Short-term docosapentaenoic acid (22:5n-3) supplementation increases tissue docosapentaenoic acid, DHA and EPA concentrations in rats

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(Received 12 February 2009 – Revised 23 June 2009 – Accepted 24 June 2009 – First published online 4 August 2009)

The metabolic fate of dietary n-3 docosapentaenoic acid (DPA) in mammals is currently unknown. The aim of the present study was to determine the extent of conversion of dietary DPA to DHA and EPA in rats. Four groups of male weanling Sprague–Dawley rats (aged 5 weeks) were given 50 mg of DPA, EPA, DHA or oleic acid, daily for 7 d by gavage. At the end of the treatment period, the tissues were analysed for concentrations of long-chain PUFA. DPA supplementation led to significant increases in DPA concentration in all tissues, with largest increase being in adipose (5-fold) and smallest increase being in brain (1·1-fold). DPA supplementation significantly increased the concentration of DHA in liver and the concentration of EPA in liver, heart and skeletal muscle, presumably by the process of retroconversion. EPA supplementation significantly increased the concentration of EPA and DPA in liver, heart and skeletal muscle and the DHA concentration in liver. DHA supplementation elevated the DHA levels in all tissues and EPA levels in the liver. Adipose was the main tissue site for accumulation of DPA, EPA and DHA. These data suggest that dietary DPA can be converted to DHA in the liver, in a short-term study, and that in addition it is partly retroconverted to EPA in liver, adipose, heart and skeletal muscle. Future studies should examine the physiological effect of DPA in tissues such as liver and heart.

DHA: Docosapentaenoic acid: EPA: n-3 PUFA

The interest in n-3 PUFA developed rapidly after two Nobel Prize winning discoveries of particular PG, metabolites of arachidonic acid (AA), by Vane & Samuelsson(1) in the late 1960s and early 1970s. Since then there have been many studies suggesting the beneficial effects of n-3 fatty acids in reducing risk of cardiovascular events, diabetes, inhibiting tumour growth of tumour cells, modulating gene expression and reducing risk of cardiovascular events, diabetes, inhibiting inflammation to yield energy (ATP) and CO2(9). Many studies have reported that feeding relatively high levels of ALA in either animals or human subjects leads to increased DPA levels, but not DHA levels, suggesting the steps between DPA and DHA are rate-limiting steps in this metabolic pathway(10,11). In contrast, ingested DHA is rapidly and efficiently deposited in brain, liver and other tissues(12). There is not much literature available on the fate of DPA. Two cell culture studies have looked at the effect of DPA supplementation in endothelial cells and hepatocytes, respectively, and reported that DPA supplementation increases both DPA and EPA levels but not DHA in these cells(13,14). However, no animal studies have been conducted to investigate the conversion of pure DPA to DHA in mammals, presumably because DPA has only recently become available for in vivo studies (in milligram amounts). DPA is found in common foods like fish, fish oil, lean red meat and n-3-enriched eggs(15); therefore, it is important to understand the metabolic fate of DPA. In the present paper, we report the effect of DPA supplementation on DPA, EPA and DHA concentrations in rat tissues. The hypothesis being tested was that dietary supplementation of DPA will increase both tissue DHA and EPA levels. The novelty of the present study is that it focuses on the metabolism of DPA in a rodent model, which has not been investigated before.
**Materials and methods**

**Animals and diets**

Thirty-two 4-week-old male weanling Sprague–Dawley rats were randomly divided into four groups of eight animals. The rats were maintained on an ad libitum normal chow diet with water throughout the study. The total lipid content of the chow diet used was 5.7 (g/100 g of wet weight) and the three main unsaturated fatty acids present in the chow diet were oleic acid (29.7%), linoleic acid (31.2%) and ALA (3.4%); EPA, DPA and DHA were not detected. The rats were pair housed and allowed 1 week to acclimatise. The rats were then administered 50 mg of DPA, EPA, DHA or oleic acid (Nu-Chek Prep, Inc., Elysian, MN, USA) by daily oral gavaging for 7 d. The dose and duration used in the present study were based on evidence from previously published studies that have successfully used doses of 90–100 mg/d of PUFA, for rats weighing up to 140–220 g. These studies demonstrated that changes in fatty acid composition of long-chain PUFA occurred within 3 d of supplementation\(^{16–19}\). In the present study, we used 50 mg of fatty acids for animals weighing 68–101 g. Thus, it was expected that the dose of 50 mg of fatty acids given for 7 d would be sufficient to detect changes in tissue fatty acid composition.

The weight of the animals was recorded every day and on the 8th day the animals were sacrificed by lethal injection of Lethobarb (Virbac, Peakhurst, NSW, Australia). Brain, heart, epididymal fat (adipose), skeletal muscle and liver were removed from the animals, washed in ice-cold saline (0.9% NaCl solution) and then dried on paper towel. After weighing, the tissues were wrapped in foil and stored at −80°C for fatty acid analysis.

**Lipid analysis**

The tissues were minced and the tissue lipids were extracted by chloroform–methanol, 2:1 as described by Sinclair et al.\(^{(20)}\). An aliquot of the total lipids from each tissue, plus an internal standard of docosatrienoic acid (22:3; Nu-Chek Prep, Inc.), was reacted with 2% H\(_2\)SO\(_4\) in methanol for 3 h at 80°C to form the fatty acid methyl esters; they were passed through a silica Sep-Pak to remove cholesterol and then the fatty acid methyl esters were separated by capillary GLC using a 50 m x 0.32 mm (inner diameter) fused silica-bonded phase column (BPX70, SGE, Melbourne, Vic, Australia). The column oven was programmed to rise after 3 min at 125–220°C at 8°C/min with a He gas flow rate of 43 cm/s as the carrier gas. Fatty acids were identified by comparison with standard mixtures of fatty acid methyl esters and the results were calculated using response factors derived from chromatographing standards of known composition (Nu-Chek Prep, Inc.).

**Statistical analysis**

Data analysis was performed using SPSS version 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). Significant differences between dietary groups were tested using a one-way ANOVA for each type of fatty acid for both fatty acid analysis and gene expression. Post hoc comparisons were made using the least significant difference test with a significance level of 0.05.

**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 La Trobe University Animal Ethics Committee (AEC07-53-P) and Deakin University Animal Welfare Committee (AEX 23/2008).
compared with values of less than 3 mg/g in other tissues (Table 1). In liver, muscle and brain tissue, the concentration of DHA was between 1 and 3 mg/g; for DPA, the concentration ranged from 0-1 to 2 mg/g, while for EPA the range was from 0-02 to 0-4 mg/g tissue. The rats supplemented with DHA showed a significant increase in tissue DHA content in all the five tissues studied. The largest proportional increase in DHA occurred in adipose (3-4-fold) and skeletal muscle (2-4-fold) and the least change was in brain (1-1-fold). It was also observed that DHA supplementation led to a significant increase in EPA concentration in liver.

DPA supplementation resulted in statistically significant accumulation of DPA in all tissues analysed except adipose tissue. DPA supplementation also led to a significant increase in EPA concentrations in liver, heart and skeletal muscle and a non-significant increase in adipose tissue. Interestingly, DPA supplementation also led to a significant increase in DHA concentration in liver.

Supplementation with EPA led to a significant increase in tissue EPA and DPA concentrations in liver, heart and skeletal muscle and a non-significant increase in adipose tissue. EPA supplementation also increased DHA concentrations significantly in liver. All three n-3 PUFA led to a significant decrease in AA concentrations in liver and heart (Figs. 2 and 3).

Discussion

The aim of the present study was to examine the effect of DPA supplementation on LCPn-3 proportions in the tissues of animals fed 50 mg of n-3 fatty acids per day for 7 d. It was observed that the primary site of DPA deposition was adipose followed by heart, liver and skeletal muscle. Adipose was also the main site for deposition of fed EPA and DHA in the present study. Fu & Sinclair\(^{(10)}\) reported that major sites of EPA and DPA deposition, in guinea pigs fed with diets containing 17.3 % ALA (of total diet lipid) for 4 weeks, were adipose, skin and carcass.

It was observed that DPA supplementation increased DPA concentration in liver, heart, skeletal muscle and brain. It was also observed that DPA supplementation led to a significant increase in EPA concentrations in liver, heart and skeletal muscle and a non-significant increase in EPA concentrations in adipose tissue suggesting the retroconversion of DPA into EPA \emph{in vivo}. The process of retroconversion was first described by Stoffel \textit{et al}.\(^{(21)}\) for DHA, and subsequent work by Christensen \textit{et al}.\(^{(22,23)}\) in human fibroblasts indicated the retroconversion of both DHA and DPA was likely to involve the peroxisomal acyl-CoA oxidase. Retroconversion of DPA into EPA has also been reported in endothelial cells, fetal skin fibroblasts and hepatocytes\(^{(13,14,24)}\). The extent of ‘apparent’ retroconversion of DPA to EPA (\(\Delta\text{EPA} \times 100/(\text{DPA} + \text{EPA})\)) was 28 % in liver, 19 % in adipose tissue, 12 % in skeletal muscle, 4 % in heart and negligible in brain.

It was also observed from the present study that EPA-fed animals showed a significant increase in EPA and DPA concentrations in the liver, heart and skeletal muscle. These data confirm the findings of previously published cell culture studies, which showed that EPA is converted into DPA in endothelial and liver cells\(^{(13,14)}\). It is evident from the present study that EPA and DPA are interconverted in the body and therefore DPA may act as a source of EPA in the body and vice versa. This is particularly relevant in adipose tissue that had the highest concentrations of these PUFA and consequently adipose may act as a reservoir of these fatty acids. Supplementation with EPA had no effect on brain EPA accumulation of DPA in all tissues analysed except adipose tissue. DPA supplementation also led to a significant increase in EPA concentrations in liver, heart and skeletal muscle and a non-significant increase in adipose tissue. Interestingly, DPA supplementation also led to a significant increase in DHA concentration in liver.

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OA, oleic acid; AA, arachidonic acid; DPA, docosapentaenoic acid.

\(^{a,b,c}\) Mean values within a row with unlike superscript letters were significantly different (\(P<0.05\)).

\(^{*}\) Fatty acid composition of various tissues from rats supplemented with 50 mg of OA, EPA, DPA or DHA for 7 d. Results are expressed as mg/g of tissue (\(n=8\)). Data were analysed using one-way ANOVA and post hoc comparisons were made using the least significant difference test.
levels that are known to be very low (in the present study 0.02 mg/g). It has recently been suggested that low levels of EPA in brain phospholipids compared with DHA may be the result of its rapid β-oxidation upon uptake by the brain\(^{(25)}\).

Another very significant finding of the present study was that supplementation of rats with DPA led to a significant increase in liver DHA concentrations and a non-significant increase in brain, compared with the control group-fed oleic acid. The DPA-fed group showed a 60% increase in liver DHA compared with the group-fed DHA. Liver is regarded as a major site for PUFA synthesis\(^{(9)}\) and since this was a short-term study only, there may have been insufficient time for the increased liver DHA to be delivered to the other tissues via plasma lipoprotein transport. The two previously published cell culture studies that have looked at DPA supplementation failed to demonstrate an increase in tissue DHA levels. It is not clear whether this was due to a limited ability of these particular cells to desaturate PUFA or to competition between \(24:5n-3\) and ALA for metabolism to DHA\(^{(26)}\).

In the present study, the DHA-fed group was regarded as a control group to judge the effectiveness of increases in DHA concentration in the EPA- and DPA-fed groups. There was an increase in the tissue DHA concentrations in the DHA-fed group with the largest rise occurring in adipose tissue (3.4-fold), followed by skeletal muscle (2.4-fold), heart (2.1-fold), liver (1.76-fold) and brain (1.1-fold). It was no surprise that significant increases in DHA were observed as this has been observed by many groups in a variety of dietary supplementation studies\(^{(26–28)}\). It was also observed that DHA supplementation increased the EPA levels in liver suggesting retroconversion into EPA.

In the present study, DPA, EPA and DHA supplementation also led to a significant decrease in tissue AA levels in liver and heart and a non-significant decrease in muscle and adipose. This could be explained by the fact that LCP\(n-3\) are known to have an inhibitory effect on \(\Delta6\)- and \(\Delta5\)-desaturases\(^{(29,30)}\), which are involved in synthesis of AA from linoleic acid. Also supplementation of cells with

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**Fig. 2.** Fatty acid composition of liver tissue of rats supplemented with 50 mg oleic acid (OA), EPA, docosapentaenoic acid (DPA) or DHA for 7 d. (a) OA concentration in liver tissue; (b) arachidonic acid (AA) concentration in liver tissue; (c) EPA concentration in liver tissue; (d) DPA concentration in liver tissue; (e) DHA concentration in liver tissue. Results are expressed as mg/g of tissue (n 8). Values are means, with standard deviations represented by vertical bars. Data were analysed using one-way ANOVA and post hoc comparisons were made using the least significant difference test. a,b,c Mean values with unlike letters were significantly different (\(P < 0.05\)).
the LCPn-3 could have led to competition for the enzyme acyl-CoA transferase thereby decreasing the incorporation of AA into phospholipids\(^{(31)}\).

To our knowledge, so far there have been very few studies that have investigated the biochemical effects of DPA. We expect this might be due to the relatively limited availability and high cost of pure DPA. But in light of the present literature, it can be speculated that the physiological consequences of accumulation of DPA in tissues may be related to accumulation of DPA, EPA and DHA from DPA and to inhibition of AA metabolism. Two studies are worth mentioning since both reported an effect of pure DPA on platelet function through AA pathway inhibition\(^{(32,33)}\). One study reported that platelets metabolise 22:5n-3 into 11- and 14-hydroxy DPA via an indomethacin-insensitive pathway\(^{(32)}\). They also reported that when DPA was released along with AA in platelets, it inhibited cyclo-oxygenase enzyme thereby reducing the thromboxane B\(_2\) and 5,8,10-heptadecatrienoic acid production from AA. Akiba \textit{et al.}\(^{(33)}\) looked at the effects of DPA on platelet aggregation and AA metabolism in rabbit platelets and compared them with those of EPA and DHA. The results showed that n-3 fatty acids inhibited collagen- or AA-stimulated platelet aggregation dose dependently, and that DPA was the most potent inhibitor. These results suggest that DPA possesses potent activity for interfering with the cyclo-oxygenase pathway and accelerating the lipoxygenase pathway.

In conclusion, the data presented in the present study demonstrated that oral consumption of dietary DPA in young male rats increased the concentration of DHA in liver but not other tissues. Furthermore, DPA was partially retroconverted to EPA in liver, muscle, adipose and heart. Future studies should investigate the physiological and biochemical effects of DPA ingestion compared with that of EPA and DHA.

Acknowledgements

The present research was funded by Meat and Livestock Australia (Project code: D.MHN.0022) and by cluster funds from Faculty of Health, Medicine, Nursing and Behavioural...
Sciences, Deakin University. There is no conflict of interest of any personal, financial or academic nature, among the authors. Contribution of authors. G. K. and A. J. S. designed the study. D. P. B. coordinated and carried out the animal procedures with G. K. D. B. performed the fatty acid analysis. G. K. performed the statistical analysis and wrote the manuscript. A. J. S., M. G., D. C.-S. and D. P. B. along with G. K. contributed to the final version of the manuscript.

References