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# Effects of EPA supplementation on plasma fatty acids composition in hypertriglyceridemic subjects with FABP2 and PPAR $\alpha$ genotypes

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## Abstract

**Background:** Fatty acid binding protein 2 (FABP2) and peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) are involved in cellular uptake and metabolism of fatty acids. Polymorphism of FABP2 and PPAR $\alpha$  may influence plasma levels of fatty acids in those who take supplemental eicosapentaenoic acid (EPA). The purpose of this study was to study the potential associations between the Ala54/Thr polymorphism in FABP2 protein and the Leu162/Val in exon 5 and G/C in intron 7 of PPAR $\alpha$  with plasma fatty acids composition after EPA supplementation.

**Methods:** Twenty three FABP2 Ala54 and twenty three Thr54 carriers with hypertriglyceridemia were enrolled in this study. Participants took 2 g of pure EPA daily for 8 wks. Plasma fatty acids composition was determined and changes from the baseline were measured.

**Results:** Although EPA supplementation increased the level of plasma EPA and  $\omega$ -3 fatty acids in both carriers of FABP2 and PPAR $\alpha$  genes, these effects were more pronounced in Thr54 and Val162 carriers. EPA supplementation decreased the level of some n-6 fatty acids such as arachidonic acid.

**Conclusion:** EPA consumption has more favorable effects on blood n-3 fatty acids and can change the level of plasma n-3 fatty acids, particularly EPA. Because the FABP2 Thr54 polymorphism appears to be prevalent in hypertriglyceridemic subjects, increasing EPA intake in these subjects could be an effective strategy for preventing cardiovascular diseases. Finally, diets and micronutrient recommendations should be individualized for high risk people.

**Keywords:** Plasma fatty acids composition, Eicosapentaenoic acid, Polymorphism, Fatty acid binding protein-2, Peroxisome proliferator-activated receptor

## Introduction

Dietary fat intake is believed to contribute to development of chronic diseases, in particular cardiovascular disease [1]. No biomarkers reflect the absolute fat intake, however, measuring fatty acids concentrations in various biological samples reflect to some extent, the proportional intake of fatty acids [2]. Fatty acids can be measured as free fatty acids in serum, components of circulating triglycerides, components of erythrocyte membranes, phospholipids or cholesterol esters, or adipose tissue from different sites. The amount of serum or plasma fatty acids reflects the

composition of dietary intakes of the past few hours (triglyceride) or the past few days (cholesterol ester and phospholipids fatty acids) [3].

Changes in plasma fatty acids composition reflect abnormalities in lipoprotein metabolism and dietary habits and have been widely studied in many animal and epidemiological [4,5] and clinical human studies [6,7].

Fatty acids of the n-3 family, particularly the long-chain n-3 fatty acids, are important nutrients throughout the life. Several epidemiological studies have shown that n-3 fatty acids in blood differ significantly among individuals [8-10]. This family of fatty acids has been historically associated with a lower risk of cardiovascular disease, including stroke [11] and coronary heart disease [12,13]. In children, cardiovascular benefits have been attributed

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to long-chain n-3 fatty acids [14,15]. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) two well-known long-chain n-3 fatty acids are important for their protective effects on cardiovascular disease; increased dietary intake of them has resulted in decreased cardiac mortality in a large secondary prevention study [16].

The composition of serum fatty acids can not only be used as an indicator of dietary fat quality [17], but can also be used as a biomarker for assessing metabolic and cardiovascular disease risk [18,19]. Intestinal fatty acid binding protein 2 (FABP2) is a small cytosolic protein involved in intracellular fatty acid (FA) transfer and metabolism. Peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) is involved in glucose and lipid metabolism and thus may have a role in development of dyslipidemia, atherosclerosis, obesity, insulin resistance, and type II diabetes mellitus. We have shown that FABP2 genotypes influence the lipid-lowering effects of EPA supplementation in hypertriglyceridemic subjects [20]. We, therefore, conducted this study to determine the potential associations between the Ala54/Thr polymorphism in FABP2 protein and the Leu162/Val in exon 5 and G/C in intron 7 of PPAR $\alpha$  with plasma fatty acids composition after EPA supplementation.

## Subjects and methods

### Subjects

Participants were selected from the hypertriglyceridemic subjects referred from Tehran Central Laboratories to Endocrinology and Metabolism Research Center (EMRC). The inclusion criteria were a serum TG level >200 mg/dL (>2.3 mmol/L), and a fasting blood glucose of <110 mg/dL (<6.2 mmol/L). Those who had received lipid lowering agents, oral contraceptive pills, diuretics, sex hormones, thyroid medications, or omega-3 supplement, and patients with a history of gastrointestinal diseases, and smokers were excluded from the study.

After determination of their FABP2 genotypes, the first 23 eligible subjects who were found as Ala54 carriers and the first 23 eligible Thr54 carriers were enrolled in the study. Participants took two grams per day of pure EPA for eight weeks (four gel caps, each containing 500 mg ethyl ester EPA 90%, courtesy of Minami Nutrition, Edegem, Belgium). Two capsules were taken in the morning and two in the evening. The participants were followed weekly at the EMRC; a checklist for weekly consumption of capsules was filled and capsules for the next week were given to them. All of the subjects consumed controlled diet (Percentage of energy from carbohydrate, fat, and protein diets were similar).

A blood sample was drawn from each participant following a 14-hour overnight fasting at the baseline and after eight weeks of EPA supplementation. Height and weight were measured by a Seca scale (Germany) with

light clothing and no shoes on. Body mass index (BMI) was then calculated. Waist circumference was measured with a flexible tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteal region.

The study was approved by Ethics Committee of EMRC, *Tehran University of Medical Sciences* (TUMS). All participants were informed of the nature of the study and gave a written informed consent. The biochemical analyses were carried out at EMRC laboratory, TUMS. Genetic studies were conducted at the Department of Medical Genetics, TUMS. The plasma fatty acids composition was determined at the Department of Medicinal Chemistry and Pharmaceutical Sciences Laboratory, TUMS.

We calculated the sample size as follow:

$$\alpha = 0.5 \quad 1 - \beta = 0.80 \quad P \text{ value} < 0.05$$

$$n = \left( \left( [Z_{1-\alpha/2}] + [Z_{1-\beta}] \right) / d \right)^2 d = 0.61$$

$$n = 20$$

### Laboratory analyses

Plasma samples and sera were separated from blood samples by centrifuging at 4°C and 1800 g for 15 min and stored in 1-mL aliquots in sterile tubes at -80°C until used. Serum and plasma lipid and lipoprotein levels were measured as described previously [20].

### Plasma fatty acid extraction and gas chromatography

Fatty acid extraction was done by Folch method [21] with some modifications. Plasma was homogenized in chloroform: methanol (2:1 vol/vol containing 50 mg/L butylated hydroxy toluene); normal saline was added to the solution, shaken vigorously and allowed for phase separation. The upper layer was drawn off by aspiration and washed several times; the lower phases were collected. Extracted lipids were dried under a stream of nitrogen. The dried lipids were soaponified by the method described previously [22]. Soaponified fatty acids were transesterified by boron trifluoride (BF<sub>3</sub>) in methanol. BF<sub>3</sub> was added to the sample and incubated at 100°C in a water bath for an hour. After cooling to room temperature, hexane, HCl and water were added, shaken vigorously, centrifuged, and the upper phase was taken into a new tube and dried with nitrogen. Before injecting to the instrument, methanol and ethylated margaric acid (as an internal standard) were added to samples. Fatty acids methyl esters (FAMES) were measured by gas chromatography. A capillary column with 60 m length, 0.25 mm internal diameter and 0.2  $\mu$ m film thickness on an HP 6890 GC equipped with flame ionization detector was used to qualify and quantify FAMES. The initial column temperature was set at 195°C for

2 min, which increased to 205°C by increments of 2°C/min, then to 214°C by 1°C/min, then to 240°C by 15°C/min and held for 10 min. Helium was used as the carrier gas at an initial flow rate of 1 mL/min for 8 min, which increased to 1.3 mL/min for 4.2 min and then to 1.9 mL/min. The detector temperature was set at 300°C and the injector temperature at 250°C. FAMES were identified by comparison with the retention times of Supelco 37 component FAME mix standard. We focused on PUFAs in chromatogram and excluded short- and medium-chain saturated fatty acids from chromatogram. Different concentrations of FAME mix with added ethylated margaric acid were injected to gas chromatography machine (GC) to obtain the standard curve for each fatty acid. The peak area of a given fatty acid was divided by the peak area of the internal standard (ethylated margaric acid) then with respect to the standard curve, concentrations of fatty acids in plasma were estimated.

#### Genotyping

##### *Ala54Thr (Gene ID: 2169)*

Genomic DNA was extracted using the Flexi Gene DNA kit (Qiagen, GmbH, Hilden, Germany) as described previously [20]. A 180-bp DNA fragment containing the G to A nucleotide substitution in exon 2 (codon 54) of the FABP2 gene (*Ala54Thr*) was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described previously [20].

##### *Leu162Val (gene ID: 55465)*

The *Leu162Val* mutation of the PPAR $\alpha$  gene is caused by a C to G transversion at nucleotide 484 in exon 5. The PCR-RFLP method was used as described earlier [23].

#### Intron 7

The PCR-RFLP method was used to determine intron 7 polymorphism (mutation) as described previously [23].

#### Statistical analyses

The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. To normalize the continuous variables not normally distributed, a log transformation was applied. The mean plasma fatty acids concentrations between the two study groups with different FABP2 genotypes were compared by independent sample *Student's t* test.

Since only few subjects with *Thr54/Thr* were found among the participants, they were pooled with *Ala54/Thr* subjects and analyses were carried out on the pooled data. Results are presented as Means $\pm$ SE unless otherwise noted. Analyses were performed by SPSS<sup>®</sup> for Windows<sup>®</sup> ver 11.5. A p value <0.05 was considered statistically significant.

#### Results

The baseline characteristics of subjects were described previously [20,23]. Table 1 shows the plasma fatty acids compositions in hypertriglyceridemic subjects.

Table 2 shows plasma fatty acids levels of studied subjects stratified by their FABP2 genotypes. Concentrations of EPA (p<0.001), DHA (p<0.055), and some of n-3 fatty acids (p<0.001) were higher in those with *Thr54* polymorphism than *Ala54* after EPA supplementation. Changes in levels of other fatty acids did not significantly differ between subjects with G or A alleles.

Plasma fatty acids levels in hypertriglyceridemic subjects with *Val162* polymorphism in PPAR $\alpha$  genotypes are shown in Table 3. The concentrations of EPA (p<0.001),

**Table 1 Plasma fatty acids composition in hypertriglyceridemic subjects**

Fatty Acids	Concentration in plasma $\mu$ g/mL n=46	Fatty Acids	Concentration in plasma $\mu$ g/mL n=46
Miristic acid (C14:0)	20.95 $\pm$ 0.6	13,16-Docosadienoic acid (DDA, C22:2 n-6)	8.28 $\pm$ 1.2
Palmitic acid (C16:0)	152.07 $\pm$ 44.7	alpha-Linolenic acid	4.9 $\pm$ 17.9
Stearic acid (C18:0)	105.91 $\pm$ 12.1	11,14,17-Eicosatrienoic acid (C20:3 n-3)	4.12 $\pm$ 0.9
Arachidic acid (C20:0)	12.4 $\pm$ 7.7	Eicosapentaenoic acid (C20:5, n-3)	2.5 $\pm$ 1.2
Behenic acid (C22:0)	13.2 $\pm$ 1.3	Docosahexaenoic acid (C22:6, n-3)	11.9 $\pm$ 8.2
Oleic acid (C18:1)	131.69 $\pm$ 20.2	Sum of saturated fatty acids	304.5 $\pm$ 32.1
11-Eicosenoic acid(C20-1)	9.41 $\pm$ 1.5	Sum of monounsaturated fatty acids	164.79 $\pm$ 26.2
Nervonic acid (C24:1)	11.45 $\pm$ 5.36	Sum of polyunsaturated fatty acids	360.5 $\pm$ 50.2
Linoleic acid (C18:2)	200.5 $\pm$ 35.2	Sum of W-6 fatty acids	110.3 $\pm$ 218.2
Gamma linolenic acid (C18:3, n-6)	7.4 $\pm$ 3.4	Sum of W-3 fatty acids	20.6 $\pm$ 11.4
11,14-Eicosadienoic acid (C20:2, n-6)	21.65 $\pm$ 6.9	Total fatty acids	777.96 $\pm$ 95.6
Dihomo gamma Linolenic acid (C20:3 n-6)	8.27 $\pm$ 1.2	Arachidonic/DGLA+EPA	23.7 $\pm$ 21
Arachidonic Acid	52.1 $\pm$ 23.0	W6:W3 ratio	10.0 $\pm$ 1.6

Values are means $\pm$ SE.

**Table 2 Plasma fatty acids concentration after 8 weeks of EPA supplementation in hypertriglyceridemic subjects stratified by FABP2 genotypes**

Fatty acids	Concentration in plasma µg/mL		Paired t-test P value			Paired t-test P value	Difference between pre- and post-treatment		p
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment		Ala54 (n=23)	Thr54 <sup>§</sup> (n=23)	
	Ala54 (n=23)			Thr54 <sup>§</sup> (n=23)					
Eicosapentaenoic acid (C20:5, n-3)	1.45±0.4	6.66±0.8	0.001*	4.65±1.0	67.96±12.9	0.001*	5.20±0.8	61.76±12.3	0.001**
Docosahexaenoic acid (C22:6, n-3)	10.47±1.5	16.12±2.0	0.06*	22.22±5.9	19.63±1.9	NS	5.65±2.9	29.94±13.1	0.055
Sum of saturated fatty acids	251.03±32.5	286.76±47.4	NS	357.96±43.5	286.76±453.5	0.006*	35.72±55.7	153.01±66.8	0.07
Sum of monounsaturated fatty acids	105.38±17.8	173.1±39.6	0.001*	224.2±34.5	2306.3±2261.6	0.001*	67.72±41.6	2175.3±2157.9	0.07
Sum of polyunsaturated fatty acids	174.93±34.6	322.75±61.5	0.08*	442.41±65.8	800.55±74.7	0.05*	147.82±81.1	351.38±82.5	0.08
Sum of W6 fatty acids	156.96±32.9	289.0±57.9	NS	403.4±63.6	662.24±65.2	0.001*	132.04±76.5	251.55±76.9	NS
Sum of W3 fatty acids	15.97±2.3	33.75±4.1	0.006*	38.97±6.9	138.3±16.2	0.05*	15.78±5.1	99.82±16.5	0.001**
Total fatty acids	531.35±70.5	782.62±138.7	NS	1024.57± 120.7	24326.97± 22645.9	0.005*			
W6:W3 ratio	7.65±0.9	7.58±0.9	NS	12.37±2.2	5.54±0.5	0.005*	-0.073±1.6	-6.76±2.2	0.02**
Miristic acid (C14:0)	20.46±0.6	21.73±0.61	NS	21.46±0.56	23.38±0.96	0.05*	1.27±0.96	1.77±0.79	NS
Palmitic acid (C16:0)	104/94±65/2	152.29±30.7	NS	199.2±24.3	319.6±33.5	0.01*	47.35±39.3	116.7±40.9	NS
Stearic acid (C18:0)	92.96±12.0	96.13±15.5	NS	118.85±12.0	152.27±25.9	0.05*	31.17±18.2	28.5±33.2	NS
Oleic acid (C18:1)	76.95±14.3	143.12±35.2	NS	186.43±27.6	316.83±34.9	0.004	46.17±40.2	124.92±47.3	NS

Values are mean±SEM.

<sup>§</sup>Because the number of subjects in the Thr54/Thr group was small, data from the Ala54/Thr group were combined with data from the Thr54 (Ala54/Thr Thr54/Thr) groups.

\*\*Significant difference between Ala54 and Thr54 groups.

\*Significant differences between post- and pre-intervention values.

NS: No significant difference.

**Table 3 Plasma fatty acids concentration after 8 weeks of EPA supplementation in hypertriglyceridemic subjects stratified by PPARα genotypes**

Fatty acids	Concentration µg/mL		Paired t-test P value	Concentration µg/mL		Paired t-test P value	Difference between pre and post treatment		Independent t-test P value
	Pre-treatment	Post-treatment		Pre-treatment	Post - treatment		Leu (n=38)	Val (n=8)	
	Leu (n=38)			Val (n=8)					
Eicosapentaenoic acid (C20:5, n-3)	3.04±0.7	21.9±3.9	0.001*	3.5±1.2	104.35±28.9	0.05*	18.9±3.5	100.9±28.5	0.001**
Docosahexaenoic acid (C22:6, n-3)	15.5±3.7	28.7±7	0.01*	19.03±4.3	58.7±16.3	NS	13.15±7.8	39.63±17.4	
Sum of saturated fatty acids	288.5±26	367.0±37.8	NS	371.53±60.6	542.9±113.5	NS	78.5±44.6	171.38±139.4	0.05**
Sum of monounsaturated fatty acids	152.36±22.5	277.1±38.5	0.01*	219.5±51.7	596.6±59.3	NS	542.9±41.7	594.4±59.3	NS
Sum of polyunsaturated fatty acids	259.3±37.5	482.2±58.2	0.01*	509.23±60.1	887.18±149.4	0.05*	222.94±66.6	378.9±127.7	NS
Sum of W6 fatty acids	231.98±35.2	416.5±51.2	0.01*	476.5±129.7	707.5±135.6	NS*	184.6±61.6	231.1±120.3	NS
Sum of W3 fatty acids	27.32±4.3	65.73±9.5	0.001*	32.76±8.6	180.65±26.8	0.01	38.3±9.1	147.89±25.5	0.001**
Total fatty acids	700.16±79.1	1126.34±128	0.01*	1100.28±202.8	61063.12±594.5	NS	426.18±142.9	5999.85±594.7	NS
Arachidonic/DGLA+EPA	2.68±1.0	1.41±0.2	NS	112.8±109.8	0.89±0.2	NS	-1.3±0.9	-11.9±109.8	NS
W6:W3 ratio	8.59±0.8	6.89±0.6	0.05*	15.91±5.5	4.57±0.9	NS	-1.7±1.1	-3.5±1.5	0.01**

Values are means±SE.

\*\*Significant difference between Leu and Val groups.

\*Significant differences between post- and pre-intervention values.

NS: No significant differences.

**Table 4 Plasma fatty acids concentration after 8 weeks of EPA supplementation in hypertriglyceridemic subjects stratified by PPARα (GG/GC) genotypes**

Fatty acids	Concentration µg/mL		Paired t-test <i>p value</i>	Concentration µg/mL		Paired t-test <i>p value</i>	Difference between pre- and post-treatment GG (n=24)	Difference between after and before values GC (n=22)	Independent t-test <i>p value</i>
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment				
	GG (n=24)			GC (n=22)					
Eicosapentaenoic acid (C20:5, n-3)	1.83±0.62	15.35±5.2	0.001*	4.49±0.9	60.24±12.4	0.001*	12.52±4.9	55.74±12.1	0.001**
Docosahexaenoic acid (C22:6, n-3)	11.26±1.7	26.49±6.6	0.04*	21.31±5.9	42.38±11.6	0.003*	15.23±6.9	21.07±12.8	NS
Sum of saturated fatty acids	262.08±35.3	243.09±53.9	NS	247.81±30.9	459.06±51.9	<0.009*	81.01±52.2	111.25±60.4	NS
Sum of monounsaturated fatty acids	112.44±20.1	217.36±42.9	0.03*	218.71±33.7	229.6±22.6	0.002*	103.9±42.0	227.4±22.6	NS
Sum of polyunsaturated fatty acids	237.53±57.6	437.02±81.5	NS	277.32±56.6	684.23±79.2	0.001*	199.53±99.8	206.9±93.3	NS
Sum of W6 fatty acids	217.9±55.7	382.97±73.0	NS	329.88±53.6	562.5±67.0	0.001*	165.08±92.0	222.62±83.0	NS
Sum of W3 fatty acids	19.62±2.9	54.1±11.1	0.009*	37.46±6.8	121.73±17.4	0.001*	34.45±11.2	84.27±18.7	0.02**
Total fatty acids	613.03±92.3	997.5±172.1	0.06*	942.4±116.2	24105.8±226.5	<0.001*	384.47±17.04	23161.9±226.4	NS
Arachidonic/DGLA+EPA	42.24±39.9	1.54±0.2	NS	2.14±0.5	1.08±0.2	NS	-41.7±39.9	-1.06±0.6	NS
W6:W3 ratio	9.65±2.2	7.56±0.9	NS	102.26±1.1	5.3±0.5	0.007*	-2.09±2.4	-4.97±1.3	NS

Values are means±SE.

\*\*Significant difference between GG and GC carriers.

\*Significant difference between post- and pre-intervention values.

NS: No significant difference.

and n-3 fatty acids ( $p < 0.001$ ) were significantly higher and the n-6:n-3 ratio ( $p < 0.01$ ) was significantly lower in Val162 than in Leu162 polymorphism. Changes in levels of other fatty acids did not significantly differ between Leu or Val carriers. The levels of EPA ( $p < 0.001$ ) and n-3 fatty acids ( $p < 0.02$ ) were significantly different between GG and GC groups (Table 4).

## Discussion

We found that EPA supplementation could increase the level of plasma EPA, in both FABP2 and PPAR $\alpha$  genotypes with more effects on subjects with either Thr or Val162 alleles. These results are in keeping with the hypothesis which indicates that presence of the Thr54 allele may increase the binding affinity of FABP2 to long-chain fatty acids (LCFAs) [24]. Furthermore, enhanced intestinal absorption of fatty acids, higher levels of plasma lipids and the consequent enhanced lipid oxidation rates would inhibit *in vivo* tissue sensitivity to insulin. It was later confirmed, in a healthy white population with normal glucose tolerance, that the Thr54 allele was associated with insulin resistance [25].

In fact, in subjects with Thr54 allele, EPA supplementation results in absorption of EPA by enterocytes, which leads to a higher plasma EPA concentration. Although EPA is the precursor of DHA, we did not observe any increase in plasma level of DHA which might be due to poor enzymatic conversion of EPA to DHA. Arterburn, *et al.*, previously reported that n-3 fatty acids consumption increased their levels in plasma. They showed that supplementation of adults with 4 g/day pure EPA ethyl ester results in significant increase in EPA concentration in whole plasma and plasma or serum phospholipids, but no increase was seen in DHA concentration, which is consistent with retro conversion of DHA to EPA [26-28]. In the present study, the levels of some fatty acids in plasma were changed. After EPA supplementation, the level of EPA, n-3 fatty acids, MUFA, PUFA and some saturated fatty acids such as myristic, palmitic, oleic, and stearic increased in both Thr54 and Ala54 carriers, the increase was more pronounced in Thr54 groups. These results were approved the Thr54 hypothesis which states increased fatty acids uptake and transport by Thr54 carriers. King, *et al.*, reported that with consuming two different fat diets the level of fatty acids composition would be different [29]. There are some evidence that the level of n-6 fatty acids will decrease after consumption of n-3 fatty acids [26,30-32]. In the current study, the level of some plasma n-6 fatty acids such as arachidonic acid (AA) decreased in both Ala54 and Thr54 carriers after EPA supplementation; although we could not observe any interaction between EPA consumption and genotype. A decrease in the level of plasma AA after n-3 consumption has been reported previously [32-34]. Hlavaty, *et al.*,

reported that supplementing diet with n-3 fatty acids decreases plasma level of some n-6 fatty acids [35]. Berstad, *et al.*, reported that n-3 supplementation decreases plasma AA level [33]. Polymorphism in codon 54 had no significant effect on serum fatty acids composition in adults Finns [36]. In Pima, no significant difference between the long-chain fatty acids amount in adipose and muscle tissues was observed between Ala54 and Thr54 carriers [37]. One study showed that in obese children who were Thr54 carriers, EPA consumption decreased the amount of plasma AA level. In the current study, AA concentrations were lower in Thr54 than Ala54 carriers after EPA supplementation. A decrease in n-6:n-3 fatty acids ratio was observed in both FABP2 and PPAR $\alpha$  genotypes after EPA supplementation too. Although the n-6:n-3 fatty acids ratio decreased in both FABP2 and PPAR $\alpha$  genotypes, these effects were more pronounced in Thr54 and Val162 than in Ala54 and Leu162 carriers. On the other hand, the ratio of AA:EPA decreased in both Thr54 and Ala54 after EPA supplementation, but no significant differences were observed between the two carriers. For nucleus receptors n-3 fatty acids are stronger ligands than n-6 fatty acids. In Greenland and Japanese people this ratio decreased in both Thr54 and Ala54 carriers after EPA supplementation [38,39].

There is increasing scientific evidence that genetic factors, conferring either protection or risk, also contribute importantly to the incidence of these diseases. SNPs are of particular interest because they can influence disease in a complex but largely unknown manner by interacting with environmental and lifestyle factors.

We showed that EPA supplementation could change the blood fatty acids composition, and thus it could be beneficial for lowering some plasma fatty acids. Since we observed more pronounced changes in blood fatty acids in Thr and Val than in Ala and Leu carriers, we suggest EPA supplementation to be used based on people genotypes.

In conclusion, EPA consumption has more favorable effects on blood n-3 fatty acids and can change the level of plasma n-3 fatty acids, particularly EPA. Because the FABP2 Thr54 polymorphism appears to be prevalent in hypertriglyceridemic subjects, increasing EPA intake in these subjects could be an effective strategy for preventing cardiovascular diseases. Finally, for high-risk people diet and micronutrients recommendation should be individualized.

## Competing interests

All authors declare that they have no conflict of interests.

## Authors' contributions

HP contributed to conception of the idea and study design, interpretation of data, performing all experiments and writing the manuscript. MA provided assistance in study design of the GC analysis. MRE helped with statistical

analysis and interpretation of data. SH helped in editing the manuscript. SAM provided assistant in the design of the study. All authors have read and approved the final form of the manuscript.

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#### References

1. Kris-Etherton PM, Harris WS, Appel LJ: **Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease.** *Circulation* 2002, **106**(21):2747–2757.
2. Arab L: **Biomarkers of fat and fatty acid intake.** *J Nutr* 2003, **133**(Suppl 3):925S–932S.
3. Kohlmeier L: **Future of dietary exposure assessment.** *Am J Clin Nutr* 1995, **61**(3 Suppl):702S–709S.
4. Rossner S, Walldius G, Bjorvell H: **Fatty acid composition in serum lipids and adipose tissue in severe obesity before and after six weeks of weight loss.** *Int J Obes* 1989, **13**(5):603–612.
5. Phinney SD, Tang AB, Thurmond DC, Nakamura MT, Stern JS: **Abnormal polyunsaturated lipid metabolism in the obese Zucker rat, with partial metabolic correction by gamma-linolenic acid administration.** *Metabolism: clinical and experimental* 1993, **42**(9):1127–1140.
6. Scaglioni S, Verduci E, Salvioni M, Bruzzese MG, Radaelli G, Zetterstrom R, Riva E, Agostoni C: **Plasma long-chain fatty acids and the degree of obesity in Italian children.** *Acta Paediatr* 2006, **95**(8):964–969.
7. Agostoni C, Riva E, Bellu R, Vincenzo SS, Grazia BM, Giovannini M: **Relationships between the fatty acid status and insulinemic indexes in obese children.** *Prostaglandins Leukot Essent Fatty Acids* 1994, **51**(5):317–321.
8. Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH: **Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment.** *Lipids* 2000, **35**(12):1305–1312.
9. Heude B, Ducimetiere P, Berr C: **Cognitive decline and fatty acid composition of erythrocyte membranes—The EVA Study.** *Am J Clin Nutr* 2003, **77**(4):803–808.
10. Tully AM, Roche HM, Doyle R, Fallon C, Bruce I, Lawlor B, Coakley D, Gibney MJ: **Low serum cholesteryl ester-docosahexaenoic acid levels in Alzheimer's disease: a case-control study.** *Br J Nutr* 2003, **89**(4):483–489.
11. He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, Goldbourt U, Greenland P: **Fish consumption and incidence of stroke: a meta-analysis of cohort studies.** *Stroke: a journal of cerebral circulation* 2004, **35**(7):1538–1542.
12. Whelton SP, He J, Whelton PK, Muntner P: **Meta-analysis of observational studies on fish intake and coronary heart disease.** *Am J Cardiol* 2004, **93**(9):1119–1123.
13. He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, Greenland P: **Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies.** *Circulation* 2004, **109**(22):2705–2711.
14. Engler MM, Engler MB, Malloy M, Chiu E, Besio D, Paul S, Stuehlinger M, Morrow J, Ridker P, Rifai N, Mietus-Snyder M: **Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY study.** *Int J Clin Pharmacol Ther* 2004, **42**(12):672–679.
15. Engler MM, Engler MB, Malloy MJ, Paul SM, Kulkarni KR, Mietus-Snyder ML: **Effect of docosahexaenoic acid on lipoprotein subclasses in hyperlipidemic children (the EARLY study).** *Am J Cardiol* 2005, **95**(7):869–871.
16. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial: **Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico.** *Lancet* 1999, **354**(9177):447–455.
17. Aro A: **Fatty acid composition of serum lipids: is this marker of fat intake still relevant for identifying metabolic and cardiovascular disorders?** *Nutr Metab Cardiovasc Dis* 2003, **13**(5):253–255.
18. Riccardi G, Giacco R, Rivellese AA: **Dietary fat, insulin sensitivity and the metabolic syndrome.** *Clinical nutrition (Edinburgh, Scotland)* 2004, **23**(4):447–456.
19. Vessby B: **Dietary fat and insulin action in humans.** *Br J Nutr* 2000, **83**(Suppl 1):S91–96.
20. Pishva H, Mahboob SA, Mehdipour P, Eshraghian MR, Mohammadi-Asl J, Hosseini S, Karimi F: **Fatty acid-binding protein-2 genotype influences lipid and lipoprotein response to eicosapentaenoic acid supplementation in hypertriglyceridemic subjects.** Burbank, Los Angeles County, Calif: Nutrition.
21. Folch J, Lees M, Sloane Stanley GH: **A simple method for the isolation and purification of total lipides from animal tissues.** *J Biol Chem* 1957, **226**(1):497–509.
22. Wang Y, Sunwoo H, Cherian G, Sim JS: **Fatty acid determination in chicken egg yolk: a comparison of different methods.** *Poult Sci* 2000, **79**(8):1168–1171.
23. Pishva H, Mahboob SA, Mehdipour P, Eshraghian MR, Mohammadi-Asl J, Saeed Hosseini M, Rahmany M: **Association between the FABP2 Ala54Thr, PPARa Leu162/Val, and PPARa intron7 polymorphisms and blood lipids, ApoB and ApoCIII in hypertriglyceridemic subjects in Tehran.** *J Clin Lipidol* 2009, **3**(2009):2187–2194.
24. Baier LJ, Sacchetti JC, Knowler WC, Eads J, Paolisso G, Tataranni PA, Mochizuki H, Bennett PH, Bogardus C, Prochazka M: **An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance.** *J Clin Invest* 1995, **95**(3):1281–1287.
25. Kim CH, Yun SK, Byun DW, Yoo MH, Lee KU, Suh KI: **Codon 54 polymorphism of the fatty acid binding protein 2 gene is associated with increased fat oxidation and hyperinsulinemia, but not with intestinal fatty acid absorption in Korean men.** *Metabolism: clinical and experimental* 2001, **50**(4):473–476.
26. Grimsgaard S, Bonna KH, Hansen JB, Nordoy A: **Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids.** *Am J Clin Nutr* 1997, **66**(3):649–659.
27. Woodman RJ, Mori TA, Burke V, Puddey IB, Watts GF, Beilin LJ: **Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension.** *Am J Clin Nutr* 2002, **76**(5):1007–1015.
28. Nestel P, Shige H, Pomeroy S, Cehun M, Abbey M, Raederstorff D: **The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans.** *Am J Clin Nutr* 2002, **76**(2):326–330.
29. King IB, Lemaitre RN, Kestin M: **Effect of a low-fat diet on fatty acid composition in red cells, plasma phospholipids, and cholesterol esters: investigation of a biomarker of total fat intake.** *Am J Clin Nutr* 2006, **83**(2):227–236.
30. Arterburn LM, Hall EB, Oken H: **Distribution, interconversion, and dose response of n-3 fatty acids in humans.** *Am J Clin Nutr* 2006, **83**(6 Suppl):1467S–1476S.
31. Haban P, Zidekova E, Klvanova J: **Supplementation with long-chain n-3 fatty acids in non-insulin-dependent diabetes mellitus (NIDDM) patients leads to the lowering of oleic acid content in serum phospholipids.** *Eur J Nutr* 2000, **39**(5):201–206.
32. Horrobin DF: **Interactions between n-3 and n-6 essential fatty acids (EFAs) in the regulation of cardiovascular disorders and inflammation.** *Prostaglandins Leukot Essent Fatty Acids* 1991, **44**(2):127–131.
33. Berstad P, Seljeflot I, Veierod MB, Hjerkin EM, Arnesen H, Pedersen JL: **Supplementation with fish oil affects the association between very long-chain n-3 polyunsaturated fatty acids in serum non-esterified fatty**



- acids and soluble vascular cell adhesion molecule-1. *Clin Sci (Lond)* 2003, **105**(1):13–20.
34. Holler C, Auinger M, Ulberth F, Irsigler K: **Eicosanoid precursors: potential factors for atherogenesis in diabetic CAPD patients?** *Perit Dial Int* 1996, **16**(Suppl 1):S250–253.
  35. Hlavaty P, Kunesova M, Gojova M, Tvrzicka E, Vecka M, Roubal P, Hill M, Hlavata K, Kalouskova P, Hainer V, Zak A, Drbohlav J: **Change in fatty acid composition of serum lipids in obese females after short-term weight-reducing regimen with the addition of n-3 long chain polyunsaturated fatty acids in comparison to controls.** *Physiological research/Academia Scientiarum Bohemoslovaca* 2008, **57**(Suppl 1):S57–65.
  36. Vidgren HM, Sipilainen RH, Heikkinen S, Laakso M, Uusitupa MI: **Threonine allele in codon 54 of the fatty acid binding protein 2 gene does not modify the fatty acid composition of serum lipids in obese subjects.** *Eur J Clin Invest* 1997, **27**(5):405–408.
  37. Pratley RE, Baier L, Pan DA, Salbe AD, Storlien L, Ravussin E, Bogardus C: **Effects of an Ala54Thr polymorphism in the intestinal fatty acid-binding protein on responses to dietary fat in humans.** *J Lipid Res* 2000, **41**(12):2002–2008.
  38. Hirai A, Hamazaki T, Terano T, Nishikawa T, Tamura Y, Kamugai A, Jajiki J: **Eicosapentaenoic acid and platelet function in Japanese.** *Lancet* 1980, **2**(8204):1132–1133.
  39. Hegele RA, Young TK, Connelly PW: **Are Canadian Inuit at increased genetic risk for coronary heart disease?** *J Mol Med (Berlin, Germany)* 1997, **75**(5):364–370.

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