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Dietary methods and biomarkers of omega 3 fatty acids: a systematic review

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Abstract

The aims of the present study were to review the validity of dietary methods used to measure the usual long chain (LC) omega-3 polyunsaturated fatty acid (*n*-3 PUFA) intake of a population and to assess the usefulness of different biomarkers of *n*-3 PUFA in healthy humans. Two systematic literature searches were conducted until May 2011 to update previous systematic reviews. The first literature search aimed to find studies validating the methodology used for measuring the dietary intake of *n*-3 PUFA. The second search aimed to find human intervention studies in which *n*-3 PUFA status changed after 2 weeks of *n*-3 PUFA supplementation. Sixteen studies were identified for inclusion in the first review. Correlation coefficients between fatty acids in subcutaneous fat or blood lipids and dietary intake of *n*-3 PUFA from different questionnaires were similar. Subcutaneous fat has been reported as the best reference method for some authors, and these studies showed moderate correlation coefficients with no dietary intake method being superior to any other. As for the evaluation of biomarkers of docosahexaenoic acid (DHA, 22 : 6 *n*-3) and eicosapentaenoic acid (EPA, 20 : 5*n*-3) status in response to supplementation, the new search reaffirmed and reinforced the evidence supporting that plasma phospholipid DHA, erythrocyte DHA, and platelet DHA were all effective and robust biomarkers of DHA status. Our findings only confirmed earlier studies and did not provide evidence for reaching new conclusions.

Key words: Polyunsaturated fatty acids: Omega-3: Dietary intake: Nutritional status: Dietary methods: Biomarkers

Introduction

Nutritional epidemiological research requires addressing issues of measurement errors and inter and intra-individual variability, which are specific for each nutrient. Public health decisions must rely on valid and precise estimates of nutrient intake and status. There is a need to reach a consensus about the best available methods for assessing nutrient intake and status at the population level. In this article a literature review of dietary methods used to assess intake of *n*-3 PUFA is presented. Also biomarkers for *n*-3 PUFA status were analysed. The effect of dietary fats on health and disease has been of interest for many decades. The various health benefits of consuming the LC *n*-3 PUFA particularly eicosapentaenoic acid (EPA, 20 : 5*n*-3) and docosapenteanoic acid (DHA, 22 : 6*n*-3), have been widely reported[(1)]. The LC *n*-3 PUFA are obtained predominantly from fish, seafood, meat, and eggs[(1)]. However, various dietary supplements containing several hundred milligrams of LC *n*-3 PUFAs per dose are commonly available. Many clinical studies have assessed the effect of LC *n*-3 PUFA supplementation in restoring health and maintaining well-being. The majority of these reviews concluded that, although there was some indication of the beneficial effect of LC *n*-3 PUFA supplementation, further studies were needed to establish efficacy of their use. To date, there is lack of a universally accepted biomarker that reflects increased LC *n*-3 PUFA status in response to increased dietary intake or supplementation. It is even more important in epidemiologic studies assessing health effects of LC *n*-3 PUFA status in populations over a long-term period to understand which biomarkers truly reflect LC *n*-3 PUFA status. To assess the reliability of biomarkers in reflecting LC *n*-3 PUFA intake, it is necessary to review biomarker data from studies reporting a change in LC *n*-3 PUFA status. On the other hand it is also necessary to know the validity and reproducibility of dietary intake estimations of LC *n*-3 PUFA from different questionnaires with regard to the appropiatte biomarkers. Therefore, the aims of this paper were to review the validity of methods used to measure the usual *n*-3 PUFA intake of a population and additionally, to assess the usefulness of different biomarkers of LC *n*-3 PUFA status in healthy humans.

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Methods

This article includes two updated systematic reviews. Both systematic literature searches were performed between March and May 2011. Two previous systematic reviews covering the objectives of both searches were conducted in 2007 and 2009 within the European Network of Excellence EURopean micronutrient RECommendations Aligned (EURRECA)[2].

For the first search updating the validity of methods to assess usual *n*-3 PUFA intake, the literature search was conducted in Medline, OvidSP and EMBASE using the following terms: 'omega-3 fatty acid', 'fish oils', 'biomarker', 'nutritional assessment', and 'fat intake' including MESH-terms. In total 286 articles were selected using Medline, 358 were selected from OvidSP and 330 were identified from EMBASE.

To select the articles to be included in the present review the following exclusion criteria were used: (a) studies conducted exclusively in diseased or institutionalised persons, (b) studies relating diseases to food consumption or nutrient intake, (c) intervention studies and other therapeutic studies with nutrients or drugs related to the metabolism of these nutrients, (d) studies in animals, (e) studies written in languages other than English or Spanish, (f) studies using single 24-hour recall or non validated FFQ, (g) studies related to fish consumption, (h) studies in infants and children, and (i) studies using another dietary method as a reference tool.

Nine hundred seventy four titles and abstracts were identified via the electronic search from the three different databases. After excluding duplicate studies, a total of 87 appeared to be potentially relevant, and we attempted to obtain them in full-text version. The literature lists in the selected papers were checked and consequently 3 more articles were included. From the 90 articles, 8 of them were chosen to update Table 1 elaborated in the original article from 2009[3]. In total 19 studies were reviewed (11 were already in the first review and 8 were consequently added). To assess the quality of the different calibration/validation studies a quality score system was developed[4]. This has been described in previous publications by Serra-Majem *et al.*[4] and Øverby *et al.*[3].

For the second search of the present article aiming to assess the utility of biomarkers for *n*-3 PUFA, another OvidSP and MEDLINE search was developed in order to refresh the search strategy developed by Fekete *et al.*[5] in the systematic review of recovery studies. In this case, the search targeted intervention/recovery studies of *n*-3 PUFA using text terms with appropriate truncation and relevant indexing terms. The following strategy was applied: (*n*-3 LCPUFA terms) and (intervention study terms) and (human studies) and was limited to the last 4 years. The inclusion criteria was the same as that used by Fekete *et al.* (2007)[5]. Six hundred and twenty one titles and abstracts were identified via electronic search. From these, only 8 studies were selected to update studies included in the initial study conducted by Fekete *et al.*[5]

Biomarkers

Eighteen different biomarkers were used to characterize changes in LC *n*-3 PUFA status. Discussion is only included for those biomarkers used in more than 3 different studies. Data for each study included in the present analysis is described in Table 2. The effects of LC *n*-3 PUFA supplementation on each biomarker are detailed in Table 3. The main focus is directed towards the effect of DHA supplementation on biomarkers reflecting changes in DHA values. Plasma phospholipid DHA as well as erythrocyte and platelet DHA appear to be reliable and robust biomarkers as shown in Table 3.

Results

Dietary method studies

Details of the 19 papers selected are given in Table 1. In the 19 articles included in the review, 15 different food frequency questionnaires (FFQ) were validated. All FFQs were designed to capture the usual diet. Some questionnaires specifically asked only about *n*-3-PUFA rich food[1,6], while others covered the whole diet with 66–360 food items included in the questionnaire[7–17]. A diet history questionnaire had been validated in one study[18]. Weighed records had been validated in 4 studies[12,19,20,21].

In the presented studies the numbers of participants varied from 24 to 4439. The age distribution ranged from 18 to 86 years, with mean ages from 45 to 65 years. In total 15 different FFQs and dietary records or recalls in 5 different settings (varying number of days and season) were validated against subcutaneous fat, serum or plasma fatty acids.

Subcutaneous fat. Adipose tissue fatty acids were determined using chromatography and calculating the area under the curve for each of the fatty acids. All studies using fatty acids in tissue reported the same procedure with only slight modifications[7,8,10,13,14,19].

Five different FFQ were validated against adipose tissue[7,8,10,13,14]. All these correlations were significant. Furthermore Marckmann *et al.*[19] validated weighed records (3 × 7 d) against subcutaneous fat. Only DHA crude correlations were significant. Finally Knutsen *et al.*[13] validated eight different 24-h recalls of intake of ALA, EPA and DHA against subcutaneous fat. They found high adjusted correlations for ALA, while the correlations for EPA and DHA were lower (Table 1).

Blood component concentrations. After extraction and isolation the serum/plasma phospholipids were quantified by gas liquid chromatography after methylation[6,8,9,11,12,15–18,20–23]. Some expressed the serum phospholipids as mg fatty acid/l serum[22], while most used percent of total fatty acid methyl esters[8,18,20] or both[11]. For detailed descriptions refer to each particular study.

Eleven different FFQ were validated against erythrocytes, plasma or serum[1,6,8,9,11,12,15–17,22,24]. Sullivan *et al.*[1] validated fatty acid estimated from a FFQ against both fatty acid from erythrocytes and from plasma. All the correlations were significant. Andersen *et al.*[8], Hjartaker *et al.*[22] and Hodge *et al.*[11] reported significant correlations of approximately 0·50–0·60 between dietary intake of EPA and DHA estimated from the FFQ and concentrations of EPA and DHA in serum or plasma. Arsenault *et al.*[9] reported adjusted correlations

Table 1. Description of the studies included in this review validating intake of *n*-3 fatty acids (sorted by publication date). Crude and adjusted correlations for dietary methods vs. reference methods in the studies included

Author	Year pub	Country	Subjects (*n*)	Age	Dietary method which was validated	Reference method	Results
Hunter *et al.*[7]	1992	USA	118	45–65 years	FFQ1 and FFQ2	Subcutaneous fat aspirates from the lateral buttock	Crude: FFQ1: EPA: 0·43†,*** FFQ2: EPA: 0·48†,*** Energy adjusted: FFQ1: EPA: 0·47*** FFQ2: EPA: 0·47***
Marckmann *et al.*[19]	1995	Denmark	24	20–29 years	Three × 7 d weighed food records	Fatty acid composition of subcutaneous fat	Crude: EPA 0·40§ DHA 0·66§,***
Ma *et al.*[17]	1995	USA	3570	45–64 years	FFQ	Plasma cholesterol ester	Crude: ALA: 0·21§ EPA: 0·23§ DHA: 0·42§
Godley *et al.*[10]	1996	USA	36 erythrocyte controls, 33 adipose tissue controls	46–86 years	FFQ	Erythrocyte membrane	Crude: EPA: 0·36† DHA: 0·19†
						Adipose tissue	Crude: EPA: 0·33† DHA: 0·42†
Hjartaker *et al.*[22]	1997	Norway	234	40–42 years	FFQ	Serum phospholipids fatty acid composition	Crude: EPA: 0·58†,‡,*** DHA: 0·53†,‡,***
Andersen *et al.*[8]	1999	Norway	119 adipose tissue samples, 135 blood samples	20–55 years	FFQ	Subcutaneous adipose tissue of the buttock	Crude: ALA: 0·42†,*** EPA: 0·52†,*** DPA: 0·39†,*** DHA; 0·49†,***
						Serum fatty acid	Crude: ALA: 0·28†,*** EPA: 0·51†,*** DPA: 0·38†,*** DHA; 0·52†,***
Norrish *et al.*[16]	1999	New Zealand	480 age-matched community controls	69·1 (SD 0·3) years	FFQ	Erythrocyte phosphatidylcholine	Crude: EPA: 0·26† DHA: 0·32†
Sasaki *et al.*[18]	2000	Japan	42 men, 44 women	19–58 years	DHQ	Serum fatty acids	Crude (men/women): ALA: −0·1/0·26§ EPA: 0·64/0·61§,*** DPA: 0·00/0·17§ DHA: 0·46/0·46§,* Marine origin: *n*-3: 0·48/0·58§,*** Energy adjusted (men/women): ALA: -0·22/0·36 EPA: 0·64/0·65 DPA: 0·07/0·20 DHA: 0·44*/0·59*** Marine origin: *n*-3:0·51***/0·69***

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Table 1. *Continued*

Author	Year pub	Country	Subjects (*n*)	Age	Dietary method which was validated	Reference method	Results
Kobayashi *et al.*[20]	2001	Japan	87	50–59 years	7 d food weighed record	Serum phospholipids fatty acid	Crude: Total *n*-3: 0·66§,*** ALA: 0·07§ EPA: 0·75§,*** DPA: 0·49§,*** DHA: 0·50§,*** Energy adjusted‖: Total *n*-3 0·76§,*** ALA: 0·09§ EPA: 0·89§,*** DPA: 0·54§,*** DHA: 0·61§,***
Baylin *et al.*[14]	2002	Costa Rica	367 men, 136 women	Men 56 (SD 11) years, women 60 (SD 10) years	FFQ	Adipose tissue of the buttock	Crude: ALA: 0·34†,*** EPA: 0·15†,*** DPA: 0·03† DHA: 0·18†,***
Kuriki *et al.*[21]	2003	Japan	15 men, 79 women	35–55 years	7 d weighed record	Plasma fatty acids	Only adjusted presented (men/women): ALA: 0·35*/0·19 EPA: 0·57*/0·60*** DHA: 0·57*/0·3*** Adjusted for age and BMI
Knutsen *et al.*[13]	2003	USA	49 black and 72 white	48 (SD 15·2) years	FFQ	Adipose tissue from the buttock	Crude (black/white): ALA: 0·29*/0·49
					Eight different 24 h recalls		Crude (black/white): ALA: 0·51/0·41 EPA: 0·19/−0·04 DHA:0·32/0·05 Energy adjusted (black/white)¶: ALA: 0·68***/0·62*** EPA:0·23/−0·05 DHA:0·54***/0·06
Sullivan *et al.*[1]	2006	Australia	53	19–58 years	FFQ	Red blood cell fatty acids	Crude: Total *n*-3 PUFA: 0·50†,* EPA: 0·40†,* DPA: 0·05† DHA: 0·39†,*
						Plasma fatty acids	Crude: Total LC *n*-3 PUFA: 0·54†,* EPA: 0·54†,* DPA : 0·09† DHA: 0·48†,*
Hodge *et al.*[11]	2007	Australia	4439	40–69 years	FFQ	Plasma phospholipid fatty acids	Crude: Total *n*-3 %: 0·31† ALA: 0·07† EPA: 0·18† DHA: 0·4† Energy adjusted††: Total *n*-3: 0·57† ALA: 0·24† EPA: 0·40† DHA: 0·78†,
McNaughton *et al.*[12]	2007	Australia	43	28–75 years	FFQ	Plasma phospholipid fatty acids	Crude: Total *n*-3 0·38†,* ALA: 0·00† EPA: 0·21† DPA: −0·05† DHA: 0·32†,*

Table 1. *Continued*

Author	Year pub	Country	Subjects (*n*)	Age	Dietary method which was validated	Reference method	Results
					Weighed record	Plasma phospholipid fatty acids	Crude: Total *n*-3 PUFA: 0·33†,* ALA: 0·09† EPA: 0·22† DPA: 0·25† DHA: 0·43†,*
Sun *et al.*[15]	2007	USA	306	43–69 years	FFQ	Plasma fatty acids	Crude: Total *n*-3: (*n* 130): 0·31†,* ALA: 0·23† EPA: (*n* 130): 0·27†,* DPA: 0·01† DHA: 0·47† Energy adjusted§§: Total *n*-3: (*n* 130): 0·30†,** ALA: 0·23†,** EPA: (n = 130): 0·21†,* DPA: −0·03† DHA: 0·48†,**
						Erythrocyte	Crude: Total *n*-3: (*n* 132): 0·42†,** ALA: 0·17 † EPA: (*n* 132): 0·23†,** DPA: 0·02† DHA: 0·54 † Energy adjusted§§: Total *n*-3: (*n* 132): 0·41†,** ALA: 0·18†,** EPA: (*n* 132): 0·38†,** DPA: 0·01†,* DHA: 0·56†,**
Arsenault *et al.*[9]	2008	USA	129 normal clinical diagnosis	72·6 (SD 8·0) years	FFQ	Plasma phospholipid fatty acids	Only adjusted presented‖‖: EPA: 0·38§ DHA: 0·49§
Astorg *et al.*[23]	2008	France	276 men, 257 women	Men 45–60 years, women 35–60 years	15 d 24 h records	Plasma fatty acids	Crude (men/women): ALA: 0·06/0·05† EPA: 0·24/0·27†,*** DPA: 0·08/0·07† DHA: 0·25/0·27†,***
Sublette *et al.*[6]	2010	USA	61	18–73 years	FFQ	Plasma fatty acids	Crude: ALA: 0·22† EPA: 0·38†,*** DHA: 0·50†,****

DHQ, diet history questionnaire; ALA, α-linolenic acid; DPA, docosapentanoic acid.
Significance: * $P<0·05$; ** $P<0·01$; *** $P<0·001$; **** $P<0·0001$.
† Spearman correlation.
‡ Marine intake of *n*-3 FA.
§ Pearson correlation.
‖ Deattenuated with the within-to-between person variance ratio for intake of FA.
¶ Corrected after attenuation correction factor.
†† Corrected for the reliability coefficients of FFQ and phospholipids.
§§ Adjusted for age at blood drawing, BMI, current weight, smoking status, postmenopausal status, postmenopausal hormone use, period of blood assay, and fasting status at blood drawing.
‖‖ Adjusted for age and total energy intake.

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Table 2. Basic characteristics of the included studies on recovery interventions for *n*-3 PUFA biomarkers[1]

Author/year	Country	Age	Sex	No. included	Short description of intervention	Latest time point	No. in intervention	No. in control at latest time	Study design	Biomarkers reported
Allard (1997)[37]	Canada	20–60 y	M	40	3·06 g EPA + 2·26 g DHA	6 wk	18	19	RCT p	PPL
Barceló-Coblijn (2008)[38]	Canada	>48	X	62	1·2 g ALA; 2·4 g ALA; 3·6 g ALA; 0·6 g (fish oil EPA-DHA), 1,2 g (fish oil EPA-DHA), 0·6 g/d EPA or DHA + 1·2 g/d ALA	12 wk	12 + 10 + 10 + 11 + 10	9	RCT p	RBC, PPL
Bønaa (1992)[39]	Norway	34–60 y	X	156	3·3 g EPA + 1·8 DHA	10 wk	72	74	RCT p	PPL
Brady (2004)[40]	United Kingdom	35–70 y	M	29	1·47 g EPA + 1 g DHA	6 wk	15 + 14	15 + 14	B/A	Plat
Christensen (1999)[41]	Denmark	38 ± 11 y	X	60	3 g EPA + 2·9 g DHA; 0·9 g EPA + 0·8 g DHA	12 wk	20 + 20	20	RCT p	G, Plat
Cleland (1992)[42]	Australia	Adults	M	32	1·6 g EPA + 0·32 g DHA	4 wk	13 + 15	13 + 15	B/A	NPL
Conquer (1998)[43]	Canada	30–34 y	X	22	0·75 g DHA; 1·5 g DHA	6 wk	6 + 7	6	RCT p	PPL, NEFA
Conquer (1999)[44]	Canada	29·5 ± 1·5 y	M	20	1·3 g EPA + 1·7 g DHA	6 wk	9	10	RCT p	PPL, NEFA
Damsgaard (2007)[45]	Denmark	9 mo	X	94	0·57 g EPA + 0·38 g DHA	12 wk	24	24	RCT p	E
DeLany (1990)[46]	USA	19–31 y	M	15	1·13 g EPA + 0·7 g DHA; 4·53 g EPA + 2·73 g DHA	5 wk	5 + 4	5	RCT p	PPL
Dunstan (2004)[47]	Australia	32·4 ± 0·5 y	F	98	1·1 g EPA + 2·2 g DHA	17 wk	36	37	RCT p	E
Dyerberg (2004)[48]	Denmark	20–60 y	M	58	0·79 g EPA + 0·5 g DHA	8 wk	24	26	RCT p	Plat
Engström (2003)[49]	Sweden	26–65 y	X	16	0·11 g EPA + 0·18 g DHA; 0·45 g EPA + 0·39 g DHA	3 wk	8 + 8	8 + 8	B/A	PPL
Hagve (1993)[50]	Norway	19–22 y	F	16	3·3 g EPA + 1·8 g DHA	4 wk	8	8	RCT p	EPL
Harris (2007)[51]	USA	21–49 y	F	23	0·104 g EPA + 0·378 g DHA	16 wk	11	12	RCT p	RBC, PPL
Helland (2006)[52]	Norway	19–35 y	F	341	0·8 g EPA + 1·18 g DHA	17 wk	158	151	RCT p	PPL
Higgins (2001)[53]	Ireland	19–63 y	X	62	0·52 g EPA + 0·33 g DHA; 0·34 g EPA + 0·22 g DHA; 0·17 g EPA + 0·11 g DHA	16 wk	14 + 16 + 17	14	RCT p	P, LDL
Hodge (1993)[54]	Australia	30·6 y	F	7	0·55 g EPA + 0·39 g DHA	2 wk	7	7	B/A	PPL, PCE, PTG, HDL PL
Hoffman (2004)[55]	USA	6 mo	X	55	0. 083 g DHA	26 wk	25	26	RCT p	E
Itomura (2005)[56]	Japan	9–12 y	X	179	0·12 g EPA 1 0·52 g DHA	12 wk	26	23	RCT p	EPL
Katan (1997)[57]	Netherlands	56·2 ± 16·5 y	M	58	2·43 g EPA + 0·49 g DHA; 1·62 g EPA + 0·33 g DHA; 0·81 g EPA + 0·16 g DHA	52 wk	14 + 15 + 15	14	RCT p	PCE, E
Kew (2004)[58]	United Kingdom	23–65 y	X	42	4·7 g EPA + 0·73 g DHA; 0·85 g EPA + 4·9 g DHA	4 wk	11 + 11	11	RCT p	PPL, N
Khan (2003)[59]	United Kingdom	40–65 y	X	56	0·02 g EPA + 0·94 g DHA	32 wk	28	28	RCT p	PPL
Laidlaw (2003)[60]	Canada	36–68 y	F	8	2·32 g EPA + 1·68 g DHA	4 wk	8	8	B/A	PPL
Mann (2010)[61]	Australia	20–50 y	X	27	0·21 g EPA + 0·03 g DPA + 0·81 g DHA; 0·34 g EPA + 0·23 g DPA + 0·45 g DHA	2 wk	10 + 8	6	RCT p	Plat
Mantzioris (1994)[62]	Australia	25–44 y	M	15	1·62 g EPA + 1·08 g DHA	4 wk	15	15	B/A	PPL, PCE, PTG
McDaniel (2010)[63]	USA	18–45 y	X	30	1·6 g EPA + 1·2 g DHA	4 wk	16	14	RCT	PPL
Miles (2004)[64]	United Kingdom	21–44 y	M	50	2·1 g EPA + 0·9 g DHA; 1·1 g EPA + 0·5 g DHA; 0·8 g EPA + 0·3 g DHA; 0·6 g EPA + 0·2 g DHA	12 wk	10 + 10 + 10 + 10	10	RCT p	PPL, PCE, PTG, PBMC
Mills (1995)[65]	Canada	21–41 y	X	18	0·74 g EPA + 0·51 g DHA	6 wk	8	9	RCT p	E ghosts

Table 2. *Continued*

Author/year	Country	Age	Sex	No. included	Short description of intervention	Latest time point	No. in intervention	No. in control at latest time	Study design	Biomarkers reported
Milte (2007)[66]	Australia	53 y	X	75	0,52 g DHA + 0,12 g EPA; 1,04 g DHA + 0,24 g EPA; 1,56 g DHA + 0,36 g EPA	12 wk	17 + 20 + 19	19	RCT p	RBC
Minns (2010)[67]	USA	18–36 mo	X	86	0·043 g DHA; 0·13 g DHA	9 wk	29 + 29	28	RCT p	RBC, PPL
Montgomery (2003)[68]	United Kingdom	Pregnant	F	100	0. 04 g EPA + 0·2 g DHA	25 wk	30	29	RCT p	E, P
Murphy (2007)[69]	Australia	20–65 y	X	86	0·125 g EPA + DHA	24 wk	38	32	RCT p	RBC
Neubronner (2011)[70]	Germany	30–75 y	X	129	1·01 g EPA + 0,67 g DHA	24 wk	41 + 45	43	RCT p	RBC
Otto (2000)[71]	Netherlands	20–45 y	F	75	0·06 g EPA + 0·27 g DHA; 0·12 g EPA + 0·53 g DHA; 0·29 g DHA; 0·57 g DHA	4 wk	15 + 15 + 1-4 + 12	15	RCT p	PPL, EPL
Otto (2000)[72]	Netherlands	20–38 y	F	24	0·57 g DHA	4 wk	12	12	RCT p	PPL, EPL
Palozza (1996)[73]	Italy	25–46 y	X	40	4·1 g EPA + 3·6 g DHA; 2·7 g EPA + 2·4 g DHA; 1·4 g EPA + 1·1 g DHA	26 wk	10 + 10 + 10	10	RCT p	P, E
Park (2002)[74]	USA	37–43 y	X	33	4 g EPA or 4 g DHA	4 wk	10 + 10	11	RCT p	Plat
Rees (2006)[75]	United Kingdom	18–70 y	M	169	1·35 g EPA + 0·3 g DHA; 2·7 g EPA + 0·6 g DHA; 4·5 g EPA + 0·9 g DHA	12 wk	39 + 38 + 38	40	RCT p	PPL, PBMC PL
Sanders (2006)[76]	United Kingdom	29–35 y	X	80	1·5 g DHA	4 wk	40	39	RCT p	P, EPL
Sanjurjo (2004)[77]	Spain	31–34 y	F	20	0·04 g EPA + 0·2 g DHA	14 wk	8	8	RCT p	P
Smuts (2003)[78]	USA	16–35 y	F	48	0·184 g DHA	14 wk	18	19	RCT p	PPL, PTG, EPL
Sørensen (1998)[79]	Denmark	29–60 y	M	50	0·37 g EPA + 0·54 g DHA	4 wk	21	24	RCT p	LDL
Stark (2000)[80]	Canada	43–60 y	F	36	2·4 g EPA + 1·6 g DHA	4 wk	18	17	RCT p	PPL
Surai (2000)[81]	United Kingdom	26–59 y	X	44	0·21 g DHA	8 wk	20	20	RCT p	PPL, PCE, PTG, NEFA
Thies (2001)[82]	United Kingdom	56–69 y	X	24	0·7 g DHA; 0·72 g EPA + 0·28 g DHA	12 wk	8 + 7	8	RCT p	PBMC PL
Vognild (1998)[83]	Norway	16–69 y	X	228	0·7 g EPA + 1 g DHA; 0·5 g EPA + 0·6 g DHA; 0·5 g EPA + 0·8 g DHA; 1 g EPA + 1·5 g DHA; 1·3 g EPA + 1·8 g DHA	12 wk	35 + 36 + 3-8 + 36 + 3-4	36	RCT p	P, Plat
Wallace (2000)[84]	Ireland	20–26 y	F	25	0·35 g EPA + 0·32 g DHA	4 wk	13 + 12	13 + 12		Plat
Yaqoob (2000)[85]	United Kingdom	39–49 y	X	16	2·1 g EPA + 1·1 g DHA	12 wk	8	8	RCT p	PPL, PBMC

1 M, exclusively male group; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RCT, randomized controlled trial; p, parallel; PPL, plasma phospholipids; X, mixed sex group; ALA, a-linolenic acid; B/A, before-after study; Plat, total platelets; G, total granulocyte; NPL, neutrophil phospholipid; NEFA, nonesterified fatty acids; E, total erythrocytes; F, exclusively female group; EPL, erythrocyte phospholipids; P, total plasma; PCE, plasma cholesteryl esters; PTG, plasma triacylglycerols; PL, phospholipids; N, total neutrophils; DPA, docosapentanoic acid; PBMC, peripheral blood mononuclear cells

between dietary intake of fatty acids estimated from the FFQ in controls and concentrations of fatty acid in plasma of 0·38 for EPA and 0·49 for DHA. Godley *et al.*[10] reported correlations between dietary intake of EPA and DHA estimated from the FFQ and concentrations of EPA and DHA in erythrocyte membrane ranging from 0·19 to 0·36. This is the smallest value found for DHA in the blood biomarkers. Sublette *et al.*[6] reported significant correlations between dietary intake of ALA, EPA and DHA estimated from the FFQ and concentrations of ALA, EPA and DHA in plasma of 0·22 for ALA, 0·38 for EPA and 0·50 for DHA, which was the highest value found for the different biomarkers utilized. Sun *et al.*[15] reported a significant adjusted correlation of 0·56 between dietary intake of DHA estimated from the FFQ and concentrations of DHA in erythrocytes.

One dietary history questionnaire was also validated against serum fatty acid and high crude (r 0·46) and adjusted (r 0·59) correlations were reported for intake of EPA for men[18]. This questionnaire was self-administered and was somewhat similar to a FFQ.

Three studies have validated weighed records (all with seven or more days) against serum, erythrocytes or plasma fatty acids[12,20,21]. Kobayashi *et al.*[20] presented a very high correlation coefficient for EPA, crude (r 0·75) and adjusted (r 0·89), as well as the best adjusted correlations for DHA and total n-3 PUFA from weighed records validated against serum fatty acids (r 0·61). Kuriki *et al.*[21] obtained adjusted correlations for dietary intake of EPA measured with weighed records against plasma concentrations of EPA (r 0·57) and for DHA (r 0·57). McNaughton *et al.*[12] showed a crude correlation of 0·43 for DHA measured with weighed records validated against DHA concentration in plasma and a lower correlation coefficient for EPA (r 0·22). Similar correlations were observed when the intake was measured with a FFQ (DHA r 0·32 and EPA r 0·21). All three studies presented low correlations for ALA[12,20,21].

Biomarker study

A total of 8 new studies were incorporated to the 41 previously included papers identified in the study by Fekete *et al.*[5].

Details of the biomarkers analysed are given in Table 2 wich include 49 recovery studies of n-3 PUFA biomarkers. In this study, as summarized in Table 3, *Total plasma lipid DHA* appears to be a good biomarker of DHA status, which reacts rapidly to supplementation and is sensitive to supplementation dose. It appears to be reliable in adults, mixed sex studies, and those with moderate baseline DHA status, but it is not clear for which other population subgroups its application can be reliable. Moreover, *plasma phospholipid DHA* appears to be a good biomarker of DHA status. It reacts rapidly to supplementation and is also sensitive to supplementation dose. This biomarker appears to respond appropriately in adults, males, females, those with low, moderate, or high baseline DHA status, those who used marine oil, seafood, or single cell oils, and in those whose dose amounts were ≤2500 mg/d of DHA. There were insufficient studies to assess the effectiveness of plasma phospholipid DHA in other population subgroups. With reference to *Plasma phospholipid EPA,* it appeared to be a good biomarker for EPA status in men and women and those who had low, moderate,

Table 3. Primary analyses (pooled data on the longest duration and the highest supplementation dose) for each identified biomarker[1]

Biomarker	No. of studies (no. of included participants)	Pooled effect size, WMD (95 % CI) [2]	I^2	Appears effective as a biomarker?[3]
Plasma DHA	6 (262)	1·13 (0·54, 1·71)	88·7	Yes
Plasma phospholipid DHA	24 (1023)	2·51 (1·97, 3·00)	95·0	Yes
Plasma phospholipid EPA	16 (759)	4·07 (2·90, 5·24)[4]	99·0	Yes
Plasma triacylglycerol DHA	5 (116)	0·86 (0·08, 1·65)	92·1	Yes
Plasma cholesteryl ester DHA	5 (110)	0·42 (0·13, 0·71)	92·2	Yes
Plasma nonesterified DHA	3 (72)	1·35 (0·11, 2·59)	95·0	Yes
Erythrocyte DHA	12 (570)	2·43 (1,04, 3·91)	95·0	Yes
Erythrocyte phospholipid DHA	6 (229)	0·97 (0·50, 1·43)	72·3	Yes
Young erythrocyte ghosts DHA	1 (17)	−1·00 (−4·07, 2·07)[5]	N/A	Unclear
Old erythrocyte ghosts DHA	1 (17)	1·70 (0·32, 3·08)[5]	N/A	Unclear
Platelet DHA	9 (251)	1·29 (0·92, 1·56)	81·9	Yes
Granulocyte DHA	1 (40)	0·60 (0·32, 0·88)	N/A	Unclear
Neutrophil DHA	1 (20)	2·80 (0·01, 5·59)	N/A	Unclear
Neutrophil phospholipid DHA	2 (28)	0·04 (−0·15, 0·23)	N/A	Unclear
PBMC DHA	2 (36)	0·06 (−0·36, 0·48)	0	Unclear
PBMC phospholipid DHA	3 (94)	0·70 (−0·66, 2·06)	93·9	Unclear
LDL DHA	2 (73)	0·60 (0·59, 0·61)	0	Unclear
HDL phospholipid DHA	1 (7)	0·80 (0·07, 1·53)	N/A	Unclear

Modified and updated from Fekete *et al.*[5].

1 WMD, weighted mean difference; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; N/A, no available data; PBMC, peripheral blood mononuclear cell.

2 All studies are in %DHA of total fatty acids unless otherwise stated.

3 To claim that a biomarker was effective (reflected change in status) within a review, 3 conditions needed to be met: (1) statistical significance within a forest plot (95 % CI did not include 0 or $P<0·05$); (2) ≥3 trials contributed data; and (3) between intervention and control arms in the studies contributing data there were ≥50 participants. To claim that a biomarker was ineffective, 4 conditions had to be met: (1) lack of statistical significance within a forest plot (95 % CI included 0 or $P\geq0·05$); (2) ≥3 trials contributed data; (3) between intervention and control arms in the studies contributing data there were ≥50 participants; and (4) study results were approximately similar (heterogeneity levels were acceptable so that $I^2 < 50\,\%$).

4 %EPA of total fatty acid.

5 μg/mg protein.

British Journal of Nutrition

or high baseline EPA, which reacts rapidly to supplementation and is sensitive to supplementation dose.

Plasma triacylglycerol DHA could be a good biomarker of DHA status, but there were insufficient studies to allow exploration of which population groups it may be most effective in. *Plasma cholesteryl ester DHA* appears to be a good biomarker of DHA status at lower-dose supplementation, but it is not clear within which population groups it is effective or whether it works well at higher doses of supplementation. *Plasma nonesterified fatty acid DHA* may be a good biomarker of DHA status, but there were insufficient studies to allow for the exploration of appropriateness of its use in different population groups and doses. *Erythrocyte membrane total lipid DH* appears to be a good biomarker of DHA status, and the data suggest that there is a dose response. Although it seems to be an effective biomarker in infants for most doses, confirmation is not possible due to limited data. *Erythrocyte membrane phospholipid DHA* appears to be a good biomarker of DHA status and although it seems to be an effective biomarker in adults, children and adolescents, as well as in pregnant or lactating women and at most doses, this cannot be confirmed due to limited data. *Total platelet lipid DHA* could be a good biomarker of DHA status, but there was no apparent dose response. For *Peripheral blood mononuclear cell phospholipid DHA*, the response of peripheral blood mononuclear cell phospholipid DHA values to DHA supplementation did not appear to be a good biomarker of DHA status. For *other potential biomarkers of DHA status,* evidence was insufficient for young erythrocyte ghost DHA, old erythrocyte ghost DHA, granulocyte DHA, neutrophil DHA, neutrophil phospholipid DHA, peripheral blood mononuclear cell total lipid DHA, LDL DHA, and HDL phospholipid DHA.

Discussion

In a validation study, the reference method used should be as accurate as possible[8]. A validation study is also called a relative validation/calibration study when one dietary method is compared to another dietary method, most often FFQ *v.* several days of food records. The limitations with this approach are the considerable individual day-to-day variation, which reduces the possibility of obtaining a true measure of usual intake with few recording days, as well as reporting bias since both self-administered dietary assessment questionnaires and dietary records are based on self-reporting[8]. FFQs often overestimate intake of energy and nutrients, while food records often underreport energy and nutrient intakes[24,25]. As such, we thought it best to exclude those questionnaires where validation was made against another dietary measurement tool. An alternative to relative validations is the use of biomarkers, whose primary advantage is that these measurements are objective and the sources of errors for a biomarker and a dietary assessment method are independent[8,26]. The *n*-3 PUFA are largely exogenic, meaning that there is no synthesis of *n*-3 PUFA in the body and that intake via diet and supplements are the major source, making the correlations with biomarkers easier[11,27]. There are several choices of a biomarker for the measurement of LC *n*-3 PUFA, and those presented in this review were fatty acids in adipose tissue, erythrocytes and plasma. Adipose tissue fatty acids are generally considered the best source of assessing long-term fatty acid intake[13,27]. Erythrocytes may be a useful marker as they can provide an indication of the previous 120-d intake of LC *n*-3 PUFA[1]. Plasma fatty acids reflect intake of fatty acids over the past few days or more[11]. Most of the included studies have presented the correlations, both crude and adjusted. The correlation coefficients obtained from the validation studies can reflect the capability of the method to rank individuals according to fatty acid intake.

Subcutaneous fat

Fatty acids estimated from six different FFQ[7,8,10,13,14], one weighed record[19] and one recall[13] were validated against subcutaneous fat, which the literature describes as the best reference method. The correlation coefficients observed in all the studies were in the range of 0·40–0·66 for ALA, EPA and DHA. In summary, none of the dietary methods validated against subcutaneous fat and presented here seem to be superior than the others in relation to ranking the dietary intake of *n*-3 PUFA. Two articles related to subcutaneous fat were found for this updated review, the correlation coefficient range for EPA was 0·15–0·33 and for DHA 0·18–0·42. This suggests a weaker correlation when compared to the previous correlation reported by Marckman *et al.*[19] which showed the highest correlation coefficient (0·66) amongst all the included studies.

Blood component composition

Dietary intake of *n*-3 PUFA estimated from eleven different FFQ[1,6,8–12,15–17,22], one diet history questionnaire[18] and three weighed record studies[12,20,21] was validated against fatty acids in serum, plasma or erythrocytes. Both fatty acids in plasma, erythrocytes and serum were found to be good biomarkers of LC *n*-3 PUFA[1,22]. The correlation coefficients observed between the intake of fatty acids measured by most FFQs[1,7,8], the diet history questionnaire[1], and the weighed records[21] *v.* fatty acid in blood parameters were at the same range (*r* 0·40–0·60). The best correlation was observed in the study by Kobayashi *et al.*[20] comparing the dietary intake of fatty acids from weighed records with fatty acids in serum phospholipids (EPA, *r* 0·89). However, there was no clear tendency among the three studies comparing fatty acids from weighed records with fatty acids in blood[12,20,21]. As such, it seems that weighed records were the best method to measure *n*-3 PUFA intake.

Most correlation coefficients from the studies comparing dietary intake with fatty acids in blood parameters were in the same range as the ones observed for fatty acids in adipose tissue (*r* 0·40–0·60). There were two studies with a lower correlation[12,23] and one with a correlation higher than this range[20]. For ALA most studies presented low correlations between dietary intake and blood parameters in both previous and updated versions of this review[6,15,17,23]. In the present updated review, the correlation coefficient range for

erythrocyte EPA was 0·23–0·38 and for erythrocyte DHA 0·19–0·56 which suggests an acceptable and a reasonable good correlation coefficient respectively[10,15]. Regarding plasma phospholipid EPA the correlation range was 0·21–0·38 and for DHA 0·25–0·50[6,15,23], again showing an acceptable and reasonably good correlation coefficient, respectively. It is important to highlight that none of the current correlation coefficients found in this updated review were higher than those previously reported in the original article; however the same levels were maintained. This implies that any additional validation study will unlikely produce higher correlation estimates between questionnaires and biomarkers.

The estimation of summarised crude and adjusted correlations for all the validation studies of FFQs using biomarkers as the reference method indicates that the FFQ gives 'acceptable' values for total *n*-3 PUFA, EPA and DHA. The summarised crude and adjusted correlations for the two studies validating weighed records against biomarkers indicate 'acceptable' estimates for total *n*-3 PUFA, while the estimates obtained a higher ranking of 'good' for EPA and DHA. As expected, the weighed records seem to be superior to the FFQ in reference to estimating intakes of EPA and DHA.

Biomarkers were more accurate than different dietary methods to rank individuals. One limitation with food records is that subjects are prone to underestimate their food intake when they keep food records[24]. The true food consumption of *n*-3 FA most likely lies somewhere between the weighed records and the FFQ. According to the systematic review, none of the dietary assessment methods used to assess *n*-3 PUFA seem to be highly superior to another, with weighed records being slightly better than FFQs. Most studies presented correlation coefficients ranging from 0·40 to 0·60. This review also confirmed the view that employing an FFQ to assess *n*-3 PUFA requires that it be validated against reliable and valid biomarkers, and that validation studies of dietary methods for measuring intakes of *n*-3 FA could be improved.

Additionally, after analyzing the 18 different potential biomarkers of LC *n*-3 PUFA status reported in Table 3, we could argue that plasma phospholipid DHA, erythrocyte DHA and platelet DHA were all effective biomarkers of DHA status. With regard to other biomarkers (plasma DHA, plasma triacylglycerol DHA, plasma cholesteryl ester DHA, plasma nonesterified DHA, erythrocyte phospholipid DHA, and plasma phospholipid EPA) we could not find any additional evidence to support modifying or promoting previously published statements from Fekete *et al.*[5].

Still and all, it is worth noting that the health benefits of increasing LC *n*-3 PUFA dietary intake need to be evaluated in RCTs investigating specific clinical outcomes. There are some clear limitations found. First, the number of studies reporting data on different potential biomarkers is limited. This situation reduced our ability to explore which population subgroups or in which types of intervention the biomarkers are effective. Second, we were able to focus on the effect of supplementing DHA only, whereas LC *n*-3 PUFA supplementation usually consists of a complex mixture of LC *n*-3 PUFAs that may interconvert with each other[27]. Third, the dose-response curve of the incorporation of DHA (or any other fatty acid) may differ between the distinct blood component constituents[28,29]; hence, it may be assumed, with good reason, that the uniform time duration and dose categories may have differently influenced the evaluation of the biomarkers.

Although several clinical studies have investigated the response of various biomarkers to modified *n*-3 fatty acid intake[28–33] and important theoretical considerations have also been published[34,35,36], we still do not have enough data available in the literature. Which biomarker might be sensitive enough to detect changes of a given dose of LC *n*-3 PUFA supplementation in a given clinical condition or population group? Further research is needed to characterize and to understand the meaning of the different correlations between intake estimates and biomarkers of LC n3 PUFA in distinct population groups and environments.

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British Journal of Nutrition

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