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

Short term DPA (22:5n-3) supplementation increases tissue DPA, DHA and EPA concentration in rats

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Abbreviations: ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LCPn-3 long chain n-3 PUFA; OA, oleic acid; AA, Arachidonic acid;

ABSTRACT

The metabolic fate of dietary n-3 docosapentaenoic acid (DPA) in mammals is currently unknown. The aim of this study was to determine the extent of conversion of dietary DPA to docosahexaenoic acid (DHA) and eicosapentaenoic acid (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 days by gavage. At the end of the treatment period the tissues were analysed for concentrations of long chain polyunsaturated fatty acids (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. This data suggests that dietary DPA can be converted to DHA in the liver, in a short term study, and that in addition it is partly retro-converted 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.

INTRODUCTION

The interest in n-3 polyunsaturated fatty acids (PUFA) developed rapidly after two Nobel Prize winning discoveries of particular prostaglandins, metabolites of arachidonic acid, by Vane and Samuelson in the late 60's and early 70's [(1)]. Since then there have been many studies suggesting the beneficial effects of n-3 fatty acids in reducing risk of cardiovascular events, diabetes, inhibiting growth of tumour cells, modulating gene expression and anti inflammatory activity [(2-7)]. The parent n-3 PUFA is alpha-linolenic acid (ALA; 18:3n-3), which is found in high concentration in some plant oils. In mammals, some of the ingested ALA is metabolised to long chain n-3 PUFA (LCPn-3) namely eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) by a series of desaturations and elongations (see Fig 1). This metabolic processing of ALA to DHA is inefficient [(8)] and much of the ingested ALA is either deposited in tissue adipose stores as ALA or catabolised by mitochondrial beta-oxidation to yield energy (ATP) and carbon dioxide [(9)]. Many studies have reported that feeding relatively high levels of ALA in either animals or humans 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 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 this 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 this 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-wk-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/100g of wet weight) and the three main unsaturated fatty acids present in the chow diet were oleic acid (OA) (29.7%), linoleic acid (31.2%) and ALA (3.4%); EPA, DPA and DHA were not detected. The rats were pair housed and allowed one week to acclimatise. The rats were then administered 50mg of DPA, EPA, DHA or OA (Nu-Chek Prep, Inc, USA) by daily oral gavaging for 7 days. The dose and duration used in this study was based on evidence from previously published studies which have successfully used doses of 90-100mg/d of PUFA, for rats weighing up to 140-220g. These studies demonstrated that changes in fatty acid composition of long chain PUFA occured within 3 days of supplementation [16-19]. In the present study, we used 50mg of fatty acids for animals weighing 68-101g. Thus, it was expected that the dose of 50mg of fatty acids given for 7 days 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, 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 -80ºC 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.USA), was reacted with 2% H_2SO_4 in methanol for 3 hours at 80ºC 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 gas liquid chromatography using a 50 m x 0.32 mm (I.D.) fused silica bonded phase column (BPX70, SGE, Melbourne, Australia). The column oven was programmed to rise after 3 min at 125° to 220°C at 8°C/min with a helium flow rate of 43 cm/sec 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 (NuChek Prep., Inc, USA).

Statistical analysis

Data analysis was performed using SPSS v15.0 for Windows (SPSS Inc., Chicago, IL). 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 LSD (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).

RESULTS

Body and tissue weights

There was no significant difference in the body weights of animals between various dietary groups at the start and the end of the study. The mean (±SD) body weights of rats at the start and end of study were 75.9 ± 5.8 and 122.4 ± 17.4 grams, respectively. There were no significant differences in the tissue weights between treatments.

Tissue fatty acid concentrations

Adipose tissue contained the highest concentration of each of the three long chain n-3PUFA with amounts ranging from 30 to 57 mg/g tissue compared with values of less than 3 mg/g in other tissues (Table 1). In liver, muscle & brain tissue, the concentration of DHA was between 1 to 3 mg/g; for DPA, the concentration ranged from 0.1 to 2mg/g while for EPA the range was from 0.02 to 0.4mg/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 the three n-3 PUFA led to a significant decrease in AA concentrations in liver and heart.

DISCUSSION

The aim of this study was to examine the effect of DPA supplementation on LCPn-3 proportions in the tissues of animals fed 50mg of n-3 fatty acids per day for 7 days. 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 this study. Fu and Sinclair, 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 [(10)].

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 *in vivo*. The process of retroconversion was first described by Stoffel et al for DHA [(21)] and subsequent work by Christensen et al in human fibroblasts indicated the retroconversion of DHA and DPA were both likely to involve the peroxisomal acyl-CoA oxidase [(22, 23)]. 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 (ΔEPA*100/Δ[DPA+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 our 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 our 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 which 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 levels which are known to very low (in this study 0.02mg/g). It has recently been suggested that low levels of EPA in brain phospholipids compared to DHA may be the result of its rapid beta oxidation upon uptake by the brain [25].

Another very significant finding of our 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 OA. 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 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 this 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 occuring 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 this 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 n-3LCP are known to have an inhibitory effect on delta 6 and delta 5 desaturases (29, 30), which are involved in

synthesis of AA from linoleic acid. Also supplementation of cells with 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 which 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 metabolic consequenses of accumulation of DPA in tissues may be related 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 metabolize 22:5n-3 into 11- and l4-hydroxy docosapentaenoic acids via an indomethacin-insensitive pathway [32]. They also reported that when DPA is released along with AA in platelets it inhibited cycloxygenase (COX) enzyme thereby reducing the thromboxin B2 and 5,8,10-heptadecatrienoic acid (HHT) production from AA. Akiba et al (2000) looked at the effects of DPA on platelet aggregation and AA metabolism in rabbit platelets and compared them with those of EPA and DHA[33]. 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 COX pathway and accelerating the lipoxygenase pathway.

In conclusion, the data presented in our 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 & biochemical effects of DPA ingestion compared with that of EPA and DHA.

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Contribution of authors: Gunveen Kaur and Andrew Sinclair designed the study. Denovan Begg co-ordinated and carried out the animal procedures with Gunveen Kaur. Daniel Barr performed the fatty acid analysis. Gunveen Kaur performed the gene expression analysis and statistical analysis of all the data. Gunveen Kaur wrote the manuscript. Andrew Sinclair, Manohar Garg, David Cameron-Smith and Denovan Begg along with Gunveen Kaur, contributed to the final version of the manuscript.

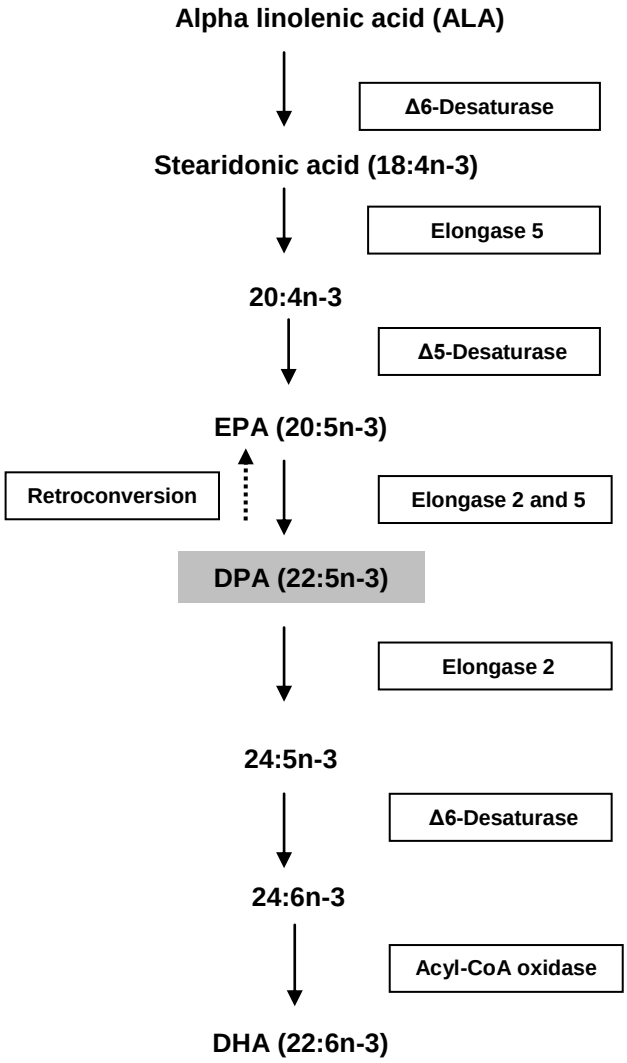

Figure 1: Pathway for metabolism of ALA to long chain n-3 PUFA.
In mammals, some of the ingested ALA is metabolised to long chain n-3 fatty acids by series of elongations and desaturations. The figure shows the enzymes involved in this pathway. PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid and DHA, docosahexaenoic acid.

TABLE 1: Tissue fatty acid concentrations in brain, adipose and skeletal muscle of animals in various dietary groups (mean ± SD)

Fatty acids	OA Control		EPA Group		DPA Group		DHA Group	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Brain								
OA	2.97^{a}	0.03	3.03^{ab}	0.05	3.12^{b}	0.05	3.10^{ab}	0.06
AA	2.01^{a}	0.05	2.01^{a}	0.04	2.03^{a}	0.03	1.98^{a}	0.05
EPA	0.02^{a}	0.00	0.02^{a}	0.00	0.02^{a}	0.00	0.03^{a}	0.00
DPA	0.08^{a}	0.01	0.10^{b}	0.01	0.11^{b}	0.01	0.07^{a}	0.00
DHA	2.50^{a}	0.05	2.62^{ab}	0.03	2.65^{ab}	0.06	2.70^{b}	0.08
Adipose								
OA	948.33^{a}	64.66	1031.55^{a}	119.07	823.57^{a}	62.55	703.85^{b}	50.27
AA	40.48^{a}	4.64	36.28^{a}	5.79	31.88^{a}	2.95	29.28^{a}	3.73
EPA	3.87^{a}	0.50	30.42^{a}	7.95	14.37^{a}	4.32	5.29^{a}	0.90
DPA	11.00^{a}	2.48	29.96^{a}	9.07	55.68^{a}	13.73	23.48^{a}	5.43
DHA	16.62^{a}	1.20	23.20^{ab}	3.16	27.01^{ab}	3.03	57.04^{a}	10.23
Skeletal Muscle								
OA	3.98^{a}	0.68	3.95^{a}	0.62	3.35^{a}	0.48	3.62^{a}	0.45
AA	1.48^{a}	0.08	1.26^{a}	0.03	1.23^{a}	0.03	1.22^{a}	0.02
EPA	0.03^{a}	0.00	0.23^{b}	0.04	0.11^{bc}	0.01	0.04^{ac}	0.01
DPA	0.27^{a}	0.27	0.50^{b}	0.03	0.87^{c}	0.06	0.21^{a}	0.02
DHA	0.55^{a}	0.04	0.66^{b}	0.03	0.61^{ab}	0.02	1.31^{c}	0.05

Fatty acid composition of various tissues from rats supplemented with 50 mg of OA, EPA, DPA or DHA for 7 days. Results are expressed as mg/g of tissue (n=8). Mean±SD values within a row with unlike superscripts were significantly different ($P<0.05$). Data was analysed using one way ANOVA and post hoc comparisons were made using LSD. OA, Oleic acid; EPA, eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, Docosahexaenoic acid.

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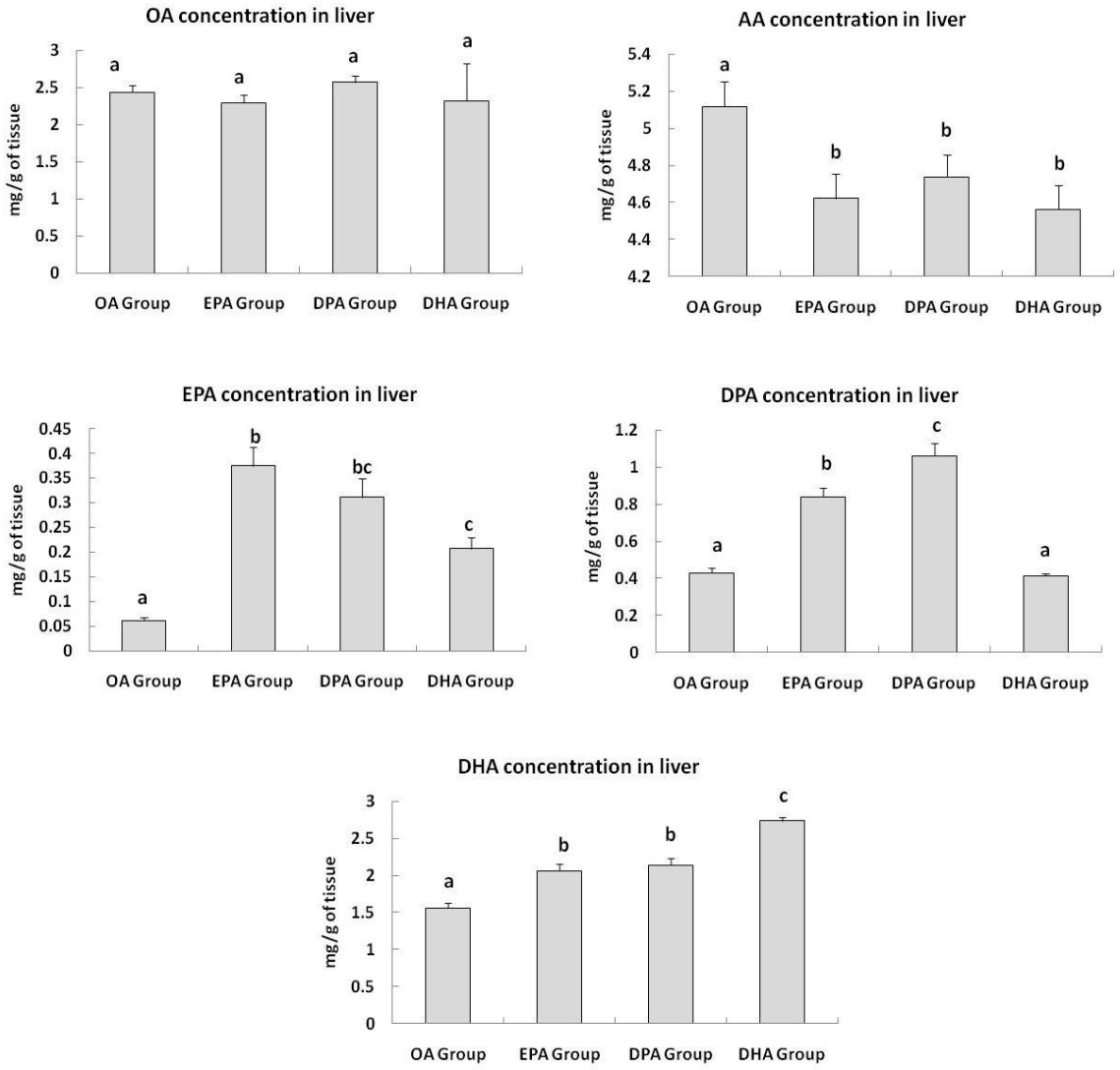

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345 Figure 2: Fatty acid composition of liver tissue of rats supplemented with 50 mg of OA, EPA,
346 DPA or DHA for 7 days. Results are expressed as mg/g of tissue (n=8). Data was analysed
347 using one way ANOVA and post hoc comparisons were made using LSD. Different
348 superscripts represent significant difference from control OA group.
349 OA, Oleic acid; EPA, eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA,
350 Docosahexaenoic acid.

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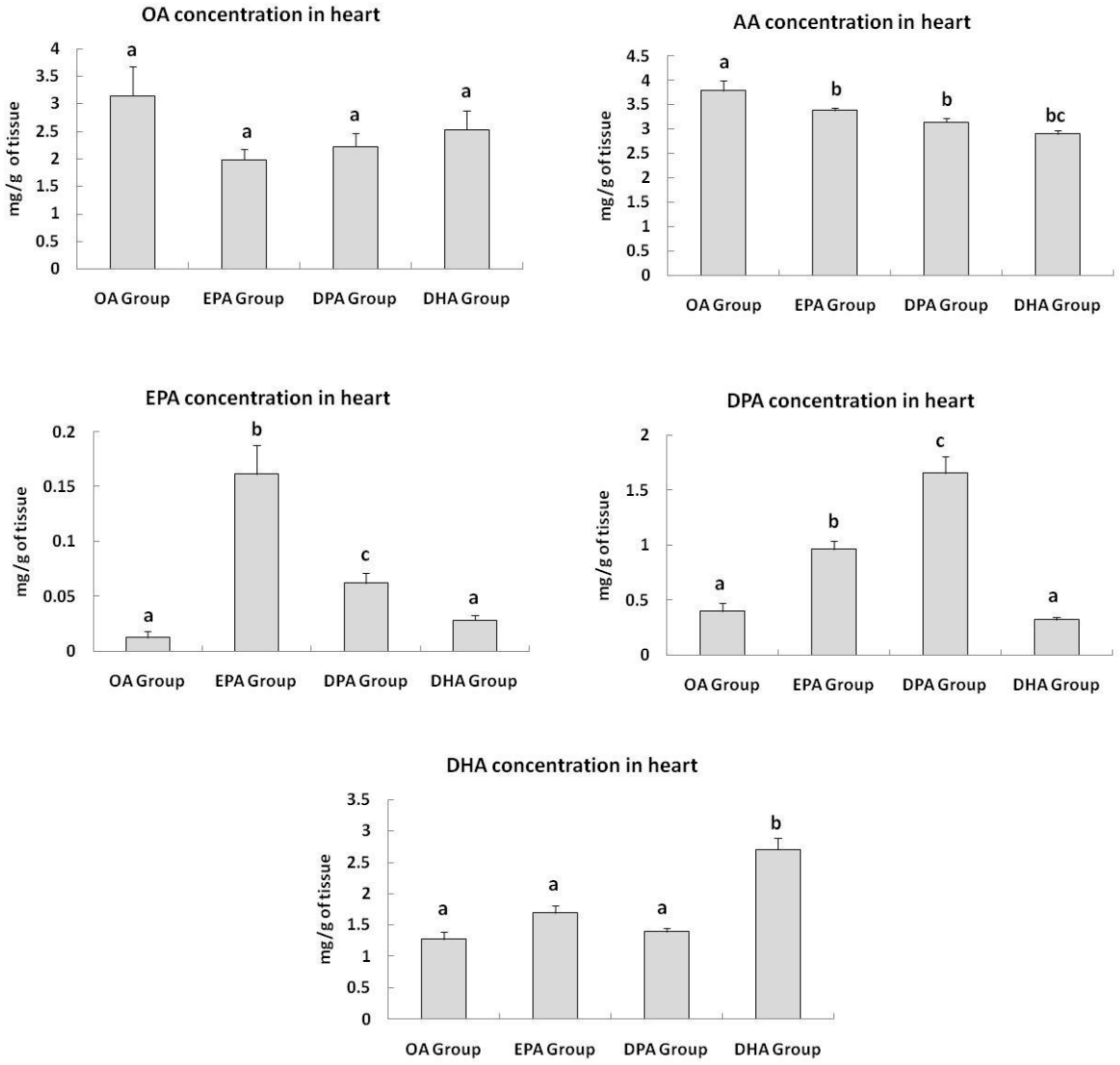

Figure 3: Fatty acid composition of heart tissue of rats supplemented with 50 mg of OA, EPA, DPA or DHA for 7 days. Results are expressed as mg/g of tissue (n=8). Data was analysed using one way ANOVA and post hoc comparisons were made using LSD. Different superscripts represent significant difference from control OA group.
OA, Oleic acid; EPA, eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, Docosahexaenoic acid.

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