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Short communication

Comparison of the bioavailability of docosapentaenoic acid (DPA, 22:5n-3) and eicosapentaenoic acid (EPA, 20:5n-3) in the rat

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ABSTRACT

Based on the results from a human study which showed significantly reduced incorporation of DPA compared with EPA into chylomicrons, this study was designed to test if dietary DPA was significantly less absorbed than EPA. Male Sprague Dawley rats were randomly assigned to three groups of six, and were fed a semi-synthetic high fat diet (23.5% fat) for 9 days. The test omega 3 fatty acids (EPA and DPA, 250 mg/animal/day, free fatty acid form) or olive oil (250 mg/animal/day) were added to the high fat diet on days 5, 6 and 7. Dietary EPA and DPA appeared in the faeces on days 6, 7 and 8, with the total amount of DPA excreted being 4.6-fold greater than that of EPA. The total amount of faecal fat did not differ significantly between the groups. At the conclusion of the study (day 9), it was found that liver DPA, EPA and total n-3 LC-PUFA levels were significantly increased by both DPA and EPA feeding compared with the olive oil fed control group. In the heart, DPA feeding increased the DPA content and both DPA and EPA feeding increased the total n-3 LC-PUFA levels. This study showed that DPA and EPA, both provided in free form, are metabolised differently, despite being chemically similar.

1. Introduction

Eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and docosapentaenoic acid (DPA, 22:5n-3) are provided from dietary sources, mainly fish, fish oil products and to a lesser extent from ruminant meats. Traditionally, whilst EPA and DHA have been abundantly studied, little interest has been paid to DPA. However, recent studies in rats [1–3] and humans [4,5] suggest that EPA and DPA are metabolised differently.

In rats, short-term supplementation with pure DPA significantly increased the concentration of DHA in liver and the concentration of EPA in the liver, heart and skeletal muscle, presumably by the process of retroconversion [1]; with the retroconversion from DPA to EPA being especially apparent in the kidney [3]. The synthesis, metabolism and biological effects of DPA have been reviewed by Kaur et al. [6] and thereafter, two studies have investigated the metabolism of DPA in humans. In a randomized cross-over double blinded study by Miller et al. [4], 10 female participants were provided a total of 8 g of pure DPA or pure EPA over a 7-day period. This study proved that DPA and EPA had different and specific incorporation patterns into plasma lipids and erythrocyte lipids in humans. Linderborg et al. [5] applied lipidomics to a postprandial cross-over study in which 2 g of either pure EPA or pure DPA (as free fatty acids, FFA) were served with the breakfast to 10 healthy female subjects with a 1-week wash out between different supplements. It was found that following the breakfast containing DPA plus olive oil, there was a significantly reduced chylomicronemia compared with that observed in the EPA-olive oil breakfast and the control breakfast with olive oil alone. Lipidomics of chylomicron triacylglycerols (TAG) revealed that the EPA and DPA were incorporated into different TAG molecular species, namely EPA into 18:1:18:1:EPA and DPA into 16:0:18:1:DPA, with a significantly lower incorporation of DPA compared with EPA in the chylomicron TAG. They also reported that 3 out of 10 participants felt sick in the stomach after having the DPA breakfast, and this led them to hypothesise that DPA might interfere with lipid digestion, absorption or clearance. However, in this study the lipid content of the faeces was not analysed.

The present short-term study aimed to investigate whether DPA and EPA were metabolised equally or differently and thus, it was specificaly designed to assess the relative loss of DPA or EPA in faeces after equal dietary intake, and whether there was the incorporation of dietary DPA or EPA into tissues.

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2. Materials and methods

2.1. Diet and study design

Twenty four 6-week old healthy male Sprague-Dawley rats were housed in pairs and acclimatised for a week on ad libitum normal chow diet. The rats were then randomly divided into four groups of six rats each. The first group was sacrificed at day 0 for baseline data. The other groups were fed a specially prepared high fat diet (Speciality Feed Company, Western Australia; 22.5% protein, 23.5% fat, 10% total saturated fat, 8.3% monounsaturated fat, 4.5% linoleic acid, 0.5% α-linolenic acid, and no detectable arachidonic acid, EPA, DPA or DHA) for 9 days; on days 5, 6 and 7 the groups of rats were fed either EPA, DPA or olive oil (250 mg/animal/day), soaked onto the high fat pellets. Using a micropipette, 500 mg of the two pure fatty acids or olive oil was applied daily for each pair of rats, before feed administration onto 6 pellets (equating to 4 g total). These fortified pellets were then supplied as first feed in the morning to their respective allocated cages and it was verified that all fortified pellets were completely consumed by the rats within 1 h since administration, daily. Subsequently, all animals were provided with the remainder of their normal daily ration (36 g). The rats continued on the high fat diet on days 8 and 9. EPA (>99%; w/w) and DPA (>99%; w/w) were supplied in the FFA form (Nu-Chek Prep, Inc., MN, USA). Olive oil (Lupi, Italy) was used as a control. Daily, throughout 9-day period, the rats were weighed, their food intake was monitored, and the faeces were collected. After nine days, the rats were humanely euthanized with CO_2 overdose. Blood was drawn and the heart and liver were removed and frozen at −80 °C.

The design of this fat absorption/excretion study was based on that reported by Jandacek et al. [7]. In that study, it was shown that non-absorbable substances, such as olestra and calcium soaps of fatty acids were poorly absorbed compared with safflower oil, over a 2 to 3-day test period. The method was reported to be reproducible and consistent in both rats and mice. Thus, the number of animals in this study was set at 6 per group and duration of the experimental feeding at three days. In the study, the entire faecal output was collected from each cage (2 animals) and the amount of fat in the faeces was analysed on a per cage basis, for each day.

The study was performed following the Australian code of practice for care and use of animals for scientific purposes, and the study was approved by Deakin University Animal Welfare Committee (G28-2012).

2.2. Lipid analysis

Aliquots of liver, heart, plasma and faeces samples were homogenised, and the lipids were extracted by dichloromethane–methanol (2:1), essentially as described by Folch et al. [8]. Fatty acids derived from the lipids were methylated using modified methods of Christie [9]. In brief, an aliquot of fatty acids derived from the lipids plus an internal standard of tricosanoic acid (C23:0 >99%; Nu-Chek Prep, Inc.) were reacted with acetyl chloride/methanol for 1 h at 100 °C to form fatty acid methyl esters (FAME). The resulting FAME were separated and quantified by using a Shimadzu GC-2010 (Kyoto, Japan) equipped with a BPX70 capillary column (60 m × 0.25 mm internal diameter, 0.25 μm film thickness (SGE Analytical Science, Australia), a splitless injector and a flame ionisation detector. Each of the fatty acids was identified relative to known external standards (Tricosanoic acid, Sigma-Aldrich, Inc., St. Louis, MO, USA, and Nu-Chek Prep, Elysian, MN, USA), and the resulting peaks were then corrected by the theoretical relative FID response factors [10] and quantified relative to the internal standard.

2.3. Statistical analysis

Data analysis was performed with Minitab Statistical Software (Version 14; Minitab Inc., USA). Significant differences between experimental groups were tested using one way ANOVA. Paired tests were performed with Tukey's test. Statistical significance was indicated by $p < 0.05$.

3. Results

3.1. Body weight

There was no significant difference ($p=0.203$) in the body weights of the rats in the three experimental groups at the end of the study. The average body weights (±SD) of animals in olive oil, DPA and EPA groups were 379 g (±6), 366 g (±19) and 375 g (±9), respectively.

3.2. The lipid content and fatty acid composition of the faeces

There were no significant differences in the total amount of faecal fat between the three groups over the 9 day study (Table 1). The average percentage of lipids in the faeces (±SD) at day 9 in the olive oil, DPA and EPA groups was 2.89 (±0.13), 2.92 (±0.15) and 2.95 (±0.12), respectively.

The fatty acid content of the faeces was affected by diet (Table 2). The content of DPA and EPA both increased significantly on days 6, 7 and 8 following feeding of these fatty acids on days 5–7. However, the oleic acid content was not affected by the 3-day provision of olive oil. The EPA supplementation was also associated with a small, but significant increase in the DHA content in faeces (days 7 and 8) compared with the olive oil or DPA supplementation (data not shown).

3.3. Plasma fatty acid content

There were no significant differences in the content of DPA or DHA between the groups measured at day 9 (Table 3). The level of EPA was significantly higher in DPA and EPA-fed groups compared with olive oil group. There was no difference between treatments for the 20:4n-6 content in plasma.

3.4. Liver and heart fatty acid content

Both EPA and DPA supplementations significantly increased the DPA and EPA concentrations in liver compared with the olive oil supplementation (Table 4). There were similar levels of these fatty acids in both EPA and DPA-fed groups. The total amount of n-3 LC-

Table 1
The amount of lipids in the faeces of rats fed high fat diet enriched with DPA, EPA or olive oil (250 mg/animal/day) at days 5–7.

Day/group	Olive oil feeding	DPA feeding	EPA feeding
1	2.95 ± 0.00	2.97 ± 0.24	2.93 ± 0.34
2	3.02 ± 0.12	2.92 ± 0.18	3.01 ± 0.07
3	3.03 ± 0.07	2.73 ± 0.15	2.92 ± 0.29
4	2.99 ± 0.13	2.99 ± 0.08	2.92 ± 0.09
5	2.70 ± 0.05	2.94 ± 0.05	2.72 ± 0.12
6	3.08 ± 0.11	3.17 ± 0.06	2.88 ± 0.10
7	3.18 ± 0.05	3.28 ± 0.08	3.12 ± 0.10
8	3.29 ± 0.11	3.21 ± 0.10	3.18 ± 0.06
9	2.89 ± 0.13	2.92 ± 0.15	2.95 ± 0.12
p Value	0.166	0.236	0.296

Values are g lipids in 100 g faeces wet weight ± SD, $n=6$. There are no significant differences between groups and experimental days.

Table 2
DPA, EPA and OA content of rats faeces (mg/g wet weight) before (day 4), during (Day 5,6 and 7) and after (Day 8 and 9) dietary supplementation of DPA, EPA and olive oil (250 mg/animal/day), respectively.

Group/FA	FA excreted	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	*p* Value
DPA feeding	DPA	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.44 ± 0.04^{b}	0.29 ± 0.02^{b}	0.28 ± 0.10^{b}	0.03 ± 0.01^{a}	0.000
EPA feeding	EPA	nd[1]	nd[1]	0.04 ± 0.00^{a}	0.09 ± 0.02^{a}	0.09 ± 0.02^{a}	nd[1]	0.000
Olive oil feeding	OA	0.65 ± 0.07^{a}	0.53 ± 0.04^{a}	0.60 ± 0.06^{a}	0.67 ± 0.02^{a}	0.66 ± 0.05^{a}	0.42 ± 0.03^{b}	0.000

Results are expressed as mean ± SD, $n=6$. Values with different letters differ significantly in each row ($p < 0.05$).

[1] Not detected.

Table 3
Selected plasma fatty acids (μg/ml plasma) at day 9 of rats fed DPA, EPA or olive oil (250 mg/day) at days 5–7.

Group	Oleic acid	EPA	DPA	DHA	n-3 LC-PUFA	20:4n-6	22:5n-6	n-6 LC-PUFA
DPA feeding	376 ± 112	13 ± 8^{b}	25 ± 7	84 ± 17	123 ± 90	472 ± 70	nd[2a]	472 ± 70
EPA feeding	525 ± 168	14 ± 3^{b}	28 ± 8	105 ± 24	140 ± 20	553 ± 116	nd[2a]	553 ± 116
Olive oil feeding	654 ± 9	nd[2a]	18 ± 8	101 ± 41	120 ± 10	611 ± 105	13 ± 2^{b}	638 ± 127
Baseline data[1]	718 ± 22	nd[2a]	18 ± 7	80 ± 30	98 ± 9	432 ± 98	14 ± 4^{b}	449 ± 87
p Value	0.173	0.013	0.328	0.525	0.432	0.352	0.046	0.330

The rats were maintained on a high fat diet throughout 9 days of the study. n-3 LC-PUFA: DPA+EPA +DHA; n-6 LC-PUFA: 20:4n-6+22:4n-6+22:5n-6. Values are means ± SD, $n=6$. Values with different letters differ significantly in each column ($p < 0.05$).

[1] Data from day 0.
[2] Not detected.

Table 4
Selected liver and heart fatty acids (mg/g of tissue) of rats fed high fat diet with DPA, EPA or olive oil (250 mg/animal/day) at days 5–7.

Group	Oleic acid	EPA	DPA	DHA	n-3 LC-PUFA	20:4n-6	22:5n-6	n-6 LC-PUFA
Liver								
DPA feeding	10.88 ± 2.17^{b}	0.20 ± 0.07^{b}	0.64 ± 0.11^{b}	1.62 ± 0.29^{ab}	2.47 ± 0.50^{b}	4.34 ± 0.47^{b}	0.06 ± 0.02^{a}	4.61 ± 0.50^{b}
EPA feeding	11.64 ± 4.10^{b}	0.27 ± 0.19^{b}	0.66 ± 0.14^{b}	2.05 ± 0.73^{b}	2.98 ± 0.66^{b}	4.74 ± 0.37^{b}	0.07 ± 0.02^{a}	5.03 ± 0.43^{b}
Olive oil feeding	13.54 ± 3.16^{b}	0.08 ± 0.03^{a}	0.32 ± 0.10^{a}	1.50 ± 0.42^{ab}	1.91 ± 0.40^{a}	4.79 ± 0.34^{b}	0.13 ± 0.04^{b}	5.27 ± 0.27^{b}
Baseline data[1]	2.75 ± 0.32^{a}	0.06 ± 0.00^{a}	0.17 ± 0.00^{a}	1.12 ± 0.14^{a}	1.35 ± 0.09^{a}	3.50 ± 0.10^{a}	0.07 ± 0.01^{a}	3.66 ± 0.10^{a}
p Value	0.000	0.004	0.017	0.014	0.032	0.000	0.012	0.000
Heart								
DPA feeding	1.59 ± 0.25	nd[2]	1.33 ± 0.27^{b}	2.56 ± 0.92^{ab}	3.32 ± 0.46^{b}	3.77 ± 0.27	0.23 ± 0.03	4.18 ± 0.44
EPA feeding	1.80 ± 0.24	0.08 ± 0.05	0.85 ± 0.08^{a}	2.94 ± 0.64^{b}	3.87 ± 0.30^{b}	4.16 ± 0.84	0.26 ± 0.04	4.42 ± 0.85
Olive oil feeding	1.50 ± 0.22	nd[2]	0.74 ± 0.14^{a}	2.29 ± 0.64^{ab}	3.03 ± 0.18^{a}	4.30 ± 0.16	0.32 ± 0.02	4.99 ± 0.18
Baseline data[1]	2.65 ± 1.05	nd[2]	0.54 ± 0.04^{a}	1.98 ± 0.38^{a}	3.10 ± 0.16^{a}	3.33 ± 0.50	0.33 ± 0.09	3.99 ± 0.75
p Value	0.236	0.157	0.002	0.037	0.042	0.100	0.098	0.165

The rats were maintained on a high fat diet throughout 9 days of the study. n-3 LC-PUFA: DPA+EPA +DHA; n-6 LC-PUFA: 20:4n-6+22:4n-6+22:5n-6. Values are means ± SD, $n=6$. Values with different letters differ significantly in each column ($p < 0.05$).

[1] Data from day 0.
[2] Not detected.

PUFA (namely DPA, EPA and DHA concentrations pooled) was significantly increased by both EPA and DPA feeding compared with the olive oil feeding. There was no difference between treatments for the 20:4n-6 content in liver.

In the heart, DPA concentration was significantly increased by the DPA feeding compared with the EPA and olive oil feeding. There were no significant increases in EPA or DPA concentrations following EPA supplementation. However, both EPA and DPA feeding significantly increased the total n-3 LC-PUFA concentration in the heart compared with the olive oil feeding or the baseline data. There was no difference between treatments for the 20:4n-6 content in heart.

4. Discussion

The present study sought to establish if short-term DPA feeding increased the excretion of DPA in faeces in rats relative to EPA. It was found that significantly more DPA was excreted in the DPA-fed group (0.4% of the dose fed) than EPA in the EPA-fed group (0.07% of the dose fed). It is interesting that despite the manifest poor apparent digestibility of DPA, the overall lipid digestibility seemed unaffected, as the total amount of lipids excreted in faeces was constant in all treatments and during the entire duration of the trial.

This 4-fold increase in DPA excretion should clearly contribute to a reduced bioavailability of the DPA for possible subsequent tissue deposition, in comparison with EPA.

The increased faecal excretion of DPA might offer empirical evidence explaining the reduced DPA incorporation into chylomicrons in the human study [5]. The reason for the increased DPA excretion is not clear, however the incorporation of FFA into chylomicrons involves a number of steps including absorption across gut mucosa, activation of the FFA into the CoA form and acylation to allow TAG formation.

In the plasma, which was sampled at the conclusion of the study (day 9), the EPA content was increased for both the DPA and EPA-fed groups. Furthermore, an increased level of DPA was recorded in both liver and heart, 2 days after the feeding of the DPA ceased. Additionally, there was an increased level of EPA in the liver in the DPA-fed group, confirming the previously suggested occurrence of active retroconversion of DPA to EPA [1,3]. An interesting difference between DPA and EPA was noted in the

heart, as DPA significantly increased heart DPA levels, whereas EPA did not increase heart EPA levels.

Both DPA and EPA feeding increased the total n-3 LC-PUFA content of liver and heart to a similar extent, showing that the increased excretion of DPA, and thus reduced bioavailability, paradoxically did not affect its deposition in tissues, relative to EPA. A possible explanation for this could be that part of the absorbed EPA was then used in other metabolic (further bioconversion and/or eicosanoids production) or catabolic (β-oxidation for energy production) processes, and thus resulted in similar tissue deposition/retention of that recorded for DPA, which was less absorbed, but then proportionally more efficiently retained. Accordingly, it was recently reported, that DHA and DPA were conserved from β-oxidation to a significantly greater extent than EPA and oleic acid at 6 h in rats [2].

In this study, neither EPA nor DPA feeding significantly increased the DHA level in the liver or the heart compared with the olive oil group, though it should be admitted that the high variability in tissue levels for DHA reduced the possibility for detecting significant increases in DHA. In a previous 7-day study in rats with pure DPA, it was shown that there was a significantly increased concentration of DHA in liver [1]. Recent research has started to uncover the complexity of elongation of DPA to 24:5n-3 in different species [11].

In humans, DPA and EPA have demonstrated different and specific incorporation patterns in plasma lipids, red blood cell lipids and chylomicrons. In the study of Miller et al. [4], the DPA and EPA did not partition identically into the plasma lipid fractions (cholesterol ester, phospholipid, TAG) or into erythrocyte phospholipids to the same extent. Furthermore, Linderborg et al. [5] revealed that the EPA and DPA were incorporated into the chylomicrons TAG to different extents (EPA more than DPA) and into different TAG molecular species. In the present study, there were different patterns of uptake of DPA and EPA in the heart, which further emphasises different rates and/or degrees of metabolism of these two n-3 fatty acids.

In the present study and the human study reported [5], the DPA was fed in the FFA form. There have been no studies which have compared the bioavailability in humans of DPA-FFA versus other n-3 LC-PUFA. However, several studies have compared the bioavailability of n-3 FFA-forms with n-3 ethyl ester and TAG forms. In most cases, the FFA form was significantly more bioavailable [12–14]. Thus, it is plausible to suggest that the results observed in the present study, are not related to the fact the two n-3 fatty acids were provided as FFA.

Together, the data from rat and human studies are pointing to significant differences in how mammals process DPA compared with EPA, a finding which was not predicted from earlier work on n-3 LC-PUFA. In summary, in answer to the research question posed, the evidence available thus far, suggests that dietary DPA is less available then EPA, and proportionally more excreted in faeces. However, EPA is then more prone to catabolic processes compared with DPA, resulting in similar deposition rates for the two n-3 LC-PUFA into tissues.

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