



**Evidence-based research in pediatric nutrition:
focus on the supplementation of
polyunsaturated fatty acids and prebiotics**

Ph.D. thesis

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Introduction

The number of systematic reviews is increasing rapidly, also in the field of nutrition and pediatric nutrition. In the field of nutrition, systematic reviews are essential for accurate summarizing of the evidence on nutrient status or baseline exposure to nutrients (either from food or from supplement intake) in a population, on bioequivalence or bioavailability of nutrients and their different chemical forms, on biological functions of different nutrients and on dose-response relationships.

Background

Long-chain-polyunsaturated fatty acids (LCPUFA) have important functions in cell membranes as indispensable building stones for human development and optimal health. Docosahexaenoic acid (DHA) and arachidonic acid (AA) are considered to be the most important functional LCPUFA. They can be provided directly from the diet or can be synthesized from their essential fatty acid precursors alpha-linolenic acid (ALA) and linoleic acid (LA). Among other enzymes, delta-6-desaturase and delta-5-desaturase are required for the formation of the longer-chain metabolites of both n-3 and n-6 series. The competitive desaturation of the n-3 and n-6 series of fatty acids by delta-6-desaturase is of major significance, because this step is considered to be the rate-limiting step of the pathway. The activity of this enzyme is modulated by hormones and by interactions of substrates and metabolic products. Sex hormones may be also among the factors which influence the activity of enzymes playing a role in the synthesis of long-chain polyunsaturated fatty acids.

Phenylketonuria (PKU) is a hereditary disease characterized by deficiency of the liver enzyme phenylalanine (Phe) hydroxylase. A special diet low in Phe has to be started immediately after suspecting the diagnosis in the newborn and they should be maintained until the exact diagnosis set up. This type of strict metabolic control usually provides not only lower amounts of vitamins and minerals, but also lower amounts of saturated and polyunsaturated fatty acids. In infancy and childhood, LCPUFA are important for normal neurodevelopment, a deficiency may contribute to disturbances in the function of the central nervous system, which are detectable as early as in preschool and school-age children with PKU.

Acute respiratory tract infections are the most common reason for people to seek medical help in developed countries. Strategies to prevent acute infections include the administration of different immunostimulants, vitamins and trace; recently, also probiotics were described to be effective in reducing the incidence rate of upper respiratory tract infections and that of diarrhea. In the case of probiotic supplementation, a large number (billions) of living probiotic bacteria have to be administered on a daily basis to ensure the continuous colonisation of the intestine and reach the desired health benefits; prebiotics may be an alternative and easier way to reach the same positive effects that usually are subscribed to probiotics. Food ingredients are classified as prebiotic when they meet three major criteria: first, they escape digestion in the upper gastrointestinal tract and reach the colon intact; second, they are fermented by the intestinal microflora; and third, they selectively stimulate the growth and/or activity of those intestinal bacteria which are associated with health and wellbeing.

Aims

I. To systematically review the gender-specific differences between women and men in the contribution of LCPUFAs to the fatty acid composition of the lipid pools most often reported in the literature for the characterization of fatty acid status, i.e. plasma phospholipids (PL), plasma cholesteryl esters (CE), plasma triacylglycerols (TG), total plasma lipids, erythrocyte and platelet lipids as well as subcutaneous adipose tissue from the abdomen or buttock.

II. To assess whether significant depletion of LCPUFA can be detected in PKU patients on diet and whether LCPUFA supplementation is an effective way to increase the availability of LCPUFA in PKU patients.

III. To systematically review the available literature on the efficacy of prebiotics in the prevention of acute infectious diseases in the pediatric age group.

I. Gender differences in long-chain polyunsaturated fatty acid status of healthy subjects: a systematic review and meta-analysis

Methods

Ovid MEDLINE (www.ovid.com), Scopus (www.scopus.elsevier.com), and the Cochrane Library CENTRAL database (www.thecochranelibrary.org) were searched from inception to February 2011 using text terms with appropriate truncation and relevant indexing terms. The search was in the form [n–3 LCPUFA terms] or [n–6 LCPUFA terms] and [biomarker terms] and [gender terms] and [differ*] and [human studies].

To be included into the review, a study needed to meet all of the following characteristics: 1. a study carried out in humans, 2. at least 14 participants included, 3. n–3 or n–6 LCPUFA status is reported in both males and females, 4. healthy individuals with normal weight were included, or population-based surveys was carried out in that the majority of participants were considered healthy, 5. omnivorous participants were included, 6. there was no dietary intervention or drug therapy before sample collection, 7. investigators measured at least 12 fatty acids by gas-liquid chromatography so the percentage distribution data contained the principal fatty acids of the fatty acid spectrum and presumably reflected realistic proportion of fatty acids in the given lipid fraction.

Data for each study included were extracted into a Microsoft Office Excel 2007 database file. Statistical analyses were performed using the Review Manager 5.1 Software (The Cochrane Collaboration, Oxford, United Kingdom). Mean differences (MD) was used for the analysis of continuous data. P values of less than 0.05 were considered to indicate statistical significance.

Results

Altogether 7925 titles and abstracts were identified via the electronic search or were found in the reference lists of the review articles. Threehundred and fiftysix of them appeared to be potentially relevant, so we attempted to collect them as full text articles. 343 full-text articles were available for detailed assessment for inclusion. Finally, 86 comparisons reported in 51 papers fulfilled the inclusion criteria.

Among the studies reviewed there were some methodological differences in the analytical methods used for the determination of fatty acid composition of the different biomarkers. The blood samples collected were stored in a deep frozen state, but the storage temperature was different (-20°C, -30°C, -40°C, -70°C or -80°C). Lipid extraction was carried out by chloroform and methanol in most, but not all the studies. The separation of different lipid fractions was performed by thin layer chromatography. Fatty acid analysis was carried out by gas-liquid chromatography in all studies. In some studies a packed column was used instead of capillary columns (capillary column: 34 papers, packed column: 10 papers, column type not reported: 7 papers)

In this review we focused on the following 8 PUFAs: LA, gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DHGLA) and AA from the n-6 series and ALA, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and DHA from the n-3 series.

Primary analysis showed significantly higher contribution of the n-6 essential fatty acid, LA and the n-6 long-chain metabolite, AA to plasma total lipids of women compared to men. As to n-3 fatty acids, the values of the principal LCPUFA, DHA were significantly higher (Figure 1), while the values of its precursor, DPA were significantly lower in women compared to men. However, with the exception of LA and DHA, considerable heterogeneity was seen among the results of the individual studies.

Plasma PL compositional data were reported for the largest number of men (n = 4097) and women (n = 4444). There was one cross-sectional study among the included articles, from which we were able to include 16 subgroups stratified according to geographic areas. Primary analysis revealed significantly higher contribution of both AA and DHA (Figure 2) to the fatty acid composition of plasma PLs in women, while in LA and ALA there was no gender difference.

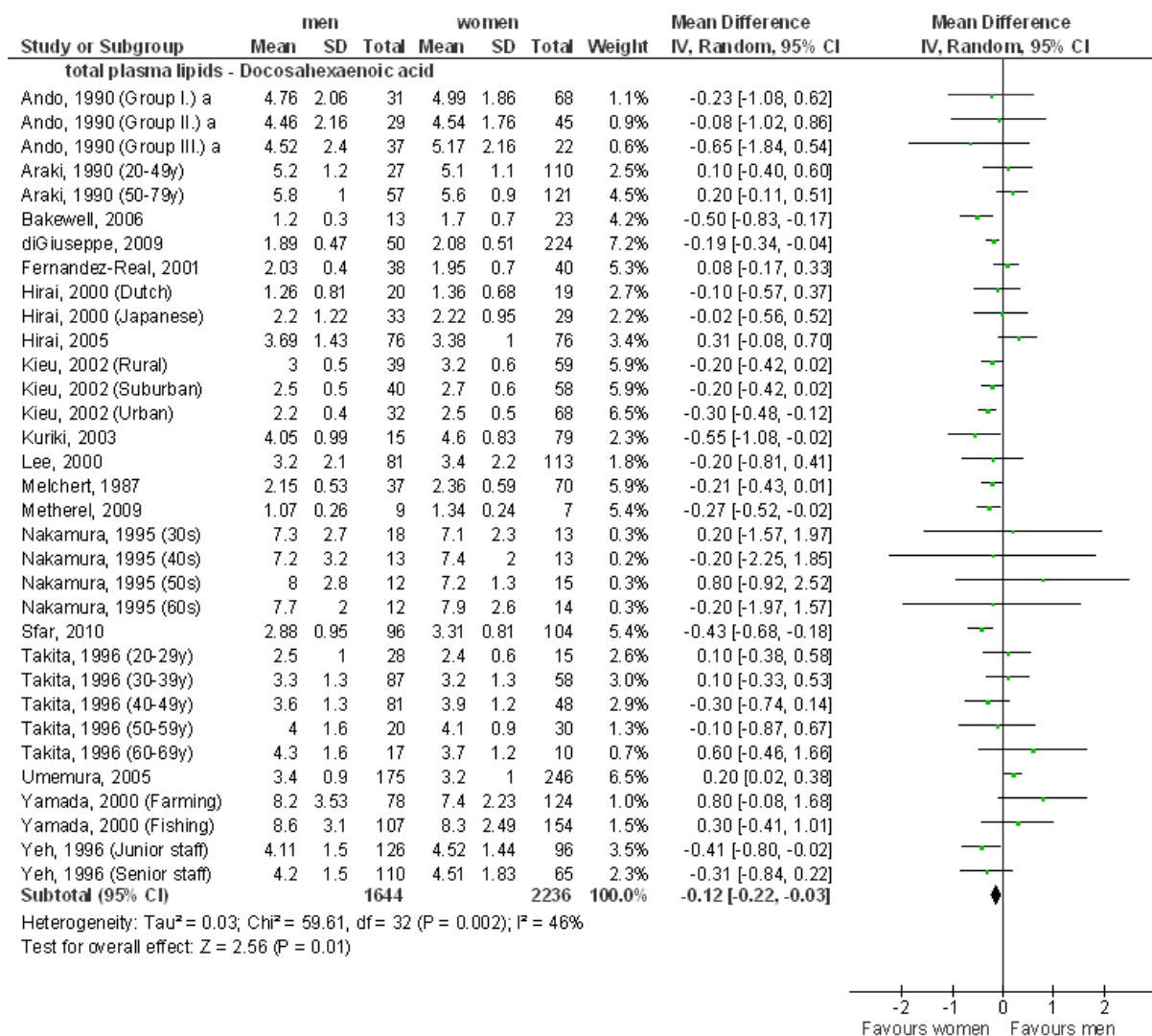


Figure 1. MD in the percent contribution of DHA to total plasma lipids of healthy male and female subjects

Plasma CE fatty acids were reported in 8 publications. There was no gender difference in AA and DHA values, but GLA and DHGLA were found significantly higher in men as compared to women.

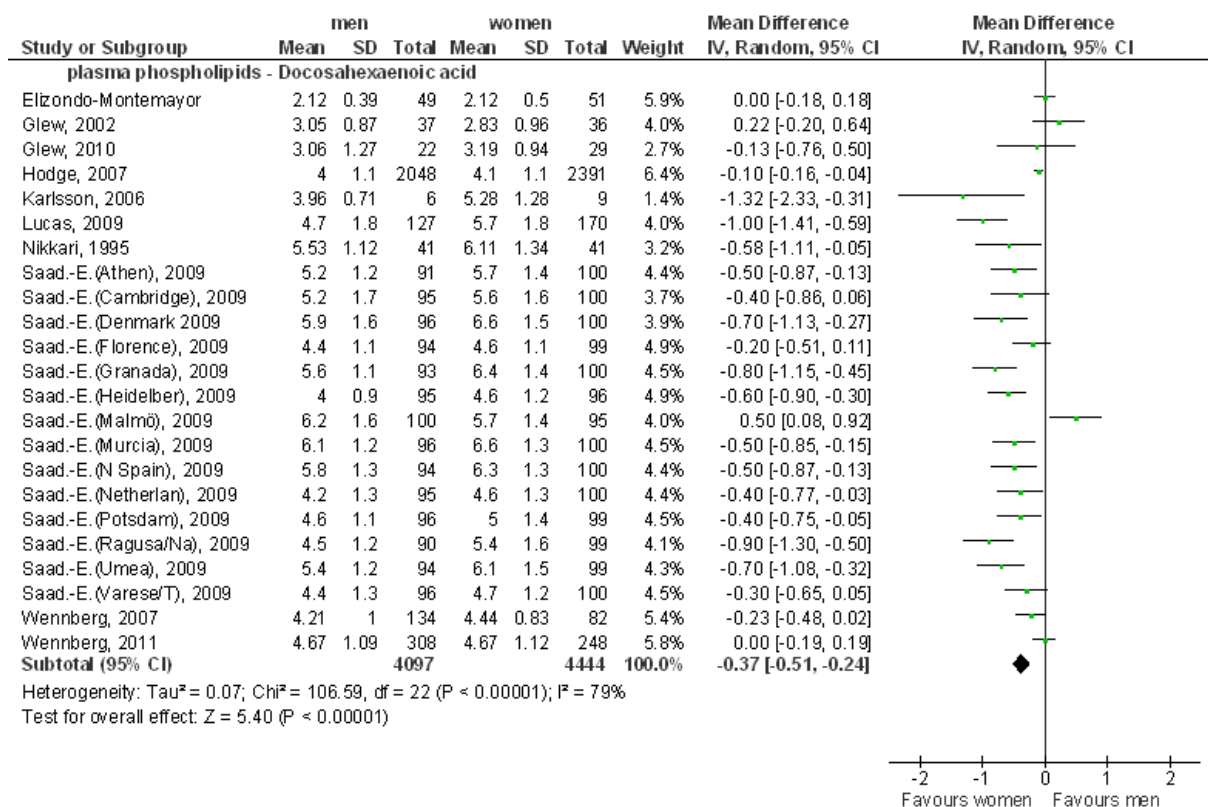


Figure 2. MD in the percent contribution of DHA to plasma PL of healthy male and female subjects

Five publications reported fatty acid composition of plasma TG in both men and women. The primary analysis showed no difference between the two sexes in any of the fatty acids discussed.

There were 9 publications reporting fatty acid composition of the erythrocyte membrane total lipids in both sexes. The primary analysis showed significant difference only in DHA values, which were higher in women than in men.

One paper reported erythrocyte phosphatidylcholine and phosphatidylethanolamine fatty acid composition in 5 subgroups; in erythrocyte PC the values of EPA were significantly higher in women than in men, whereas in erythrocyte PEA there was no difference between the two sexes in any of the fatty acids discussed.

In adipose tissue the values of LA and DHGLA were significantly higher in women than in men, while the values of GLA, AA, and from the n-3 series the values from EPA were significantly higher in men than in women.

II. Long-chain polyunsaturated fatty acid status in patients with phenylketonuria as compared to healthy controls: a systematic review and meta-analysis

Methods

To be included into the review, a study was required to meet the following characteristics: 1. be a study on patients of any age with PKU on low-protein diet and 2. be a case-control study reporting n-3 and/or n-6 LCPUFA status in patients and in healthy controls; or randomized controlled trial (RCT) investigating the effect of LCPUFA supplementation on LCPUFA status of patients with PKU.

Ovid MEDLINE, Scopus, LILACS (www.bireme.br) and the Cochrane Library CENTRAL database were searched from inception to February 2011. The search was repeated in a reduced form in May 2012. The search was in the form [n-3 LCPUFA terms] or [n-6 LCPUFA terms] and [PKU terms]. We did not apply any language restriction.

Statistical analyses were performed using the Review Manager 5.1 Software, with the random-effects model. The confidence interval (CI) was established at 95%. P values of less than 0.05 were considered to indicate statistical significance. Statistical heterogeneity was assessed using the I^2 statistics.

Results

Altogether 618 titles and abstracts were identified; 101 of them appeared to be potentially relevant and were ordered as full-text papers; finally, we included nine case-control studies and six RCTs in this systematic review, with a total of 713 and 273 participants, respectively.

In the case-control studies included, fatty acid composition of the following 5 biomarkers was reported: total plasma lipids, plasma phospholipids, plasma cholesteryl esters, total erythrocyte membrane lipids and erythrocyte phospholipids.

In this review we focused on LA and AA from the n-6 series and ALA, EPA and DHA from the n-3 series.

Seven papers reported fatty acid composition of plasma total lipids. Analysis showed significantly lower contribution of AA, EPA and DHA (for DHA data see Figure 4) to plasma total lipids of patients with PKU compared to healthy controls, while in the values of the essential precursors, LA and ALA there was no significant difference between the two groups.

Analysis of data from the four papers reporting fatty acid composition of plasma phospholipids showed significantly lower EPA and DHA (Figure 3) values in patients with PKU than in controls. No significant differences were found between the two groups with respect to AA, LA or ALA values. Comparison of erythrocyte membrane total lipid fatty acid composition in patients with PKU and healthy controls was present in 8 studies. The primary analysis showed significantly lower EPA and DHA (Figure 4) values in patients as compared to controls; however, there was no significant difference in AA values. There was no difference in LA values between the two groups, whereas ALA values were significantly higher in patients than in controls.

Data relating to the erythrocyte phospholipid fraction were sufficient only for a meta-analysis of DHA; values were significantly lower in PKU patients than in controls (Figure 3).

There were insufficient data to conduct a meta-analysis comparing fatty acid composition of plasma cholesteryl esters in subjects with PKU and controls.

Effects of LCPUFA supplementation on DHA concentrations in PKU were analyzed in six RCTs with parallel design. In two studies participants were newborns, in three studies infants and children between 1 and 18 years were included, while one study included both children and adults. Five studies were carried out in Europe, one in the USA.

In this report we primarily focused on the effect of LCPUFA supplementation on DHA status. We found eight biomarkers used to characterize changes in DHA values. From these, only total plasma DHA was used in at least three independent studies. Although different dosages and forms of n-3 LCPUFA supplementation were used in the different studies, these were all effective in significantly increasing DHA values of different biomarkers

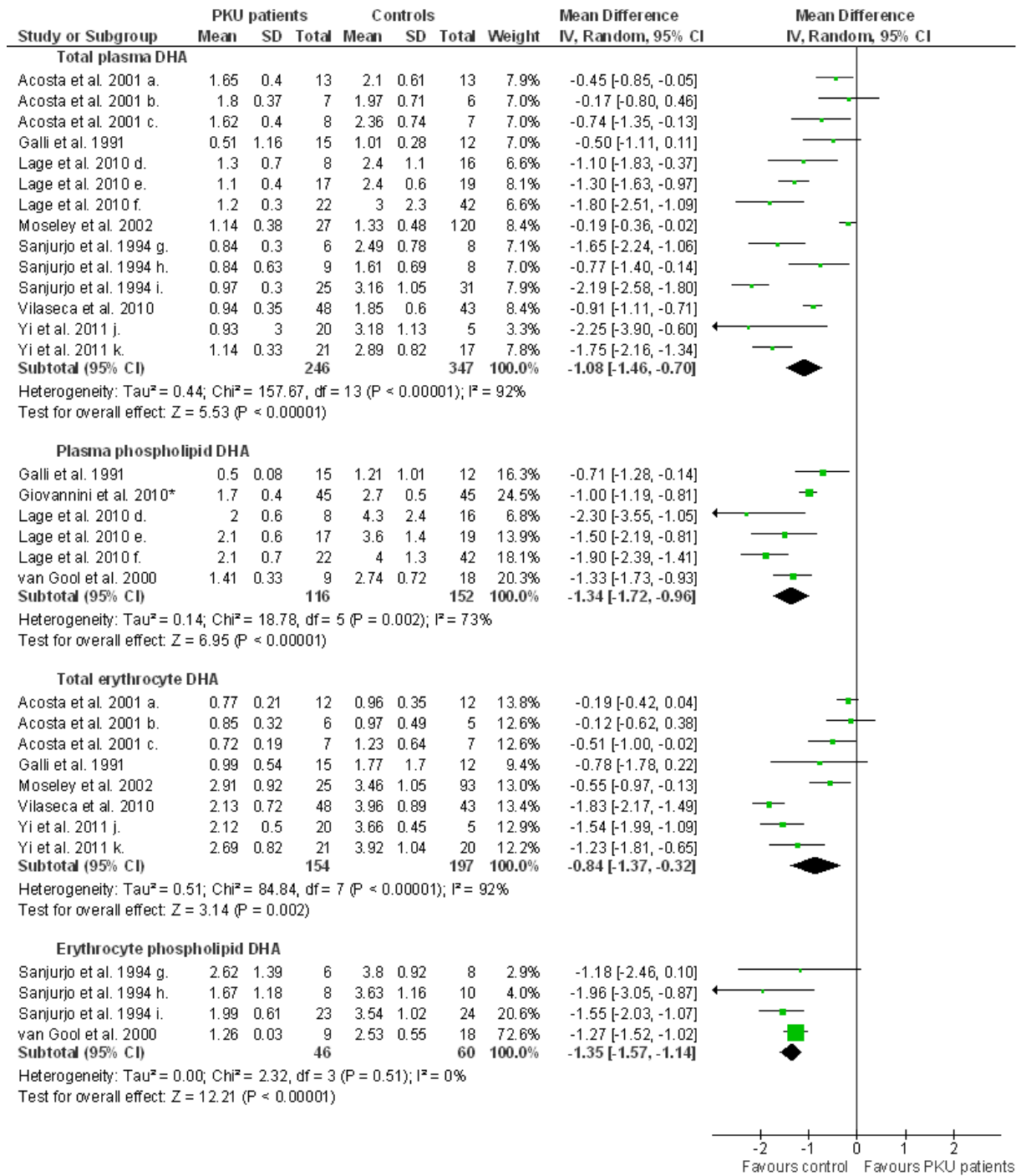


Figure 3. Case-control studies on DHA status in PKU patients versus healthy subjects, in different biomarkers

III. Prebiotics in healthy infants and children for prevention of acute infectious diseases: a systematic review and meta-analysis

Methods

To be included, a study needed to meet all of the following criteria: a) randomized allocation to treatment groups (RCT); b) carried out in healthy infants or children, aged 0-18 years; c) intervention with prebiotics (prebiotics added to food in the manufacturing process or as a separate supplement) compared to controls (placebo or no supplementation); d) a supplementation time of at least two months and an observation time of at least four months; e) using one of the following supplements: oligosaccharides, galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), fructans, inulin, oligofructose; f) describing at least one of the following outcomes: incidence of overall infections (any kind of infections), incidence of all acute respiratory tract infections (RTI), incidence of upper respiratory tract infections (URTI), incidence of lower respiratory tract infections (LRTI), incidence of otitis media, incidence of gastrointestinal infections (GITI) (defined in primary studies as 3 or more liquid/semiliquid stools per day with fever, vomiting, and/or dehydration and compromised general status), incidence of diarrhea episodes, incidence of urinary tract infections (UTI), incidence of fever episodes, incidence of infections requiring antibiotic treatment. No language restrictions were applied.

Ovid MEDLINE, Scopus, Web of Science (www.webofknowledge.com) and the Cochrane Library CENTRAL database were searched from inception to Week 3, June, 2013 for intervention studies. The search was in the form: [prebiotics terms] and [search filters for children] and [search filters for randomized controlled trials].

Meta-analysis was carried out according to the methodology for counts and rates described in The Cochrane Handbook. The results of the studies were expressed as a rate ratio, the ratio of the rate in the prebiotic group to the rate in the control group. The (natural) logarithm of the rate ratios was combined across studies using the generic inverse-variance method.

We used the I^2 statistic to estimate the variation across studies. An I^2 statistic < 40% was considered to be a low level of heterogeneity, 30% to 60% a moderate level, 50% to 90% a substantial level and 75% to 100% a considerable level. Regardless of

heterogeneity between the pooled studies, we used a random-effects model to synthesize all data.

Results

Altogether 1429 titles and abstracts were identified via the electronic search, 39 of them appeared to be potentially relevant. Finally, 5 studies reported in 8 full-text papers and 4 conference abstracts fulfilled the inclusion criteria. The studies ranged in size from 140 to 830 children randomized initially. All participants were infants and children younger than two years of age at the beginning of the intervention.

The studies involved different types of prebiotics including GOS/FOS mixture, polydextrose (PDX)/GOS mixture or oligofructose. In most of the studies included the intervention and observation time was the same and ranged between 108 days and 12 months, there was only one study in which investigators kept on observing the patients after the end of supplementation.

The incidence of any infection during the observation period was reported only in one study: infants in the scGOS/lcFOS group had significantly fewer episodes of any type of infection during the 6-month supplementation-observation period.

The incidence of fever episodes recorded by the parents of the participating infants and children up to 24 months was examined in two studies. The pooled effect of these two studies was not significant, but showed a considerable level of heterogeneity.

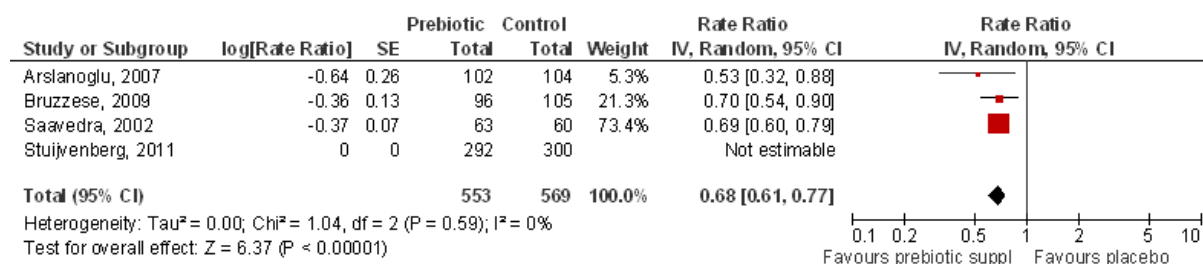


Figure 4. The rate of infections requiring antibiotic treatment (events per person/year) in infants and children supplemented with prebiotics versus placebo

For three RCTs eligible count data were available on the effect of prebiotic supplementation on the incidence of infections requiring antibiotic treatment (Figure

4). A pooled analysis of the data from these 3 studies showed a statistically significant difference in the episode rates. The level of heterogeneity between these studies was low.

Count data on any kind of gastrointestinal infections (GITI) were available for one RCT; the rate of GITI was lower in the prebiotic group, however this difference was not statistically significant. One further study reported that the mean number of episodes of gastroenteritis was significantly lower in the GOS/FOS group than in controls.

Diarrhea episodes were reported for three RCTs (Figure 5). The pooled effect estimate of these three studies shows no significant difference between the prebiotic supplemented and the placebo groups on the rate of diarrhea episodes; however, the level of heterogeneity among these studies was considerable.

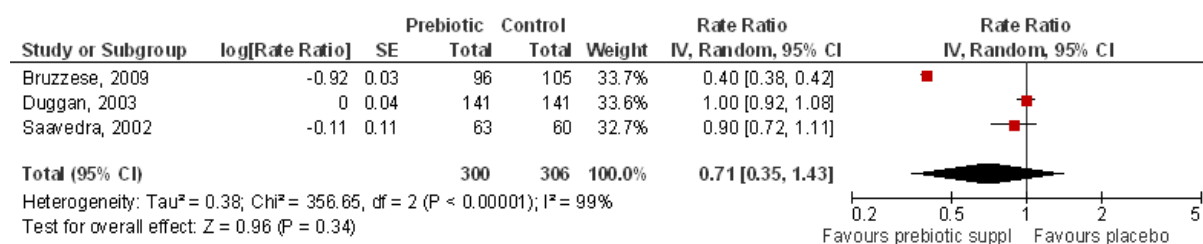


Figure 5. The rate of diarrhoea episodes (events per person/year) in infants and children supplemented with prebiotics versus placebo

Information on the incidence of upper respiratory tract infections (URTI) was available for 2 RCTs. Both studies only reported that the number of episodes of URTI was lower in the prebiotic group as compared to the control group, but the difference was in neither of the two studies significant.

Researchers in one study observed no significant difference between the prebiotic supplemented and the control group either in the rate of otitis media or in the rate of lower respiratory tract infections.

One report providing data on the effect of prebiotic supplementation on the incidence of urinary tract infections showed no significant difference in the incidence between the two groups.

Novel findings and practical applications

- 1.** Our systematic review based on 51 publications showed significantly lower contribution of both arachidonic and docosahexaenoic acid to plasma total lipids and plasma phospholipids in men than in women; in erythrocyte membrane total lipids DHA values were significantly lower in men than in women.
- 2.** In supplementation studies reporting fatty acid composition in serum PL, serum total lipids or erythrocyte membrane lipids, gender distribution should be regarded as significant potential confounding variable.
- 3.** The data systematically reviewed here indicate that in patients with PKU n-3 LCPUFA supply is insufficient. However, data from RCTs suggest that DHA status in patients with PKU may be reflectively improved by dietary supplementation.
- 4.** There is only limited evidence about the optimal LCPUFA supplementation dosage in the different age groups. It is of clinical importance to investigate whether LCPUFA deficiency is present in all age groups, especially in adolescents and adults who usually follow more relaxed diets.
- 5.** Currently available evidence suggests that the preventive use of prebiotics decreases the rate number of infections requiring antibiotic therapy in infants and children aged 0-24 months. Data from only one randomized controlled study indicate that prebiotics may also be effective in decreasing the rate of overall infections.
- 6.** Further studies should be carried out in the 3-18 years old age group to answer the question whether prebiotics can be considered for the prevention of acute infectious diseases also in the older pediatric age group.

List of publications

Publications on which the thesis was based

Lohner S, Fekete K, Marosvölgyi T, Decsi T: *Gender differences in long-chain polyunsaturated fatty acid status: systematic review of 51 publications*. Ann Nutr Metab. 2013;62(2):98-112 (IF₂₀₁₃: **2.747**)

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Cumulative impact factor of publications: **18.668**

Cumulative impact factor of publications as first author: **14.812**

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Poster presentation

- **Szimonetta Lohner**, Katalin Fekete, Tamás Marosvölgyi, Tamás Decsi: Is gender a relevant modifying factor influencing the polyunsaturated fatty acid composition of serum and erythrocyte membrane lipids in all age groups? 47th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, 2013 May, London