Systematic reviews in the field of clinical nutrition

Ph.D. thesis

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Contents

Abbreviations

B/A	before-after study
CI	confidence interval
MD	mean difference
RCT	randomized controlled trials
SMD	standardized mean difference
LCPUFA	long-chain polyunsaturated fatty acid
n-6 LCPUFAs:	
LA	linoleic acid (C18:2n-6)
GLA	γ-linolenic acid (C18:3n-6)
DHGLA	dihomo-γ-linolenic acid (C20:3n-6)
AA	arachidonic acid (C20:4n-6)
n-3 LCPUFAs:	
ALA	α-linolenic acid (C18:3n-3)
EPA	eicosapentaenoic acid (C20:5n-3)
DPA	docosapentaenoic acid (C22:5n-3)
DHA	docosahexaenoic acid (C22:6n-3)

1. Introduction

"...it is necessary, while formulating the problems of which in our further advance we are to find the solutions, to call into council the views of those of our predecessors who have declared any opinion on this subject, in order that we may profit by whatever is sound in their suggestions and avoid their errors."

Aristotle, De Anima

The volume of the medical literature has increased spectacularly over recent decades; over 2 million articles published yearly in more than 20,000 journals (Hemingway 2009). On top of this, researchers, therapists, healthcare managers and policy makers often have to deal with unclear or contradictory results. Traditional, narrative reviews have been a part of the medical literature for a long time. Although narrative reviews may be useful to receive a general overview on a topic; they are inadequate to answer specific clinical questions. Systematic reviews can fulfil this need, providing high quality and comprehensive summaries. The main differences between narrative reviews and systematic reviews are summarized in **Table 1**.

Table 1: Differences between narrative and systematic reviews

Feature	Narrative reviews	Systematic reviews (minimum criteria)
Question	May not clear and concise	Clearly formulated
Sources and search	Non-specified, potentially biased	Comprehensive sources and explicit search strategy
Selection	Non-specified, potentially biased	Criterion-based, uniformly applied
Quality of the studies	Variable	Rigorous critical appraisal
Data synthesis	Qualitative and subjective	Qualitative or if possible, quantitative (meta-analysis)
Inference	Sometimes evidence-based, but more often based on personal opinions	Evidence-based

Adopted and modified from González-de Dios et al. 2005.

The value of evidence can be ranked on the basis of the quality of the study, and it ranges from expert opinions to systematic reviews of randomized controlled trials (RCTs), which represent the highest level of evidence (**Figure 1**). The systematic review can be defined as "a review of the evidence on a clearly formulated question that uses systematic and explicit methods to *identify, select* and *critically appraise* relevant primary research, and to *extract and analyse data* from the studies that are included in the review" (Summerbell and Moore 2007). The primary goal of the systematic reviews is to summarize the current best evidence on a topic, as well as

to determine the gaps and limitations in evidence and identify priorities for future research.

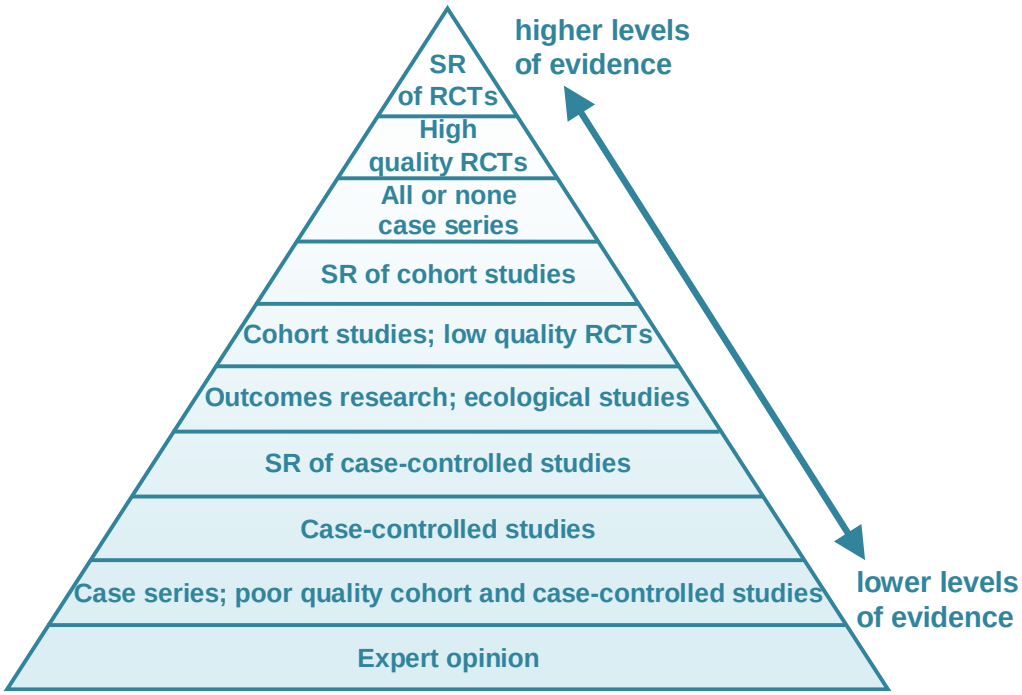

Figure 1: Hierarchy of evidence

Abbreviations: SR, systematic review; RCTs, randomized controlled trials.

Adopted and modified from Phillips et al. 2009.

Meta-analysis is the use of statistical techniques in systematic reviews to combine and summarize the results of individual studies; thus to increase the statistical power of studies with small sample size and improve the precision of the estimation of the effect. The first meta-analysis was performed by the influential English mathematician, Karl Pearson in 1904 (Rosenthal and DiMatteo 2001), and the term "meta-analysis" was coined by a social scientist and modern statistician, Gene V. Glass in 1976 (Gregson et al. 2002). Thousands of systematic reviews and meta-analyses have been published in recent years, and standards for reporting them have been developed (Moher et al. 1999, Liberati et al. 2009).

1.1. The process of systematic review

1.1.1. Defining the review question and developing criteria for including studies

Developing a clear and focused question is the first and probably the most important step in writing systematic reviews. This question should include four parts, referred to as **PICO** question: **p**atients, population or problem; **i**ntervention, independent

variable or exposure; **c**omparison; and **o**utcomes of interest or dependent variable (Stone 2002). These four components may define much of the eligibility criteria for selecting the studies; however, topic-specific criteria are often necessary, e.g. study design, minimum intervention period, or baseline nutritional status. Precise inclusion of the review question and selection criteria into the study protocol can help to minimise bias and to ensure that results are reproducible.

1.1.2. Searching for studies

Systematic searches should include multiple electronic databases; MEDLINE (Medical Literature Analysis and Retrieval System Online), EMBASE (Excerpta Medica), and Cochrane Library are the most frequently used databases. Depending on the review topic, there are many specialized databases which can also be used. Development of a search strategy is an interactive process, and it is usually built up in a number of test searches. In order to construct an effective combination of search terms, it is necessary to break down the review question into elements. Thereafter, these PICO elements with appropriate synonyms, abbreviations, related terms, variant spellings and common misspellings can be combined with Boolean operators (AND, OR, NOT). Handsearching the pertinent journals, searching the reference lists of the included studies; moreover, personal communications with experts in the field can also be proposed in order to identify all the relevant studies.

1.1.3. Study selection

The selection of studies is usually conducted in two stages: the initial step is to screen the titles and abstracts in order to identify the potentially relevant papers, and the following step is to screen the full text of these selected papers for detailed assessment against the inclusion criteria. When the information is insufficient to make decision about inclusion or exclusion, it can be helpful to contact the authors for more details. Flow diagram showing the number of included/excluded studies is a simple and useful way to document the study selection process, and the reasons for exclusion can also be reported.

1.1.4. Data extraction and quality assessment

Data extraction is the process for obtaining the necessary information about study characteristics and findings from the studies. Because each review is different, data

extraction requirements and data extraction forms vary across reviews. Data extraction is closely linked to quality assessment; they are often undertaken at the same time. Studies are usually carried out with different degrees of methodological deficiencies; therefore the assessment of quality can give indication of the robustness of evidence derived from the included studies. Many scoring systems can be used to assess the quality (Jüni et al. 1999); however, none of these systems are widely accepted.

1.1.5. Data synthesis and meta-analysis

Data synthesis can be done qualitatively; this is so-called descriptive systematic review, or quantitatively by using statistical techniques such as meta-analysis. A common criticism of meta-analyses is that they combine “apples and oranges” (Moayyedi 2004). Indeed, when the included studies are too heterogeneous, only descriptive synthesis should be undertaken instead of meta-analysis. Heterogeneity can result from either methodological (e.g. study design, study quality) or clinical (e.g. age, sex, study location, dose of the intervention) differences between studies. Nevertheless, when meta-analysis is possible, descriptive synthesis may also be required and can be effectively incorporated into the quantitative review.

Two main statistical models can be used to combine data: the fixed-effects model and the random-effects model. Fixed-effects model assumes that every study is evaluating a common treatment effect. In other words, the effect of treatment, allowing for the play of chance, is the same in all studies. This model is generally used if there is no heterogeneity in the meta-analysis. The random-effects model is an alternative approach of meta-analysis; it assumes that the true treatment effect may vary across the individual studies because the difference between studies. If there is any concern about heterogeneity, the random-effects model is proposed (Zlowodzki et al. 2007).

Dichotomous or binary outcome data arise when the outcome is one of two possibilities. The most frequently used measures for dichotomous data are the odds ratio, the risk ratio, the risk difference and the number needed to treat. Continuous outcomes can take any value on a scale that is continuously variable, and can be presented either as a mean difference (MD) or as a standardized mean difference (SMD). The MD measures the absolute differences between the mean values in two

groups. The SMD can be used when the studies assess the same outcome, but measure it using different scales (Zlowodzki et al. 2007).

Forest plot (also known as ‘blocks and lines plot’, ‘confidence interval plot’ or ‘blobbogram’) is typically used to present a good visual summary of the results of the meta-analysis (**Figure 2**). Individual studies are represented by a horizontal line extending either side of a square at the point estimate of treatment effect. The ends of the line represent the confidence interval (CI) (usually 95%, but other intervals can also be used). The area of each square is proportional to the weight assigned to that study in the meta-analysis. The pooled effects size from the meta-analysis is at the bottom of the plot, represented by a diamond. The centre of the diamond represents the pooled point estimate, and the horizontal tips of the diamond represent its CI.

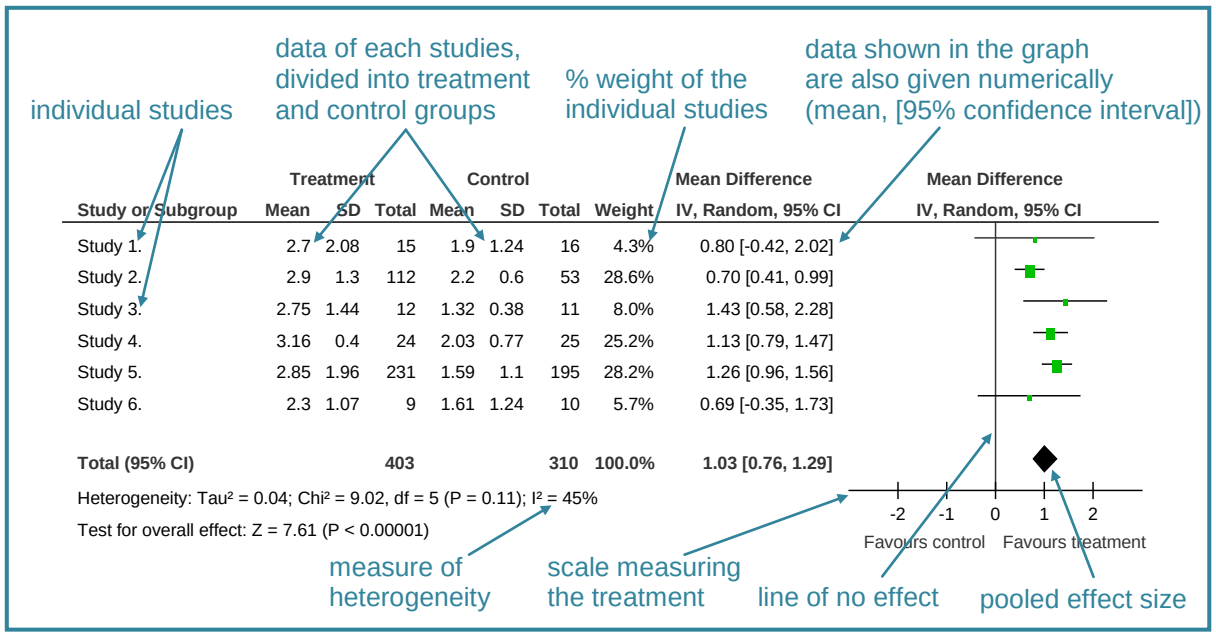

Figure 2: Hypothetical example of a forest plot with continuous outcome parameter

(analysis done with the Review Manager 5.1 software)

If the horizontal line for any study does not cross the line of no effect, it means that there is a significant difference between treatment and control groups. If the horizontal line for any study reaches or crosses the line of no effect, it means that there is no significant difference between the two groups. The same statement is true for the pooled effect size.

2. Aims

As the importance of nutrition is increasingly recognised, clinical nutrition is now integrated into the mainstream medical treatment. In parallel with this, the use of systematic reviews for nutrition-related topic becomes more widespread. Currently, if the results of a study show no relation between micronutrient status and health outcome, it is not clear if the lack of relation is due to the biomarker being an inadequate measure of status or the absence of any relation between status and the health outcome. Use of biomarkers that reflect changes in micronutrient status can facilitate the understanding of the relationship between micronutrient intake and status and it follows from this, the relationship between micronutrient status and health outcome (Hooper et al. 2009).

The risk of low dietary zinc intake, consequently zinc deficiency is widespread problem affecting between one-third and one-half of the world's population (Brown et al. 2001). In European populations, severe primary zinc deficiency is extremely uncommon, but marginal deficiency is likely to be much more prevalent (Gibson et al. 2008). The lack of a reliable, responsive, and specific indicator of zinc status means that the diagnosis of marginal zinc deficiency is difficult. Therefore, our aim was to perform a systematic review to assess the usefulness of the biomarkers of zinc status in healthy humans to determine which biomarkers appropriately reflect changes in zinc status in response to supplementation or depletion.

In contrast to various micronutrients, it is not fatty acid deficiency that generates public interest in n-3 long-chain polyunsaturated fatty acid (LCPUFA) supplementation, rather the potential health benefits attributed to enhanced n-3 LCPUFA intake. However, there are no generally accepted, gold standard biomarkers that reflect n-3 LCPUFA status in a specific and sensitive way (Baylin and Campos 2006). Accordingly, our aim was to conduct a systematic review of intervention studies with n-3 LCPUFA in healthy humans to identify the biomarkers of status that reliably reflect change in n-3 LCPUFA intake.

3. Assessing potential biomarkers of zinc status in humans: a systematic review

3.1 Introduction

Zinc is well established as an essential micronutrient for human health because it has numerous structural and biochemical functions at the cellular and subcellular level, which include enzyme function, DNA and RNA metabolism, protein synthesis, gene expression, cell growth and differentiation, and cell-mediated immunity. The ubiquitous nature of zinc in human biological systems indicates the widespread consequences and the complexity of inadequate dietary supply of zinc and zinc depletion.

Rich sources of dietary zinc include meat, fish, seafood, nuts, seeds, legumes, and whole-grain cereals (**Table 2**). However, plant sources are considered to be less bioavailable because of the presence of phytic acid that binds to zinc-forming insoluble complexes, which thus inhibits zinc's absorption (Brown et al. 2001).

Table 2: Zinc and phytate content in foods and estimated amount of absorbable zinc

Food group	Zinc content (mg/100 g)	Phytate content (mg/100 g)	Phytate:zinc molar ratio	Absorbable zinc (mg/100 g)
Liver, kidney (beef, poultry)	4.2–6.1	0	0	2.1–3.1
Meat (beef, pork)	2.9–4.7	0	0	1.4–2.4
Poultry	1.8–3.0	0	0	0.9–1.5
Seafood	0.5–5.2	0	0	0.2–2.6
Eggs	1.1–1.4	0	0	0.6–0.7
Dairy products	0.4–3.1	0	0	0.2–1.6
Seeds, nuts	2.9–7.8	1760–4710	22–88	0.3–0.8
White bread	0.9	30	3	0.4
Whole-grain cereals	0.5–3.2	211–618	22–53	0.1–0.3
Legumes	1.0–2.0	110–617	19–56	0.1–0.2
Refined cereal grains	0.4–0.8	30–439	16–54	0.1
Tubers	0.3–0.5	93–131	3–27	<0.1–0.2
Vegetables	0.1–0.8	0–116	0–42	<0.1–0.4
Fruits	0–0.2	0–63	0–?	<0.1–0.2

Diets with a phytate:zinc molar ratio less than 5 have relatively good zinc bioavailability (45% to 55% of zinc is absorbed); between 5 and 15 have medium zinc bioavailability (30% to 35% of zinc is absorbed); greater than 15 have relatively poor zinc bioavailability (10% to 15% of zinc is absorbed). Adopted and modified from Brown et al. 2001.

Current recommendations for dietary zinc intake in adults range from 7 mg/day (UK Reference Nutrient Intake) to 11 mg/day (US Recommended Dietary Allowance) (Geissler and Powers 2005). This broad range reflects in part the variation in requirements due to differences in the bioavailability of zinc from different national diets and also the difficulties associated with estimating the requirements for optimal health, which depends on a reliable indicator of status (King 1990).

Unlike other micronutrients such as iron, there is no storage form of zinc in the body that can be readily mobilized when intakes are inadequate, which emphasizes the need for a regular dietary supply (King et al. 2001). A highly effective homeostatic mechanism responds to alterations in zinc intake, upregulating absorption and conserving losses via the gastrointestinal tract and kidneys when intakes fall. By using isotope tracer techniques, it was predicted that when dietary zinc fell from 12.2 to 0.23 mg/day in a group of adult men, fractional zinc absorption could increase to virtually 100%, with urinary excretion falling from 0.36 to 0.006 mg/day and fecal excretion falling from 11.8 to 0.23 mg/day (King et al. 2001). When homeostatic mechanisms fail to ensure that requirements are met, clinical symptoms of zinc deficiency ensue. Severe deficiency is associated with stunted growth, immune dysfunction, and poor wound healing. These symptoms of severe zinc deficiency are most dramatically observed in acrodermatitis enteropathica, a congenital condition in which the infant is born with impaired gastrointestinal zinc transport, which limits the ability to absorb zinc (Atherton et al. 1979).

3.2 Methods

3.2.1. Inclusion criteria

To be included, a study needed to meet all of the following criteria: 1) be an intervention study in humans (including supplementation and/or depletion studies) without restriction in study design, which could include RCTs, controlled clinical trials, and before-after studies (B/A); 2) report the zinc status in humans at baseline and after supplementation or depletion; 3) minimum duration of supplementation of 2 wk; 4) report the daily dose of the zinc supplement; 5) use one of the following supplements: zinc sulphate; zinc acetate; zinc gluconate, or zinc methionine; and 6)

involve healthy participants who had not recently used mineral or vitamin supplements.

3.2.2. Search strategy

Electronic searches were performed with Ovid MEDLINE (www.ovid.com), EMBASE (Ovid) (www.ovid.com), and the Cochrane Library CENTRAL (www.thecochranelibrary.com) database, which were searched from inception to October 2007 for intervention studies by using text terms with appropriate truncation and relevant indexing terms. The search was in the form: [zinc terms] and [intervention study terms] and [human studies]. The OVID Medline search strategy can be found in **Table 3**, and the strategies for the other databases were based on the relevant OVID Medline strategy. We did not apply any language restriction.

Table 3: Search strategy for Ovid MEDLINE from 1950 to October Week 3, 2007

#	Search History	Results
1	Zinc Sulfate/	1082
2	((zinc$ or Zn$) adj1 sulphate$).mp.	569
3	((zinc$ or Zn$) adj1 sulfate$).mp.	1681
4	Zinc Acetate/	99
5	((zinc$ or Zn$) adj1 acetate$).mp.	396
6	((zinc$ or Zn$) adj1 gluconate$).mp.	138
7	((zinc$ or Zn$) adj1 methionine$).mp.	56
8	1 or 2 or 3 or 4 or 5 or 6 or 7	2543
9	exp clinical trials/ or feasibility studies/ or intervention studies/ or pilot projects/	267783
10	interven$.mp.	303162
11	Dietary Supplements/	13542
12	supplement$.mp.	133439
13	9 or 10 or 11 or 12	671914
14	8 and 13	542
15	((zinc$ or Zn$) adj5 deplet$).mp.	581
16	14 or 15	1109
17	(animals not humans).sh.	3197957
18	16 not 17	652

Abbreviations: $, truncation symbol; sh, subject heading word; adj, adjacent operator; mp = title, original title, abstract, name of substance word, subject heading word.

An Ovid MEDLINE search was conducted for reviews of the methods of assessing zinc status; 6 of these reviews (Wood 2000; Brown 1998, Brown 2002; Hotz et al. 2003; Hambidge 2003; Thompson 1991) were collected in full text, and the reference lists were checked. Studies that appeared to be intervention studies but that had not been already assessed for inclusion were collected.

One expert, Rosalind Gibson (University of Otago), was asked if she could suggest additional intervention studies for the review. She suggested additional articles for assessment and these were then subjected to the same criteria listed above before they were accepted for inclusion.

3.2.3. Data collection and synthesis

Titles and abstracts were screened for inclusion by a single reviewer. The full text of all articles collected was screened for inclusion by using an inclusion and exclusion form by 2 independent reviewers. Where the 2 reviewers disagreed, the study was discussed and a consensus decision was reached, or a third reviewer was asked to arbitrate. Data for each included study were extracted into a Microsoft Access 2003 database file (Microsoft Corp, Redmond, WA) by a single reviewer. In doubtful cases, studies were discussed with the review team before beginning full data extraction and, in some cases, study authors were contacted for clarification. When necessary, units of measurement were converted to a standard form to facilitate comparison across studies.

We used formal inclusion/exclusion criteria and applied standard operation procedures for data extraction, validity assessment and meta-analysis (Hooper et al. 2009). Meta-analysis was carried out with Cochrane software, Review Manager version 4.2 (Cochrane Collaboration; www.cochrane.org), with random-effects model. A statistically significant result indicated that the marker was indeed responding to supplementation and/or depletion. Levels of the heterogeneity were noted (heterogeneity was considered significant where $P < 0.1$ on the chi-square test or $I^2 > 50\%$).

Because there was a danger of categorizing some biomarkers as ineffective when there actually was a shortage of data, such that one would not expect a statistically significant effect size on pooling, we would declare a biomarker effective (statistically significant pooled effect size; $P < 0.05$) or ineffective (statistically insignificant pooled effect size; $P \geq 0.05$) only where the pooling included ≥3 studies and ≥50 participants overall. Where there were <3 studies or <50 participants, it was stated that there were insufficient data to make a decision (Hooper et al. 2009).

3.3. Results

3.3.1. Study inclusion

The flow diagram for this review is shown in **Figure 3**. A total of 1334 titles and abstracts were screened after electronic and bibliographic searches or were recommended by experts. Of these, 182 appeared potentially relevant and were collected as full-text articles to be assessed for inclusion, and 180 full-text articles were assessed (2 articles could not be traced); 48 studies were found to fulfill the inclusion criteria. One article had elements of both an RCT and a B/A in the study design (Abdulla and Svensson 1979) and one article contained a supplementation and a depletion study (Prasad et al. 1996). These data were analyzed as 2 separate studies, giving a total of 48 studies from 46 publications: 24 described RCT studies and 24 described B/A studies. In some cases, studies were further subdivided into data sets, when, for example, the study cohort was assigned to groups receiving different amounts of supplementation.

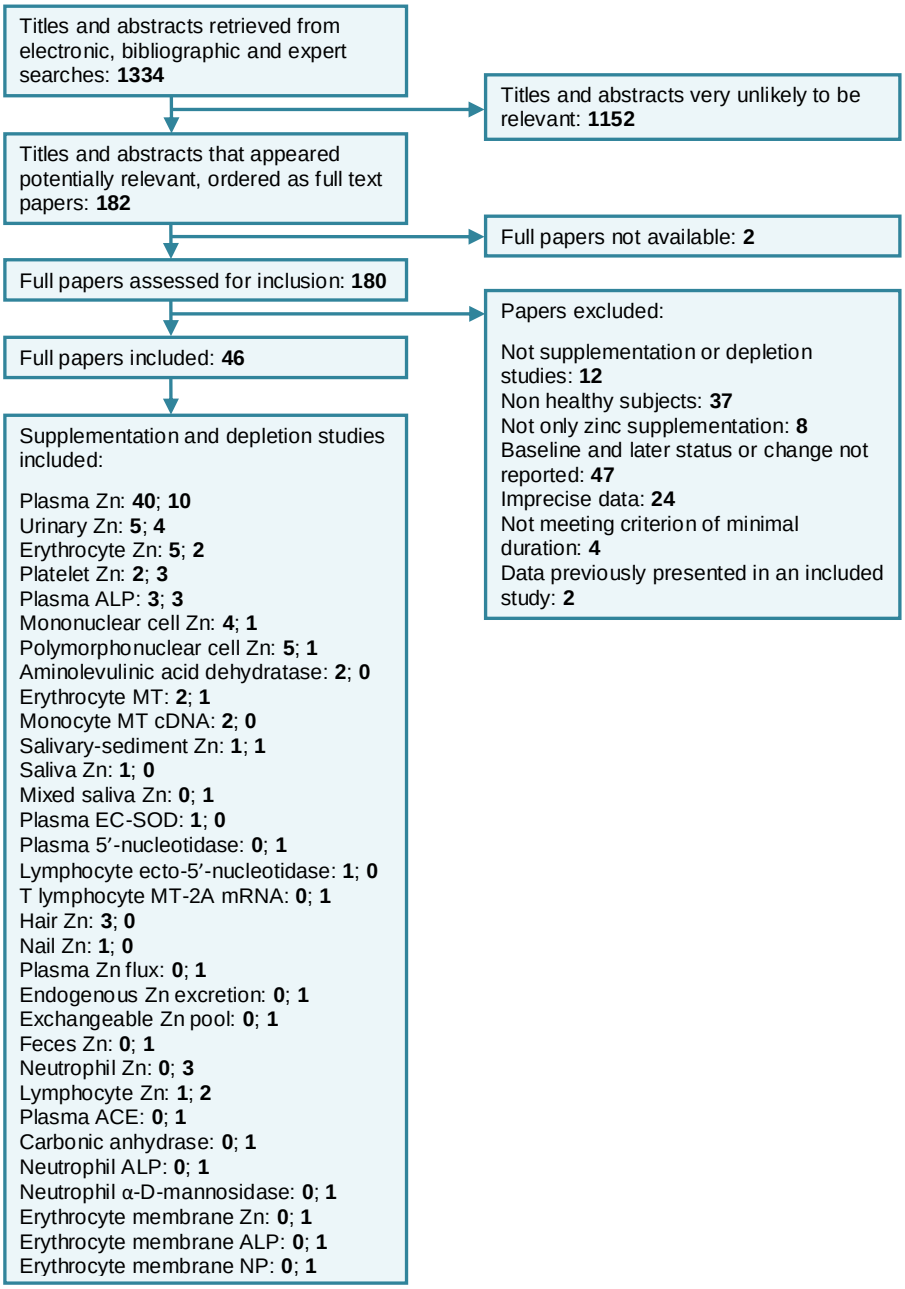

Figure 3: Flow diagram of systematic literature search on biomarkers of zinc status

Abbreviations: ALP, alkaline phosphatase; MT, metallothionein; EC-SOD, extracellular superoxide dismutase; ACE, angiotensin-converting enzyme; NP, neutral phosphatase.

3.3.2. Quality of included studies

The characteristics of the studies included in the analysis are presented in **Table 4** (supplementation studies) and **Table 5** (depletion studies). In terms of the distribution of the age of the population groups studied, 67% (32/48) of the studies were in healthy adults, and 19% (9/48) in elderly people.

Table 4: Basic characteristics of the included supplementation studies

Studies	Country(s); age; sex; no. included	Short description of intervention; latest time point; no. in intervention; no. in control at latest time	Micronutrient type	Study design	Biomarkers reported
Abdulla and Svensson 1979 *1*	Sweden; 25 yrs; X; 12	135 mg Zn; 12 wks; 7; 5	Zinc sulphate	RCT p	Pl Zn; ALAD
Abdulla and Svensson 1979 *2*	Sweden; 25 yrs; X; 7	45 mg Zn; 12 wks; 7	Zinc sulphate	B/A	Pl Zn; ALAD
Abdulla and Suck 1998	India; Pakistan; 40 yrs; X; 45	15 mg Zn; 30 mg Zn; 45 mg Zn; 6 wks; 15 + 15 + 15	Zinc gluconate	B/A	Pl Zn
Barrie et al. 1987	USA; students; X; 15	50 mg Zn; 4 + 4 wks; 15; 15	Zinc gluconate	RCT c	Pl Zn; E Zn; Hair Zn
Black et al. 1988	USA; 19-29 yrs; M; 45	50 mg Zn; 75 mg Zn; 12 wks; 13 + 9; 9	Zinc gluconate	RCT p	Pl Zn; Urinary Zn
Bodgen et al. 1988	USA; 71 yrs; X; 103	15 mg Zn; 100 mg Zn; 12 wks; 32 + 32; 32	Zinc acetate	RCT p	Pl Zn; MNC Zn; Pl ALP; PMNC Zn; Plat Zn
Crouse et al. 1984	USA; 20-55 yrs; M; 44	28.7 mg Zn; 8 wks; 11 + 12; 10 + 11	Zinc sulphate	RCT p	Pl Zn
Demetree et al. 1980	USA; 27-34 yrs; M; 10	100 mg Zn; 3 wks; 5; 5	Zinc sulphate	RCT p	Pl Zn
Donangelo et al. 2002	USA; 20-28 yrs; F; 11	22 mg Zn; 6 wks; 11	Zinc gluconate	B/A	Pl Zn; Urinary Zn
Duchateau et al. 1981	Belgium; 20-60 yrs; F; M; 83	150 mg Zn; 4 wks; 20 + 20 + 20 + 23	Zinc sulphate	B/A	Pl Zn
Field et al. 1987	UK; 71-93 yrs; F; 15	50 mg Zn; 100 mg Zn; 150 mg Zn; 4 wks; 5+5+5	Zinc sulphate	B/A	Pl Zn; MNC Zn; PMNC Zn;
Fischer et al. 1984	Canada; adults; M; 26	50 mg Zn; 6 wks; 13; 13	Zinc gluconate	RCT p	Pl Zn
Gatto and Samman 1995	Australia; 24,3±4.2 yrs; M; 10	50 mg Zn; 4 + 4 wks; 10; 10	Zinc sulphate	RCT c	Pl Zn
Grider et al. 1990	USA; 25-32 yrs; M; 6	50 mg Zn; 9 wks; 6	Zinc gluconate	B/A	E MT
Gupta et al. 1998	India; 50±10.65 yrs; X; 20	150 mg Zn; 6 wks; 20	Zinc sulphate	B/A	Pl Zn
Hayee et al. 2005	Bangladesh; 51.62±10.49 yrs; X; 20	150 mg Zn; 6 wks; 20	Zinc sulphate	B/A	Pl Zn
Heckmann et al. 2005	Germany; 41-82 yrs; X; 50	20 mg Zn; 12 wks; 24; 26	Zinc gluconate	RCT p	Pl Zn; Saliva Zn
Hininger-Favier et al. 2007	France; UK; Italy; 55-85 yrs; X; 387	15 mg Zn; 30 mg Zn; 26 wks; 126 + 131; 130	Zinc gluconate	RCT p	Pl Zn; E Zn; Urinary Zn; Pl ALP
Hodkinson et al. 2007	Northern Ireland; 55-70 yrs; X; 101	15 mg Zn; 30 mg Zn; 26 wks; 28 + 34; 31	Zinc gluconate	RCT p	Pl Zn; E Zn; Urinary Zn
Hollingsworth et al. 1987	USA; 66-85 yrs; X; 8	100 mg Zn; 12 wks; 8	Zinc sulphate	B/A	Pl Zn; L ecto-5'-NT

Table 4 (Continued)

Studies	Country(s); age; sex; no. included	Short description of intervention; latest time point; no. in intervention; no. in control at latest time	Micronutrient type	Study design	Biomarkers reported
Hunt et al. 1985	USA; 16 yrs; F; 138	20 mg Zn; 19 wks; 56; 47	Zinc sulphate	RCT p	Pl Zn
Medeiros et al. 1987	USA; 19-29 yrs; M; 31	50 mg Zn; 75 mg Zn; 12 wks; 13 + 9; 9;	Zinc gluconate	RCT p	Pl Zn; Urinary Zn; Hair Zn
O'Brien et al. 2007	USA; 31±4 yrs; F; 26	15 mg Zn; 26 wks; 16; 10	Zinc sulphate	RCT p	Pl Zn
Pachotikarn et al. 1985	USA; 18-29 yrs; M; 23	50 mg Zn; 6 wks; 23	Zinc gluconate	B/A	Pl Zn
Palin et al. 1979	USA; 16.8±5.1 yrs; X; 17	23 mg Zn; 8 wks; 7; 10	Zinc sulphate	RCT p	Pl Zn
Peretz et al. 1993	Belgium; 24-46 yrs; X; 9	45 mg Zn; 9 wks; 9	Zinc gluconate	B/A	Pl Zn; MNC Zn; PMNC Zn
Prasad et al. 1996	USA; 64±9 yrs; M; 9	30 mg Zn; 26 wks; 5	Zinc gluconate	B/A	Pl Zn; L Zn; PMNC Zn
Samman and Roberts 1987	Australia; 28 yrs; F; M; 47	150 mg Zn; 6 + 6 wks; 41; 41	Zinc sulphate	RCT c	Pl Zn
Shaaban et al. 2005	Egypt; pregnant; F; N/A	10 mg Zn; 8 wks; 30; 30	Zinc sulphate	RCT p	Nail Zn; Hair Zn
Stur et al. 1996	Austria; 71 yrs; X; 112	45 mg Zn; 104 wks; 38; 42	Zinc sulphate	RCT p	Pl Zn
Sullivan and Cousins 1997	USA; 19-35 yrs; M; 20	50 mg Zn; 2 wks; 10; 10	Zinc gluconate	RCT p	Pl Zn; Monocyte MT cDNA
Sullivan et al. 1998	USA; 19-35 yrs; M; 25	50 mg Zn; 2 wks; 11; 11	Zinc gluconate	RCT p	Pl Zn; Monocyte MT cDNA; E MT
Swanson et al. 1988	Switzerland; 64-95 yrs; X; 34	30 mg Zn; 4 wks; 17; 17	Zinc acetate	RCT p	Pl Zn; MNC Zn; PMNC Zn; Plat Zn
Tamura et al. 1996	USA; 13-39 yrs; F; 135	25 mg Zn; 17 wks; 70; 65	Zinc sulphate	RCT p	Pl Zn; E Zn
Tamura et al. 2001	USA; pregnants; F; 63	25 mg Zn; 20 wks; 31; 32	Zinc sulphate	RCT p	Pl Zn; E Zn; Pl ALP; PL EC-SOD
Weismann et al. 1977	Denmark; 17-37 yrs; X; 39	135 mg Zn; 12 wks; 13; 12	Zinc sulphate	RCT p	Pl Zn
Yadrick et al. 1989	USA; 25-40 yrs; F; 9	50 mg Zn; 10 wks; 9	Zinc gluconate	B/A	Pl Zn; Salivary-sediment Zn

Abbreviations: M, exclusively male group; F, exclusively female group; X, mixed group; RCT p, randomized controlled trial – parallel; RCT c, randomized controlled trial – crossover study; B/A, before/after study; N/A, no available data; Pl, plasma; ALAD, amino levulinic acid dehydratase; E, erythrocytes; MNC, mononuclear cells; Pl ALP, plasma alkaline phosphatase; PMNC, polymorphonuclear cells; Plat, platelet; E MT, erythrocyte metallothionein; L, lymphocyte; ecto-5'-NT, ecto-5'-nucleotidase; monocyte MT cDNA, monocyte metallothionein cDNA; EC-SOD, plasma extracellular superoxide dismutase.
1, study 1.; *2*, study 2.

Table 5: Basic characteristics of the included depletion studies

Studies	Country(s); age; sex; no. included	Short description of intervention; latest time point; no. in intervention; no. in control at latest time	Study design	Biomarkers reported
Allan et al. 2000	USA; 27-47 yrs; M; 7	4.6 mg Zn; 10 wks; 7	B/A	Pl Zn; TL MT-2A mRNA
Bales et al. 1994	USA; 59-78 yrs; X; 15	3.97±0.21 mg Zn; 2 wks; 15	B/A	Pl Zn; Pl ALP; Pl 5'NT
Freeland-Graves et al. 1981	USA; 23-44 yrs; F; 12	3.2 mg Zn; 3 wks; 12	B/A	Pl Zn; Mixed saliva Zn; Salivary-sediment Zn
Lowe et al. 2004	USA; 20-35 yrs; M; 5	0,23 mg Zn; 6 wks; 5	B/A	Pl Zn; Pl ALP; EZE; Pl Zn flux; Urinary Zn; EZP
Lukaski et al. 1984	USA; 23-57 yrs; M; 5	3.6 mg Zn; 17 wks; 5	B/A	Pl Zn
Mahajan et al. 1992	USA; 21-30 yrs; M; 8	3.2-5.6 mg Zn; 24 wks; 8	B/A	Pl Zn; Plat Zn; L Zn; Neutr Zn
Milne et al. 1987	USA; 50-63 yrs; F; 5	2,6 mg Zn; 17 wks; 5	B/A	Pl Zn; Plat Zn; MNC Zn; E Zn; Neutr Zn; CA; Urinary Zn; Feces Zn; Pl ALP; Pl ACE
Pinna et al. 2002	USA; 27-47 yrs; M; 8	4.6 mg Zn; 10 wks; 8	B/A	Pl Zn
Prasad et al. 1996	USA; 27 yrs; M; 4	4.9 mg Zn; 20 wks; 4	B/A	L Zn; PMNC Zn
Ruz et al. 1992	Canada; 25.3±3.3 yrs; M; 15	4 mg Zn; 7 wks; 14	B/A	Pl Zn; Urinary Zn; Neutr Zn; Neutr ALP; Neutr αDM; Plat Zn; EM Zn; EM ALP; EM NP
Thomas et al. 1992	USA; 22-35 yrs; M; 5	3.2 mg Zn; 6 wks, 5	B/A	Pl Zn; E Zn; E MT; Urinary Zn

Abbreviations: M, exclusively male group; F, exclusively female group; X, mixed group; B/A, before-after study; Pl, plasma; TL MT-2A mRNA, T lymphocyte metallothionein-2A mRNA; Pl ALP, plasma alkaline phosphatase; Pl 5'NT, plasma 5'-nucleotidase; EZE, endogenous zinc excretion; EZP, exchangeable zinc pool; Plat, platelet; L, lymphocytes; Neutr, neutrophils; MNC, mononuclear cells; E, erythrocytes; CA, carbonic anhydrase; Pl ACE, plasma angiotensinconverting enzyme; PMNC, polymorphonuclear cells; Neutr ALP, neutrophil alkaline phosphatase; Neutr αDM, neutrophil α-D-mannosidase; EM, erythrocyte membrane; EM ALP, erythrocyte membrane alkaline phosphatase; EM NP, erythrocyte membrane neutral phosphatase; E MT, erythrocyte metallothionein.

There were 5 studies in pregnant or lactating women (Hunt et al. 1985; O'Brien et al. 2007; Shaaban et al. 2005; Tamura et al. 1996; Tamura et al. 2001), one study in postmenopausal women (Milne et al. 1987), and one study in children and adolescents (Palin et al. 1979). There were no studies in infants, and none that selected for immigrant or low-socioeconomic groups. As discussed in the methodology article (Hooper et al. 2009), quality assessment was undertaken as part of the data extraction process. A summary of the reasons for dropping out in the intervention group, the methods of randomization, and compliance checking are summarized in **Table 6**. In the majority of studies, the reasons for dropping out and the method and outcome of compliance testing were not reported. In studies that claimed to be randomized, only 2 of 26 described the methods used.

3.3.3. Biomarkers identified and evaluated

In the 48 studies included in this review, a total of 32 potential zinc biomarkers, 17 biomarkers of zinc status in zinc supplementation trials, and 25 biomarkers in zinc depletion trials were identified (**Figure 3**). A summary of all the biomarkers identified, including the number of studies, participants, and the results of the primary analysis where relevant is presented in **Table 7**. A large proportion of the studies included in this review measured plasma or serum zinc concentration, and for simplicity, here the term ''plasma'' will be used to refer to both. The results of the secondary analysis of biomarkers for which there were sufficient data for subgroup analysis are described below.

3.3.3.1. Plasma zinc concentration

Plasma zinc concentration was the most frequently investigated marker of zinc status, with a total of 40 data sets from 35 supplementation studies, which involved 1375 participants and 10 data sets from 10 depletion studies involving 79 participants. Combining data from the depletion and supplementation studies, primary analysis revealed an overall significant ($P < 0.00001$) response of plasma zinc concentration to dietary zinc intake (MD: 2.88 μmol/L; 95% CI: 2.24, 3.51; I^2 = 93.6%) (**Table 7**). However, the high level of heterogeneity in this primary analysis means that the biomarker responded differently in the included studies; the reasons for these differences were explored through subgrouping of studies by their different characteristics.

Table 6: Validity of included studies

Studies	Randomized? Method of randomization	Reasons for dropouts by intervention group (no. of subjects)	Method for checking; Results of compliance check	Risk of bias
Abdulla and Svensson 1979 *1*	Randomized; N/A	No information on dropouts	N/A; N/A	Moderate or high
Abdulla and Svensson 1979 *2*	Non-randomized	No information on dropouts	N/A; N/A	Moderate or high
Abdulla and Suck 1998	Randomized; N/A	No information on dropouts	N/A; N/A	Moderate or high
Allan et al. 2000	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Bales et al. 1994	Non-randomized	No information on dropouts	N/A; N/A	Moderate or high
Barrie et al. 1987	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Black et al. 1988	Randomized; N/A	Lack of compliance, illness (8)	N/A; N/A	Moderate or high
Bodgen et al. 1988	Randomized, random number tables	No information on dropouts	Count of returned capsules; 87%	Moderate or high
Crouse et al. 1984	Randomized; N/A	No dropouts	Daily records;>95%	Moderate or high
Demetree et al. 1980	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Donangelo et al. 2002	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Duchateau et al. 1981	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Field et al. 1987	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Fischer et al. 1984	Randomized; N/A	No information on dropouts	N/A; N/A	Moderate or high
Freeland-Graves et al. 1981	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Gatto and Samman 1995	Randomized; N/A	No dropouts	Count of returned capsules; 98%	Moderate or high
Grider et al. 1990	Non-randomized	No information on dropouts	N/A; N/A	Moderate or high
Gupta et al. 1998	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Hayee et al. 2005	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Heckmann et al. 2005	Randomized; software program	No dropouts	N/A; N/A	Moderate or high
Hininger-Favier et al. 2007	Randomized; N/A	No information on dropouts	Count of returned capsules; ≥98%	Moderate or high
Hodkinson et al. 2007	Randomized; N/A	Reasons not reported (N/A)	N/A; N/A	Moderate or high
Hollingsworth et al. 1987	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Hunt et al. 1985	Randomized; N/A	Lack of compliance (14)	N/A; N/A	Moderate or high
Lowe et al. 2004	Non-randomized	Reasons not reported, lack of compliance (7)	N/A; N/A	Moderate or high
Lukaski et al. 1984	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Mahajan et al. 1992	Non-randomized	No dropouts	N/A; N/A	Moderate or high

Table 6. (Continued)

Studies	Randomized? Method of randomization	Reasons for dropouts by intervention group (no. of subjects)	Method for checking; Results of compliance check	Risk of bias
Medeiros et al. 1987	Randomized; N/A	Lack of compliance, illness (8)	N/A; N/A	Moderate or high
Milne et al. 1987	Non-randomized	No dropouts	Strict control; N/A	Moderate or high
O'Brien et al. 2007	Randomized; N/A	No dropouts	Count of returned capsules; >90%	Moderate or high
Pachotikarn et al. 1985	Non-randomized	Lack of compliance, mononucleosis (2)	N/A; N/A	Moderate or high
Palin et al. 1979	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Peretz et al. 1993	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Pinna et al. 2002	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Prasad et al. 1996 *3*	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Prasad et al. 1996 *4*	Non-randomized	Reasons not reported (4)	N/A; N/A	Moderate or high
Ruz et al. 1992	Non-randomized	No dropouts	Strict control; Satisfactory	Moderate or high
Samman and Roberts 1987	Randomized; N/A	Side effects (6)	N/A; N/A	Moderate or high
Shaaban et al. 2005	Randomized; N/A	Reasons not reported (N/A)	N/A; N/A	Moderate or high
Stur et al. 1996	Randomized; N/A	Side effects, personal reasons (18)	N/A; ≥80%	Moderate or high
Sullivan and Cousins 1997	Randomized; N/A	No dropouts	By consumption in the presence of a dietitian	Moderate or high
Sullivan et al. 1998	Randomized; N/A	No information on dropouts	N/A; N/A	Moderate or high
Swanson et al. 1988	Randomized; N/A	No dropouts	Count of returned capsules; Excellent	Moderate or high
Tamuta et al. 1996	Randomized; N/A	No information on dropouts	Count of returned capsules; 78%	Moderate or high
Tamuta et al. 2001	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Thomas et al. 1992	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Weismann et al. 1977	Randomized; N/A	Side effects, lack of compliance (7)	Count of returned capsules; N/A	Moderate or high
Yadrick et al. 1989	Non-randomized	No information on dropouts	N/A; N/A	Moderate or high

Abbreviations: N/A, no available data; *1*, study 1.; *2*, study 2.; *3*, depletion study; *4*, supplementation study.

Low risk of bias meant that the study was randomized, the randomization method was at least partially described, reasons for and numbers of dropouts were stated (or there were no dropouts), and the method used to assess compliance and some assessment of compliance were reported. All other studies were considered at moderate or high risk of bias.

Table 7. Primary analyses (the greatest duration and the greatest supplementation dose) for each of the identified biomarkers for supplementation with zinc and zinc depletion

Biomarker	No. of studies (no. of included participants)	Pooled effect size, MD (95%CI)	Heterogeneity I^2 (%)	Appears effective as a biomarker?
Plasma Zn (μmol/L)	50 (1454)	2.88 [2.24, 3.51]	93.6	Yes
Urinary Zn (mmol/mol creatinine) *supplementation*	5 (373)	0.31 [0.20, 0.43]	0	Yes
Urinary Zn (μmol/day) *depletion*	4 (30)	3.89 [1.01, 6.76]	92.9	Unclear
Erythrocyte Zn (μmol/L)	7 (537)	2.20 [-4.58, 8.98]	0	No
Platelet Zn (nmol/10^9 cells)	5 (105)	0.09 [-1.12, 1.30]	76.0	No
Plasma alkaline phosphatase (IU/L)	6 (410)	4.14 [-2.38, 10.65]	56.6	No
Mononuclear cell Zn (μmol/10^{10} cells)	5 (95)	-0.05 [-0.21, 0.11]	37.7	No
Polymorphonuclear cell Zn (μmol/10^{10} cells)	6 (101)	0.05 [-0.13, 0.22]	83.3	No
Aminolevulinic acid dehydratase (IU/L RBC)	2 (19)	7.88 [-7.90, 23.66]	89.4	Unclear
Erythrocyte metallothionein (μg MT/g protein) *supplementation*	2 (25)	121.82 [-22.65, 266.29]	90.7	Unclear
Erythrocyte metallothionenin (nmol/g protein) *depletion*	1 (5)	0.30 [-0.43, 1.03]	N/A	Unclear
Monocyte metallothionein cDNA (pg cDNA/ng RNA)	2 (40)	1.02 [0.48, 1.56]	0	Unclear
Salivary-sediment Zn (μmol/g dry wt)	2 (14)	0.27 [-0.07, 0.60]	N/A	Unclear
Saliva Zn (mg/dL)	1 (50)	2.82 [-2.67; 8.31]	N/A	Unclear
Mixed saliva Zn (μmol/L)	1 (7)	-0.73 [-2.49, 1.03]	N/A	Unclear
Plasma extracellular superoxide dismutase (IU/mL)	1 (52)	0.50 [-1.46, 2.46]	N/A	Unclear
Plasma 5'-nucleotidase (Shinowara Units)	1 (15)	1.75 [0.54, 2.96]	N/A	Unclear
Lymphocyte ecto-5'-nucleotidase (nmol/hr/10^6 cells)	1 (6)	-0.60 [-3.91, 2.71]	N/A	Unclear
T lymphocyte metallothionein-2A mRNA (fg MT-2A mRNA/pg β-actin mRNA)	1 (7)	6.60 [-1.77, 14.97]	N/A	Unclear
Hair Zn (ppm)	3 (93)	13.24 [11.91, 14.56]	0	Yes
Nail Zn (ppm)	1 (60)	24.10 [4.69, 43.51]	N/A	Unclear
Plasma Zn flux (mmol/day)	1 (5)	3.74 [2.42, 5.06]	N/A	Unclear
Endogenous Zn excretion (μmol/day)	1 (5)	36.70 [33.96, 39.44]	N/A	Unclear
Exchangeable Zn pool (mmol)	1 (5)	0.92 [0.27, 1.57]	N/A	Unclear
Feces Zn (μmol/day)	1 (5)	60.39 [57.00, 63.78]	N/A	Unclear
Neutrophil Zn (μg/10^{10} cells)	3 (26)	7.44 [-15.71, 30.58]	95.0	Unclear
Lymphocyte Zn (μmol /10^{10} cells)	3 (18)	-0.36 [-1.61, 0.90]	99.7	Unclear
Plasma angiotensin-converting enzyme (IU/L)	1 (5)	-19.40 [-38.34, -0.46]	N/A	Unclear
Carbonic anhydrase (IU/g Hgb)	1 (5)	-0.10 [-0.89, 0.69]	N/A	Unclear
Neutrophil alkaline phosphatase (nmol product/h/mg protein)	1 (15)	-122.80 [-294.85, 49.25]	N/A	Unclear
Neutrophil α-D-mannosidase (nmol product/h/mg protein)	1 (15)	-5.30 [-58.75, 48.15]	N/A	Unclear
Erythrocyte membrane Zn (μmol/g protein)	1 (15)	0.05 [-0.11, 0.21]	N/A	Unclear
Erythrocyte membrane alkaline phosphatase (nmol product/min/mg protein)	1 (15)	0.15 [-0.04, 0.34]	N/A	Unclear
Erythrocyte membrane neutral phosphatase (nmol product/min/mg protein)	1 (15)	0.00 [-0.15, 0.15]	N/A	Unclear

Abbreviations: N/A, no available data; MD, mean difference; CI, confidence interval.

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 contributing data, and 3) ≥50 participants contributing data in the intervention and control arm. To claim that a biomarker was ineffective, 3 conditions had to be met: 1) lack of statistical significance within a forest plot (95% CI included 0 or P ≥0.05); 2) ≥3 trials contributing data; 3) ≥50 participants contributing data in the intervention and control arm.

The summary of the subgroup analysis of the response of plasma zinc concentration to zinc supplementation and depletion is given in **Table 8**. The data included in this analysis were mostly collected from studies in adults and the elderly. The response of plasma zinc concentration to intervention was significant in adults, the elderly, pregnant and lactating women, men, women, mixed-sex groups and in those with a low or moderate zinc status at baseline, but there were insufficient data to draw firm conclusions on children and adolescents or on postmenopausal women. Data from 2 studies suggest that individuals with high baseline status do not respond to supplementation, but further studies are required. Sulfate, gluconate, and acetate all elicited a significant response, although there were only 2 studies of acetate. Subgroup analysis revealed a significant fall in plasma zinc concentration in response to marginally depleted diet; all levels of zinc supplementation resulted in a significant increase in plasma zinc concentration and the MD in plasma zinc concentration increased in a dose-dependent manner (**Figure 4**). Overall, plasma zinc appears to be a good marker of zinc status in all subgroupings for which we have sufficient studies to judge.

3.3.3.2. Urinary zinc excretion

Data on the response of urinary zinc excretion to changes in dietary intake were extracted from 5 supplementation and 4 depletion studies, comprising 6 studies in adults (47 individuals from supplementation studies and 25 individuals from depletion studies), 2 studies in elderly people (both supplemented, with a total of 326 participants), and one depletion study in postmenopausal women (5 participants). However, the units used for supplementation studies (mmol/mol creatinine) differed from those in the depletion studies (μmol/day), so only the supplementation studies could be pooled. Primary analysis (highest dose arm and longest duration for each included study) of the supplementation studies revealed a significant effect of zinc intake on urinary zinc excretion (MD: 0.31 mmol/mol creatinine; 95% CI: 0.20, 0.43) without important heterogeneity (I^2 = 0%) and was supported by depletion studies (MD: 3.89 μmol/day; 95% CI: 1.01, 6.76; I^2 = 93%) (**Table 7**).

Table 8: Subgroup analysis of the results of the systematic review of data on changes in plasma or serum zinc after supplementation with zinc and zinc depletion

Analysis	Pooled effect size, MD (95% CI)	Study design			Heterogeneity I^2	Biomarker useful?
		RCT supplementation	B/A supplementation	B/A depletion		
	µmol/L	*no. of studies included (no. of participants)*			*%*	
All studies (primary outcome)	2.88 [2.24, 3.51]	25 (1157)	15 (218)	10 (79)	93.6	Yes
Children and adolescents	0.22 [-4.34, 4.78]	1 (17)	N/A	N/A	N/A	Unclear
Pregnancy and lactation	0.37 [0.32, 0.43]	4 (325)	N/A	N/A	0	Yes
Adults	3.50 [2.47, 4.54]	15 (311)	12 (197)	8 (59)	91.5	Yes
Post-menopausal women	-1.00 [-2.72, 0.72]	N/A	N/A	1 (5)	N/A	Unclear
The elderly	2.78 [1.28, 4.28]	5 (504)	3 (21)	1 (15)	89.7	Yes
Males	3.23 [2.13, 4.33]	10 (189)	3 (51)	7 (53)	83.8	Yes
Mixed	2.98 [1.47, 4.49]	10 (623)	6 (799)	1 (15)	95.3	Yes
Females	1.76 [0.98, 2.53]	5 (345)	6 (88)	2 (11)	84.2	Yes
Low status at baseline[1]	0.43 [0.07, 0.79]	5 (324)	1 (11)	N/A	48.6	Yes
Moderate status at baseline[1]	3.24 [2.36, 4.11]	18 (789)	14 (207)	10 (79)	91.2	Yes
High status at baseline[1]	2.66 [-1.01, 6.32]	2 (44)	N/A	N/A	0	Unclear
Zinc sulphate	3.55 [2.36, 4.74]	14 (564)	9 (143)	X	94.7	Yes
Zinc gluconate	2.47 [1.63, 3.32]	9 (495)	6 (75)	X	78.3	Yes
Zinc acetate	3.95 [2.96, 4.95]	2 (98)	N/A	X	0	Unclear
<1 mg Zn/day	8.70 [7.05, 10.35]	X	X	1 (5)	N/A	Unclear
1 to 2.9 mg Zn/day	-1.00 [-2.72, 0.72]	X	X	1 (5)	N/A	Unclear
3 to 5 mg Zn/day	1.59 [0.28, 2.89]	X	X	8 (69)	89.1	Yes
15 to 25 mg Zn/day	0.7 [0.38, 1.03]	9 (771)	2 (26)	X	49.3	Yes
26 to 50 mg Zn/day	2.61 [1.88, 3.34]	13 (621)	8 (91)	X	76.3	Yes
51 to 100 mg Zn/day	4.21 [3.15, 5.26]	4 (110)	2 (13)	X	0	Yes
101 to 150 mg Zn/day	4.94 [2.18, 7.70]	4 (119)	7 (128)	X	91.4	Yes

Abbreviations: X, this design category is not applicable to the subgroup analysis; RCT, randomized controlled trial; B/A, before/after trial; MD, mean difference; CI, confidence interval; N/A, no available data.

[1], The normal range of plasma zinc concentration is 11–19 µmol/L (Smith et al. 1979).

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 contributing data, and 3) ≥50 participants contributing data in the intervention arm and control arm. To claim that a biomarker was ineffective, 3 conditions had to be met: 1) lack of statistical significance within a forest plot (95% CI included 0 or P ≥0.05); 2) ≥3 trials contributing data; 3) ≥50 participants contributing data in the intervention arm and control arm.

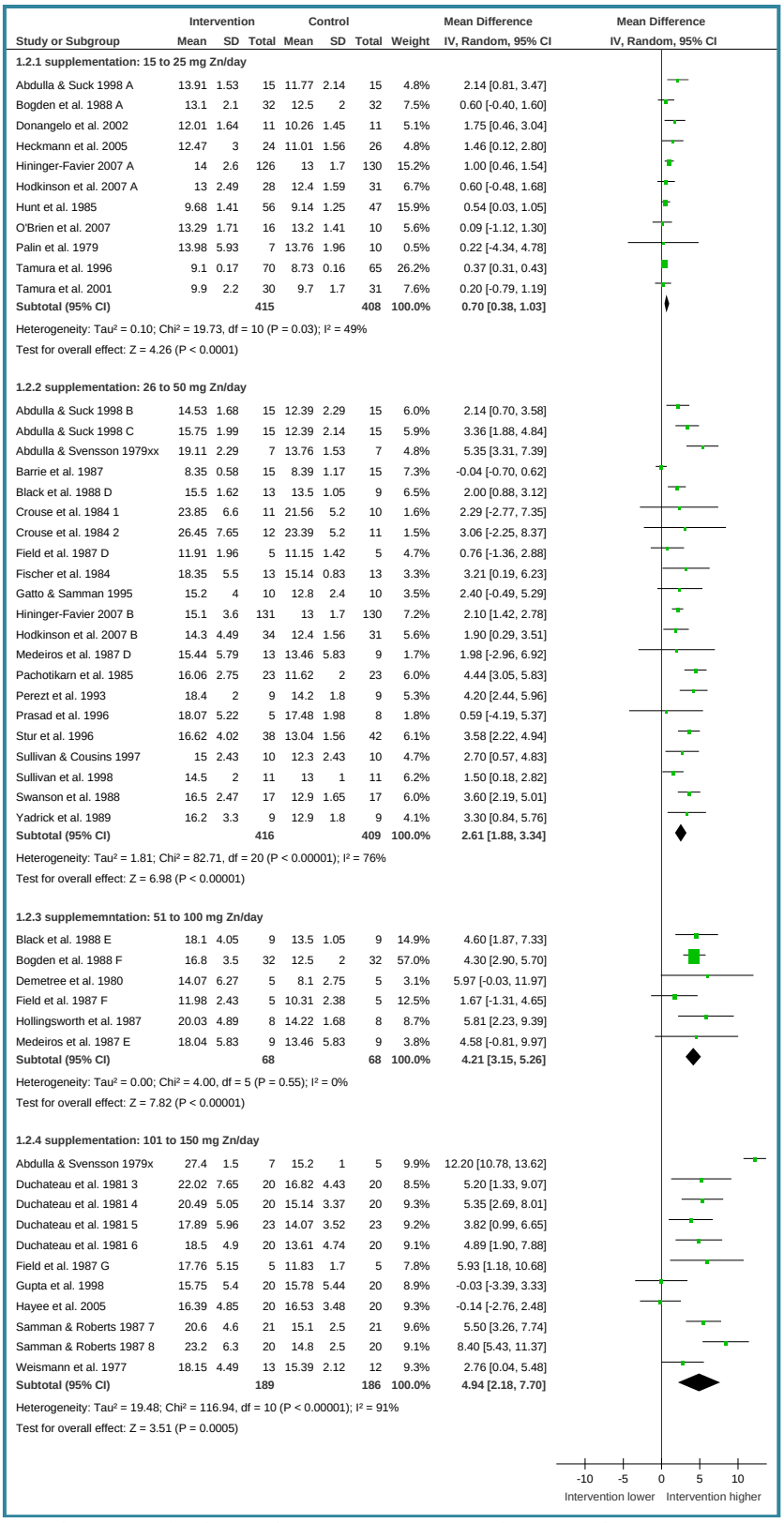

Figure 4: Secondary analysis of the response of plasma zinc concentration (µmol/L) to zinc supplementation with subgrouping by dose (mg/day)

Specification of the groups as represented in the original articles: x, study 1; xx, study 2; A, 15-mg Zn/day group; B, 30-mg Zn/day group; C, 45-mg Zn/day group; D, 50-mg Zn/day group; E, 75-mg Zn/day group; F, 100-mg Zn/day group; G, 150-mg Zn/day group; 1, endurance-trained male group; 2, sedentary male group; 3, male group (20–40 yrs); 4, female group (20–40 yrs); 5, female group (20–40 yrs + oral contraceptive); 6, female group (40–50 yrs); 7, male group; 8, female group.

Significant responses were recorded in studies of adults, the elderly, males, mixed-sex groups, females and in those with a low or moderate zinc status at baseline; however, there were enough studies (≥3) and participants (≥50) to declare urinary zinc a useful marker of zinc status only in those with moderate status at baseline (**Table 9**). All of the supplementation trials that measured urinary zinc concentration used zinc gluconate, with 3 data sets (326 participants) in the 15–25 mg Zn/day range, 4 data sets (370 participants) in the 26–50 mg Zn/day range, and 2 data sets (36 participants) in the 51–100 mg Zn/day range. A statistically significant increase in urinary zinc excretion was seen in response to all 3 dose ranges, but only the 15–25 and the 26–50 mg Zn/day subgroups included enough studies to declare the marker useful. The data do suggest a dose response (**Figure 5**).

Table 9: Subgroup analysis of the results of the systematic review of data on changes in urinary zinc after supplementation with zinc

		Study design			
Analysis	**Pooled effect size MD (95% CI)**	**RCTs**	**B/A**	**Heterogeneity I^2**	**Biomarker useful?**
	mmol/mol creatinine	*no. of studies (no. of participants)*		*%*	
All studies (primary outcome)	0.31 [0.20, 0.43]	4 (362)	1 (11)	0	Yes
Adults	0.33 [0.10, 0.56]	2 (36)	1 (11)	0	Unclear
The elderly	0.31 [0.18, 0.44]	2 (326)	N/A	0	Unclear
Males	0.61 [0.07, 1.15]	2 (36)	N/A	0	Unclear
Mixed	0.31 [0.18, 0.44]	2 (326)	N/A	0	Unclear
Females	0.27 [0.02, 0.52]	N/A	1 (11)	N/A	Unclear
Low status at baseline	0.27 [0.02, 0.52]	N/A	1 (11)	N/A	Unclear
Moderate status at baseline	0.33 [0.20, 0.45]	4 (362)	N/A	0	Yes
Zinc gluconate	0.31 [0.20, 0.43]	4 (362)	1 (11)	0	Yes
15 to 25 mg Zn/day	0.15 [0.04, 0.25]	2 (315)	1 (11)	0	Yes
26 to 50 mg Zn/day	0.32 [0.20, 0.45]	4 (370)	N/A	0	Yes
51 to 100 mg Zn/day	0.62 [0.07, 1.16]	2 (36)	N/A	0	Unclear

See details below **Table 8**.

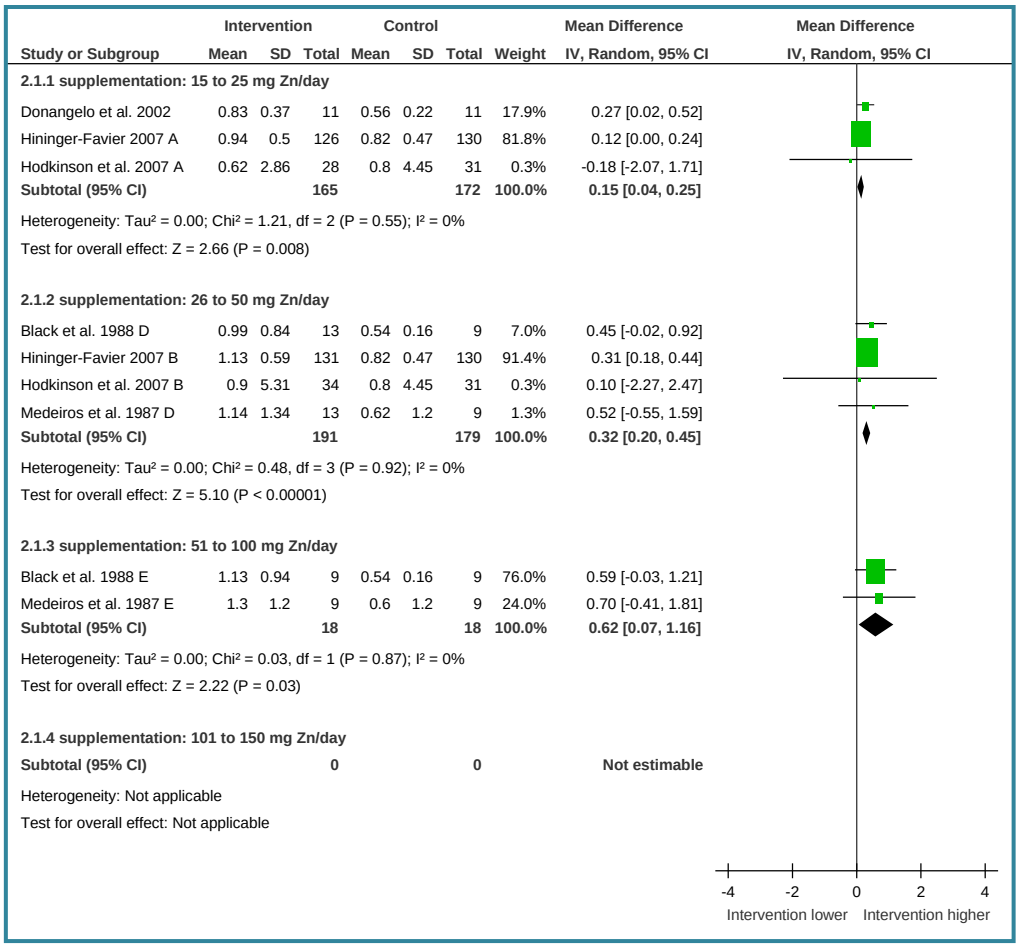

Figure 5: Secondary analysis of the response of urinary zinc excretion to zinc supplementation (mmol/mol creatinine) with subgrouping by dose (mg/day)

Specification of the groups as represented in the original articles: A, 15-mg Zn/day group; B, 30-mg Zn/day group; D, 50-mg Zn/day group; E, 75-mg Zn/day group.

3.3.3.3. Erythrocyte zinc concentration

A total of 5 supplementation and 2 depletion studies reported values for erythrocyte zinc concentration. Neither primary analysis (MD: 2.20 µmol/L; 95% CI: -4.58, 8.98; I^2 = 0%) nor any individual study suggested a response of this biomarker to changes in zinc intake. For more subgroup analysis details see **Table 10**. These data suggest that erythrocyte zinc concentration is not a useful marker of zinc status

3.3.3.4. Platelet zinc concentration

Five studies that measured platelet zinc concentration (2 supplementation RCTs and 3 depletion B/A studies) were identified. The primary analysis, combining data from supplementation and depletion studies, did not reveal a significant response to changes in dietary zinc intake (MD: 0.09 nmol/10^9 cells; 95% CI: -1.12, 1.30; I^2 = 76%) (**Table 7**).

Table 10: Subgroup analysis of the results of the systematic review of data on changes in erythrocyte zinc after supplementation with zinc and zinc depletion

Analysis	Pooled effect size MD (95% CI)	Study design		Heterogeneity I^2	Biomarker useful?
		RCT supplementation	B/A depletion		
	µmol/L	*no. of studies (no. of participants)*		*%*	
All studies (primary outc ome)	2.20 [-4.58, 8.98]	5 (527)	2 (10)	0	No
Pregnancy and lactation	3.80 [-8.51, 16.10]	2 (186)	N/A	0	Unclear
Adults	-1.36 [-13.34, 10.62]	1 (15)	1 (5)	0	Unclear
Post-menopausal women	-6.00 [-28.49, 16.49]	N/A	1 (5)	N/A	Unclear
The elderly	5.30 [-12.49, 23.09]	2 (326)	N/A	39.2	Unclear
Males	-2.40 [-22.36, 17.56]	N/A	1 (5)	N/A	Unclear
Mixed	3.62 [-6.82, 14.07]	3 (341)	N/A	11.6	No
Females	1.54 [-9.26, 12.33]	2 (186)	1 (5)	0	No
Low status at baseline	1.96 [-7.55, 11.47]	3 (201)	N/A	0	No
Moderate status at baseline	2.45 [-7.22, 12.12]	2 (326)	2 (10)	0	No
Zinc sulphate	3.80 [-8.51, 16.10]	2 (186)	X	0	Unclear
Zinc gluconate	3.62 [-6.82, 14.07]	3 (341)	X	11.6	No
1 to 2.9 mg Zn/day	-6.00 [-28.49, 16.49]	X	1 (5)	N/A	Unclear
3 to 5 mg Zn/day	-2.40 [-22.36, 17.56]	X	1 (5)	N/A	Unclear
15 to 25 mg Zn/day	25.17 [-6.01, 56.36]	4 (501)	X	91.2	No
26 to 50 mg Zn/day	3.62 [-6.82, 14.07]	3 (341)	X	11.6	No

See details below **Table 8**.

3.3.3.5. Plasma alkaline phosphatase activity

Six studies investigating the response of plasma alkaline phosphatase activity to changes in zinc intake were included: 3 supplementation (RCTs) and 3 depletion (B/A) studies. Overall, the primary analysis, combining the supplementation and depletion trials, revealed no significant effect of zinc intakes on plasma alkaline phosphatase activity (MD: 4.14 IU/L; 95% CI: -2.38, 10.65; I^2 = 56.6%), which suggests that this is not a useful zinc biomarker. Subgrouping also did not reveal any groups in which this was a clearly useful biomarker of zinc status (**Table 11**).

3.3.3.6. Mononuclear cell zinc concentration

Five studies, including 95 participants, assessed the effect of the change in zinc intake on mononuclear cell zinc concentration. Pooling these 5 studies suggested that this is not a useful biomarker of zinc status (MD: -0.05 µmol/10^{10} cells; 95% CI: -0.21, 0.11; I^2 = 38%) (**Table 7**).

Table 11: Subgroup analysis of the results of the systematic review of data on changes in plasma alkaline phosphatase activity after supplementation with zinc and zinc depletion

Analysis	Pooled effect size MD (95% CI)	Study design		Heterogeneity I^2	Biomarker useful?
		RCT supplementation	B/A depletion		
	IU/L	*no. of studies (no. of participants)*		*%*	
All studies (primary outcome)	4.14 [-2.38, 10.65]	3 (385)	3 (25)	56.6	No
Pregnancy and lactation	12.00 [-11.81, 35.81]	1 (60)	N/A	N/A	Unclear
Adults	21.80 [8.91, 34 .69]	N/A	1 (5)	N/A	Unclear
Post-menopausal women	-5.20 [-16.82, 6.42]	N/A	1 (5)	N/A	Unclear
The elderly	1.22 [-2.39, 4.83]	2 (325)	1 (15)	0	No
Males	21.80 [8.91, 34.69]	N/A	1 (5)	N/A	Unclear
Mixed	1.22 [-2.39, 4.83]	2 (325)	1 (15)	0	No
Females	0.13 [-15.46, 15.72]	1 (60)	1 (5)	38.3	Unclear
Low status at baseline	12.00 [-11.81, 35.81]	1 (60)	N/A	N/A	Unclear
Moderate status at baseline	3.68 [-3.26, 10.63]	2 (325)	3 (25)	63.2	No
Zinc sulphate	12.00 [-11.81, 35.81]	1 (60)	X	N/A	Unclear
Zinc gluconate	0.90 [-3.47, 5.27]	1 (261)	X	N/A	Unclear
Zinc acetate	6.00 [-21.65, 33.65]	1 (64)	X	N/A	Unclear
<1 mg Zn/day	21.80 [8.91, 34.69]	X	1 (5)	N/A	Unclear
1 to 2.9 mg Zn/day	-5.20 [-16.82, 6.42]	X	1 (5)	N/A	Unclear
3 to 5 mg Zn/day	1.67 [-4.92, 8.26]	X	1 (15)	N/A	Unclear
15 to 25 mg Zn/day	4.43 [-1.60, 10.45]	3 (380)	X	0	No
26 to 50 mg Zn/day	0.90 [-3.47, 5.27]	1 (261)	X	N/A	Unclear
51 to 100 mg Zn/day	6.00 [-21.65, 33.65]	1 (64)	X	N/A	Unclear

See details below **Table 8**.

3.3.3.7. Polymorphonuclear cell zinc concentration

Five supplementation trials and one depletion study that measured polymorphonuclear cells as a biomarker of zinc status were identified. Population groups represented in the studies included adults (2 studies) and elderly persons (4 studies). Individual studies were variable, which suggests both significantly positive and negative effects of increased zinc status on polymorphonuclear cell zinc concentration. Neither primary (MD: 0.05 µmol/10^{10} cells; 95% CI: -0.13, 0.22; I^2 = 83.3) nor subgroup analyses (**Table 12**) revealed any significant response of this biomarker to changes in zinc intake. Our data suggest that polymorphonuclear cell zinc concentration is not a useful marker of zinc status.

Table 12: Subgroup analysis of the results of the systematic review of data on changes in polymorphonuclear cell zinc after supplementation with zinc and zinc depletion

Analysis	Pooled effect size WMD (95% CI)	Study design			Heterogeneity I^2	Biomarker useful?
		RCT supplementation	B/A supplementation	B/A depletion		
	µmol/10^{10} cells	*no. of studies (no. of participants)*			*%*	
All studies (primary outcome)	0.05 [-0.13, 0.22]	2 (74)	3 (23)	1 (4)	83.3	No
Adults	0.17 [0.06, 0.29]	N/A	1 (9)	1 (4)	32.6	Unclear
The elderly	-0.05 [-0.35, 0.26]	2 (74)	2 (14)	N/A	88.0	No
Males	0.21 [0.13, 0.30]	N/A	1 (9)	1 (4)	0	Unclear
Mixed	0.03 [-0.22, 0.29]	2 (74)	1 (9)	N/A	81.4	No
Females	-0.56 [-0.99, -0.13]	N/A	1 (5)	N/A	N/A	Unclear
Moderate status at baseline	0.05 [-0.13, 0.22]	2 (74)	3 (23)	1 (4)	83.3	No
Zinc sulphate	-0.56 [-0.99, -0.13]	N/A	1 (5)	X	N/A	Unclear
Zinc gluconate	0.16 [0.07, 0.25]	N/A	2 (18)	X	0	Unclear
Zinc acetate	0.01 [-0.44, 0.46]	2 (74)	N/A	X	83.8	Unclear
3 to 5 mg Zn/day	0.23 [0.10, 0.36]	X	X	1 (4)	N/A	Unclear
15 to 25 mg Zn/day	-0.03 [-0.21, 0.15]	1 (46)	N/A	X	N/A	Unclear
26 to 50 mg Zn/day	0.14 [0.02, 0.26]	1 (28)	3 (23)	X	34.7	Yes
51 to 100 mg Zn/day	-0.20 [-0.35, -0.05]	1 (46)	1 (5)	X	N/A	Unclear
101 to 150 mg Zn/day	0.00 [-0.43, 0.43]	N/A	1 (5)	X	N/A	Unclear

See details below **Table 8**.

3.3.3.8. Hair zinc concentration

Data were analyzed from 3 RCT supplementation studies, which included a total of 93 adult participants with either low or moderate baseline status and intakes in the ranges of 15–25, 26–50, and 51–100 mg Zn/day. Primary analysis revealed that hair zinc concentration was significantly elevated after supplementation (MD: 13.24 ppm; 95% CI: 11.91, 14.56; I^2 = 0%) (**Figure 7**). Insufficient data precluded subgroup analyses.

3.3.3.9. Other potential markers

We found at least one study each to assess the effects of zinc supplementation or depletion on the following potential zinc biomarkers: aminolevulinic acid dehydratase, erythrocyte metallothionein, monocyte metallothionein cDNA, salivary-sediment zinc, salivary zinc, mixed-saliva zinc, plasma extracellular superoxide dismutase, plasma 5'-nucleotidase, lymphocyte ecto-5'-nucleotidase, T lymphocyte metallothionein -2A mRNA, nail zinc, plasma zinc flux, endogenous zinc excretion, exchangeable zinc pool, fecal zinc, neutrophil zinc, lymphocyte zinc, plasma angiotensin-converting enzyme, carbonic anhydrase, neutrophil alkaline phosphatase, neutrophil α-D-mannosidase, erythrocyte membrane zinc, erythrocyte membrane alkaline phosphatase, and erythrocyte membrane neutral phosphatase. However, there were not enough eligible studies of these markers to allow us to decide whether they were effective markers of zinc status (**Table 7**).

3.4. Discussion

Data were extracted and analyzed on 32 potential biomarkers from 46 publications. Plasma zinc concentration responded to dietary manipulation in adults, women, men, pregnant and lactating women, the elderly, and those at low and moderate baseline zinc status and in both depletion and supplementation studies. Urinary zinc excretion also appeared to respond to change in zinc status for all groups for which we had data, but with fewer studies there were fewer subgroupings with enough studies to make a clear decision about urinary zinc response. Hair zinc concentration also responded to zinc supplementation, but there were insufficient studies to assess in which subgroups these may be effective markers. For platelet, polymorphonuclear

cell, mononuclear cell, and erythrocyte zinc concentration and alkaline phosphatase activity, there were sufficient data to judge them as likely to be ineffective as biomarkers of zinc status.

The majority of studies identified and included in this review were zinc supplementation studies that covered a broad range of zinc intake levels, which ranged from intakes that could be achieved through diet alone to pharmacologic doses at values of >10 times the European and US dietary recommendations. Fewer zinc depletion studies were identified, and participant numbers were low compared with the supplementation trials because of the practical and ethical difficulties of conducting depletion trials.

The search for a reliable indicator for zinc has been problematic because the effective regulation of zinc homeostasis buffers the functional response to dietary deficiency and excess. The total amount of zinc present in the human body ranges from 1.5 to 2.5 mg, most of which is found intracellularly within skeletal muscle tissue (57%), bone (29%), and other tissues, including skin and organs (Jackson 1989). The zinc located within these tissues has a relatively slow turnover rate and is not readily responsive to changes in dietary zinc intake. Kinetic studies suggest that only a small proportion of total body zinc (≈10%) represents the ‘‘functional pool’’ (King 1990; Foster et al. 1979; Miller et al. 1994), which is composed of zinc, located within the liver and other tissues, that exchanges rapidly with the plasma. When this functional pool is depleted, zinc deficiency ensues (King 1990).

All studies included in the analysis were undertaken in apparently healthy individuals. It is well established that plasma zinc concentration can fall in response to factors unrelated to zinc status or dietary zinc intake, including infection, inflammation, stress, or trauma. Conversely, tissue catabolism during starvation can release zinc into the circulation, causing a transient increase in circulating zinc levels. Furthermore, postprandial falls in plasma zinc concentration of ≤22% have been reported (Hambidge et al. 1989; Lowe et al. 1998). Clearly, the interpretation of plasma zinc concentration requires knowledge of all of these possible confounders.

This review highlights a number of gaps in the field of zinc research. More high-quality studies are required to assess the effects of most potential zinc biomarkers

and in a variety of populations. Ideally, these would be highly controlled studies or RCTs, with clearly presented methodology indicating that they are at low risk of bias. Studies need to describe their randomization methodology, clearly describe the numbers of dropouts or exclusions and the reasons for their cessation of inclusion, check compliance, and report the results of such checks. Further research is needed to evaluate potentially useful biomarkers, including enzymes and other zinc-binding proteins that were measured in only 1 or 2 studies and for which conclusions were unable to be drawn. Although stable isotope studies are expensive, technically demanding, and unsuitable for large population studies, they may be useful tools for evaluating more accessible potential biomarkers and to develop new biomarkers. There were some notable gaps in the availability of data from certain population groups; in particular, there was a complete absence of data regarding infants and immigrant population groups and a paucity of studies of zinc status in adolescents.

4. Assessing potential biomarkers of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review

4.1. Introduction

Long-chain polyunsaturated fatty acids (LCPUFAs) are important components of membrane lipids in all tissues. The most important of them are the n-6 (omega-6) essential fatty acid, linoleic acid (C18:2n-6, LA), and the n-3 (omega-3) essential fatty acid, α-linolenic acid (C18:3n-3, ALA), as well as their longer-chain metabolites, arachidonic acid (C20:4n-6, AA), eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) (**Figure 6**). LCPUFAs increase the fluidity, flexibility and permeability of cell membranes, the number of receptors and the affinity of receptors to their substrates: hormones, growth factors, and proteins. Moreover, some LCPUFAs are also precursors of several second messengers (Das 2006). N-6 fatty acids, mainly AA and dihomo-γ-linolenic acid (C20:3n-6), are predominantly precursors of proinflammatoric prostaglandins, thromboxans and leucotriens, while n-3 fatty acids, mainly EPA, are precursors of antiinflammatory eicosanoids. AA and DHA are concentrated in the central nervous system, as well as in the retina, heart and skeletal muscle, and play an important role in the maintenance of normal development and normal neural functions (Das 2006).

Mammals, including humans, cannot synthesize essential fatty acids; therefore they have to consume them in the diet from dietary sources. The enzymatic reactions of Δ-6- and Δ-5 desaturation and elongation of essential fatty acids convert LA to AA and ALA to EPA. While AA is the major product of the n-6 fatty acid family, EPA is an intermediate, which needs further elongation, Δ-6 desaturation and peroxysomal β-oxidation to be converted into the biologically most important product, DHA (**Figure 6**) (Infante and Huszagh 1997).

Vegetables are good sources of essential fatty acids; however, their preformed long-chain metabolites are found mainly in animal foods. Food products of terrestrial animals are rich in n-6 fatty acids, whereas sea fishes are rich in n-3 fatty acids (Kris-Etherton et al. 2007). Although oily fish and various other seafoods are excellent dietary sources of EPA and DHA, dietary intake of n-3 LCPUFA (including DHA) in

the United States is only ≈110 mg/day in women and ≈170 mg/day in men (Gebauer et al. 2006). However, various dietary supplements containing several hundred milligrams of n-3 LCPUFAs per dose are widely available, so it is relatively easy to achieve a 10-fold increase in daily n-3 LCPUFA intake.

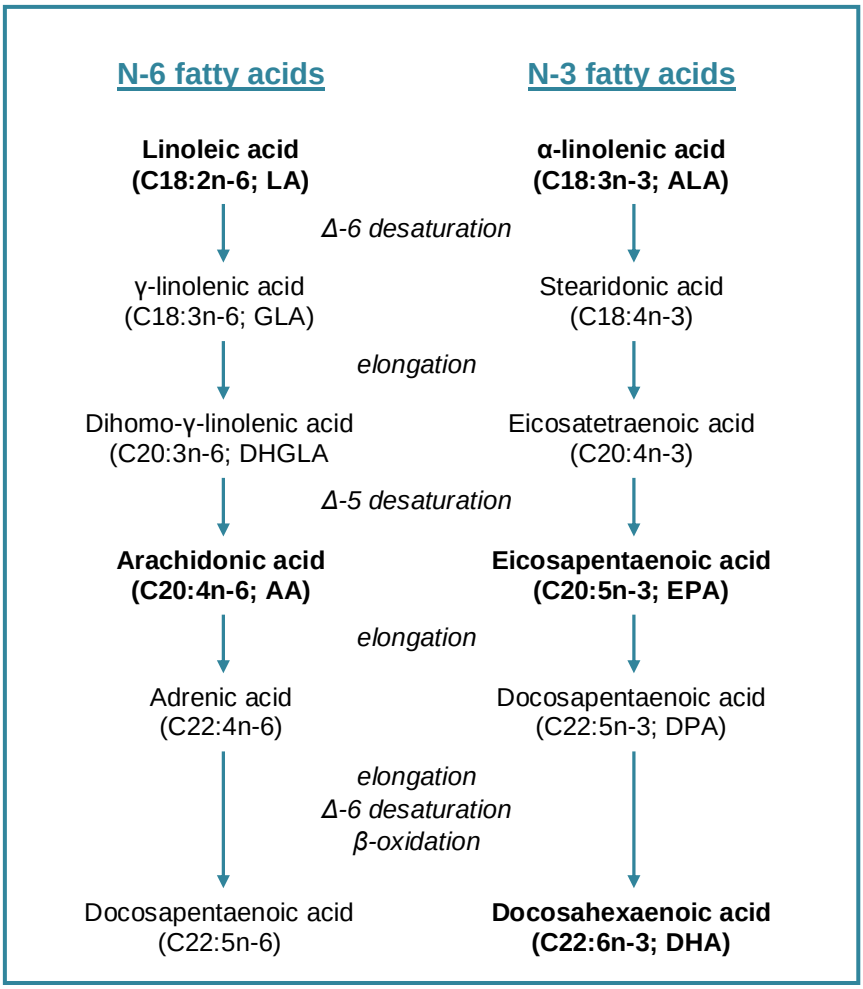

Figure 6: Metabolism of the n-6 and n-3 fatty acids

Adopted and modified from Giovannini et al. 1995.

Many clinical studies have assessed the effect of n-3 LCPUFA supplementation in restoring health and maintaining well-being, and the medical relevance of the topic is underpinned by ≥15 Cochrane Database Systematic Reviews of n-3 LCPUFA supplementation. These systematic reviews assessed the affect of dietary n-3 LCPUFA supplementation on preterm (Schulzke et al. 2011) and full-term infants (Simmer et al. 2008) as well as the potential role of n-3 LCPUFA adjuvant therapy in type 2 diabetes mellitus (Hartweg et al. 2008), cardiovascular disease (Hooper et al. 2004), cancer cachexia (Dewey et al. 2007), Crohn's disease (Turner et al. 2009), ulcerative colitis (Turner et al. 2007; De Ley et al. 2007), cystic fibrosis (Oliver et al.

2007), intermittent claudication (Sommerfield et al. 2007), asthma (Thien et al. 2002), schizophrenia (Irving et al. 2006), dementia (Lim et al. 2006), bipolar disorder (Montgomery et al. 2008), and multiple sclerosis (Farinotti et al. 2007) and in kidney transplant recipients (Lim et al. 2007). The majority of these reviews concluded that, although there is some indication of the beneficial effect of n-3 LCPUFA supplementation, further studies are needed to establish efficacy of intervention.

One of the practical difficulties of designing and carrying out clinical trials investigating the effect of n-3 LCPUFA supplementation is the lack of a generally accepted biomarker that reflects increased n-3 LCPUFA status in response to enhanced dietary intake. This link is essential to deciding whether the negative outcome of a controlled trial, ie, lack of functional change in response to supplementation, can be related to either the basic hypothesis on clinical effect, lack of subject compliance, or the inability of dietary intervention to evoke changes in the fatty acid composition of relevant biological compartments within the body. It is even more important in epidemiologic studies assessing health effects of n-3 LCPUFA status in populations over long periods to understand which biomarkers truly reflect n-3 LCPUFA status.

4.2. Methods

4.2.1. Inclusion criteria

To be included in the review, a study needed to meet all of the following characteristics: 1) an intervention study in humans (RCT, controlled clinical trial, or B/A study); 2) reported n-3 LCPUFA status at baseline and after supplementation; 3) involved supplementation with marine oils (fish, whale, or seal oil), seafoods (caviar or oily fish), single cell oils, or DHA-enriched eggs; 4) minimum duration of supplementation of 2 wk; 5) described a daily dose of n-3 LCPUFA supplement; and 6) included participants who were healthy individuals.

4.2.2. Search strategy

Ovid MEDLINE (www.ovid.com), EMBASE (Ovid) (www.ovid.com), and the Cochrane Library CENTRAL database (www.thecochranelibrary.com) were searched from inception to September 2007 for intervention studies of n-3 LCPUFA using text terms

with appropriate truncation and relevant indexing terms. The search was in the form, [n-3 LCPUFA terms] and [intervention study terms] and [human studies]. The OVID Medline search strategy can be found in **Table 13**, and the strategies for the other databases were based on the relevant OVID Medline strategy. We did not apply any language restriction.

An Ovid MEDLINE search was conducted for reviews of n-3 LCPUFA status assessment. Ten reviews were collected in full text and the reference lists checked (Arab 2003; Lands 1995; Baylin and Campos 2006; Katan et al. 1991; Fleith and Clandinin 2005, Carlson 1994; Innis 1992; Cantwell 2000; Morris 2003; Arterburn et al. 2006). Studies that appeared to be intervention studies but that had not been already assessed for inclusion were collected.

Three experts on n-3 LCPUFAs were contacted (Carlo Agostoni, University of Milan; Berthold Koletzko, University of Munich; and Cristina Campoy, University of Granada). The aim of the review was explained and our current list of included studies forwarded with a request to suggest any additional studies they believed may have been appropriate for inclusion. These were assessed for inclusion according to the criteria listed above.

Table 13: Search strategy for Ovid MEDLINE from 1950 to September Week 2, 2007

#	Search History	Results
1	Eicosapentaenoic Acid/	2615
2	"eicosapent?enoic acid$".mp.	4224
3	EPA.mp.	5110
4	Docosahexaenoic Acids/	3135
5	"docosahex?enoic acid$".mp.	5140
6	DHA.mp.	4360
7	"docosapent?enoic acid$".mp.	393
8	DPA.mp.	1159
9	fish oils/ or cod liver oil/ or fatty acids, omega-3/	8069
10	"fish oil$".mp.	6008
11	"cod liver oil$".mp.	562
12	"icosapent?enoic acid$".mp.	22
13	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12	19650
14	randomized controlled trial.pt.	242634
15	controlled clinical trial.pt.	76223
16	randomized controlled trials.sh.	51000
17	random allocation.sh.	59065
18	double blind method.sh.	93460
19	single blind method.sh.	11340
20	14 or 15 or 16 or 17 or 18 or 19	411123
21	13 and 20	1906
22	("n-3" adj4 "FA$").mp.	4783
23	("n-3" adj4 "PUFA$").mp.	1435
24	("n3" adj4 "fatty$").mp.	64
25	("n3" adj4 "PUFA$").mp.	24
26	"omega-3".mp.	6367
27	exp fatty acids, omega-3/ or exp fish oils/	12358
28	omega3$.mp.	240
29	22 or 23 or 24 or 25 or 26 or 27 or 28	15568
30	deplet$.mp.	103076
31	29 and 30	265
32	21 or 31	2161
33	(animals not humans).sh.	3181772
34	32 not 33	1774

Abbreviations: $, truncation symbol; ?, optional wild card character; adj, adjacent operator; exp, exploded subject heading word; pt, publication type; sh, subject heading word; mp = title, original title, abstract, name of substance word, subject heading word.

4.2.3. Data collection and synthesis

Titles and abstracts were screened for inclusion by a single reviewer. The high number of items to be evaluated made duplicate assessment at this stage unfeasible. The full text of all articles collected was screened for inclusion by using an

inclusion/exclusion form completed by 2 independent reviewers. Where the 2 reviewers disagreed, the study was discussed and a consensus decision reached where possible. If this was not possible, then a third reviewer was asked to arbitrate. Data for each included study were extracted into a Microsoft Access 2003 database file (Microsoft Corp, Redmond, WA) by a single reviewer. In doubtful cases, studies were discussed with the review team before beginning full data extraction. Where necessary, units of measurement were recalculated to percentage contribution of n-3 LCPUFA to total fatty acid composition of the relevant lipid fraction (% weight/weight). The data for the given fatty acid fraction were divided into tertiles, which were used to define low, moderate, and high DHA status. If data from more than one lipid fraction were published in the same study, DHA status was defined by using plasma phospholipid fatty acid data.

We used formal inclusion/exclusion criteria and applied standard operation procedures for data extraction, validity assessment and meta-analysis (Hooper et al. 2009). Meta-analysis was carried out with Cochrane software, Review Manager version 4.2 (Cochrane Collaboration; www.cochrane.org), with random-effects model. A statistically significant result indicated that the marker was indeed responding to supplementation. Levels of the heterogeneity were noted (heterogeneity was considered significant where $P < 0.1$ on the chi-square test or $I^2 > 50\%$).

Because there was a danger of categorizing some biomarkers as ineffective when there actually was a shortage of data, such that one would not expect a statistically significant effect size on pooling, we would declare a biomarker effective (statistically significant pooled effect size; $P < 0.05$) or ineffective (statistically insignificant pooled effect size; $P \geq 0.05$) only where the pooling included ≥3 studies and ≥50 participants overall. Where there were <3 studies or <50 participants, it was stated that there were insufficient data to make a decision (Hooper et al. 2009).

4.3. Results

4.3.1. Study inclusion

The flow diagram of the literature search for this review is shown in **Figure 7**. Altogether 2733 titles and abstracts were identified via the electronic search, 255 of

them appeared to be potentially relevant, and we attempted to collect them as full-text articles. Fifteen full articles (6%) could not be collected, but 240 full-text articles were available for detailed assessment for inclusion. Finally, 41 studies (divided into 45 arms) reported in 41 publications fulfilled the inclusion criteria (**Table 14**). In the majority of cases, exclusion of articles was due to incomplete data presented; occasionally, an article did not describe an intervention or included nonhealthy subjects.

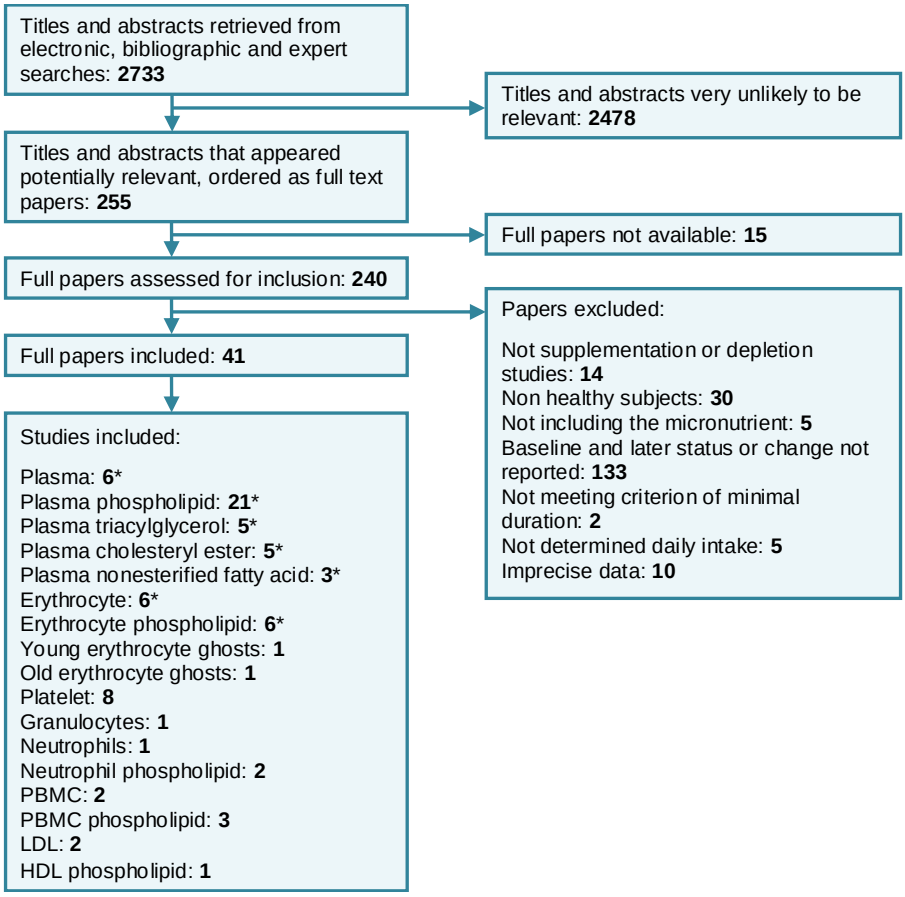

Figure 7: Flow diagram of the systematic literature search

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; PBMC, peripheral blood mononuclear cells.
*Number of studies reporting eicosapentaenoic acid values differs from the number reporting docosahexaenoic acid values. Eicosapentaenoic acid data were available as follows: plasma, 5 studies; plasma phospholipid, 16 studies; plasma triacylglycerol, 3 studies; plasma cholesteryl ester, 4 studies; plasma nonesterified fatty acid, 1 study; erythrocyte,5 studies; and erythrocyte phospholipid, 3 studies.

Table 14: Basic characteristics of the included studies

Studies	Country(s); age; gender; no. included	Short description of intervention; latest time point; no. in intervention; no. in control at latest time	Study design	Biomarkers reported
Allard et al. 1997	Canada; 20-60y; M;40	3.06 g EPA + 2.26 g DHA; 6 wk; 18; 19	RCT p	PPL
Bonaa et al. 1992	Norway; 34-60y; X;156	3.3 g EPA + 1.8 DHA; 10 wk; 72; 74	RCT p	PPL
Brady et al. 2004	UK; 35-70y; M; 29	1.47 g EPA + 1 g DHA; 6 wk; 15 + 14	B/A	Plat
Christensen et al. 1999	Denmark; 38±11y; 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 et al. 1992	Australia; adults; M; 32	1.6 g EPA + 0.32 g DHA; 4 wk; 13 + 15	B/A	NPL
Conquer and Holub 1998	Canada; 30-34y; X; 22	0.75 g DHA; 1.5 g DHA; 6 wk; 6 + 7; 6	RCT p	PPL; NEFA
Conquer et al. 1999	Canada; 29.5y; M; 20	1.3 g EPA + 1.7 g DHA; 6 wk; 9; 10	RCT p	PPL; NEFA
Damsgaard et al. 2007	Denmark; 9 mo; X; 94	0.57 g EPA + 0.38 g DHA; 12 wk; 24; 24	RCT p	E
DeLany et al. 1990	USA;19-31y; M; 15	1.13g EPA+0.7 g DHA; 4.53 g EPA+2.73 g DHA; 5 wk; 5 + 4; 5	RCT p	PPL
Dustan et al. 2004	Australia; 32.4y; F; 98	1.1 g EPA + 2.2 g DHA; 17 wk; 36; 37	RCT p	E
Dyerberg et al. 2004	Denmark; 20-60y; M;58	0.79 g EPA + 0.5 g DHA; 8 wk; 24; 26	RCT p	Plat
Engström et al. 2003	Sweden; 26-65y; X; 16	0.11 g EPA + 0.18 g DHA; 0.45 g EPA+0.39 g DHA; 3 wk; 8 + 8	B/A	PPL
Hagve et al. 1993	Norway; 19-22y; F; 16	3.3 g EPA + 1.8 g DHA; 4 wk; 8; 8	RCT p	EPL
Helland et al. 2006	Norway; 19- 35y; F; 341	0.8 g EPA + 1.18 g DHA; 17 wk; 158; 151	RCT p	PPL
Higgins et al. 2001	Ireland; 19-63y; X; 62	0.52g EPA+0.33g DHA; 0.34g EPA+0.22g DHA; 0.17g EPA+0.11g DHA; 16 wk; 14 + 16 + 17; 14	RCT p	P; LDL
Hodge et al. 1993	Australia; 30.6y ; F; 7	0.55 g EPA + 0.39 g DHA; 2 wk; 7	B/A	PPL;PCE;PTG; HDL PL
Hoffman et al. 2004	USA; 6 mo; X; 55	0. 083 g DHA; 26 wk; 25; 26	RCT p	E
Itomura et al. 2005	Japan; 9-12y; X; 179	0.12 g EPA + 0.52 g DHA; 12 wk; 26; 23	RCT p	EPL
Katan et al. 1997	NL; 56.2±16.5y; 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 et al. 2004	UK; 23-65y; 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 et al. 2003	UK; 40-65y; X; 56	0.02 g EPA + 0.94 g DHA; 32 wk; 28; 28	RCT p	PPL
Laidlaw and Holub 2003	Canada; 36-68y; F; 8	2.32 g EPA + 1.68 g DHA; 4 wk; 8	B/A	PPL
Mantzioris et al. 1994	Australia; 25-44y; M; 15	1.62 g EPA + 1.08 g DHA; 4 wk; 15	B/A	PPL; PCE; PTG

Table 14. (Continued)

Studies	Country(s); age; gender; no. included	Short description of intervention; latest time point; no. in intervention; no. in control at latest time	Study design	Biomarkers reported
Miles et al. 2004	UK; 21-44y; 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; PMBC
Mills et al. 1995	Canada; 21-41y; X; 18	0.74 g EPA + 0.51 g DHA; 6 wk; 8; 9	RCT p	E ghosts
Montgomery et al. 2003	UK; pregnant; F; 100	0. 04 g EPA + 0.2 g DHA; 25 wk; 30; 29	RCT p	E; P
Otto et al. 2000*	NL; 20-45y; 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 + 14 + 12; 15	RCT p	PPL; EPL
Otto et al. 2000**	NL; 20-38y; F; 24	0.57 g DHA; 4 wk; 12; 12	RCT p	PPL; EPL
Palozza et al. 1996	Italy; 25-46y; 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 and Harris 2002	USA; 37-43y; X; 33	4 g EPA or 4 g DHA; 4 wk; 10 + 10; 11	RCT p	Plat
Rees et al. 2006	UK; 18-70y; 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; PMBC PL
Sanders et al. 2006	UK; 29-35y; X; 80	1.5 g DHA; 4 wk; 40; 39	RCT p	P; EPL
Sanjurjo et al. 2004	Spain; 31-34y; F; 20	0.04 g EPA + 0.2 g DHA; 14 wk; 8; 8	RCT p	P
Smuts et al. 2003	USA; 16-35y; F; 48	0.184 g DHA; 14 wk; 18; 19	RCT p	PPL; PTG; EPL
Sørensen et al. 1998	Denmark;29-60y; M;50	0.37 g EPA + 0.54 g DHA; 4 wk; 21; 24	RCT p	LDL
Stark et al. 2000	Canada; 43-60y; F; 36	2.4 g EPA + 1.6 g DHA; 4 wk; 18; 17	RCT p	PPL
Surai et al. 2000	UK; 26-59y; X; 44	0.21 g DHA; 8 wk; 20; 20	RCT p	PPL; PCE; PTG; NEFA
Thies et al. 2001	UK; 56-69y; X; 24	0.7 g DHA; 0.72 g EPA + 0.28 g DHA; 12 wk; 8 + 7; 8	RCT p	PMBC PL
Vognild et al. 1998	Norway; 16-69y; X; 228	0.7g EPA+1g DHA; 0.5g EPA+0.6g DHA; 0.5g EPA+0.8g DHA; 1g EPA+1.5g DHA; 1.3g EPA+1.8g DHA; 12 wk; 35+36+38+36+34;36	RCT p	P; Plat
Wallace et al. 2000	Ireland; 20-26y; F; 25	0.35 g EPA + 0.32 g DHA, 4 wk; 13 + 12	B/A	Plat
Yaqoob et al. 2000	UK; 39-49y; X; 16	2.1 g EPA + 1.1 g DHA; 12 wk; 8; 8	RCT p	PPL, PMBC

Abbreviations: M, exclusively male group; F, exclusively female group; X, mixed group; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RCT p, randomized controlled trial – parallel; B/A, before/after study; P, total plasma; PPL, plasma phospholipid; PCE, plasma cholesteryl ester; PTG, plasma triacylglycerol; Plat, total platelet; N, total neutrophil NPL, neutrophil phospholipid; G, total granulocyte; E, total erythrocyte; EPL, erythrocyte phospholipid; NEFA, nonesterified fatty acid; LDL, low-density lipoprotein; HDL PL, high-density lipoprotein phospholipid; PMBC PL, peripheral blood mononuclear cell phospholipid.

*, Otto et al. Nutr Res 2000.; **, Otto et al. Prostaglandins Leukot Essent Fatty Acids 2000.

4.3.2. Quality of included studies

Of the 41 studies included, 34 were parallel RCTs (83%) and 7 were B/A studies; no non-randomized controlled clinical trials were included (**Table 14**). The majority of studies were carried out in Europe (26 studies) and North America (10 studies). The studies ranged in size from 7 to 341 participants. Fourteen studies were 2–4 wk duration, 9 studies were 5–10 wk, 13 studies were 11–17 wk, and 5 studies were 6 mo. The supplement was marine oil or seafood in 35 studies, single cell oil in 5, and DHA-rich eggs in 3 studies. The most common placebo was vegetable oil (25 studies). The studies used a wide range of supplementation doses, from 83 mg/day DHA (Hoffman et al. 2004) to 4900 mg/day DHA (Kew et al. 2004).

Aspects of methodologic quality are described in **Table 15**. The method of randomization was poorly described in the majority of studies. There were only a moderate number of dropouts in most of the studies, but reasons often were not reported. Attempts were made to assess compliance objectively in 24 studies, but the results of the checks, i.e. levels of compliance, were not comprehensively reported (in only 12 studies was there at least some attempt to describe it) (**Table 15**). Overall the risk of bias was low in only 5 studies (Allard et al. 1997; Higgins et al. 2001; Hoffman et al. 2004; Itomura et al. 2005; Kew et al. 2004).

4.3.3. Biomarkers identified and evaluated

Within the 41 included studies, 18 different biomarkers were used to characterize changes in n-3 LCPUFA status. We discuss in detail only those biomarkers that were used in 3 independent studies; however, the effects of n-3 LCPUFA supplementation on every biomarker are detailed in **Table 16**. In this thesis, we focus primarily on the effect of DHA supplementation on biomarkers reflecting changes in DHA values.

Table 15: Validity of included studies

Studies	Randomized? Method of randomization	Reasons for dropouts by intervention group (no. of subjects)	Method for checking; Results of compliance check	Risk of bias
Allard et al. 1997	Randomized; random number tables	Lack of compliance, side effects (2)	Assessed biochemical parameters; very good	Low
Bonaa et al. 1992	Randomized; N/A	Abdominal discomfort (6)	Count of returned capsules, assessed biochemical parameters; N/A	Moderate or high
Brady et al. 2004	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Christensen et al. 1999	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Cleland et al. 1992	Randomized; N/A	Lack of compliance (4)	Count of returned capsules; >94%	Moderate or high
Conquer and Holub 1998	Randomized; N/A	Lack of compliance, personal reasons (3)	Count of returned capsules, assessed biochemical parameters; N/A	Moderate or high
Conquer et al. 1999	Randomized; N/A	Lack of compliance (1)	Count of returned capsules; assessed biochemical parameters; N/A	Moderate or high
Damsgaard et al. 2007	Randomized; N/A	Reasons not reported (N/A)	N/A; 88%	Moderate or high
DeLany et al. 1990	Randomized; stratified by cholesterol levels	Appendectomy (1)	N/A; N/A	Moderate or high
Dustan et al. 2004	Randomized; N/A	Significant disease, nausea (7)	Count of returned capsules; N/A	Moderate or high
Dyerberg et al. 2004	Randomized; N/A	Lack of compliance, personal reasons (5)	N/A; good	Moderate or high
Engström et al. 2003	Randomized; N/A	No dropouts	Count of returned food, assessed biochemical parameters; N/A	Moderate or high
Hagve et al. 1993	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Helland et al. 2006	Randomized; computer generated	Reasons not reported (N/A)	N/A; N/A	Moderate or high
Higgins et al. 2001	Randomized; stratified by plasma PTG levels	Lack of compliance (1)	Count of returned capsules, assessed biochemical parameters; 93-95%	Low
Hodge et al. 1993	Non-randomized	No dropouts	N/A; N/A	Moderate or high
Hoffman et al. 2004	Randomized; computer generated	Constipation, refusal to eat solid foods (3)	Count of returned food; 93%	Low
Itomura et al. 2005	Randomized; stratified by age, BMI and gender	Lost to follow-up, got cold, did not agree for blood sampling (62)	Count of returned food; >90%	Low
Katan et al. 1997	Randomized; N/A	No dropouts	Count of returned capsules; excellent	Moderate or high
Kew et al. 2004	Randomized; stratified by age, BMI and PTG levels	No dropouts	Count of returned capsules; >90%	Low
Khan et al. 2003	Randomized; N/A	No information on dropouts	Count of returned supplement, assessed biochemical parameters; generally good	Moderate or high
Laidlaw and Holub 2003	Non-randomized	No dropouts	Assessed biochemical parameters; N/A	Moderate or high

Table 15. (Continued)

Studies	Randomized? Method of randomization	Reasons for dropouts by intervention group (no. of subjects)	Method for checking; Results of compliance check	Risk of bias
Mantzioris et al. 1994	Non-randomized	No dropouts	Count of returned capsules; N/A	Moderate or high
Miles et al. 2004	Randomized; N/A	Reasons not reported (N/A)	N/A; N/A	Moderate or high
Mills et al. 1995	Randomized; N/A	Reason not reported (1)	N/A; N/A	Moderate or high
Montgomery et al. 2003	Randomized; N/A	Lack of compliance, nausea gravidarum, loss of contact (19)	Count of returned capsules; N/A	Moderate or high
Otto et al. 2000*	Randomized; N/A	Lack of compliance, side effects, moving away (4)	Count of returned capsules; N/A	Moderate or high
Otto et al. 2000**	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Palozza et al. 1996	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Park and Harris 2002	Randomized; N/A	Reasons not reported (2)	N/A; N/A	Moderate or high
Rees et al. 2006	Randomized; N/A	Lack of compliance, inconvenience, car accident, myocardial infarction, diarrhea, constipation (N/A)	Count of returned capsules; N/A	Moderate or high
Sanders et al. 2006	Randomized; stratified by gender	No dropouts	Count of returned capsules; N/A	Moderate or high
Sanjurjo et al. 2004	Randomized; computer generated number table	Reasons not reported (2)	N/A; N/A	Moderate or high
Smuts et al. 2003	Randomized; N/A	Reasons not reported (9)	Count of returned eggs; N/A	Moderate or high
Sorensen et al. 1998	Randomized; N/A	Reasons not reported (2)	Records of margarine use, assessed biochemical parameters; 100%	Moderate or high
Stark et al. 2000	Randomized; N/A	No dropouts	N/A; N/A	Moderate or high
Surai et al. 2000	Randomized; stratified by age and gender	Reasons not reported (2)	N/A; N/A	Moderate or high
Thies et al. 2001	Randomized; N/A	Stomach upset (1)	Self-reporting questionnaire, assessed biochemical parameters; N/A	Moderate or high
Vognild et al. 1998	Randomized; N/A	Reasons not reported (N/A)	N/A; N/A	Moderate or high
Wallace et al. 2000	Randomized; N/A	No dropouts	Verbally assessed; satisfactory	Moderate or high
Yaqoob et al. 2000	Randomized; N/A	No dropouts	Self-reporting questionnaire, assessed biochemical parameters; N/A	Moderate or high

Abbreviations: N/A, no available data; BMI, body mass index; PTG, plasma triacylglycerol.

*, Otto et al. Nutr Res 2000; **, Otto et al. Prostaglandins Leukot Essent Fatty Acids 2000.

Low risk of bias meant that the study was randomized, the randomization method was at least partially described, reasons for and numbers of dropouts were stated (or there were no dropouts), and the method used to assess compliance and some assessment of compliance were reported. All other studies were considered at moderate or high risk of bias.

Table 16: Primary analyses (the longest duration and the highest supplementation dose) for each of the identified biomarkers for supplementation with n-3 long-chain polyunsaturated fatty acids

Biomarker	No. of studies (no. of included participants)	Pooled effect size, MD (95% CI)[1]	Heterogeneity I^2 (%)	Appears effective as a biomarker?
Plasma DHA	6 (262)	1.13 [0.54, 1.71]	88.7	Yes
Plasma phospholipid DHA	21 (923)	2.45 [1.87, 3.02]	94.0	Yes
Plasma phospholipid EPA	16 (759)	4.07 [2.90, 5.24][2]	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	6 (277)	2.33 [0.86, 3.81]	94.0	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][3]	N/A	Unclear
Old erythrocyte ghosts DHA	1 (17)	1.70 [0.32, 3.08][3]	N/A	Unclear
Platelet DHA	8 (235)	1.25 [0.87, 1.64]	79.9	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	No
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

Abbreviations: MD, mean difference; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; N/A, no available data; PBMC, peripheral blood mononuclear cell; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

[1], all studies are in %DHA of total fatty acids unless otherwise stated; [2], %EPA of total fatty acid; [3], µg/mg protein.

To claim that a biomarker was effective (reflected change in status) within a review, 3 conditions needed to be meet: 1) statistical significance within a forest plot (95% CI did not include 0 or P <0.05), 2) ≥3 trials contributing data, and 3) ≥50 participants contributing data in the intervention and control arm. 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 ≥0.05); 2) ≥3 trials contributing data; 3) ≥50 participants contributing data in the intervention and control arm.

4.3.3.1. Total plasma lipid DHA

Six RCTs contained eligible data on the effect of DHA supplementation on the percentage contribution of DHA to the fatty acid composition of total plasma lipids. The primary analysis, to assess whether the biomarker was responding to change in DHA status, of the largest dose and longest duration of supplementation within each publication showed a statistically significant increase in each study and also in the pooled response (MD: 1.13% DHA; 95% CI: 0.54, 1.71; 262 participants; I^2 = 89%). This suggested that total plasma lipid DHA is an effective biomarker of DHA status (**Table 16**). However, the high level of heterogeneity in this primary analysis means that the biomarker responded differently in the included studies; the reasons for these differences were explored through subgrouping of studies by their different characteristics.

When the data were subgrouped according to dose of supplementation (**Figure 8**), even the lowest-dose category (<300 mg DHA/day) showed a statistically significant

effect (P = 0.0007) over 14–25wk. There appeared to be an almost linear increase in total plasma DHA values with increasing supplementation dose: the percentage of DHA in plasma lipids was >8-fold higher when supplementing with DHA doses >2500 mg DHA/day was compared with doses <300 mg DHA/day (comparison of subgroup MD, **Figure 8**). Total plasma DHA appeared to usefully reflect changed DHA status in studies of adults, mixed sex studies (same results as for adult studies), those with moderate baseline plasma DHA status, those supplemented with marine oil or seafood, and all those whose doses were ≤2500 mg DHA/day. (**Table 17** and **Figure 8**). Although there were several subgroups for which there were insufficient studies to be clear about how effective the biomarker was in that subgroup, there were no subgroups for which total plasma DHA clearly did not reflect changes in DHA status.

Table 17: Subgroup analysis of the results of the systematic review of data on changes in total plasma docosahexaenoic acid (DHA) to supplementation with DHA

		Study design			
Analysis	**Pooled effect size MD (95% CI)**	**RCTs**	**B/A**	**Heterogeneity I^2**	**Biomarker useful?**
	% of total fatty acid	*no. of studies (no. of participants)*		*%*	
All studies (primary outcome)	1.13 [0.54, 1.71]	6 (262)	N/A	88.7	Yes
Pregnancy and lactation	0.34 [0.16, 0.53]	2 (75)	N/A	0	Unclear
Adults	1.72 [0.80, 2.64]	4 (187)	N/A	81.6	Yes
Mixed	1.72 [0.80, 2.64]	4 (187)	N/A	81.6	Yes
Females	0.34 [0.16, 0.53]	2 (75)	N/A	0	Unclear
Low status at baseline[1]	0.37 [0.10, 0.65]	2 (87)	N/A	13.8	Unclear
Moderate status at baseline[1]	2.12 [1.22, 3.03]	3 (159)	N/A	64.0	Yes
High status at baseline[1]	0.40 [0.12, 0.68]	1 (16)	N/A	N/A	Unclear
Marine oil or seafood	0.79 [0.31, 1.26]	5 (183)	N/A	81.6	Yes
Single cell oil	2.66 [1.78, 3.54]	1 (79)	N/A	N/A	Unclear
<300 mg DHA/day	0.30 [0.13, 0.48]	3 (122)	N/A	0	Yes
300 to 1500 mg DHA/day	0.62 [0.30, 0.95]	3 (185)	N/A	0	Yes
1500 to 2500 mg DHA/day	1.85 [1.01, 2.70]	3 (195)	N/A	64.7	Yes
>2500 mg DHA/day	2.60 [0.92, 4.28]	1 (20)	N/A	N/A	Unclear

Abbreviations: RCT, randomized controlled trial; B/A, before-after study; MD, mean difference; CI, confidence interval; N/A, no available data.

[1], Data were divided into tertiles to define low, moderate and high baseline status. If data for more than one lipid fraction were published in the same study, plasma phospholipid fatty acid data were used to define DHA baseline status.

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 contributing data, and 3) ≥50 participants contributing data in the intervention and control arm. To claim that a biomarker was ineffective, 3 conditions had to be met: 1) lack of statistical significance within a forest plot (95% CI included 0 or P ≥0.05); 2) ≥3 trials contributing data; 3) ≥50 participants contributing data in the intervention and control arm.

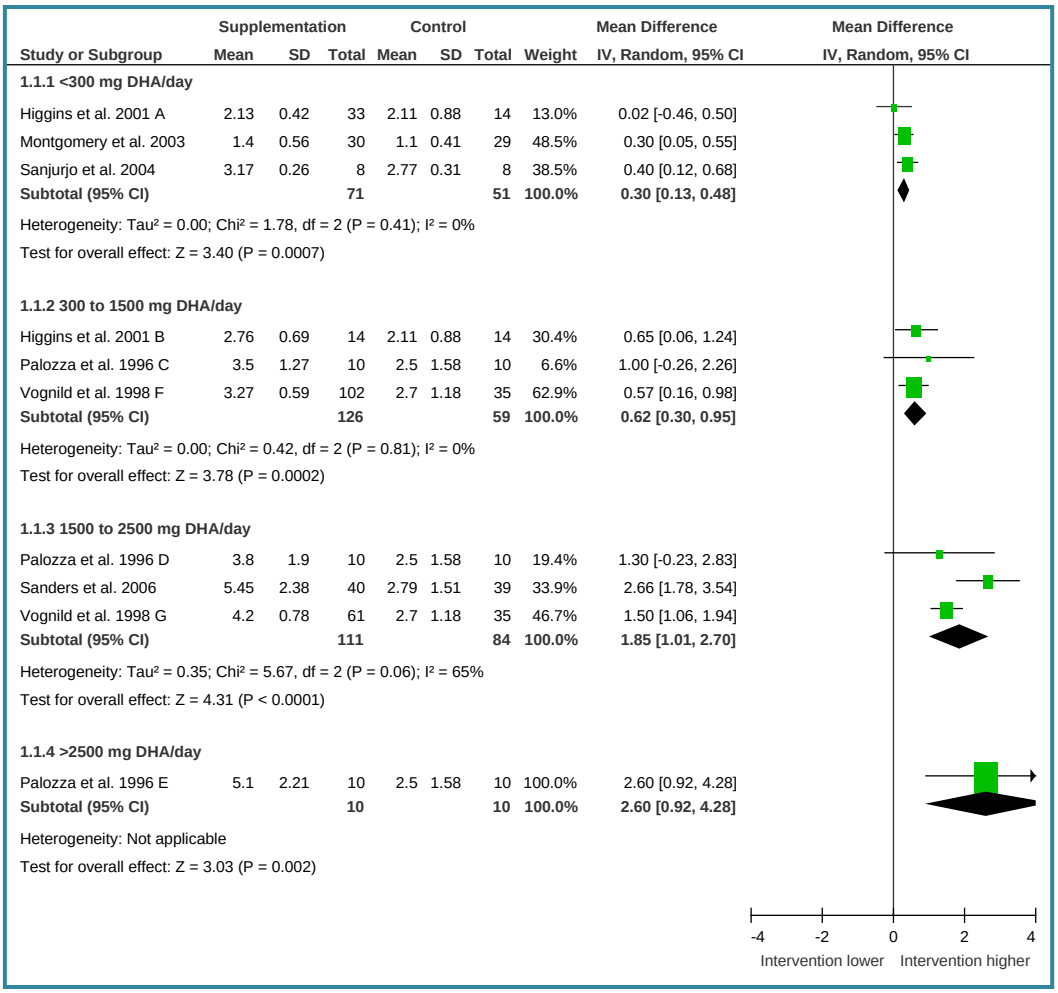

Figure 8: Response of total plasma docosahexaenoic acid (DHA) to supplementation with DHA [%DHA/total fatty acid composition (weight/weight)]

Specification of the groups as represented in the original articles: A, combined data of 0.3-g n-3 polyunsaturated fatty acid (PUFA) and 0.6-g n-3 PUFA groups; B, 0.9-g n-3 PUFA group; C, 2.5-g n-3 PUFA group; D, 5.1-g n-3 PUFA group; E, 7.7-g n-3 PUFA group; F, combined data of refined whale oil, crude whale oil, and olive oil/cod liver oil groups; and G, combined data of cod liver oil and seal oil/cod liver oil groups.

4.3.3.2. Plasma phospholipid DHA

Information on plasma phospholipid DHA was available for 21 studies (17 RCTs and 4 B/A studies). The primary analysis showed a highly significant increase of percentage DHA in plasma phospholipids (MD: 2.45% DHA; 95% CI: 1.87, 3.02; 21 studies; 923 participants; I^2 = 94%) but with high levels of heterogeneity (**Table 16**).

There were sufficient studies and participants and a sufficiently statistically significant effect on plasma phospholipid DHA to declare the biomarker effective in the following subgroups: adults; males; mixed sexes; females; those with low, moderate, or high baseline DHA status; those who used either marine oil, seafood, or single cell oils;

and those who had dose levels ≤2500 mg DHA/day. Although the response appeared to increase with increasing doses of DHA ≤2500 mg/day, additional dose increases did not appear to alter plasma phospholipid DHA further (**Table 18** and **Figure 9**). The effect was not significant in pregnancy and lactation. There were insufficient data to assess whether it was a good biomarker in all other groups. Overall, plasma phospholipid DHA appears to be a good biomarker of DHA status, which reacts rapidly to supplementation and is sensitive to supplementation dose.

Table 18: Subgroup analysis of the results of the systematic review of data on changes in plasma phospholipid docosahexaenoic acid (DHA) to supplementation with DHA

		Study design			
Analysis	**Pooled effect size MD (95% CI)**	**RCTs**	**B/A**	**Heterogeneity I^2**	**Biomarker useful?**
	% of total fatty acid	*no. of studies (no. of participants)*		*%*	
All studies (primary outcome)	2.45 [1.87, 3.02]	17 (885)	4 (38)	94.0	Yes
Pregnancy and lactation	1.44 [-0.01, 2.89]	3 (367)	N/A	95.0	No
Adults	2.47 [1.79, 3.16]	11 (439)	4 (38)	93.5	Yes
Post-menopausal women	3.10 [2.39, 3.81]	1 (35)	N/A	N/A	Unclear
The elderly	0.70 [-0.56, 1.96]	1 (31)	N/A	N/A	Unclear
Low income and immigrants	6.30 [5.29, 7.31]	1 (13)	N/A	N/A	Unclear
Males	2.72 [1.51, 3.94]	6 (163)	1 (15)	96.6	Yes
Mixed	2.57 [1.07, 4.07]	6 (293)	1 (8)	93.9	Yes
Females	2.09 [1.40, 2.78]	5 (429)	2 (15)	89.1	Yes
Low status at baseline	2.57 [1.89, 3.26]	5 (120)	N/A	60.0	Yes
Moderate status at baseline	2.64 [1.67, 3.60]	8 (257)	3 (30)	96.7	Yes
High status at baseline	1.93 [1.24, 2.63]	4 (508)	1 (8)	73.8	Yes
Marine oil or seafood	2.59 [1.99, 3.18]	12 (747)	4 (38)	92.2	Yes
Single cell oil	3.16 [0.56, 5.77]	3 (64)	N/A	97.0	Yes
DHA-rich egg	0.56 [0.16, 0.97]	2 (74)	N/A	0	Unclear
<300 mg DHA/day	0.85 [0.62, 1.09]	4 (138)	1 (8)	7.1	Yes
300 to 1500 mg DHA/day	1.99 [1.40, 2.58]	11 (686)	3 (30)	91.6	Yes
1500 to 2500 mg DHA/day	3.83 [2.78, 4.87]	5 (250)	1 (8)	92.6	Yes
>2500 mg DHA/day	2.74 [2.03, 3.44]	2 (31)	N/A	0	Unclear

See details below **Table 17**.

4.3.3.3. Plasma phospholipid EPA

In addition to the evaluation of DHA biomarkers in response to DHA intake, we also examined the response of plasma phospholipid EPA to dietary supplementation with EPA. Data on plasma phospholipid EPA were available from 16 studies (12 RCTs and 4 B/A studies). Primary analysis of the largest dose and longest duration of intervention showed significant increase of plasma phospholipid EPA (MD: 4.07% EPA; 95% CI: 2.90, 5.24; 16 studies; 759 participants; I^2 = 99%).

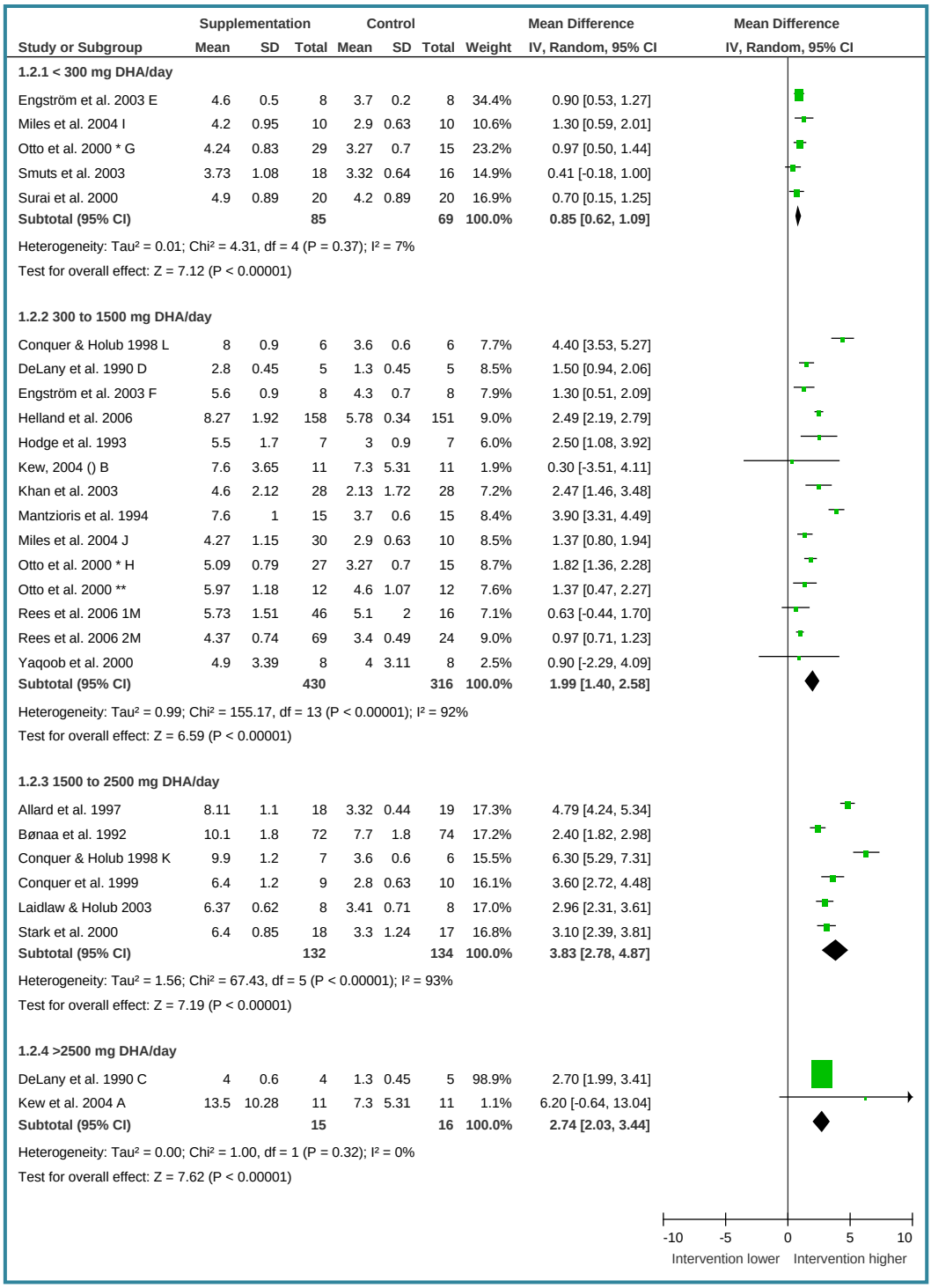

Figure 9: Response of plasma phospholipid docosahexaenoic acid (DHA) to supplementation with DHA [%DHA/total fatty acid composition (weight/weight)]

Specification of the groups as represented in the original articles: A, DHA group; B, eicosapentaenoic acid group; C, 20-g fish oil group; D, 5-g fish oil group; E, ordinary caviar paste group; F, fish oil–enriched caviar paste group; G, combined data of low fish oil and low DHA groups; H, combined data of high fish oil and high DHA groups; I, blend 3 group; J, combined data of eicosapentaenoic acid, blend 1, and blend 2 groups; K, high DHA group; L, low DHA group; and M, combined data of low, moderate, and high groups. 1 indicates the older group within the study, and 2, the younger group within the study.

*, Otto et al. Nutr Res 2000.; **, Otto et al. Prostaglandins Leukot Essent Fatty Acids 2000.

There was evidence that phospholipid EPA was an effective biomarker of EPA status in adults; males; females; mixed sex populations; those with low, moderate, or high baseline EPA status; and those given marine oil or seafood (**Table 19**). Subgroup analyses showed significant effects in all dose categories, and increased response with increased dose (although this did not decrease heterogeneity between studies a great deal) (**Figure 10**). No subgroups suggested that plasma phospholipid EPA was not an effective biomarker of EPA status, but there were insufficient studies to assess this in some groups. Overall, plasma phospholipid EPA appears to be a good biomarker of EPA status.

Table 19: Subgroup analysis of the results of the systematic review of data on changes in plasma phospholipid EPA to supplementation with EPA

		Study design			
Analysis	**Pooled effect size MD (95% CI)**	**RCTs**	**B/A**	**Heterogeneity I^2**	**Biomarker useful?**
	% of total fatty acid	*no. of studies (no. of participants)*		*%*	
All studies (primary outcome)	4.07 [2.90, 5.24]	12 (721)	4 (38)	99.3	Yes
Pregnancy and lactation	1.69 [1.52, 1.86]	1 (309)	N/A	N/A	Unclear
Adults	3.88 [2.57, 5.19]	9 (346)	4 (38)	98.7	Yes
Post-menopausal women	5.68 [5.43, 5.93]	1 (35)	N/A	N/A	Unclear
The elderly	7.20 [6.22, 8.18]	1 (31)	N/A	N/A	Unclear
Males	5.08 [3.80, 6.35]	6 (163)	1 (15)	91.8	Yes
Mixed	3.77 [0.48, 7.07]	3 (184)	1 (8)	98.0	Yes
Females	2.96 [1.04, 4.88]	3 (374)	2 (15)	99.8	Yes
Low status at baseline	3.61 [2.33, 4.89]	4 (64)	N/A	78.7	Yes
Moderate status at baseline	4.26, [1.97, 6.55]	4 (149)	3 (30)	99.7	Yes
High status at baseline	4.25 [2.12, 6.37]	4 (508)	1 (8)	98.6	Yes
Marine oil or seafood	4.07 [2.90, 5.24]	12 (721)	4 (38)	99.3	Yes
<300 mg EPA/day	0.36 [0.19, 0.54]	1 (45)	1 (8)	0	Unclear
300 to 1500 mg EPA/day	2.01 [1.61, 2.42]	7 (479)	2 (15)	82.1	Yes
1500 to 2500 mg EPA/day	4.08 [2.77, 5.38]	3 (71)	2 (23)	92.2	Yes
>2500 mg EPA/day	5.92 [4.95, 6,89]	6 (330)	N/A	98.8	Yes

See details below **Table 17**.

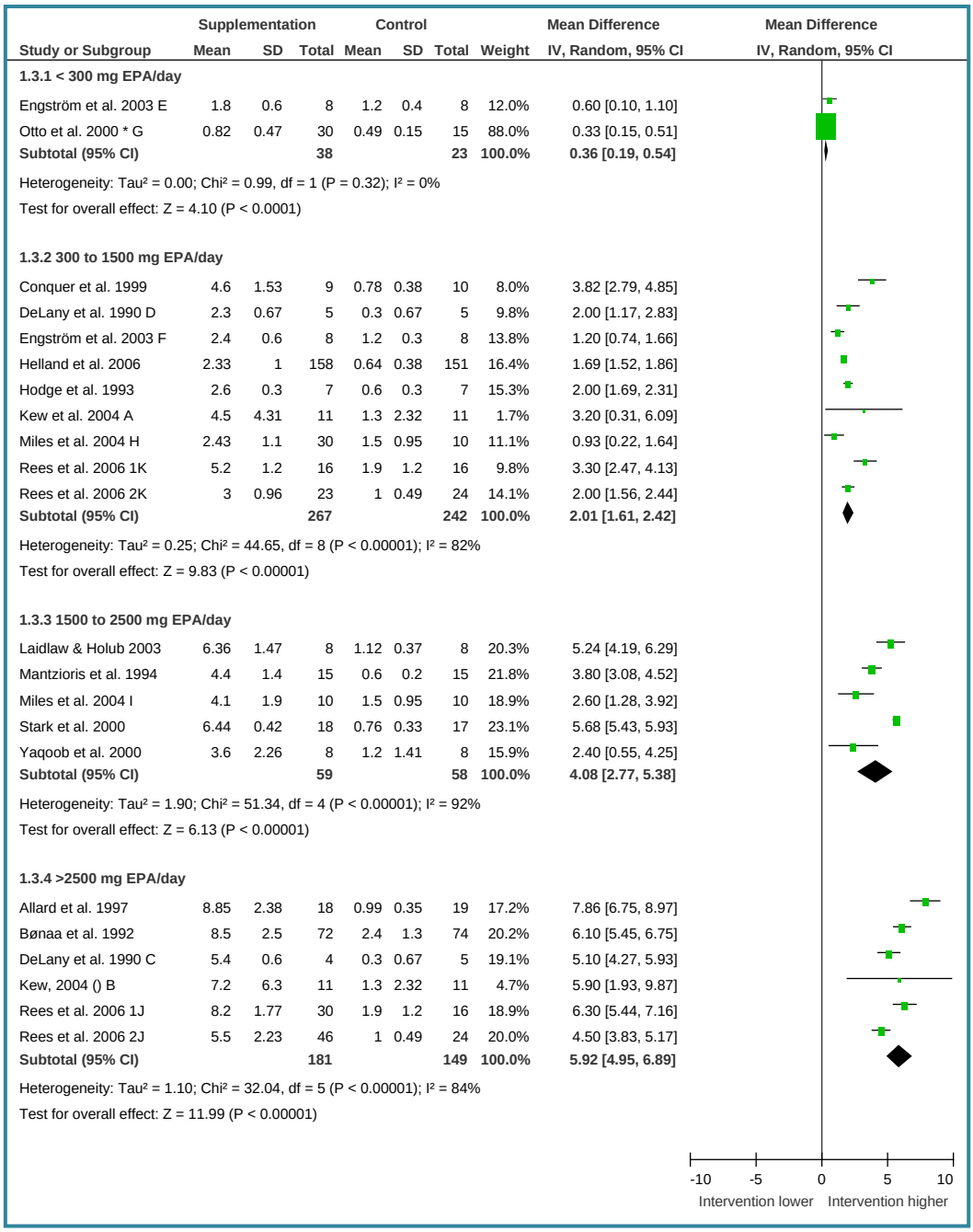

Figure 10: Response of plasma phospholipid eicosapentaenoic acid (EPA) to supplementation with EPA [%EPA/total fatty acid composition (weight/weight)]

Specification of the groups as represented in the original articles: A, docosahexaenoic acid (DHA) group; B, EPA group; C, 20-g fish oil group; D, 5-g fish oil group; E, ordinary caviar paste group; F, fish oil–enriched caviar paste group; G, combined data of low fish oil and high fish oil groups; H, combined data of blend 1, blend 2, and blend 3 groups; I, EPA group; J, combined data of moderate EPA and high EPA groups; and K, low EPA group. 1 indicates the older group within the study, and 2, the younger group within the study.

4.3.3.4. Plasma triacylglycerol DHA

There were 5 studies of changes of plasma triacylglycerol DHA values in response to DHA supplementation (3 RCTs and 2 B/A studies). The pooled response in the

primary analysis of the largest dose and longest duration showed significant increase (MD: 0.86% DHA; 95% CI: 0.08, 1.65; 5 studies; 116 participants; I^2 = 92%) but with significant heterogeneity (**Table 16**). There were too few studies to explore the causes of heterogeneity with subgrouping. Plasma triacylglycerol DHA may be a good biomarker of DHA status, but there were insufficient studies to allow exploration of which population groups it may be most effective.

4.3.3.5. Plasma cholesteryl ester DHA

Response of plasma cholesteryl ester DHA values to DHA supplementation was reported in 5 studies (3 RCTs and 2 B/A studies). Primary analysis showed a significant increase of plasma cholesteryl ester DHA values on DHA supplementation (MD: 0.42% DHA; 95% CI: 0.13, 0.71; 5 studies; 110 participants; I^2 = 92%) with significant heterogeneity (**Table 16**). Although there were no data for the >2500 or 1500–2500 mg DHA/day dose categories, we observed significant increases in the lower dose categories. There were insufficient studies for further subgrouping. 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.

4.3.3.6. Plasma nonesterified fatty acid DHA

Three RCTs assessed the contribution of DHA to plasma nonesterified fatty acids after DHA supplementation in adults. The primary analysis evaluating the largest dose and longest duration suggested a statistically significant response of plasma nonesterified fatty acid DHA (MD: 1.35% DHA; 95% CI: 0.11, 2.59; 3 studies; 72 participants; I^2 = 95%) (**Table 16**). There were insufficient studies to perform subgroup analysis. Plasma nonesterified fatty acid DHA may be a good biomarker of DHA status, but there were insufficient studies to allow exploration of appropriateness of its use for different population groups and doses.

4.3.3.7. Erythrocyte membrane total lipid DHA

Changes in the contribution of DHA to total erythrocyte membrane lipids on DHA supplementation were examined in 6 RCTs. The primary analysis showed a statistically significant increase (MD: 2.33% DHA; 95% CI: 0.86, 3.81; 6 studies; 277 participants; I^2 = 94%) with significant heterogeneity (**Table 16**). There were 2 RCTs

each in infants, pregnant or lactating women, and adults, so there were insufficient studies to be sure whether erythrocyte DHA was a good marker of DHA status in any single group. There was evidence that erythrocyte DHA was a good marker for studies of mixed sex groups with moderate baseline DHA status and those supplemented with marine oil or seafood and/or given 300–1500 mg DHA/day (**Table 20**). Inclusion of every dose of supplementation resulted in 9 substudies (**Figure 11**). Although the number of studies was insufficient to assess biomarker effectiveness in studies providing >1500 mg DHA/day, and the effect was not statistically significant (P = 0.09) in the lowest dose (<300 mg DHA/day), the forest plot suggested that erythrocyte membrane lipid DHA rose with supplementation dose. Erythrocyte membrane total lipid DHA appears to be a good biomarker of DHA status, and the data suggest that there is a dose response, which may explain some of the heterogeneity in the primary analysis. Although it appears to be an effective biomarker in infants for most doses, confirmation is not possible due to limited data.

Table 20: Subgroup analysis of the results of the systematic review of data on changes in total erythrocyte docosahexaenoic acid (DHA) to supplementation with DHA

		Study design			
Analysis	**Pooled effect size MD (95% CI)**	**RCTs**	**B/A**	**Heterogeneity I^2**	**Biomarker useful?**
	% of total fatty acid	*no. of studies (no. of participants)*		*%*	
All studies (primary outcome)	2.33 [0.86, 3.81]	6 (277)	N/A	94.0	Yes
Infants	2.40 [1.82, 2.98]	2 (99)	N/A	0	Unclear
Pregnancy and lactation	2.55 [-1.07, 6.18]	2 (130)	N/A	98.7	Unclear
Adults	1.98 [-1.34, 5.31]	2 (48)	N/A	77.7	Unclear
Males	0.40 [-1.45, 2.25]	1 (28)	N/A	N/A	Unclear
Mixed	2.47 [1.91, 3.04]	3 (119)	N/A	0	Yes
Females	2.55 [-1.07, 6.18]	2 (130)	N/A	98.7	Unclear
Low status at baseline	0.70 [0.08, 1.32]	1 (57)	N/A	N/A	Unclear
Moderate status at baseline	2.14 [0.54, 3.75]	3 (99)	N/A	64.4	Yes
High status at baseline	3.37 [1.32, 5.43]	2 (121)	N/A	94.1	Unclear
Marine oil or seafood	2.30 [0.45, 4.15]	5 (226)	N/A	95.2	Yes
DHA-rich egg	2.50 [1.69, 3.31]	1 (51)	N/A	N/A	Unclear
<300 mg DHA/day	1.24 [-0.19, 2.66]	3 (137)	N/A	85.0	No
300 to 1500 mg DHA/day	1.79 [0.60, 2.99]	3 (111)	N/A	50.0	Yes
1500 to 2500 mg DHA/day	3.34 [1.09, 5.59]	2 (93)	N/A	89.0	Unclear
>2500 mg DHA/day	3.80 [1.26, 6.34]	1 (20)	N/A	N/A	Unclear

See details below **Table 17**.

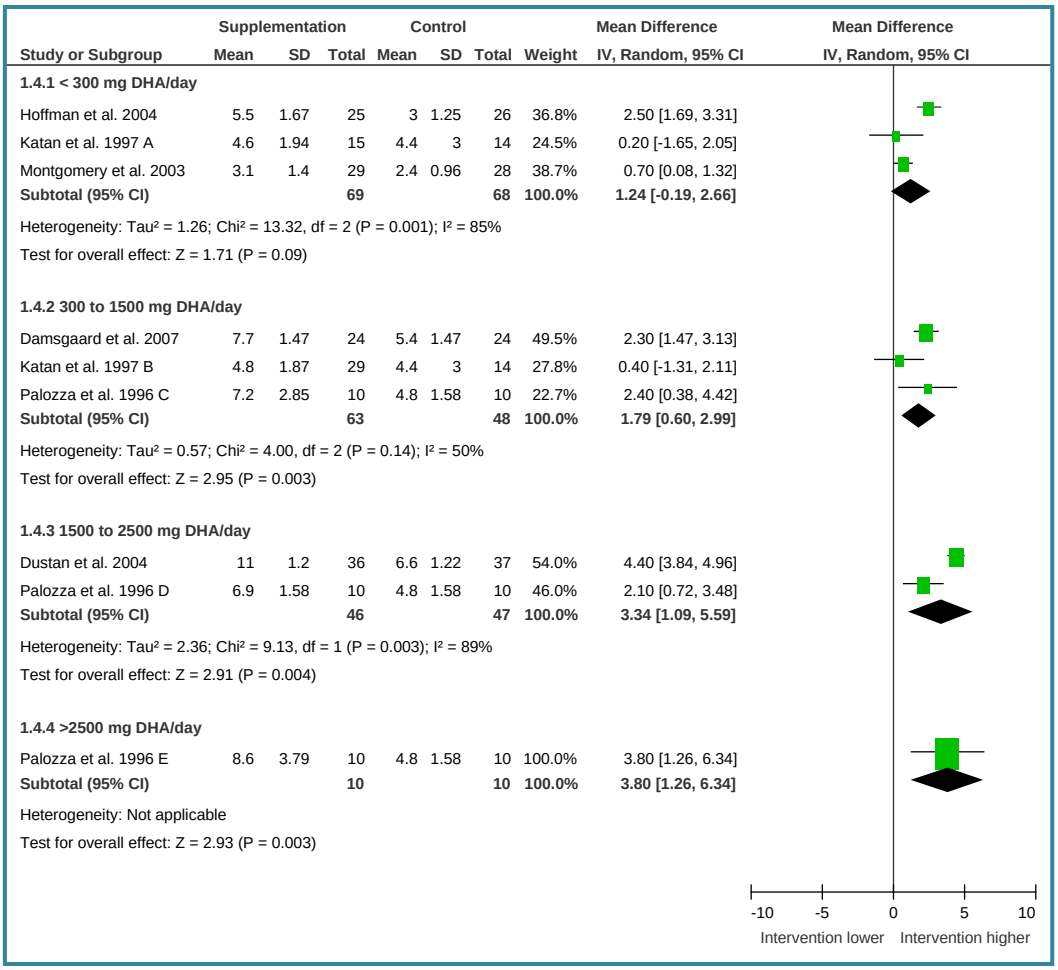

Figure 11: Response of total erythrocyte docosahexaenoic acid (DHA) to supplementation with DHA [%DHA/total fatty acid composition (weight/weight)]

Specification of the groups as represented in the original articles: A, low fish oil group; B, combined data of middle fish oil and high fish oil groups; C, 2.5-g n–3 polyunsaturated fatty acid (PUFA) group; D, 5.1-g n–3 PUFA group; and E, 7.7-g n–3 PUFA group.

4.3.3.8. Erythrocyte membrane phospholipid DHA

Data on changes of erythrocyte membrane phospholipid DHA values on DHA supplementation were available in 6 RCTs. Primary analysis of the largest dose and longest duration showed a statistically significant increase in DHA values (MD: 0.97% DHA; 95% CI: 0.50, 1.43; 6 studies; 229 participants; I^2 = 72%) with important heterogeneity (**Table 16**). Subgrouping suggested that erythrocyte phospholipid DHA is a good marker of DHA status in adults, females, those of moderate DHA status, and in those given single cell oils (**Table 21**). We included 7 substudies in the extended analysis of all doses. Although there were no data for the >2500 mg DHA/day dose category, we observed significant increases in the other 3 dose categories, although there were sufficient studies to declare the biomarker effective only in the 300–1500 mg DHA/day subgroup. Erythrocyte membrane phospholipid

DHA appears to be a good biomarker of DHA status. Although it may be an effective biomarker in adults, in children and adolescents, in pregnant or lactating women, and at most doses, this cannot be confirmed due to limited data.

Table 21: Subgroup analysis of the results of the systematic review of data on changes in erythrocyte phospholipid docosahexaenoic acid (DHA) to supplementation with DHA,

		Study design			
Analysis	**Pooled effect size MD (95% CI)**	**RCTs**	**B/A**	**Heterogeneity I^2**	**Biomarker useful?**
	% of total fatty acid	*no. of studies (no. of participants)*		*%*	
All studies (primary outcome)	0.97 [0.50, 1.43]	6 (229)	N/A	72.3	Yes
Children and adolescents	0.50 [0.02, 0.98]	1 (49)	N/A	N/A	Unclear
Pregnancy and lactation	0.68 [0.22, 1.13]	2 (58)	N/A	27.4	Unclear
Adults	1.57 [0.47, 2.67]	3 (122)	N/A	84.0	Yes
Mixed	0.95 [-0.03, 1.92]	2 (128)	N/A	77.1	Unclear
Females	1.02 [0.38, 1.66]	4 (101)	N/A	78.0	Yes
Moderate status at baseline	0.73 [0.45, 1.02]	5 (213)	N/A	30.9	Yes
High status at baseline	2.80 [1.66, 3.94]	1 (16)	N/A	N/A	Unclear
Marine oil or seafood	1.59 [-0.66, 3.84]	2 (65)	N/A	92.5	Unclear
Single cell oil	0.91 [0.55, 1.27]	3 (130)	N/A	28.6	Yes
DHA-rich egg	0.38 [-0.27, 1.03]	1 (34)	N/A	N/A	Unclear
<300 mg DHA/day	0.53 [0.24, 0.81]	2 (78)	N/A	0	Unclear
300 to 1500 mg DHA/day	0.76 [0.53, 0.99]	3 (115)	N/A	0	Yes
1500 to 2500 mg DHA/day	2.09 [0.82, 3.35]	2 (95)	N/A	70.1	Unclear

See details below **Table 17**.

4.3.3.9. Total platelet lipid DHA

Eight individual studies (4 RCTs and 4 B/A studies) reported changes of total platelet DHA values with DHA supplementation. The primary analysis showed statistically significant increases in response to supplementation (MD: 1.25% DHA; 95% CI: 0.87, 1.64; 8 studies; 235 participants; I^2 = 80%) with significant heterogeneity (**Table 16**). Although no data were available for the lowest DHA dose category, increases were seen at the other 3 doses (**Table 22 and Figure 12**). Subgrouping suggested that total platelet lipid DHA is a useful measure of DHA status for adults, males, mixed sex populations, and those at moderate DHA status. Total platelet lipid DHA may be a good biomarker of DHA status, but there was no apparent dose response, and the substantial heterogeneity could not be explained.

Table 22: Subgroup analysis of the results of the systematic review of data on changes in total platelet docosahexaenoic acid (DHA) to supplementation with DHA

Analysis	Pooled effect size MD (95% CI)	Study design		Heterogeneity I^2	Biomarker useful?
		RCTs	B/A		
	% of total fatty acid	*no. of studies (no. of participants)*		*%*	
All studies (primary outcome)	1.25 [0.87, 1.64]	4 (181)	4 (54)	79.9	Yes
Adults	1.06 [0.69, 1.44]	4 (181)	2 (25)	72.8	Yes
Low income and immigrants	1.70 [0.92, 2.48]	N/A	2 (29)	75.1	Unclear
Males	1.40 [0.70, 2.11]	1 (50)	2 (29)	86.3	Yes
Mixed	1.37, [0.63, 2.11]	3 (131)	N/A	88.8	Yes
Females	0.73 [0.03, 1.42]	N/A	2 (25)	0	Unclear
Low status at baseline	2.80 [1.80, 3.80]	1 (21)	N/A	N/A	Unclear
Moderate status at baseline	0.89 [0.70, 1.07]	3 (160)	2 (25)	13.1	Yes
High status at baseline	1.70 [0.92, 2.48]	N/A	2 (29)	75.1	Unclear
Marine oil or seafood	1.25 [0.87, 1.64]	4 (181)	4 (54)	79.9	Yes
300 to 1500 mg DHA/day	0.94 [0.53, 1.35]	3 (228)	4 (54)	83.7	Yes
1500 to 2500 mg DHA/day	0.70 [0.46, 0.94]	1 (105)	N/A	N/A	Unclear
>2500 mg DHA/day	1.91 [0.32, 3.51]	2 (61)	N/A	89.1	Unclear

See details below **Table 17**.

4.3.3.10. Peripheral blood mononuclear cell phospholipid DHA

The response of peripheral blood mononuclear cell phospholipid DHA values to DHA supplementation was reported in 3 RCTs. There was no statistically significant effect of supplementation with DHA (MD: 0.70% DHA; 95% CI: -0.66, 2.06; 3 studies; 94 participants; I^2 = 94%) (**Table 16**). Peripheral blood mononuclear cell phospholipid DHA does not appear to be a good biomarker of DHA status.

4.3.3.11. Other potential biomarkers

One or 2 studies were found to include data on young erythrocyte ghost DHA, old erythrocyte ghost DHA, granulocyte DHA, neutrophil DHA, neutrophil phospholipid DHA, peripheral blood mononuclear cell total lipid DHA, low-density lipoprotein DHA, and high-density lipoprotein phospholipid DHA. There were insufficient data to assess whether these potential biomarkers of DHA status are effective.

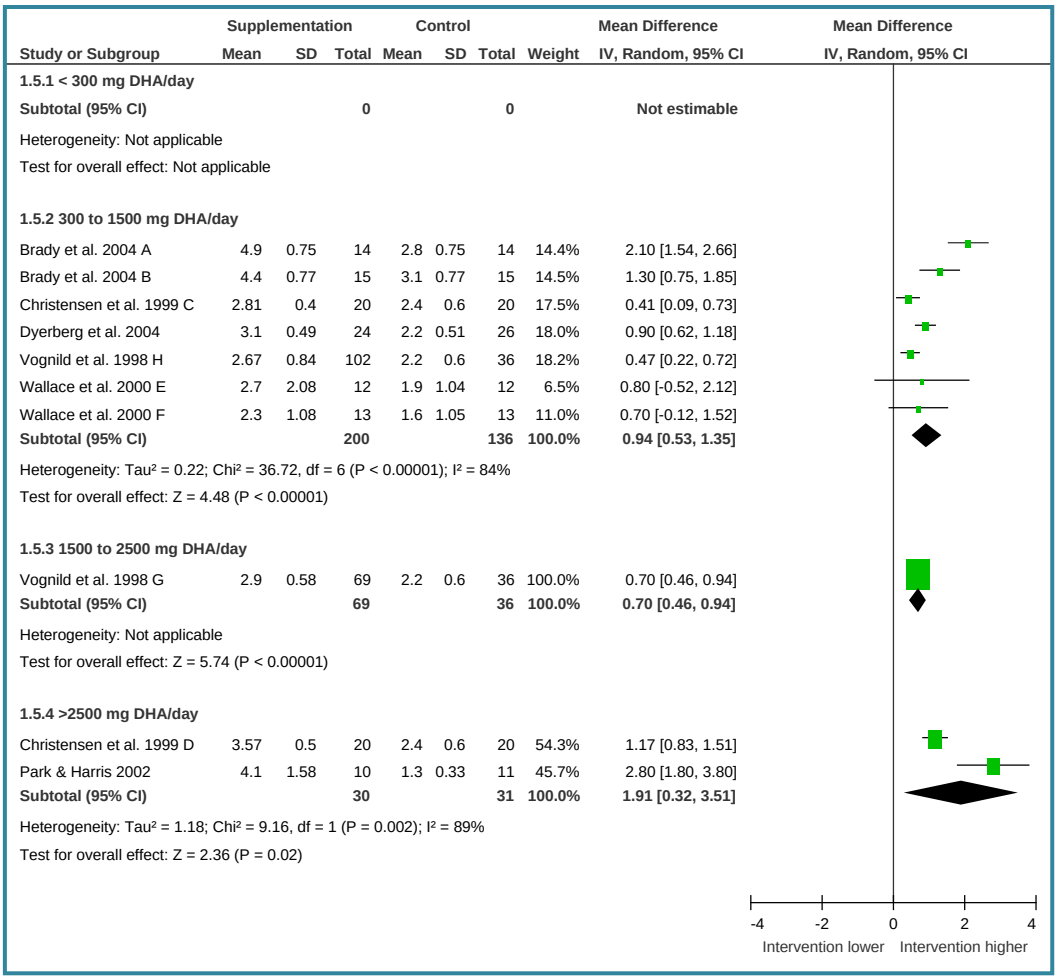

Figure 12: Response of total platelet docosahexaenoic acid (DHA) to supplementation with DHA [%DHA/total fatty acid composition (weight/weight)]

Specification of the groups as represented in the original articles: A, high n–6 group; B, moderate n–6 group; C, 2.0-g n–3 polyunsaturated fatty acid (PUFA) group; D, 6.6-g n–3 PUFA group; E, capsule group; F, food group; G, combined data of cod liver oil and seal oil/cod liver oil groups; and H, combined data of refined whale oil, crude whale oil, and olive oil/cod liver oil groups.

4.4. Discussion

We included 41 studies (34 RCTs and 7 B/A studies) reporting on 18 different potential biomarkers of n–3 LCPUFA status in this systematic review. There were sufficient data to state that plasma DHA, plasma phospholipid DHA, plasma triacylglycerol DHA, plasma cholesteryl ester DHA, plasma nonesterified DHA, erythrocyte DHA, erythrocyte phospholipid DHA, and platelet DHA were all effective biomarkers, peripheral blood mononuclear cell phospholipid DHA was an ineffective biomarker of DHA status and that plasma phospholipid EPA was an effective marker of EPA status.

For most of these biomarkers, however, only limited data were available for subgroup analysis. In contrast, we were able to evaluate the effect of n–3 LCPUFA supplementation on the fatty acid composition of plasma phospholipids on the basis of a considerable number of supplementation substudies. This finding is consistent with previous reports on the comparison of the incorporation of DHA and EPA into plasma phospholipids (Nordøy et al. 1991, Zuijdgeest-van Leeuwen et al. 1999) and supports the concept of using the enhancement of EPA values as an effective indicator of compliance in studies supplementing n–3 LCPUFAs.

There are some clear limitations with this review. First, the number of studies reporting data on different potential biomarkers was >6 for only 3 outcomes (plasma phospholipid DHA, plasma phospholipid EPA, and platelet DHA). This limited our ability to explore the reasons for heterogeneity seen in the main analyses and also limited our ability to assess for which population subgroups and types of intervention the biomarkers are effective. For instance, EPA and DHA in erythrocyte membrane lipids were found closely correlated to those measured in cardiac biopsy samples (Harris et al. 2004) and were suggested as the most reliable biomarkers of n-3 LCPUFA status (Harris 2007). However, until now, only limited data have been published on the effect of DHA supplementation on erythrocyte membrane lipids, thereby limiting evaluation in the present review. Second, we were able to focus on the effect of supplementing DHA only, whereas n-3 LCPUFA supplementation usually consists of a complex mixture of n-3 LCPUFAs that may interconvert with each other (Arterburn et al. 2006). Similarly, different food matrices can result in differences in absorption of nominally equal amounts of n-3 LCPUFAs. None of these factors could be reliably evaluated in the present review. Third, the dose-response curve of the incorporation of DHA (or any other fatty acid) may differ between compartments (Brown et al. 1991; Tremoli et al. 1995); hence, it may be assumed, with good reason, that the uniform duration and dose categories used in this review may have differently influenced the evaluation of the biomarkers. Additionally, we assessed the risk of bias to be low in only 5 included RCTs, limiting our ability to claim effectiveness.

Despite these limitations, the data generated in the present systematic review may contribute to a better understanding of the role of biomarkers in evaluating n-3 LCPUFA status in nutritional interventions. Although several clinical studies have

investigated the response of various biomarkers to modified n-3 fatty acid intake (Silverman et al. 1991, Nordøy et al. 1991; Zuijdgeest-van Leuwen et al. 1999; Brown et al. 1991; Tremoli et al. 1995; Prisco et al. 1996; Cao et al. 2006) and important theoretical considerations have also been published (Harris et al. 2004; Fokkema et al. 2002; Harris et al. 2006), we are unaware of any other systematic collection and evaluation of data available in the literature.

5. New findings of the thesis

Plasma zinc concentration responded in a dose-dependent manner to dietary manipulation in adults, women, men, pregnant and lactating women, the elderly, and those at low and moderate baseline zinc status. Urinary zinc excretion responded to zinc status overall and in all subgroups for which there were sufficient data. Hair zinc concentration also responded, but there were insufficient studies for subgroup analysis. These results indicate that in healthy individuals, plasma, urinary and hair zinc concentrations are reliable biomarkers of zinc status. Platelet, polymorphonuclear cell, mononuclear cell, and erythrocyte zinc concentration and alkaline phosphatase activity did not appear to be effective biomarkers of zinc status.

There were sufficient data to determine that plasma DHA, plasma phospholipid DHA, plasma triacylglycerol DHA, plasma cholesteryl ester DHA, plasma nonesterified DHA, erythrocyte DHA, erythrocyte phospholipid DHA, and platelet DHA were all effective biomarkers of DHA status, whereas peripheral blood mononuclear cell phospholipid DHA does not appear to be a good biomarker of DHA status and that plasma phospholipid EPA was an effective marker of EPA status. Plasma phospholipid DHA was the most frequently investigated biomarker; it appears to be a good marker of DHA status in adult men and women irrespective of DHA baseline status or supplementation dose, but ineffective marker in pregnancy and lactation; its usefulness in other population subgroups is unclear.

6. Practical applications

At the present time, plasma zinc concentration is the only biomarker of status that can be used to measure zinc status in individuals with either a low or a high supply of dietary zinc, but with many limitations and constraints. Urinary zinc excretion and hair zinc can provide useful information on zinc status in zinc-supplemented individuals, but whether these reflect zinc status in depleted individuals is not certain. It is clear that there is an urgent need to develop new biomarkers of zinc status.

Data presented in this thesis may be useful in designing novel studies on n-3 LCPUFA supplementation, especially those aimed at answering the question as to which biomarker might be sensitive enough to detect changes of a given dose of n-3 LCPUFA supplementation in a given clinical setting. There appears to be a range of useful biomarkers of DHA status in humans, but further research is needed to characterize which work best in particular population subgroups.

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9. List of publications

9.1. Publications in the topic of the thesis

Nicola M Lowe, **Katalin Fekete**, Tamás Decsi. Methods of assessment of zinc status in humans: a systematic review. Am J Clin Nutr 2009;89:2040S–2051S.

IF_{2009}: 6.307
Independent citations: 39

Katalin Fekete, Tamás Marosvölgyi, Viktória Jakobik, Tamás Decsi. Methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review. Am J Clin Nutr 2009;89:2070S–2084S.

IF_{2009}: 6.307
Independent citations: 20

9.2. Further publications

Fekete Katalin és Decsi Tamás. A cink szerepe gyermekek egészségének megőrzésében és helyreállításában. Gyermekorvos Továbbképzés 2009;8:169–172.

Katalin Fekete, Cristiana Berti, Irene Cetin, Maria Hermoso, Berthold V Koletzko, Tamás Decsi. Perinatal folate supply: relevance in health outcome parameters. Matern Child Nutr 2010;6:23–38.

IF_{2010}: 2.311
Independent citations: 0

Katalin Fekete and Tamás Decsi. Long-chain polyunsaturated fatty acids in inborn errors of metabolism. Nutrients 2010;2:965–974.

IF_{2010}: still computing
Independent citations: 0

Cumulative IF: 14.925
Total independent citations: 59

9.3. Abstracts

Katalin Fekete, Nicola M Lowe, Tamás Decsi. Systematic review of methods for assessing zinc status in clinical trials. J Pediatr Gastroenterol Nutr 2009;48:E75.

Tamás Decsi, Tamás Marosvölgyi, Viktória Jakobik, **Katalin Fekete**. Systematic review of methods for assessing n-3 long-chain polyunsaturated fatty acid status in clinical trials. J Pediatr Gastroenterol Nutr 2009;48:E131.

Fekete Katalin, Nicola M Lowe, Decsi Tamás. A cinkellátottság biomarkereinek jellemzése az intervenciós vizsgálatok szisztematikus irodalmi áttekintése alapján. Gyermekgyógyászat 2009;60:131.

Tamás Decsi, Tamás Marosvölgyi, Viktória Jakobik, **Katalin Fekete**. Methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review. Ann Nutr Metab 2009;55:88.

Nicola M Lowe, **Katalin Fekete**, Tamás Decsi. EURRECA systematic review: how robust are biomarkers of zinc status? Proc Nutr Soc 2010;69:E39.

9.4. Oral presentations

Fekete Katalin, Jakobik Viktória, Marosvölgyi Tamás, Decsi Tamás. Az n-3 hosszú szénláncú többszörösen telítetlen zsírsavak ellátottságának biomarkerei az intervenciós vizsgálatok szisztematikus irodalmi áttekintése alapján. PhD Tudományos Napok, Budapest, 2009. október 30-31.

Fekete Katalin, Nicola M Lowe, Decsi Tamás. A cinkellátottság biomarkereinek jellemzése az intervenciós vizsgálatok szisztematikus irodalmi áttekintése alapján. Fiatal Gyermekorvosok Országos Találkozója, Kőszeg, 2009. április 3-5.

Fekete Katalin, Nicola M Lowe, Decsi Tamás. A cinkellátottság biomarkereinek összefoglalása és értékelése az irodalom szisztematikus áttekintésével. Magyar Gyermekorvosok Társasága 53. Nagygyűlése, Eger, 2009. június 18-20.

Tamás Decsi, Tamás Marosvölgyi, Viktória Jakobik, **Katalin Fekete.** Methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review. 19th International Congress on Nutrition, Bangkok, Thailand, October 4-9, 2009.

Fekete Katalin és Decsi Tamás. Szisztematikus irodalmi áttekintés a folsavellátottság perinatális egészségre gyakorolt hatásáról: az EURRECA nemzetközi project. Magyar Gyermekorvosok Társasága 54. Nagygyűlése, Esztergom, 2010. szeptember 23-25.

Fekete Katalin és Decsi Tamás. A hosszú szénláncú többszörösen telítetlen zsírsavak szerepe a veleszületett anyagcsere-betegségben szenvedő gyermekek táplálkozásában. Magyar Gyermekorvosok Társasága és a Magyar Gasztroenterológiai Társaság Gyermekgasztroenterológiai Szekciójának XXVII. Tudományos Ülése, Nyíregyháza, 2010. október 1-2.

Lohner Szimonetta, **Fekete Katalin**, Marosvölgyi Tamás, Decsi Tamás. Van-e nemi különbség a plazmalipidek zsírsavösszetételében? – szisztematikus irodalmi áttekintés. Magyar Gyermekorvosok Társaságának 2011. évi Nagygyűlése, Pécs, 2011. szeptember 1-3.

Lohner Szimonetta, **Fekete Katalin**, Marosvölgyi Tamás, Decsi Tamás. Nemi eltérések a plazma- és vörösvértest membrán lipidek zsírsavösszetételében. Magyar Gyermekorvosok Társasága és a Magyar Gasztroenterológiai Társaság Gyermekgasztroenterológiai Szekciójának XXVIII. Tudományos Ülése, Hévíz, 2011. szeptember 23-24.

9.5. Poster presentations

Tamás Decsi, Tamás Marosvölgyi, Viktória Jakobik, **Katalin Fekete**. Systematic review of methods for assessing n-3 long-chain polyunsaturated fatty acid status in

clinical trials. The 42th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, Budapest, Hungary, June 3-6, 2009.

Katalin Fekete, Nicola M Lowe, Tamás Decsi. Systematic review of methods for assessing zinc status in clinical trials. The 42th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, Budapest, Hungary, June 3-6, 2009.

Nicola M Lowe, **Katalin Fekete**, Tamás Decsi. EURRECA systematic review: how robust are biomarkers of zinc status? Nutrition Society Summer Meeting 2009, “Over- and Undernutrition: Challenges and Approaches", Guildford, UK, June 29-July 2, 2009.

Katalin Fekete, Viktória Jakobik, Tamás Marosvölgyi, Tamás Decsi. Assessing potential biomarkers of eicosapentaenoic acid status in humans: a systematic review. The Power of Programming – International Conference on Developmental Origins of Health and Disease, Munich, Germany, May 6-8, 2010.

Eszter Györei, **Katalin Fekete**, Elvira Verduci, Carlo Agostoni, Tamás Decsi. Are n-6 polyunsaturated fatty acids really involved in the pathogenesis of obesity? The Power of Programming – International Conference on Developmental Origins of Health and Disease, Munich, Germany, May 6-8, 2010.

Katalin Fekete and Tamás Decsi. Long-chain polyunsaturated fatty acids in phenylketonuria: a systematic review. The 44th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, Sorrento, Italy, May 25-28, 2011.

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