Functional markers for the assessment of (sub)clinical micronutrient deficiencies. Contributions to the diagnosis of hyperhomocysteinemia and essential fatty acid deficiency

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5 Summary

Nutrition plays an important role in the pathophysiology of (chronic) disease (chapter 1). The rapidly dietary changes in the past century may at least in part be at the basis of the high risk of some typically Western diseases, including coronary heart disease (CHD), diabetes mellitus, certain cancers, chronic obstructive pulmonary disease, dementia and osteoporosis. Among the most important dietary changes are excessive macronutrient intakes (giving rise to overweight), insufficient micronutrient intakes (giving rise to subclinical deficiencies) and nutritional imbalances. In this thesis, we have focused on the diagnosis of subclinical micronutrient deficiencies and the augmentation of micronutrient status.

Many investigations have concentrated on the effects of single nutrients on risk of disease. The evidence is particularly strong for the relation between low ω3 fatty acid (FAω3) status, particularly long chain polyunsaturated FAω3 (LCPω3) status, and increased CHD risk. The evidence for a relation between low folate status and elevated CHD risk is expanding, although final proof of a causal relation awaits the outcome of randomized controlled trials with CHD endpoints. The folate-CHD relation is probably caused by an increased plasma total homocysteine concentration (tHcy) (chapter 1.3).

The recognition of the relations between micronutrients and disease has changed both the perspectives of the nutritional guidelines for whole foods and the Dietary Reference Intakes [DRIs; either recommended dietary allowances (RDA) or adequate intakes (AI)] for nutrients, from prevention of clinical nutrient deficiency towards disease prevention. For some vitamins, it is nowadays recognized that the higher DRI levels cannot always be met with intakes of the recommended diet. The finding from the most recent Dutch National Food Consumption Survey that many subjects in The Netherlands do not meet nutritional recommendations suggests that improvement of the diet may decrease the risk of (chronic) disease in the Dutch population (chapter 1.4). Dietary assessments are less suitable for establishment of micronutrient deficiencies in individuals than laboratory tests. Reference values of so-called 'static parameters' of micronutrient status are commonly employed to establish micronutrient deficiency, but these cannot be used for the evaluation of subclinical deficiencies. Cut-off values of subclinical deficiencies are preferentially based on the relation between micronutrient status and disease risk, but these relations are difficult to study. Consequently, only few of these are currently known in detail. The cut-off values may alternatively be based on the relation between so-called 'functional parameters' and either static parameters or micronutrient intakes. The choice of the parameter and its cut-off value depends the action following the test outcome, while taking benefits, hazards and costs into account (chapter 1.5). The micronutrient status may be optimized either by dietary advice, nutrient supplement intakes or food fortification. The

first two possibilities are commonly accepted for the prevention of (chronic) disease and clinical nutrient deficiency, respectively. Intervention may also be indicated when the mean nutrient intakes in populations do not meet the DRI, i.e. when (sub)populations have high risk of nutrient deficiency. This is particularly applicable when the specific nutrient is causally related with an increased risk of developing (chronic) disease or with severe clinical nutrient deficiency symptoms (chapter 1.6).

Chapter 2 is focused on homocysteine (Hcy). Increased plasma tHcy is associated with increased risk of coronary, cerebral and peripheral vascular disease. Hyperhomocysteinemia (HHcy) may e.g. be caused by low folate, vitamin B_{12} and vitamin B_6 status, and by impaired renal function. Folate status is the main determinant of plasma tHcy in Western populations. Although there is as yet no definitive proof that the relation between high plasma tHcy and cardiovascular disease (CVD) is causal, it nevertheless seems that lowest plasma tHcy confers lowest disease risk.

In chapter 2.1, we used plasma tHcy to investigate the folate, vitamin B_{12} and vitamin B_6 status in subjects from four different populations. In two of these, we focused on treatment of HHcy (2.1.3 and 2.1.4).

In chapter 2.1.1, we investigated whether formula-fed infants had different plasma tHcy compared with breast-fed counterparts, and during what time period any difference developed. For this, we determined plasma tHcy in 53 formula-fed and 15 breast-fed healthy low-birth-weight babies (≤2500 g) around days 10, 20 and 40. We found that breast- and formula-fed infants have similar plasma tHcy until 20 postnatal days and that plasma tHcy subsequently increases from day 20 to day 40 in breast-fed infants, but not in formula-fed counterparts. Plasma tHcy was inversely related with formula (day 10) and human milk (day 40) volume intakes. Hcy concentrations in human milk were found to be low. The gradually increasing plasma tHcy in breast-fed, but not in formula-fed, infants may be caused by lower folate, vitamin B_{12}, vitamin B_6 and vitamin B_2 concentrations in human milk. Low milk B-vitamins may derive from gradually developing suboptimal B vitamin status in the mother with duration of lactation. It remains to be established which of the four vitamins is primarily responsible for the encountered plasma tHcy difference between formula- and breast-fed infants.

Indian subjects, in particular subjects of Indian descent who migrated to other countries, have as yet poorly understood high CVD risk. Their risk is not accounted for by risk factors such as cigarette smoking, hypercholesterolemia or hypertension, but may be explained by high prevalence of diabetes mellitus and the combination of high triglycerides and low HDL-cholesterol, as combined in the syndrome X. A combination of low vitamin B_{12} and high plasma tHcy, caused by the Indian vegetarian diet, may also be a factor in their increased CVD risk. In chapter 2.1.2, we determined plasma tHcy and circulating vitamin B_6, vitamin B_{12} and folate concentrations in 41 Indian subjects and compared their values with those of 51 black and 26 Caucasian adults. All subjects lived in the island of Curaçao (Netherlands Antilles). Plasma tHcy and circulating vitamins were also monitored in the Indians during a five weeks supplementation study with vitamin B_6, vitamin B_{12} and

folic acid in pharmacological dosages. Compared with the other two groups, the Indians had highest prevalence of low vitamin B_{12} status (63%) and HHcy (51%). Their plasma tHcy did not change upon supplementation with vitamin B_6, but decreased upon vitamin B_{12} and to a lesser extent upon folic acid. We concluded that low folate and notably low vitamin B_{12} status cause high HHcy prevalence in the investigated Indian population, and suggest that HHcy may be a factor in their high CVD risk.

In chapter 2.1.3, we determined the optimal vitamin dose for pediatric patients with sickle cell disease (SCD), as derived from the ability of these vitamins to lower plasma tHcy. SCD is a single gene inheritable disorder that is pathophysiologically characterized by endothelial dysfunction with chronic and occasionally exacerbating components. From the grossly decreased erythrocyte (RBC) half-lives of SCD patients, it might be expected that they have higher folate needs. We previously showed that these patients indeed have subclinical folate deficiency, as derived from their high, and folic acid responsive, plasma tHcy. Lowering plasma tHcy in SCD patients by B-vitamin supplementation may reduce their inherently high risk of endothelial damage. Twenty-one pediatric SCD patients (11 HbSS, 10 HbSC; 7-16 years) participated in this 82 weeks dose-escalation study. Daily folic acid, vitamin B_{12} and vitamin B_6 supplements were gradually increased and blood was taken at 9 occasions for measurements of plasma tHcy and circulating vitamin concentrations. Lowest plasma tHcy was reached from 700 µg (3.5-7 US 1989 RDA) folic acid, 3 US 1989 RDA (4.2-6.0 µg) vitamin B_{12} and 3 US 1989 RDA (4.2-6.0 mg) vitamin B_6. In practice, this translates in daily dosages of 1 mg folic acid, 6 µg vitamin B_{12} and 6 mg vitamin B_6. We propose to prescribe this vitamin regimen to all pediatric SCD patients, since it may reduce their high risk of endothelial damage by simple and relatively inexpensive means.

Lack of proof of a causal relation between plasma tHcy and CVD precludes institution of a public health policy to improve folate status of the general population. In chapter 2.1.4, we investigated the effect of intensive nutritional education regarding a Mediterranean type of diet on plasma tHcy. The study was performed in the context of a controlled community-based CVD prevention project with 30-70 years old subjects. Their serum total cholesterol was 6-8 mmol/l and they had at least two other CVD risk factors. The intervention group (n=97) received intensive dietary education at weeks 4, 6, 12 and 40, whereas the control group (n=153) received a posted leaflet with the standard Dutch nutritional guidelines at baseline. One of the targets was to reach a 200 g/d fruit and vegetable intake difference between the intervention and the control groups. Plasma tHcy was measured at baseline and after 16 and 52 weeks. When compared with the control group and adjusted for baseline intake and gender, the intervention group increased after 16 and 52 weeks their combined fruit and vegetable intake with 77 and 62 g/d, respectively. Their plasma tHcy did not change. Also other studies have shown that nutritional education is moderately effective in changing fruit and vegetable intakes on a population level. We therefore conclude that nutritional education regarding a Mediterranean type of diet does not reduce plasma tHcy to an appreciable extent, and that this may also apply to other dietary advises. Folic acid fortified foods are likely to be more effective for plasma tHcy lowering on a population level.

In chapter 2.2, we described our four studies on the laboratory diagnosis of HHcy. There is as yet no consensus for HHcy diagnosis and treatment, mainly because the evidence for a causal relation is as yet lacking.

The upper limit of the plasma tHcy reference values, i.e. 15 µmol/l as derived from apparently healthy subjects, is generally used as cut-off value for HHcy establishment. The reference population may however include subjects with subclinical folate, vitamin B_{12} and vitamin B_6 deficiency. Since these deficiencies are associated with increased CVD risk, this 15 µmol/l cut-off value is likely to be too high for HHcy establishment in CVD risk assessments. Future demonstration of a beneficial effect of plasma tHcy lowering on CVD risk would justify the use of plasma tHcy reference values as established at optimized vitamin status. In chapter 2.2.1, we determined vitamin-optimized plasma tHcy reference values and investigated their influence on the prevalence of HHcy in healthy adults. Results were compared with HHcy prevalences as obtained by using 'European Concerted Action Project' (ECAP) cut-off values. Apparently healthy adults (n=101) received pharmacological dosages of folic acid, vitamin B_{12} and vitamin B_6 during a four weeks study period. We determined both fasting (f-tHcy) and 6h post methionine load (postload-tHcy) plasma tHcy at baseline and after 4 weeks. Baseline (4 weeks) f-tHcy and postload-tHcy reference values were 4.7-14.6 (4.1-9.3) and 18.8-49.7 (12.9-35.1) µmol/l, respectively. Mean f-tHcy and postload-tHcy decreased after 4 weeks vitamin supplementation by 3.5 (33.5%) and 8.5 (26.3%) µmol/l, respectively. The percentage subjects exhibiting significant decreases of f-tHcy following vitamin supplementation amounted to 88% (all subjects), 92% (non-vitamin users) and 72% (vitamin-users). HHcy prevalences with use of ECAP cut-off values were: 29% (all), 29% (men), 27% (pre-menopausal women) and 53% (post-menopausal women). With vitamin-optimized cut-off values they were: 58, 58, 76 and 89%, respectively. We concluded that the use of vitamin-optimized cut-off values gives rise to high HHcy pre-test probabilities in the general population and therefore precludes any meaningful role for plasma tHcy testing.

The results presented in chapter 2.2.1 not only showed that use of vitamin-optimized cut-off values increased the diagnostic value of f-tHcy, but also that it decreased the value of a postload-tHcy, when compared with use of ECAP cut-off values. These findings were studied into more detail in chapter 2.2.2. In a retrospective study design we determined the diagnostic value of f-tHcy and the added value of postload-tHcy with use of several plasma tHcy cut-off values for HHcy assessment in 177 healthy subjects and in 3,477 subjects with plasma tHcy test indications. Cut-off values were based on reference limits (f-tHcy ≤15.0; postload-tHcy ≤50.0 µmol/l), relative risk (f-tHcy ≤12.0, postload-tHcy ≤38.0; or f-tHcy ≤10.0 µmol/l) and vitamin-optimized reference limits (f-tHcy ≤9.3; postload-tHcy ≤35.1 µmol/l). Use of the American Heart Association (AHA) 10 µmol/l f-tHcy cut-off value gave HHcy prevalences of 65% in subjects with plasma tHcy test indications and 50% in healthy subjects. The combination of the vitamin-optimized reference limits for f-tHcy and postload-tHcy gave a HHcy prevalence of 79% in subjects with plasma tHcy test indications, of which only 5% was on account of increased postload-tHcy. Corresponding values for healthy subjects were 68 and 3%, respectively. The high diagnostic value of a f-tHcy with

employment of a 10 µmol/l (AHA) or 9.3 µmol/l (vitamin-optimized reference values) cut-off value, and the low added value of a postload-tHcy, supported our previous conclusion that there is no indication for plasma tHcy testing from an evidence-based point-of-view.

To determine whether fasting conditions are necessary for HHcy diagnosis, we studied intra- (CV_i) and inter- (CV_g) individual biological variation of plasma tHcy and factors that influence biological variation (chapter 2.2.3). Three-week biological variation was calculated from 9 samples of 6 adults, taken at Mondays, Wednesdays and Fridays at three different clock-times. Within-day plasma tHcy changes and variation were studied in 16 healthy adults at 8:00, 10:00, 12:00, 14:00, 16:00, 18:30 h, and in 10 of them after 3 weeks supplementation with pharmacological dosages of folic acid, vitamin B_{12} and vitamin B_6. A standardized breakfast (8:15 h) and lunch (12:15 h) was provided during these days. Between-day CV_i and CV_g were 9.9 and 25.6%, respectively. Between-day CV_i and CV_g were insignificantly highest at 8:00 h and lowest at 14:00 h. Within-day plasma tHcy decreased from 8:00h-10:00 h and was associated with protein intake in the previous evening. From the similarity between our biological variation coefficients and the standardized biological variations published by other investigators, and from the similarities of our time-specific CV_i and CV_g, we concluded that fasting is not necessary for plasma tHcy analysis. The relation between within-day plasma tHcy changes and protein intakes prior to testing, and insignificantly lowest CV_i and CV_g after 16 h of low protein intake, indicates that protein intake standardization prior to testing might lower biological variation, but its extent is as yet unknown.

In chapter 2.2.4, we discuss the recommendations of The Netherlands Heart Foundation (NHS) for the diagnosis, screening and treatment of HHcy. With these recommendations, the NHS encourages the development of a consensus for HHcy diagnosis and treatment and provided us with an appreciated initiative that brings the Hcy-CVD risk relation to the attention of the medical profession. Their recommendation may, in our opinion, be sharpened and simplified. To increase uniformity, we suggest to use a single cut-off value for HHcy diagnosis and HHcy treatment goal. The, in our opinion, preferable f-tHcy cut-off value amounts to 10 µmol/l, since this value is a consensus based on the relation between plasma tHcy and CVD risk from several studies. Moreover, it is concordant with the P97.5 of healthy subjects after vitamin-optimization. An additional advantage is that use of this cut-off value renders virtually no added value for the methionine load test (MLT). Treatment with a folic acid, vitamin B_{12} and vitamin B_6 combination, as opposed to the recommended stepwise treatment regimen, reduces the number of clinical chemical tests, reduces the risk of masking vitamin B_{12} deficiency, and possibly increases therapy compliance.

Chapter 3 is focused on essential fatty acids (EFA). EFA include FA of the ω3 and ω6 series. It is assumed that we have evolved on a ω6/ω3 ratio of 1/1 to 5/1. FAω6 intakes have however increased in the past 100 years at the expense of FAω3 to a current ω6/ω3 ratio of over 15/1. The relation between increased FAω3 intakes and reduced CVD risk suggests that the increased FAω6 consumption has eventually caused a state of subclinical FAω3 deficiency. The LCPω3, eicosapentaenoic (EPA) and docosahexaenoic

(DHA) acids, are mainly held responsible for the beneficial effects of FAω3. These FA derive mainly from the diet, notably fish, but may also be synthesized endogenously from the parent EFA α-linolenic acid (ALA). Synthesis of LCPω3, particularly of DHA, is however limited.

The aim of the studies in chapter 3.1 (3.1.1-3.1.6) was to investigate whether LCPω3, notably DHA, are (conditionally) essential.

In chapter 3.1.1, we compared the polyunsaturated fatty acid (PUFA) status of Dutch vegans and omnivores to investigate whether different diets and LCP synthesis rates might explain disparities. For this, we investigated dietary intakes and fatty acid compositions of RBC, platelets (PLT), plasma cholesterol esters (CE) and plasma triglycerides (TG) of 12 vegans and 15 age- and sex-matched omnivores. In general, vegans had lower ω3 status and higher ω6 status, including γ-linolenic acid (GLA) and dihomo-γ-linolenic acid (DGLA) but not arachidonic acid (AA). Vegans had lower AA (TG) after normalization of PUFA to 100%. Normalization of eicosanoid precursors to 100% revealed similar AA (all compartments), higher DGLA (TG) and lower EPA (all). High ω6, notably linoleic acid (LA), and low ω3, notably EPA and DHA, status in Dutch vegans derive from low dietary LCPω3 and ALA/LA ratio. Higher GLA and DGLA in their TG may reflect higher hepatic AA production rate, whereas higher AA and 22:4ω6 in omnivores indicates AA intake from meat.

In the subsequent two chapters, we investigated in two short-term supplementation studies whether GLA or cartinine alone, or in combination with ALA, augment ALA conversion to LCPω3. It has been speculated that these factors enhance LCPω3 synthesis from ALA by increasing Δ6-desaturase activity (GLA), or by stimulating FA transport across the peroxisomal membrane (carnitine). The latter would be necessary for LCP synthesis according to the pathway proposed by Infante et al. The studies were performed with subjects consuming vegan or lacto-ovo-vegetarian diets, since these subjects may have little negative feedback inhibition from dietary LCP on the conversion of ALA to EPA and DHA. Moreover, they might be expected to have low carnitine status, because of their low consumption of carnitine-rich foods such as meat.

The GLA study (chapter 3.1.2) was performed with 9 apparently healthy vegans, who were requested to consume either 2.0 g/d ALA (4 ml linseed oil) or 1.2 g/d GLA (6 ml borage oil) during the first four weeks and their combination in the subsequent four weeks. The LCPω3 contents before and after each supplementation period were determined in RBC, PLT, CE, TG and plasma phospholipids (PL). The supplements changed the dietary LA/ALA ratio (in g/g) from about 13.7 (baseline) to 6.8 (linseed oil), 14.3 (borage oil) and 6.4 (linseed+borage oil), respectively. ALA or GLA given as single supplements did not increase LCPω3 status, but their combination augmented LCPω3 (in CE) and EPA (in fasting TG) to a statistically significant, but nevertheless negligible, extent. Our results were similar to those of our previous study with omnivores. We therefore concluded that neither GLA, nor negative feedback inhibition by dietary LCP, is an important factor in the inability to augment notably DHA status by dietary ALA. The reach of a DHA plateau already at low dietary ALA intakes suggests that dietary DHA causes a non-functional DHA surplus, or

alternatively, that dietary DHA is important for maintaining DHA status at a functionally relevant level (i.e. that DHA intake is essential).

Twenty apparently healthy vegans and lacto-ovo-vegetarians participated in the carnitine study (chapter 3.1.3). They were requested to consume 990 mg/d l-carnitine or 2.0 g/d ALA (4 ml/d linseed oil) during the first four weeks and their combination in the subsequent four weeks. FA compositions of RBC, PLT, CE and TG were measured at baseline and after 4 and 8 weeks. Carnitine supplementation increased plasma free and total carnitine concentrations, but did not affect EPA and DHA contents of any of the investigated compartments. EPA and DHA changes were however inversely related to initial carnitine status. Our results did not suggest that carnitine is an important limiting factor, if any, for LCPω3 synthesis. The most efficient means to augment EPA and particularly DHA status remains consumption of LCPω3 from e.g. fish or supplements.

In chapter 3.1.4, we investigated the effect of high dose EPA supplementation on DHA synthesis. For this, we investigated the plasma, RBC and PLT FA profiles of a patient with mantle cell lymphoma, who had taken 12 g/d of ethyl-EPA for 16 months. Long-term ethyl-EPA consumption did not cause adverse side effects. EPA and its elongation product 22:5ω3 were highly elevated in all compartments, but DHA was within reference range. AA was moderately reduced, but DGLA remained within limits. In spite of an LCPω3 intake higher than in most Inuit populations, AA levels remained considerably higher in this patient when compared with Inuits. The absence of effects on DGLA and DHA, and the modest effect on AA in PLT, indicate that levels of these key FA may be subject to strong homeostatic regulation.

The homeostatic regulation of DHA status may be different in men when compared with women, since it was recently shown that women of child-bearing age have higher capacity for ALA conversion to LCPω3 than men. This observation was suggested to reflect the high DHA demands of fetuses and neonates during pregnancy and lactation, respectively. In chapter 3.1.5, we investigated in a retrospective design whether gender difference in RBC EFA status were demonstrable. For this we reviewed the RBC FA compositions that were used for the assessment of reference values for EFA and FAω3 status (see chapter 3.2). The apparently healthy study groups comprised 59 babies (2-46 days old), 33 infants (3.5 years) and 61 adults (22-49 years). Babies and infants did not show differences in RBC FA that were based on gender. On the other hand, male adults had higher RBC 22:5ω3, and insignificantly lower DHA, when compared with female adults. The DHA difference became significant after inclusion of data from 8 vegan adults. No differences were observed in RBC FAω6, apart from higher RBC 22:5ω6 in women, when compared with men. The encountered lower 22:5ω3 in combination with higher DHA in women is in agreement with their higher ALA to DHA conversion capacity as noted by others. In addition, our data suggest higher conversion rate to 22:5ω6 and thereby adds to the notion that women of childbearing age exhibit increased Δ4-desaturation activity.

In the literature study presented in chapter 3.1.6, we investigated whether LCPω3, and notably DHA, are essential. Dietary intervention trials, including those presented in the previous chapters, and stable isotope studies revealed that humans are poor DHA synthesizers. There is circumstantial evidence that we evolved on a diet with higher LCP

content and ω3/ω6 ratio, and that the AA and DHA needs to expand our brain were mainly covered by the diet. Early African hominid species lived at the margins of lakes and rivers and at the seashore, eating diets abundant of EPA and DHA, but also AA, from easily caught fish and other animals living in the water vicinity. The presently low intake of ALA and LCPω3 from the Western diet is associated with CVD, inflammatory disorders, and mental and psychiatric diseases, and to suboptimal neurodevelopment in neonates. The strongest indications for a current subclinical ω3 deficiency come from randomized controlled trials with LCPω3, showing reduced mortality from CVD and improved neonatal neurodevelopment. With notably these studies in mind, we conclude that DHA is essential.

Early suspicion of EFA deficiency (EFAD) or ω3-deficiency may rather focus on FA analyses than clinical symptoms. Reference values of static markers of EFA status are of limited value for the detection of these conditions, since many subjects in the reference population have inherently high CVD risk. Within-reference range EFA status may consequently not be synonymous with low risk of (chronic) disease. There are unfortunately as yet no functional markers for EFA status that are related to increased disease risk. In chapter 3.2 we investigated new functional markers, i.e. RBC 20:3ω9 (Mead acid), 22:5ω6/20:4ω6 and 22:5ω6/22:6ω3, that can be used for the early detection of biochemical EFAD, ω3- and ω3/DHA-deficiencies, respectively. Their cut-off values were based on 97.5 percentiles of RBC FA data that derived from apparently healthy groups of omnivorous subjects (n=205) and subjects with low dietary LCP intakes (n=93; i.e. vegans and formula-fed infants). Cut-off values were evaluated by their application in an EFAD suspected group of 108, mostly malnourished, Pakistani children, three pediatric patients with chronic fat-malabsorption and one patient with a peroxisomal β-oxidation disorder. The proposed cut-off values are applicable for ages above 0.2 years, since all parameters proved age-dependent up to that age. The omnivorous and low dietary LCP groups had similar RBC 20:3ω9 and RBC 22:5ω6/20:4ω6, indicating that these parameters are independent of dietary LCP intakes. Cut-off values for EFAD and ω3-deficiency were 0.46 mol% RBC 20:3ω9 and 0.068 mol/mol RBC 22:5ω6/20:4ω6, respectively. Since the omnivorous and low dietary LCP groups had different RBC 22:5ω6/22:6ω3, we advised two cut-off values for ω3/DHA status, i.e. 0.22 mol/mol RBC 22:5ω6/22:6ω3 for ω3/DHA-marginality and 0.48 mol/mol RBC 22:5ω6/22:6ω3 for ω3/DHA-deficiency. Use of RBC 20:3ω9 and 22:5ω6/20:4ω6 cut-off values classified 20.4% of the Pakistani children as EFAD+ω3-deficient, 12.9% as EFAD+ω3-sufficient, 38.9% as EFA-sufficient+ω3-deficient and 27.8% as EFA-sufficient+ω3-sufficient. The patient with the peroxisomal disorder was classified as EFA-sufficient, ω3-sufficient (based on RBC 22:5ω6/20:4ω6) and ω3/DHA-deficient (based on RBC 22:5ω6/22:6ω3). The three other pediatric patients were classified as EFAD, ω3-deficient and ω3/DHA-deficient. We propose to use present cut-off values for EFA, ω3 and ω3/DHA status assessment for the decision to initiate PUFA supplementation, until better concepts have emerged.