

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

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5 Gunveen Kaur¹, Denovan P Begg^{2,3}, Daniel Barr³, Manohar Garg⁴, David Cameron-
6 Smith³ and Andrew J Sinclair^{1,3}

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11 ¹ Metabolic Research Unit, School of Exercise and Nutrition Sciences, Deakin
12 University, Waurn Ponds, 3217, Victoria, Australia

13 ² School of Psychological Science, La Trobe University, Bundoora, 3086, Victoria,
14 Australia

15 ³ School of Exercise and Nutrition Sciences, Deakin University, Burwood, 3126,
16 Victoria, Australia

17 ⁴ School of Health Science, University of Newcastle, Callaghan NSW 2308, Australia

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20 Running Title: Metabolism of DPA (22:5n-3)

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24 Key Words: Docosahexaenoic acid (DHA), docosapentaenoic acid (DPA),
25 eicosapentaenoic acid (EPA), n-3 polyunsaturated fatty acids (PUFA)

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32 To whom correspondence should be addressed
33 Andrew Sinclair
34 School of Exercise and Nutrition Sciences, 221 Bwd Hwy,
35 Deakin University, Burwood, 3125, Victoria, Australia.
36 Fax: +61-3-9251 7282
37 Phone +61-3-92446017
38 email andrew.sinclair@deakin.edu.au

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

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49 **ABSTRACT**

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51 The metabolic fate of dietary n-3 docosapentaenoic acid (DPA) in mammals is currently
52 unknown. The aim of this study was to determine the extent of conversion of dietary
53 DPA to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in rats. Four
54 groups of male weanling Sprague Dawley rats (aged 5 weeks) were given 50 mg of
55 DPA, EPA, DHA or oleic acid, daily for 7 days by gavage. At the end of the treatment
56 period the tissues were analysed for concentrations of long chain polyunsaturated fatty
57 acids (PUFA). DPA supplementation led to significant increases in DPA concentration
58 in all tissues, with largest increase being in adipose (5 fold) and smallest increase being
59 in brain (1.1 fold). DPA supplementation significantly increased the concentration of
60 DHA in liver and the concentration of EPA in liver, heart and skeletal muscle,
61 presumably by the process of retroconversion. EPA supplementation significantly
62 increased the concentration of EPA and DPA in liver, heart and skeletal muscle and the
63 DHA concentration in liver. DHA supplementation elevated the DHA levels in all
64 tissues and EPA levels in the liver. Adipose was the main tissue site for accumulation of
65 DPA, EPA and DHA. This data suggests that dietary DPA can be converted to DHA in
66 the liver, in a short term study, and that in addition it is partly retro-converted to EPA in
67 liver, adipose, heart and skeletal muscle. Future studies should examine the
68 physiological effect of DPA in tissues such as liver and heart.

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84 **INTRODUCTION**

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86 The interest in n-3 polyunsaturated fatty acids (PUFA) developed rapidly after two
87 Nobel Prize winning discoveries of particular prostaglandins, metabolites of arachidonic
88 acid, by Vane and Samuelson in the late 60's and early 70's ⁽¹⁾. Since then there have
89 been many studies suggesting the beneficial effects of n-3 fatty acids in reducing risk of
90 cardiovascular events, diabetes, inhibiting growth of tumour cells, modulating gene
91 expression and anti inflammatory activity ⁽²⁻⁷⁾. The parent n-3 PUFA is alpha-linolenic
92 acid (ALA; 18:3n-3), which is found in high concentration in some plant oils. In
93 mammals, some of the ingested ALA is metabolised to long chain n-3 PUFA (LCPn-3)
94 namely eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3)
95 and docosahexaenoic acid (DHA; 22:6n-3) by a series of desaturations and elongations
96 (see Fig 1). This metabolic processing of ALA to DHA is inefficient ⁽⁸⁾ and much of the
97 ingested ALA is either deposited in tissue adipose stores as ALA or catabolised by
98 mitochondrial beta-oxidation to yield energy (ATP) and carbon dioxide ⁽⁹⁾. Many
99 studies have reported that feeding relatively high levels of ALA in either animals or
100 humans leads to increased DPA levels, but not DHA levels, suggesting the steps
101 between DPA and DHA are rate limiting steps in this metabolic pathway ^(10, 11). In
102 contrast, ingested DHA is rapidly and efficiently deposited in brain, liver and other
103 tissues ⁽¹²⁾. There is not much literature available on fate of DPA. Two cell culture
104 studies have looked at the effect of DPA supplementation in endothelial cells and
105 hepatocytes, respectively, and reported that DPA supplementation increases both DPA
106 and EPA levels but not DHA in these cells ^(13, 14). However, no animal studies have been
107 conducted to investigate the conversion of pure DPA to DHA in mammals, presumably
108 because DPA has only recently become available for *in vivo* studies (in milligram
109 amounts). DPA is found in common foods like fish, fish oil, lean red meat and n-3-
110 enriched eggs ⁽¹⁵⁾ therefore it is important to understand the metabolic fate of DPA. In
111 this paper we report the effect of DPA supplementation on DPA, EPA and DHA
112 concentrations in rat tissues. The hypothesis being tested was that dietary
113 supplementation of DPA will increase both tissue DHA and EPA levels. The novelty of
114 this study is that it focuses on the metabolism of DPA in a rodent model, which has not
115 been investigated before.

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117 MATERIALS AND METHODS

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119 *Animals and diets*

120 Thirty two 4-wk-old male weanling Sprague Dawley rats were randomly divided into
121 four groups of eight animals. The rats were maintained on an *ad libitum* normal chow
122 diet with water, throughout the study. The total lipid content of the chow diet used was
123 5.7 (g/100g of wet weight) and the three main unsaturated fatty acids present in the
124 chow diet were oleic acid (OA) (29.7%), linoleic acid (31.2%) and ALA (3.4%); EPA,
125 DPA and DHA were not detected. The rats were pair housed and allowed one week to
126 acclimatise. The rats were then administered 50mg of DPA, EPA, DHA or OA (Nu-
127 Chek Prep, Inc, USA) by daily oral gavaging for 7 days. The dose and duration used in
128 this study was based on evidence from previously published studies which have
129 successfully used doses of 90-100mg/d of PUFA, for rats weighing up to 140-220g.
130 These studies demonstrated that changes in fatty acid composition of long chain PUFA
131 occurred within 3 days of supplementation⁽¹⁶⁻¹⁹⁾. In the present study, we used 50mg of
132 fatty acids for animals weighing 68-101g. Thus, it was expected that the dose of 50mg
133 of fatty acids given for 7 days would be sufficient to detect changes in tissue fatty acid
134 composition.

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

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141 *Lipid analysis*

142 The tissues were minced and the tissue lipids were extracted by chloroform/methanol
143 2:1, as described by Sinclair et al⁽²⁰⁾. An aliquot of the total lipids from each tissue, plus
144 an internal standard of docosatrienoic acid (22:3) (Nu-Chek Prep.USA), was reacted
145 with 2% H₂SO₄ in methanol for 3 hours at 80°C to form the fatty acid methyl esters;
146 they were passed through a silica sep-pak to remove cholesterol and then the fatty acid
147 methyl esters were separated by capillary gas liquid chromatography using a 50 m x
148 0.32 mm (I.D.) fused silica bonded phase column (BPX70, SGE, Melbourne, Australia).
149 The column oven was programmed to rise after 3 min at 125° to 220° C at 8° C/min with
150 a helium flow rate of 43 cm/sec as the carrier gas. Fatty acids were identified by

151 comparison with standard mixtures of fatty acid methyl esters and the results were
152 calculated using response factors derived from chromatographing standards of known
153 composition (NuChek Prep., Inc, USA).

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155 *Statistical analysis*

156 Data analysis was performed using SPSS v15.0 for Windows (SPSS Inc., Chicago, IL).
157 Significant differences between dietary groups were tested using a one-way ANOVA for
158 each type of fatty acid for both fatty acid analysis and gene expression. Post-hoc
159 comparisons were made using the LSD (least significant difference) test with a
160 significance level of 0.05.

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162 *Ethics approval*

163 All experimental procedures involving animals were performed under the ‘Australian
164 code of practice for the care and use of animals for scientific purposes’ and were
165 approved by La Trobe University Animal Ethics Committee (AEC07-53-P) and Deakin
166 University Animal Welfare Committee (AEX 23/2008).

167

168 **RESULTS**

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170 *Body and tissue weights*

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

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176 *Tissue fatty acid concentrations*

177 Adipose tissue contained the highest concentration of each of the three long chain n-
178 3PUFA with amounts ranging from 30 to 57 mg/g tissue compared with values of less
179 than 3 mg/g in other tissues (Table 1). In liver, muscle & brain tissue, the concentration
180 of DHA was between 1 to 3 mg/g; for DPA, the concentration ranged from 0.1 to 2mg/g
181 while for EPA the range was from 0.02 to 0.4mg/g tissue. The rats supplemented with
182 DHA showed a significant increase in tissue DHA content in all the five tissues studied.
183 The largest proportional increase in DHA occurred in adipose (3.4 fold) and skeletal

184 muscle (2.4 fold) and the least change was in brain (1.1 fold). It was also observed that
185 DHA supplementation led to a significant increase in EPA concentration in liver.

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

191 Supplementation with EPA led to a significant increase in tissue EPA and DPA
192 concentrations in liver, heart and skeletal muscle and a non-significant increase in
193 adipose tissue. EPA supplementation also increased DHA concentrations significantly in
194 liver. All the three n-3 PUFA led to a significant decrease in AA concentrations in liver
195 and heart.

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197 **DISCUSSION**

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

205 It was observed that DPA supplementation increased DPA concentration in
206 liver, heart, skeletal muscle and brain. It was also observed that DPA supplementation
207 led to a significant increase in EPA concentrations in liver, heart and skeletal muscle
208 and a non-significant increase in EPA concentrations in adipose tissue suggesting the
209 retroconversion of DPA into EPA *in vivo*. The process of retroconversion was first
210 described by Stoffel et al for DHA⁽²¹⁾ and subsequent work by Christensen et al in
211 human fibroblasts indicated the retroconversion of DHA and DPA were both likely to
212 involve the peroxisomal acyl-CoA oxidase^(22, 23). Retroconversion of DPA into EPA
213 has also been reported in endothelial cells, fetal skin fibroblasts and hepatocytes^{(13, 14,}
214 ²⁴⁾. The extent of “apparent” retroconversion of DPA to EPA
215 ($\Delta\text{EPA} * 100 / \Delta[\text{DPA} + \text{EPA}]$) was 28% in liver, 19% in adipose tissue, 12% in skeletal
216 muscle, 4% in heart and negligible in brain.

217 It was also observed from our study that EPA fed animals showed a significant
218 increase in EPA and DPA concentrations in the liver, heart and skeletal muscle. These
219 data confirm the findings of previously published cell culture studies which showed that
220 EPA is converted into DPA in endothelial and liver cells ^(13, 14). It is evident from our
221 study that EPA and DPA are interconverted in the body and therefore DPA may act as a
222 source of EPA in the body and vice-versa. This is particularly relevant in adipose tissue
223 which had the highest concentrations of these PUFA and consequently adipose may act
224 as a reservoir of these fatty acids. Supplementation with EPA had no effect on brain
225 EPA levels which are known to very low (in this study 0.02mg/g). It has recently been
226 suggested that low levels of EPA in brain phospholipids compared to DHA may be the
227 result of its rapid beta oxidation upon uptake by the brain ⁽²⁵⁾.

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

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

247 In this study, DPA, EPA and DHA supplementation also led to a significant
248 decrease in tissue AA levels in liver and heart and a non significant decrease in muscle
249 and adipose. This could be explained by the fact that n-3LCP are known to have an
250 inhibitory effect on delta 6 and delta 5 desaturases (29, 30), which are involved in

251 synthesis of AA from linoleic acid. Also supplementation of cells with the LCPn-3
252 could have led to competition for the enzyme acyl CoA transferase thereby decreasing
253 the incorporation of AA into phospholipids⁽³¹⁾.

254 To our knowledge, so far there have been very few studies which have
255 investigated the biochemical effects of DPA. We expect this might be due to the
256 relatively limited availability and high cost of pure DPA. But in light of the present
257 literature it can be speculated that the metabolic consequences of accumulation of DPA
258 in tissues may be related accumulation of DPA, EPA and DHA from DPA and to
259 inhibition of AA metabolism. Two studies are worth mentioning since both reported an
260 effect of pure DPA on platelet function through AA pathway inhibition^(32, 33). One
261 study reported that platelets metabolize 22:5n-3 into 11- and 14-hydroxy
262 docosapentaenoic acids via an indomethacin-insensitive pathway⁽³²⁾. They also reported
263 that when DPA is released along with AA in platelets it inhibited cyclooxygenase (COX)
264 enzyme thereby reducing the thromboxin B2 and 5,8,10-heptadecatrienoic acid (HHT)
265 production from AA. Akiba et al (2000) looked at the effects of DPA on platelet
266 aggregation and AA metabolism in rabbit platelets and compared them with those of
267 EPA and DHA⁽³³⁾. The results showed that n-3 fatty acids inhibited collagen- or AA-
268 stimulated platelet aggregation dose-dependently, and that DPA was the most potent
269 inhibitor. These results suggest that DPA possesses potent activity for interfering with
270 the COX pathway and accelerating the lipoxygenase pathway.

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

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281 Behavioural Sciences, Deakin University.

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283 authors.

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

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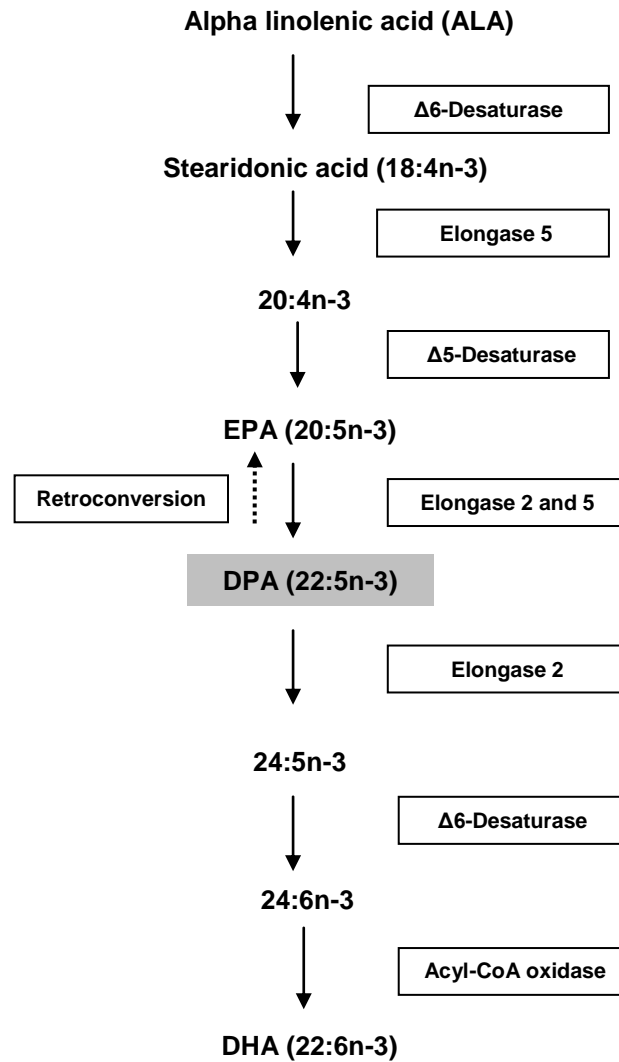
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310 Figure 1: Pathway for metabolism of ALA to long chain n-3 PUFA.

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

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324 **TABLE 1: Tissue fatty acid concentrations in brain, adipose and skeletal muscle of**
 325 **animals in various dietary groups (mean \pm 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

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 327 Fatty acid composition of various tissues from rats supplemented with 50 mg of OA, EPA,
 328 DPA or DHA for 7 days. Results are expressed as mg/g of tissue (n=8). Mean \pm SD values
 329 within a row with unlike superscripts were significantly different (P<0.05). Data was analysed
 330 using one way ANOVA and post hoc comparisons were made using LSD. OA, Oleic acid;
 331 EPA, eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, Docosahexaenoic acid.
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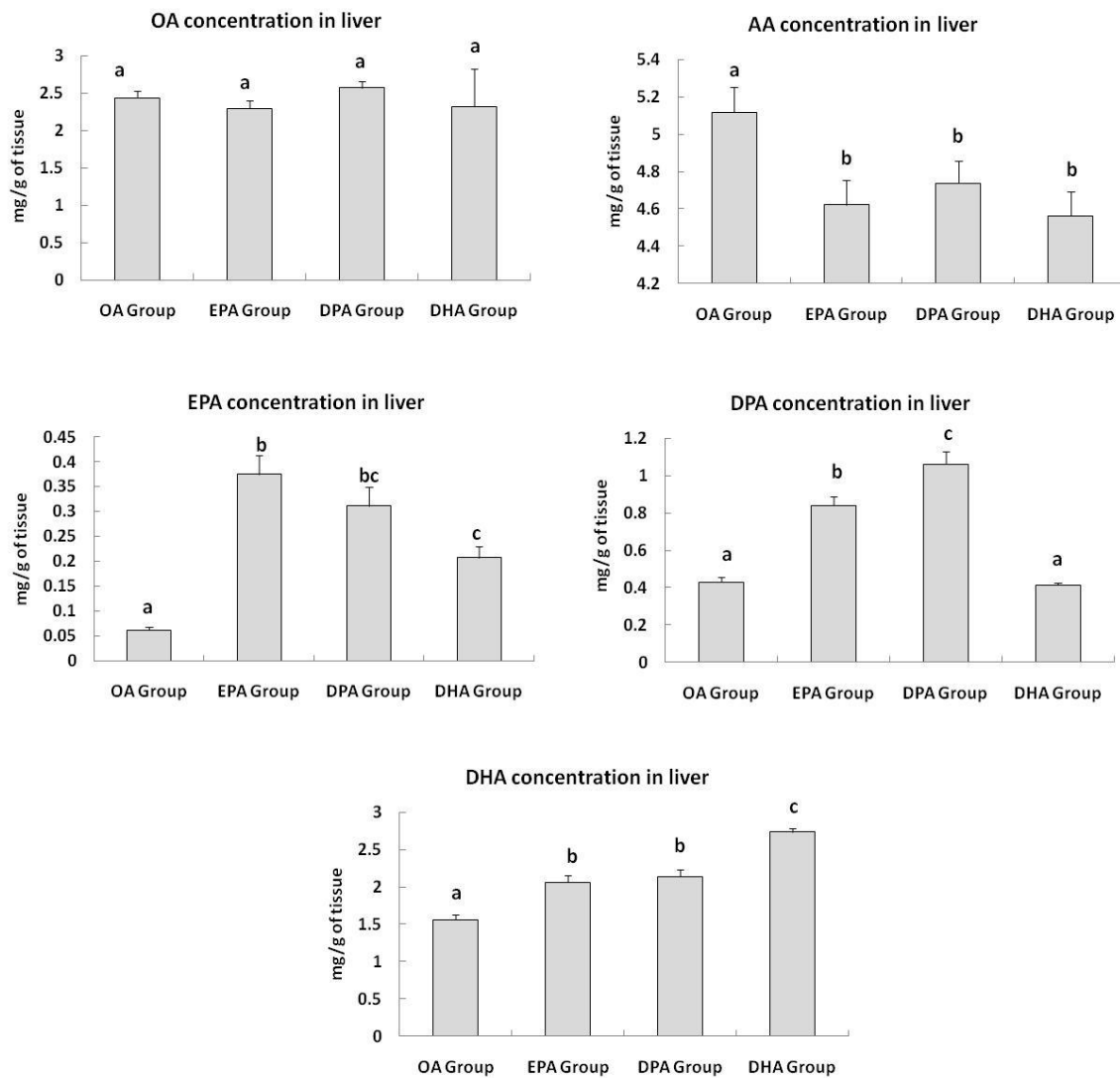
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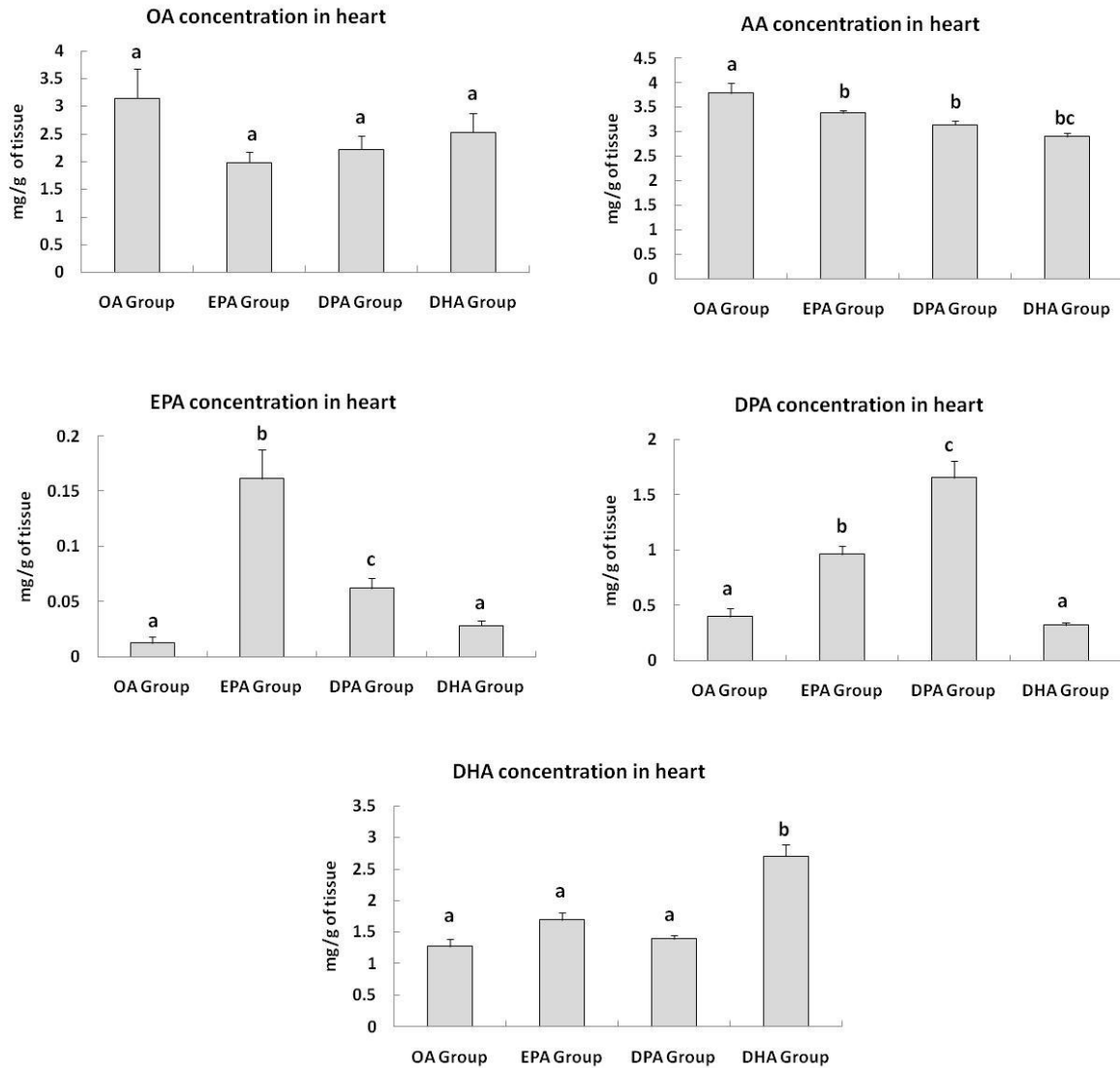
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345 Figure 2: Fatty acid composition of liver tissue of rats supplemented with 50 mg of OA, EPA,
 346 DPA or DHA for 7 days. Results are expressed as mg/g of tissue (n=8). Data was analysed
 347 using one way ANOVA and post hoc comparisons were made using LSD. Different
 348 superscripts represent significant difference from control OA group.

349 OA, Oleic acid; EPA, eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA,
 350 Docosaheaxaenoic acid.

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

361 OA, Oleic acid; EPA, eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA,
 362 Docosahexaenoic acid.

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