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Correlations between blood and tissue omega-3 LCPUFA status following dietary ALA intervention in rats

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ABSTRACT

The aim of this study was to assess relationships between the fatty acid contents of plasma and erythrocyte phospholipids and those in liver, heart, brain, kidney and quadriceps muscle in rats. To obtain a wide range of tissue omega-3 (n-3) long chain polyunsaturated fatty acids (LCPUFA) we subjected weanling rats to dietary treatment with the n-3 LCPUFA precursor, alpha linolenic acid (ALA, 18:3 n-3) for 3 weeks. With the exception of the brain, we found strong and consistent correlations between the total n-3 LCPUFA fatty acid content of both plasma and erythrocyte phospholipids with fatty acid levels in all tissues. The relationships between eicosapentaenoic acid (EPA, 20:5n-3) and docosapentaenoic acid (DPA, 22:5 n-3) content in both blood fractions with levels in liver, kidney, heart and quadriceps muscle phospholipids were stronger than those for docosahexaenoic acid (DHA, 22:6 n-3). The strong correlations between the EPA+DHA (the Omega-3 Index), total n-3 LCPUFA and total n-3 PUFA contents in both plasma and erythrocyte phospholipids and tissues investigated in this study suggest that, under a wide range of n-3 LCPUFA values, plasma and erythrocyte n-3 fatty acid content reflect not only dietary PUFA intakes but also accumulation of endogenously synthesized n-3 LCPUFA, and thus can be used as a reliable surrogate for assessing n-3 status in key peripheral tissues.

Key words: ALA%en; dietary fatty acids; tissue fatty acid profile; LCPUFA; Omega-3 Index

1. Introduction

The omega-3 (n-3) class of fatty acids has been reported to confer a number of health benefits to humans [1, 2]. As a result of these health benefits, there is increasing interest in assessing the effects of changing the n-3 fatty acid status in animal models [3-6].

The incorporation of dietary preformed or endogenously synthesized long chain polyunsaturated fatty acids (LCPUFA) into tissue membrane phospholipids such as brain, liver, heart and muscle is thought to be the mechanism to achieve the health effects of these n-3 fatty acids [7]. Although those tissues are important sites for metabolism, neural and structural development, collection of tissue samples in human and other animals is invasive and time-consuming. Blood fractions such as plasma and erythrocytes are readily collectable and have been used as markers for measuring fatty acid intakes and fatty acid metabolism in clinical trials [8-11] and animal studies [12-14]. However, there are relatively little information is available on the extent to which the fatty acid pattern of plasma or erythrocyte phospholipids correlates with the fatty acid pattern of tissue phospholipids after systematically supplementing dietary PUFA in a range made possible from existing vegetable oils. It is an important consideration since the data which exist suggest that different tissues accumulate fatty acids in their membrane at different rates [6, 15-17].

To obtain a wide range of tissue LCPUFA we subjected weanling rats to a range of isoenergetic diets varying in the level of the n-3 LCPUFA precursor, alpha linolenic acid (ALA, 18:3n-3), for 3 weeks [3]. Here we report on the relationship between the fatty acids in the plasma and erythrocyte fatty acid levels and those in a range of organ tissues. The five tissues (liver, kidney,

heart, brain and quadriceps muscle) were selected since it is known that changed in the composition of their membrane can affect the functionality of the organ [18-22].

2. Materials and methods

2.1. Animals

All procedures were performed in accordance with institutional guidelines for the use of animals and the Australian code of practice for the care and use of animal for scientific purposes. The protocol was approved by the Animal Ethics Committee, University of Adelaide (Ethics number S-092-2007). Twenty-five weaned male Hooded-Wistar rats were obtained from Laboratory Animal Services (University of Adelaide, Australia) at three weeks of age. The animals were group-housed (5 rats per cage) in a laboratory animal facility at thermal-neutrality (25°C) and a regulated humidity environment with a 12 hr light/12 hr dark cycle. The rats had free access to fresh water and to the experimental diets.

2.2. Diets

The diets used in the study all contained 5% fat (w/w), and the base diet was comprised of a modified rodent chow formulation of the AIN-93G diet (Specialty Feeds, WA, Australia). The isocaloric diets provided total energy of 3797 kcal per kg (15.9 MJ per kg) comprised of 67.8% energy (%en) of carbohydrate, 11.8%en of fat (vegetable oil) and 20.4%en of protein. The calculated energy content was based on the standard fuel values for protein (19.4%), fat (5%) and carbohydrate (64.4%) of 4, 9 and 4 kcal/g, respectively. The macronutrient content in all diets were as follows: sucrose 100 g/kg, casein 200 g/kg, fat 50 g/kg, starch (including dextrinised starch) 556 g/kg, cellulose 50 g/kg, dl-methionine 3 g/kg, AIN93 minerals 1.4 g/kg,

AIN93 vitamins 10 g/kg and other minerals 29.2 g/kg. The nutritional parameters were provided by the feed company. Five experimental diets designed to provide levels of ALA of 0.2, 0.5, 1.0, 1.9 and 2.9%en while keeping linoleic acid (LA, 18:2 n-6) constant at 1%en were produced by blending different vegetable oils. Details of the composition of these diets have been published previously [3].

Rats were randomly assigned to each of the five different dietary treatments (5 rats per treatment group). The rats were weighed at the start of the experiment, at weekly intervals during the 21 day feeding trial and on the day of sacrifice (6 weeks of age).

2.3. Blood and tissue collection

The rats were anaesthetized with 2-5% anaesthetic agent, isoflurane (Veterinary Companies of Australia Pty Ltd, NSW, Australia) by inhalation in an anaesthesia induction box. Upon loss of responsiveness and spontaneous movement, the rats were removed from the induction box and continuously anaesthetized with 0.4-3% isoflurane in air-oxygen mixture with spontaneous respiration. 4 mL of blood was collected by intra-cardiac puncture and immediately transferred into tubes coated with lithium heparin (Greiner Bio-one, Kremsmunster, Austria). Plasma and erythrocytes were separated by centrifugation of whole blood at 1500 x *g* for 5 minutes. The rats were then killed by exsanguination and cervical dislocation whilst still unconscious. Liver, kidney, heart, quadriceps muscle and brain tissues were collected from each rat, placed immediately into chilled sample vials and frozen at -20°C for later fatty acid profiling.

2.4. Lipid extraction

All solvents used in this study were analytical grade and were purchased from Ajax Finechem Pty Ltd (Auckland, New Zealand) or Chem-Supply (SA, Australia). Other chemicals and reagents were purchased from Sigma-Aldrich (MO, USA) unless specified otherwise. Total lipids from erythrocytes were extracted with chloroform/isopropanol (2:1, v/v) [23] and the total lipids from diets, plasma and tissues were extracted with chloroform/methanol (2:1, v/v) [24]. The phospholipids were separated from total lipid extracts by thin layer chromatography (TLC) on silica gel plates (Silica gel 60H; Merck, Darmstadt, Germany). A lipid class standard (Nu-Chek Prep Inc, MN, USA) was run on the plates for lipid identification. The mobile phase for TLC was petroleum spirit/ethyl ether/glacial acetic acid (180/30/2, v/v). The TLC plates were sprayed with fluorescein 5-isothiocyanate in methanol, and the lipid classes were visualized under UV light. The phospholipid bands located at the bottom of TLC were transferred into a vial containing 1% (v/v) sulphuric acid (H_2SO_4) in methanol for transmethylation. All solvents used for extraction and separation contained 0.005% (w/v) of the antioxidant, butylated hydroxyanisol (BHA).

2.5 Fatty acid methylation

All lipids and phospholipids were transesterified with 1% (v/v) H_2SO_4 in methanol at 70°C for 3 hr. After the samples were cooled, the resulting fatty acid methyl esters (FAME) were extracted into *n*-heptane and transferred into vials containing anhydrous sodium sulphate (Na_2SO_4).

2.6 Gas chromatographic analysis of FAME

FAME were separated and quantified by GC (Hewlett-Packard 6890, CA, USA) equipped with a capillary column (50 m x 0.32 mm id) coated with 0.25 µm film thickness 70% cyanopropyl

polysilphenylene-siloxane (BPX-70, SGC Pty Ltd, Victoria, Australia) and a flame ionisation detector (FID). The injector temperature was set at 250°C and the FID temperature at 300°C. The oven temperature at injection was initially set at 140°C and was programmed to increase to 220°C at a rate of 5°C per minute. Helium gas was utilized as a carrier at a flow rate of 35 cm per second in the column and the inlet split ratio was set at 20:1. The identification and quantification of FAME were achieved by comparing the retention times and peak area% values of unknown samples to those of commercial lipid standards (Nu-Chek Prep Inc) using the Hewlett-Packard Chemstation data system.

2.7 Statistical analysis

A one-way analysis of variance (ANOVA) followed by Tukey-HSD test was used if P value was less than 0.05 and the observed data followed Gaussian distributions (calculated by the Kolmogorov and Smirnov test). Kruskal-Wallis post-hoc test with Dunn's multiple comparison test was applied for non-parametric analyses if data did not pass the normality test. A probability level of 0.05 ($P<0.05$) was used in all tests. Simple linear regression models were used to analyse the relationship between phospholipid fatty acid levels in tissue and both plasma and erythrocytes. Results are presented as Pearson correlation coefficients and regression equations for the correlations between fatty acid levels in plasma or erythrocytes and tissues. Correlations between fatty acid levels in the separate tissue and either plasma or erythrocytes were compared using the method of Meng *et. al.* [25] to determine the better predictor. Analyses were carried out with SAS version 9.2 (Cary, NC, USA), SPSS version 15.0 (SPSS Inc, IL, USA) and GraphPad Instat version 3.10 (GraphPad Software, CA, USA) for Windows.

In this paper, we measured all the major phospholipid fatty acids for use in the regressions and plotted levels of 4 dominant n-3 (eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3)) and n-6 (arachidonic acid, AA, 20:4 n-6) LCPUFA in Figure 1 and represented correlations between plasma or erythrocyte phospholipid n-3 LCPUFA and tissue phospholipid n-3 LCPUFA in Figure 2.

3. Results

Feeding animals a range of dietary ALA while holding the dietary LA constant at 1%en resulted in the anticipated wide range of LCPUFA levels in most tissues examined (Fig. 1 A-D). Although the overall patterns of change in fatty acid content was similar between tissues, there were large variations in the amount of the respective fatty acids accumulated as ALA content of the diet increased (Fig. 1 A-D). The extent of the change of EPA levels was highest followed by DPA and then DHA. The level of AA decreased in response to increases in n-3 LCPUFA (Fig. 1 D).

The correlations in key n-3 LCPUFA between plasma or erythrocyte and various organ tissues are shown in Figure 2. Clear linear relationships were evident between the n-3 LCPUFA in plasma and erythrocytes levels in a variety of organs (Fig. 2).

N-3 fatty acids

Both plasma and erythrocyte phospholipid ALA, individual n-3 LCPUFA, EPA+DHA (Omega-3 Index), total n-3 LCPUFA and total n-3 were significantly ($P<0.0001$) and strongly (r=0.75-1.0) correlated with the corresponding fatty acids in liver, kidney, heart and quadriceps muscle

(Tables 1-3, 5 and Fig. 2). Slightly weaker correlations were also observed between n-3 fatty acid levels, with the exception of DPA, in both plasma and erythrocyte phospholipids with those in the brain (Tables 4 and Fig. 2). DPA levels in plasma or erythrocyte were highly correlated with brain DPA content (r=0.95, P<0.0001) (Table 4, Fig. 2 B and F). However, plasma and erythrocyte EPA (r=0.52-0.53, P<0.01) (Fig. 2 A and E), DHA (r=0.75, P<0.0001 and 0.65, P<0.001) (Fig. 2 C and G), Omega-3 Index (r=0.71, P<0.0001 and r=0.53, P<0.01) (Fig. 2 D and H), total n-3 LCPUFA (r=0.74, P<0.0001 and r=0.6, P<0.01) and total n-3 (r=0.71, P<0.0001 and r=0.59, P<0.01) had weaker correlations with the corresponding fatty acid content in the brain compared with other tissues.

Overall, both the erythrocyte and plasma fatty acid content was significantly correlated with the fatty acid content of organ tissues, however the strength of these correlations varied in a tissue-specific manner. For example, in kidney (r=0.99), heart (r=0.99) and quadriceps muscle (r=0.99), tissue ALA content was more strongly correlated with the ALA content of erythrocytes than plasma (Tables 2, 3 and 5). The Omega-3 index, total n-3 LCPUFA and total n-3 content in the kidney were also more strongly correlated with erythrocyte fatty acid levels than those in plasma (P<0.0001) (Table 2 and Fig. 2). On the other hand, the content of these same n-3 fatty acids in the brain were more strongly correlated with the fatty acid content of plasma compared to erythrocyte phospholipids (P=0.01, 0.009 and 0.008 for Omega-3 Index, total n-3 LCPUFA and total n-3 respectively) (Table 4 and Fig. 2). No significant differences were observed for the correlations between Omega-3 Index, total n-3 LCPUFA and total n-3 in the liver, heart or quadriceps muscle for the two blood fractions, with an exception of the Omega-3 Index in the heart, for which plasma Omega-3 Index was a better predictor than the Omega-3 index in

erythrocytes (P=0.026). The content of all fatty acids in plasma and erythrocyte phospholipids were strongly correlated (Table 6).

N-6 fatty acids

The LA content of both plasma and erythrocytes was significantly correlated with the LA content of all tissues examined here with the exception of the quadriceps muscle (Table 5). Both plasma and erythrocyte AA levels were correlated with AA levels in all tissues examined, with the strongest correlations observed for the liver (*r*=0.98-0.99, P<0.0001) and the weakest correlation for the brain (*r*=0.72-0.73, P<0.0001). Plasma and erythrocyte total n-6 was significantly correlated with the total n-6 content of all tissues examined (Tables 1-5)

In the liver and brain, the LA content in erythrocyte phospholipids was a stronger predictor of tissue LA levels than LA content of plasma phospholipids (*r*=0.88 vs 0.80 and *r*=0.96 vs 0.91 in liver and brain respectively, with P<0.05; Tables 1 and 4). Similarly, the AA content in erythrocyte phospholipids was a stronger predictor of AA content in the kidney than plasma AA content (*r*=0.98 vs 0.93, P=0.002) (Table 2). Erythrocyte total n-6 content was also more strongly correlated (P<0.0001) with liver, kidney and quadriceps muscle total n-6 levels compared with those in plasma phospholpids (Tables 1, 2 and 5). As expected, there were strong correlations between LA, AA and total n-6 levels in plasma and erythrocytes (Table 6).

Monounsaturated fatty acids

Both plasma and erythrocyte phospholipid monounsaturated fatty acids were correlated with the corresponding fatty acids in all tissues examined (Tables 1-3 and 5), with the exception of the

brain (Table 4), however the strength of these correlations was different for the two blood fractions. In the liver, the monounsaturated fatty acid content was more strongly correlated with the monounsaturated fatty acid level in plasma compared to those in erythrocytes. In contrast the monounsaturated fatty acid level in the plasma phospholipids was only weakly correlated with the monounsaturated fatty acid content of the kidney, heart and quadriceps muscle (Tables 1-3 and 5, respectively), and were more strongly correlated with the monounsaturated fatty acid content in erythrocyte phospholipids. There was no correlation between the monounsaturated fatty acid content of either the plasma or erythrocytes and the monounsaturated fatty acids in the brain (Table 4). Strong correlations between total monounsaturated fatty acids in plasma and erythrocytes were observed (Table 6).

Saturated fatty acids

The saturated fatty acid contents of plasma or erythrocytes and tissues were within a very narrow range, the stable levels of total saturated fatty acids in the different tissues is shown in the lack of correlation values in this fatty acid type between the tissues.

4. Discussion and conclusions

The key finding of this study is that the levels of fatty acids, particularly the n-3 LCPUFA, in various tissue phospholipids can be predicted with confidence from plasma and erythrocyte phospholipid fatty acid levels. The strong (r=~0.8-1.0) and significant (all $P<0.0001$) correlations observed between n-3 PUFA and LCPUFA and erythrocyte and plasma phospholipids and those in liver, kidney, heart and quadriceps muscle phospholipid across a wide

range of fatty acid values is striking. This validates the use of blood as a surrogate for the changes in fatty acids in organs of animals as a result of changes in dietary fatty acids [6, 26-30].

The range of dietary ALA levels used in this study resulted in the anticipated alterations in the level of n-3 LCPUFA in plasma, erythrocytes and all tissue phospholipids examined. These findings are consistent with previous reports by our group and others that PUFA in the diet directly influences the fatty acid profile of blood and various tissue phospholipids in rats fed with vegetable oil-based diets [3, 9, 31]. The LCPUFA in tissues are thought to be mainly derived from endogenously fatty acid synthesis. The strong correlation between blood and tissue LCPUFA levels was independent of the absolute levels of LCPUFA in the tissues. For example, high EPA levels were found in liver and kidney while low EPA levels were observed in heart. Despite this, the correlation between EPA levels in blood and these organs was generally high.

The n-3 fatty acids in most body tissues were strongly correlated with these fatty acids in the blood markers, the phospholipids in plasma and erythrocytes. The correlation coefficient for all n-3 fatty acids was in a range of r=0.85-1 between plasma and tissues (liver, kidney, heart and quadriceps muscle) and r=0.75-0.99 between erythrocytes and the tissues. Although all n-3 LCPUFA levels between blood and tissues examined here were closely related, the correlations were somewhat lower for DHA than other n-3 LCPUFA. We hypothesized that this may due in part to the curvilinear nature of the DHA data, and the very small range in levels of DHA in blood and tissues. The strong correlations between blood and heart that we observed in this animal study are consistent with a recent paper from our group in which we demonstrated a close relationship between blood and myocardial n-3 fatty acid over a range of intakes of vegetable oil

and fish oil in 61 subjects who were undergoing cardiac bypass surgery [32]. The data showed strong correlations between plasma or erythrocyte n-3 fatty acids with atrial n-3 fatty acids most notably *r*=0.87-0.93 for EPA, *r*=0.72-0.78 for DHA and *r*=0.80-0.85 for the Omega-3 Index [32].

In general, the n-6 fatty acids in blood highly correlated with the levels in all body organs. We found significant correlation coefficient between blood and heart LA (*r*=0.66-0.7) and AA (*r*=0.94-0.95). However, there was no correlation between LA levels in blood and LA in quadriceps muscle. Moreover, although the correlations in LA levels between blood and heart were significant, the correlation coefficients were weaker than in other tissues, perhaps due to the fact that LA levels in quadriceps muscle and heart only changed modestly with diet. The present results are supported by previous reports suggesting that skeletal muscle and heart have similar fatty acid profiles which respond similarly to dietary changes [33, 34]. Interestingly, Baur *et. al.* [35] could detect no correlation between muscle LA and erythrocyte LA levels in 61 young children undergoing elective surgery, despite a significant positive correlation between muscle and erythrocyte total n-3 fatty acids. In our earlier human study [32], blood n-6 fatty acids were significantly correlated with the corresponding atrial fatty acids with LA of *r*=0.64-0.69 and AA of *r*=0.39-0.45.

One of the common questions relating to the fatty acids in blood fractions as biomarkers is whether plasma or erythrocyte phospholipid fatty acids are the more accurate predictors of tissue LCPUFA levels. Our results suggest that there were no general trends of plasma phospholipid fatty acids being a better marker for predicting tissue LCPUFA levels than erythrocyte phospholipid fatty acids, except in the kidney where erythrocyte phospholipid PUFA and

LCPUFA showed stronger correlations with kidney phospholipid fatty acids than those in the plasma. The reason is not clear. However, such tissue specific correlation patterns were not applied to other organ tissues. Our results are consistent with the work of Skeaff *et. al.* [36] who showed a high correlation between plasma and erythrocyte fatty acid levels following dietary-induced changes over a short term (19-day) period. It has been suggested that the levels of individual fatty acids in plasma reflected those in erythrocytes in humans [37]. Baylin *et. al.* [38] observed high correlation coefficient between whole blood ALA and LA and the two PUFA in plasma. Similar findings have been reported by others [39, 40], suggesting high relationships between the blood fractions. The high correlation between the two blood fractions is explained by ready exchange of phospholipids between plasma and erythrocytes [41]. In general, the measuring erythrocyte phospholipid fatty acids, provides no clear advantage over plasma phospholipid fatty acids in most of organ tissues examined in this study.

DHA and AA are the two most abundant LCPUFA in the mammalian brain. In this study, the correlations between most phospholipid fatty acids in brain and plasma or erythrocyte fatty acids were slightly weaker and variable (r=~0.5-0.9) compared to the same fatty acids observed in other peripheral tissues. It has been reported that liver phospholipid DHA and AA with those in the brain of newborn infants during normal human development was correlated (r=0.83, $P<0.001$), suggesting liver may be capable of providing the fatty acids required by the developing brain [42]. Our rat data show significant correlations in DHA or AA levels between blood and brain (r=0.65-0.75), and also between liver and brain (r=0.73-0.79, $P<0.0001$, data not shown in the tables). It has been shown that both plasma and erythrocyte DHA levels were significantly correlated with brain DHA levels in the piglets fed with dietary DHA, despite the

correlation coefficient value was lower for the correlation between blood and brain than in other tissues [43]. Data here are consistent with other reports showing that brain membrane lipids are alterable by the type and amount of fatty acids in the diet, however, brain is more resistant to dietary fatty acid manipulation than are other tissues [15, 44]. Moreover, The involvement of the blood-brain barrier provides a protective barrier between the blood and the extracellular environment of the brain and therefore made lipid transportation into cells more complicated [45].

In conclusion, we have demonstrated that there are strong correlations between the phospholipid fatty acid composition of plasma and erythrocytes and those in the majority of organ tissues evaluated in this study in rats following a 3-week dietary ALA intervention. Both plasma and erythrocyte phospholipids were indicative of the tissue n-3 LCPUFA content of tissues across the wide range of n-3 fatty acid levels. Our finding are particular important for feeding studies involving rodents but can also be applied to farmed animals fed ALA rich diets in substitute for fish oil and fish meal in the diets.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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Figure legends

Figure 1. Proportions of phospholipid EPA (A), DPA (B), DHA (C) and AA (D) in plasma, erythrocytes, liver, kidney, heart, quadriceps muscle and brain of rats fed a range of diets with ALA levels ranging from 0.2-2.9%en. Data points are means ± SEM, n=5 per group.

Figure 2. Correlations of phospholipid EPA (A and E), DPA (B and F), DHA (C and G) and Omega-3 Index (D and H) between plasma or erythrocyte and organ tissues of rats fed a range of ALA diets, n=25.

Figure

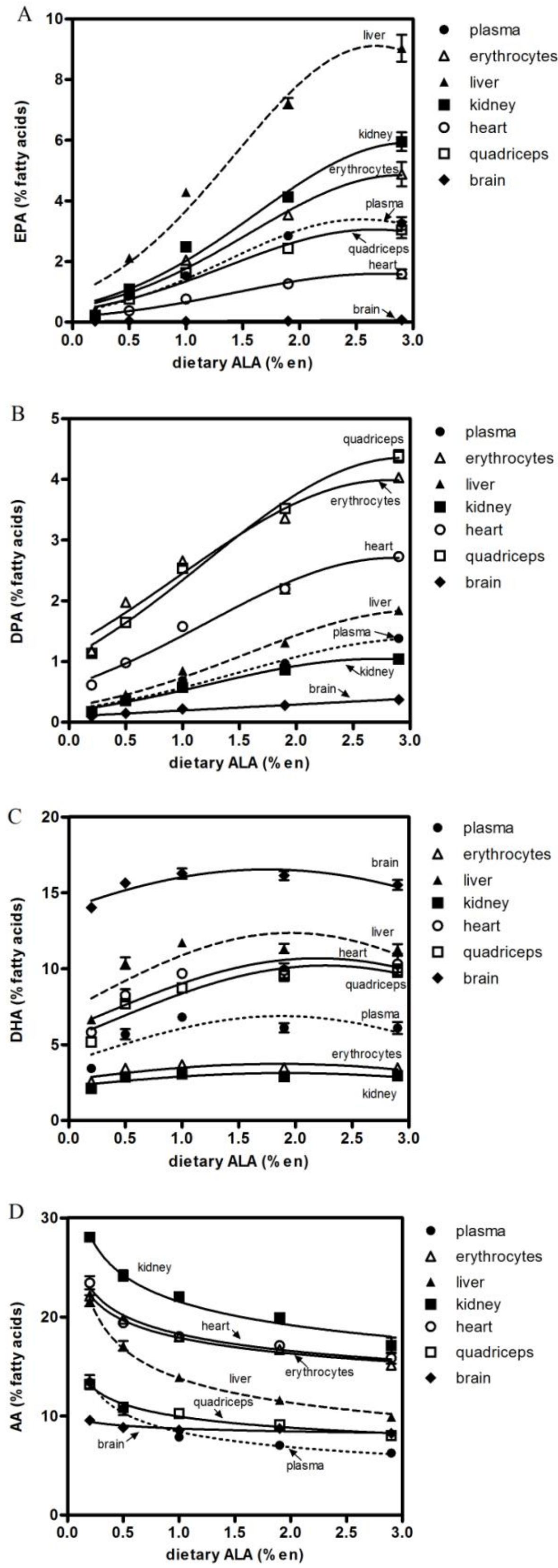

Figure 1

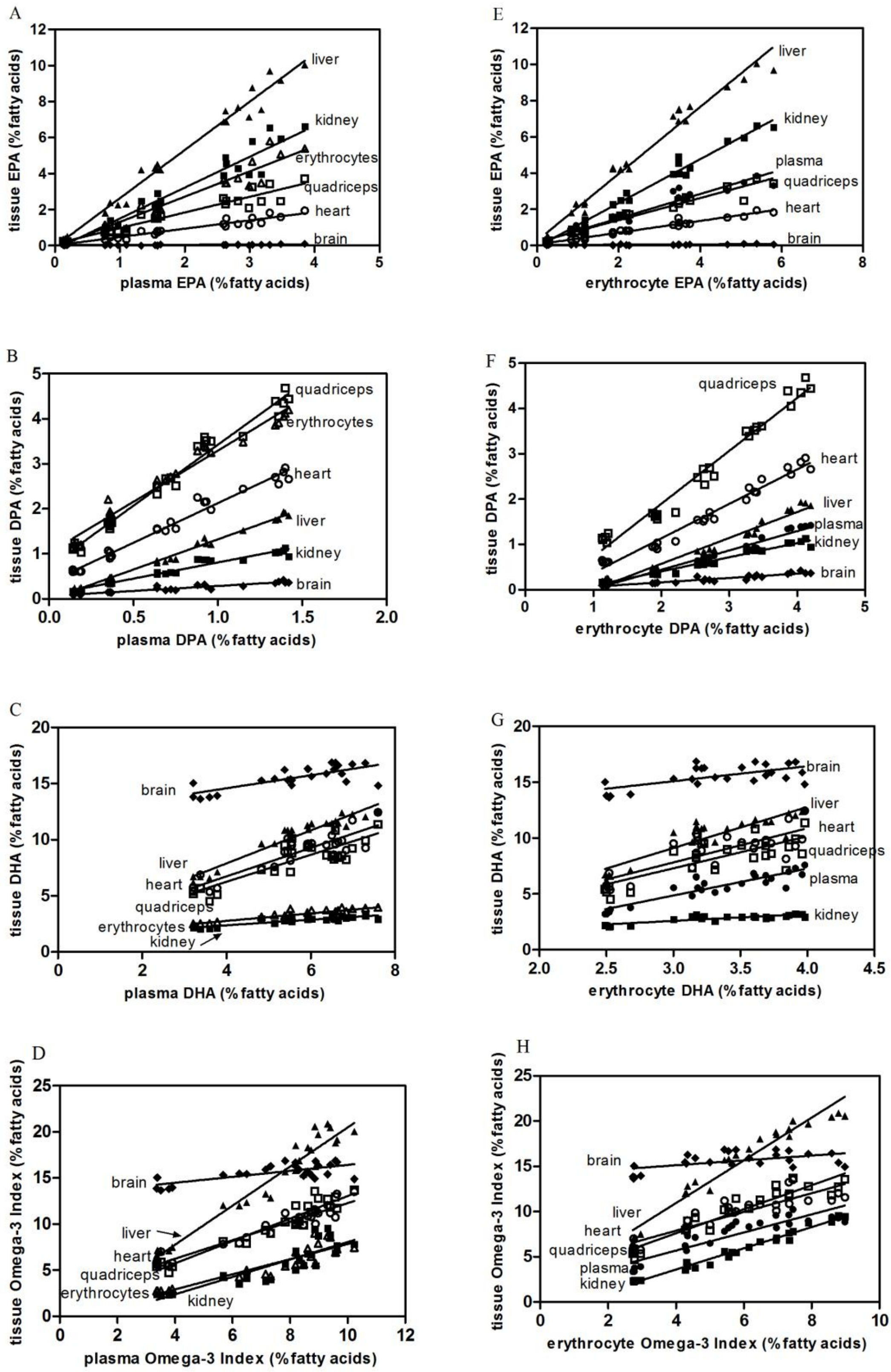

Figure 2

Table

Table 1 Correlations between liver phospholipid fatty acids and corresponding plasma and erythrocyte phospholipid fatty acids[1]

Liver Fatty Acid	Plasma Regression Equation	Plasma Correlation	Erythrocyte Regression Equation	Erythrocyte Correlation	P value Comparing Plasma and Erythrocyte Correlations
ALA	-0.00 + 0.65X	1.00****[2]	-0.03 + 0.89X	0.99****	0.1502
EPA	-0.04 + 2.68X	0.99****	0.26 + 1.85X	0.99****	0.4520
DPA	-0.02 + 1.34X	1.00****	-0.57 + 0.57X	0.98****	0.0001
DHA	2.03 + 1.47X	0.96****	-1.90 + 3.67X	0.90****	0.0113
EPA+DHA	-0.74 + 2.12X	0.97****	1.50 + 2.36X	0.98****	0.4691
Total n-3 LCPUFA	-0.54 + 2.03X	0.98****	1.18 + 1.76X	0.99****	0.2907
Total n-3	-0.17 + 1.93X	0.99****	1.42 + 1.70X	0.98****	0.7435
LA	3.76 + 0.34X	0.80****	0.77 + 1.26X	0.88****	0.0452
AA	1.48 + 1.47X	0.98****	-16.14 + 1.69X	0.99****	0.3008
Total n-6	-49.33 + 3.20X	0.82****	-17.22 + 1.61X	0.98****	<.0001
Total Monos	1.89 + 0.78X	0.95****	-2.48 + 1.02X	0.81****	0.0007
Total Saturates	5.63 + 0.74X	0.85****	14.85 + 0.58X	0.44*	0.0019

[1] n=25.

[2] *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

Table 2 Correlations between kidney phospholipid fatty acids and corresponding plasma and erythrocyte phospholipid fatty acids[1]

Kidney Fatty Acid	Plasma Regression Equation	Plasma Correlation	Erythrocyte Regression Equation	Erythrocyte Correlation	P value Comparing Plasma and Erythrocyte Correlations
ALA	0.04 + 0.74X	0.98****[2]	0.01 + 1.03X	0.99****	0.0441
EPA	-0.20 + 1.71X	0.97****	-0.08 + 1.21X	0.99****	0.0012
DPA	0.09 + 0.72X	0.98****	-0.22 + 0.31X	0.99****	0.2345
DHA	1.40 + 0.25X	0.89****	0.72 + 0.62X	0.83****	0.2536
EPA+DHA	-1.41 + 0.95X	0.88****	-1.10 + 1.17X	0.99****	<.0001
Total n-3 LCPUFA	-1.48 + 0.95X	0.92****	-1.20 + 0.89X	0.99****	<.0001
Total n-3	-1.58 + 0.96X	0.94****	-1.25 + 0.90X	0.99****	<.0001
LA	1.85 + 0.59X	0.88****	-2.47 + 2.04X	0.91****	0.4351
AA	10.82 + 1.26X	0.93****	-5.52 + 1.52X	0.98****	0.0021
Total n-6	-11.96 + 1.94X	0.66***	3.14 + 1.15X	0.93****	<.0001
Total Monos	12.82 + 0.22X	0.58**	10.32 + 0.36X	0.61**	0.7448
Total Saturates	37.07 + 0.13X	0.29	38.59 + 0.10X	0.15	0.5035

[1] n=25.

[2] *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

Table 3 Correlations between heart phospholipid fatty acids and corresponding plasma and erythrocyte phospholipid fatty acids[1]

Heart Fatty Acid	Plasma Regression Equation	Plasma Correlation	Erythrocyte Regression Equation	Erythrocyte Correlation	P value Comparing Plasma and Erythrocyte Correlations
ALA	0.07 + 0.88X	0.97****[2]	0.02 + 1.22X	0.99****	0.0077
EPA	0.02 + 0.46X	0.97****	0.06 + 0.33X	0.99****	0.0735
DPA	0.37 + 1.76X	0.99****	-0.39 + 0.76X	0.99****	0.8198
DHA	1.66 + 1.27X	0.89****	-1.37 + 3.06X	0.80****	0.0555
EPA+DHA	2.22 + 1.00X	0.96****	3.74 + 1.04X	0.89****	0.0259
Total n-3 LCPUFA	2.10 + 1.13X	0.97****	3.32 + 0.95X	0.94****	0.1696
Total n-3	2.06 + 1.12X	0.97****	3.17 + 0.97X	0.95****	0.1219
LA	10.59 + 0.47X	0.66***	6.89 + 1.67X	0.70****	0.5237
AA	10.37 + 0.93X	0.94****	-0.63 + 1.06X	0.95****	0.9046
Total n-6	-5.49 + 1.82X	0.77****	13.68 + 0.88X	0.88****	0.0756
Total Monos	3.02 + 0.66X	0.79****	-7.27 + 1.23X	0.96****	<.0001
Total Saturates	16.75 + 0.42X	0.47*	20.91 + 0.35X	0.26	0.2751

[1]n=25.

[2]*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

Table 4 Correlations between brain phospholipid fatty acids and corresponding plasma and erythrocyte phospholipid fatty acids[1]

Brain Fatty Acid	Plasma Regression Equation	Plasma Correlation	Erythrocyte Regression Equation	Erythrocyte Correlation	P value Comparing Plasma and Erythrocyte Correlations
ALA	ND[2]	ND	ND	ND	.
EPA	0.03 + 0.01X	0.52**[3]	0.03 + 0.01X	0.53**	0.7397
DPA	0.07 + 0.22X	0.95****	-0.03 + 0.10X	0.95****	0.8886
DHA	12.27 + 0.58X	0.75****	11.06 + 1.35X	0.65***	0.1231
EPA+DHA	13.19 + 0.32X	0.71****	14.05 + 0.27X	0.53**	0.0100
Total n-3 LCPUFA	13.40 + 0.30X	0.74****	14.07 + 0.21X	0.60**	0.0094
Total n-3	13.58 + 0.26X	0.71****	14.15 + 0.19X	0.59**	0.0078
LA	0.19 + 0.02X	0.91****	-0.00 + 0.09X	0.96****	0.0389
AA	7.47 + 0.15X	0.72****	5.74 + 0.17X	0.73****	0.9791
Total n-6	2.02 + 0.44X	0.54**	5.91 + 0.24X	0.71****	0.0924
Total Monos	18.89 + -0.03X	-0.06	21.42 + -0.17X	-0.24	0.1263
Total Saturates	52.41 + 0.02X	0.05	64.11 + -0.23X	-0.38	0.0380

[1]n=25.

[2]ND, not detected.

[3]*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

Table 5 Correlations between quadriceps muscle phospholipid fatty acids and corresponding plasma and erythrocyte phospholipid fatty acids[1]

Quadriceps Fatty Acid	Plasma Regression Equation	Plasma Correlation	Erythrocyte Regression Equation	Erythrocyte Correlation	P value Comparing Plasma and Erythrocyte Correlations
ALA	0.28 + 1.67X	0.97****[2]	0.19 + 2.33X	0.99****	0.0169
EPA	0.11 + 0.87X	0.96****	0.18 + 0.61X	0.98****	0.2381
DPA	0.72 + 2.71X	0.99****	-0.44 + 1.17X	0.99****	0.4777
DHA	1.45 + 1.20X	0.85****	-1.29 + 2.86X	0.75****	0.0724
EPA+DHA	0.85 + 1.22X	0.96****	2.29 + 1.33X	0.95****	0.5259
Total n-3 LCPUFA	0.60 + 1.47X	0.96****	1.81 + 1.28X	0.97****	0.4182
Total n-3	0.38 + 1.53X	0.97****	1.53 + 1.36X	0.98****	0.4517
LA	16.07 + 0.05X	0.19	15.47 + 0.22X	0.24	0.5568
AA	4.71 + 0.62X	0.94****	-2.87 + 0.72X	0.96****	0.2509
Total n-6	-15.15 + 1.93X	0.76****	2.54 + 1.03X	0.97****	<.0001
Total Monos	3.17 + 0.89X	0.76****	-12.13 + 1.75X	0.96****	<.0001
Total Saturates	36.34 + 0.04X	0.08	36.56 + 0.04X	0.05	0.8899

[1]n=25.

[2]$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$.

Table 6 Correlations between phospholipid fatty acid levels in plasma and erythrocytes[1]

Plasma Fatty Acid	Plasma Regression Equation	Correlation Between Plasma and Erythrocytes
ALA	-0.04 + 0.72X	0.99****[2]
EPA	-0.08 + 1.40X	0.97****
DPA	1.02 + 2.27X	0.98****
DHA	1.44 + 0.33X	0.90****
EPA+DHA	-0.38 + 0.82X	0.91****
Total n-3 LCPUFA	-0.49 + 1.09X	0.94****
Total n-3	-0.49 + 1.08X	0.95****
LA	2.28 + 0.28X	0.93****
AA	10.80 + 0.83X	0.95****
Total n-6	-18.00 + 1.90X	0.80****
Total Monos	8.16 + 0.548X	0.84****
Total Saturates	30.59 + 0.33X	0.5*

[1]n=25.

[2]*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.