA. Data for liver, heart, brain, kidney and muscle were analysed using one-way ANOVA (due to a normal distribution) and *post hoc* comparisons were made using the least significant difference test. Data for adipose tissue counts were analysed using the non-parametric Kruskal–Wallis test (due to a non-normal distribution), with a level of $<0·05$ considered as significant.

† The total weight of muscle and adipose tissue was estimated as described in the Methodology section.

ranged from 2·2- to 2·5-fold for the *n*-3 LC-PUFA groups, compared with the OA group.

Other fractions

For the muscle tissue, in the OA group, the TAG + NEFA fraction was clearly more labelled than the PL fraction; in contrast, in the muscle, there was approximately equal labelling of the PL and TAG + NEFA fractions for the *n*-3 LC-PUFA groups. In the case of adipose tissue, more than half of the label was found in the TAG + NEFA fraction, with a low proportion only in the PL fraction in the case of all labelled fatty acids. In all tissues examined, the spot assigned as cholesterol on the basis of the standards showed the label in this fraction ranging from 3·4 to 50 % of the radioactivity in different tissue lipids with the fatty acid isotopes. In general, there was a higher proportion of label in cholesterol for the OA group than for the *n*-3 LC-PUFA groups. In the brain, there was significantly more radioactivity in cholesterol as a proportion of the lipids detected for OA compared with DPA and DHA.

Table 2. Incorporation of radioactivity into various lipid fractions in different tissues (percentage of radioactivity in lipid fractions per tissue)*

(Mean values with their standard errors (percentage of dose), *n* 5 per group)

	OA group		EPA group		DPA group		DHA group	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Brain								
Phospholipid	35·4[a]	4·8	58·2[b]	2·9	70·9[c]	1·0	80·0[d]	0·9
Cholesterol	27·2[a]	3·4	22·2[a,b]	3·8	13·4[b,c]	2·1	9·6[c]	0·2
NEFA + TAG†	37·4[a]	3·9	19·6[b]	1·9	15·7[b,c]	2·5	10·4[c]	0·9
Liver								
Phospholipid	28·87[a]	2·2	54·8[b]	1·1	54·5[b]	2·1	58·9[b]	1·0
Cholesterol	29·1[a]	2·3	3·4[b]	0·4	6·1[c]	0·9	5·0[b,c]	1·3
NEFA + TAG†	42·1[a]	3·0	41·7[a]	1·3	39·4[a]	1·5	36·2[a]	1·4
Heart								
Phospholipid	27·3[a]	10·6	57·8[a]	6·8	73·5[b]	2·3	68·7[b]	3·1
Cholesterol	50·7[a]	14·7	26·2[a]	6·8	8·4[b]	1·6	21·5[a]	3·9
NEFA + TAG†	22·0[a]	5·4	16·0[a]	4·2	18·1[a]	2·8	9·8[b]	1·1
Kidney								
Phospholipid	19·8[a]	3·3	50·6[b]	4·8	42·9[b]	2·7	49·7[b]	2·5
Cholesterol	21·5[a]	5·0	7·0[b]	0·9	13·0[a,b]	3·5	11·6[a,b]	1·0
NEFA + TAG†	58·7[a]	4·2	42·4[b]	4·3	44·1[a,b]	4·6	38·7[b]	2·2
Muscle								
Phospholipid	17·7[a]	1·9	45·1[b]	3·3	45·8[b]	5·1	49·4[b]	4·0
Diacylglycerol	14·4[a]	3·1	11·1[a]	1·7	5·8[b]	0·7	7·7[b]	1·0
Cholesterol	9·1[a]	1·5	8·5[a]	1·5	5·0[b]	0·5	12·2[a]	2·6
NEFA + TAG†	58·8[a]	3·7	35·3[b]	1·7	43·5[b]	5·4	30·7[b]	1·6
Adipose tissue								
Phospholipid	6·6[a]	0·6	17·3[a,b]	3·4	18·8[b]	1·1	11·4[c]	0·8
Diacylglycerol	15·5[a]	2·6	17·1[a]	2·4	12·4[a]	1·3	12·5[a]	1·7
Cholesterol	19·2[a]	2·3	14·1[a]	1·2	16·2[a]	3·9	10·7[b]	3·0
NEFA + TAG†	58·8[a]	5·4	46·8[a]	4·4	52·6[a]	3·6	65·4[a]	3·2

OA, oleic acid; DPA, docosapentaenoic acid.

[a,b,c] Mean values with unlike superscript letters within a row were significantly different ($P<0·05$).

* Data were analysed using the non-parametric Kruskal–Wallis test (due to a non-normal distribution), with a level of $<0·05$ considered as significant.

† NEFA and TAG fractions were poorly separated on most TLC plates, so these two fractions were pooled for ^{14}C determinations.

In the brain, the differential was between 1·2- and 2·8-fold in favour of OA. In the liver, there was a significantly higher proportion of the label in cholesterol in the OA group than for the *n*-3 LC-PUFA groups. In the adipose, kidney and muscle, tissue lipid radioactivity in cholesterol was between 9 and 21·5 % in the OA group, but these values were not consistently different from those of the *n*-3 LC-PUFA groups.

Discussion

The present study examines the effects of the oral administration of [^{14}C]DPA to animals on the extent of β-oxidation of [^{14}C]DPA (measured by the labelling of expired $^{14}CO_2$), as well as the tissue incorporation of ^{14}C-labelled lipids. DPA and DHA were conserved from β-oxidation to a greater extent than EPA and OA at 6 h. This finding is consistent with that of Leyton *et al.*[4], who used 3·5-week-old Sprague–Dawley rats and showed that after 6 h, 48 % of the radioactivity from OA administered to the rats was recovered as $^{14}CO_2$. In the present study, it was observed that after 6 h of dosing with [^{14}C]OA, the 4-week-old Wistar rats expired 64 % of the radioactivity administered. Leyton *et al.*[4] also found a significantly greater β-oxidation of OA at 6 h compared with linoleic acid, ALA and arachidonic acid.

Previously, studies have also reported a higher β-oxidation rate of EPA compared with DHA. Madsen *et al.*[10] reported that EPA-CoA is a good substrate for mitochondrial carnitine acyl-transferase-I and DHA is a poor substrate for both mitochondrial and peroxisomal β-oxidation in *ex vivo* experiments, which could explain the high rate of β-oxidation of EPA in the present study[10]. They also reported that [1-^{14}C]EPA was oxidised to a much greater extent than [1-^{14}C]DHA in rat liver parenchymal cells, isolated peroxisomes and, especially, purified mitochondria. However, there is no literature on *in vitro* or *in vivo* mitochondrial or peroxisomal β-oxidation of DPA.

Evidence suggests that *n*-3 LC-PUFA are important regulators of PPAR, though the effects vary according to cell types, i.e. in some cells, PPAR expression is increased by *n*-3 LC-PUFA while in others it is decreased[18]. For example, studies have reported an increase in PPARγ after exposure to *n*-3 LC-PUFA in myotubes[19], in HepG2 cells[20] and adipocytes[21]. However, Lee & Hwang[22] reported that *n*-3 LC-PUFA suppressed PPAR responses in a human colon tumour cell line[22]. Although the effect of DPA on fat oxidation has not been investigated, one study that investigated the PPARα-binding affinity of various fatty acids reported that DPA induced PPARα, but EPA and DHA had a stronger and more consistent effects[23].

There is another isomer of DPA known as *n*-6 DPA. The content of *n*-6 DPA is low in most mammalian tissues, except testes tissue[24,25]. In fish and fish oils, the *n*-3 isomer of DPA is substantially higher than the *n*-6 isomer[26]. It is not known whether *n*-6 DPA would behave in the same manner as *n*-3 DPA, as no animal studies have been conducted to investigate its β-oxidation *in vivo*. However, studies in cell culture have shown that when [1-^{14}C]4,7,10,13,16–22 : 5 was incubated directly with peroxisomes, microsomes and 1-acyl-*sn*-glycero-3-phosphocholine, or generated from [3-^{14}C]6,9,12,15,18–24 : 5, its primary fate was esterification rather than continued β-oxidation[27].

While it is generally regarded that DHA is highly concentrated in the brain, the present study shows that for all *n*-3 LC-PUFA, the tissues most extensively labelled were skeletal muscle, liver and adipose tissue. Polozova & Salem[12] also found DHA to be highly incorporated in the liver, heart and brain, compared with OA, 5 min after an intravenous injection of [^{14}C]DHA in mice.

A recently published study on the whole-body distribution of labelled DHA (either TAG or PL form), using the technique of whole-body autoradiography[28], showed similar data to the present study. The percentage of the oral dose for rats aged 4 weeks (24 h post-dose with the TAG form) was 17·93 % in the liver (the result from Table 2 was 17·6 %), 22·98 % for the skeletal muscle (the present study 13·5 %), 0·72 % for the heart (the present study 0·8 %), 0·62 % for the kidney (the present study 0·7 %) and 0·21 % for the brain (the present study 0·5 %). The generally good consistency between the data from Graf *et al.*[28] and the present study supports the assumptions made in the present study on the proportions of muscle and adipose tissue in rats (for a description see the Methodology section). In addition, Graf *et al.*[28] reported a difference in the labelling between brown and white adipose tissue in favour of brown fat by 9-fold. In the present study, only white adipose tissue was examined. Graf *et al.*[28] also reported that the form of lipid that contained DHA could play a significant role in tissue distribution. The study showed that in 10-week-old rats, tissues such as liver, brain, kidney and anterior uveal tract accumulated 2- to 3-fold more [^{14}C]DHA-derived radioactivity after [^{14}C]DHA-PL dosing compared with [^{14}C]DHA-TAG dosing. In the present study, the NEFA form of DHA and the other LC-PUFA was used.

One of the indications of β-oxidation of ^{14}C-labelled fatty acids is the detection of the label into the cholesterol fraction. This fraction presumably reflects the synthesis of cholesterol derived from labelled acetyl CoA or other water-soluble metabolites, such as ketones, resulting from β-oxidation of the dosed ^{14}C-labelled fatty acids. The results in Table 2 show that there was a higher incorporation of the label in the cholesterol fraction in the OA group in most tissues, compared with all three *n*-3 LC-PUFA groups. This is consistent with the CO_2 data, which showed that OA was β-oxidised at a higher rate compared with the *n*-3 LC-PUFA. While not measured, it would be expected that SFA and MUFA would become labelled in some tissues presumably via the same process that resulted in the labelling of cholesterol[11,29–31].

In the present study, the label from the *n*-3 LC-PUFA showed a higher incorporation in the PL fraction compared with OA in all tissues. Polozova & Salem[12] also showed that rats injected with radioactive DHA showed higher radioactivity in PL and NEFA fractions in the liver tissue and lower radioactivity in the TAG fraction, 5 min post-injection, compared with OA-injected rats. In comparing the deposition of label in the PL fraction within the three *n*-3 LC-PUFA groups, it was observed that DHA showed a significantly

higher incorporation into PL in the brain, compared with EPA (by 1·4-fold) and DPA (1·1-fold). The reduced incorporation of label in expired CO_2 in the DPA and DHA groups may be due to the fact that there may be selective acylation of DPA and DHA into PL, making the substrates less readily available as substrates for β-oxidation[32,33].

Conclusions

DPA is an *n*-3 LC-PUFA commonly consumed through red meat and fish; however, the fate of ingested DPA *in vivo* has not been studied in much detail. The present study is the first to report on the whole-body metabolism of [^{14}C]DPA in animals, with the aim to examine the extent of β-oxidation and tissue incorporation of [^{14}C]DPA and [^{14}C]DHA compared with [^{14}C]EPA. The conservation of DPA from β-oxidation and higher incorporation of DPA (as with DHA) in the heart and muscle, compared with EPA, suggests that DPA might have a specific role in these tissues; further studies are required to investigate this issue, as well as to establish whether DPA is a substrate for cyclo-oxygenase and lipoxygenase enzymes *in vivo* [34,35].

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