Effect of Fish Oil Supplementation on Fatty Acid Status, Coordination, and Fine Motor Skills in Children with Phenylketonuria

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Objective To investigate effects of long-chain omega-3 polysaturated fatty acids (LC-PUFA) on motor skills in patients with phenylketonuria (PKU).

Study design Thirty-six patients with PKU (1-11 years of age, good metabolic control: plasma phenylalanine ≤360 μmol/L for ≥6 months). We determined plasma phospholipid fatty acids, and in patients >4 years of age (N = 24) the motometric Rostock-Oseretzky Scale (ROS), before and after supplementation with fish oil for 3 months (15 mg docosahexaenoic acid [DHA]/kg body weight daily). ROS was also assessed in 22 age-matched controls.

Results Patients had low n-3 LC-PUFA in plasma phospholipids (DHA, 2.37 ± 0.10%; eicosapentaenoic acid [EPA], 0.4 ± 0.03%) and poorer ROS performance than controls (motor development index [MQ] 107 ± 3 vs 117 ± 3, P = .010). Supplementation increased phospholipid n-3 LC-PUFA (DHA 7.05 ± 0.24%; EPA 3.31 ± 0.19%; P < .001), decreased n-6 LC-PUFA (arachidonic acid, 9.26 ± 0.23% vs 6.76 ± 0.16%; P < .001), and improved ROS (MQ 115 ± 3.54; P = .011, paired t test). ROS was unchanged in 11 retested controls (MQ 115 ± 5.16, P = NS, paired t test multivariate analysis of variance [MANOVA] for time by group, P = .027). Patients tolerated fish oil well. Plasma phenylalanine remained unchanged.

Conclusion In patients with PKU, fish oil supplementation enhances n-3 LC-PUFA levels and improves motor skills. (J Pediatr 2007;150:479-84)

Phenylketonuria (PKU) caused by deficient activity of hepatic phenylalanine hydroxylase affects about 1 in 7000 live newborns in Caucasians. If left untreated, children with PKU suffer from severe psychomotor retardation.1 Postnatal diagnosis by newborn screening programs and early initiation of treatment based on strictly limited natural protein intake and supplemented phenylalanine-free synthetic amino acid mixtures result in a largely normal cognitive development. However, patients with PKU continue to have subtle but measurable neurological deficits. The IQ of these patients tends to be slightly lower than that of their healthy siblings,2,3 and they show inferior average school achievements.4 Many patients suffer from disturbance of concentration ability and a prolonged reaction time.5,6 Slight impairments in specific motor functions have been shown, especially in hand-wrist steadiness, finger-hand dexterity, and hand-wrist speed.7,8

The reasons for these subtle dysfunctions remain unclear. Fluctuations of plasma phenylalanine concentrations can influence cognitive performance,6,9 but other factors seem to contribute as well. The mainstay of therapy, strict dietary treatment, may induce metabolic imbalances. Vitamin and trace element depletions have been reported in patients with PKU.10-13 Long-chain polysaturated fatty acids (LC-PUFA), particularly docosahexaenoic acid (DHA, 22:6n-3) are also poorly supplied because natural food sources of LC-PUFA such as meat, liver, fish, and eggs are rich in protein and hence avoided with the PKU diet. Therefore, patients with PKU rely almost exclusively on endogenous production of DHA and other LC-PUFA by elongation and desaturation of the precursors linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3). Low plasma or
serum concentrations of LC-PUFA, especially of DHA, have been described in patients with classic PKU.\textsuperscript{14,15}

LC-PUFA are essential components of all cell membranes and modulate membrane functions. Animal studies have established that LC-PUFA availability modulates visual and behavioral development.\textsuperscript{16,17} In controlled trials supplementation of healthy formula-fed infants with preformed LC-PUFA led to beneficial effects on visual and psychomotor development in some but not all reported studies.\textsuperscript{18-20} Little is known on potential effects of LC-PUFA status on neurological functions beyond infancy.

We hypothesized that children with PKU who have a poor LC-PUFA supply may benefit from supplementation with fish oil providing n-3 LC-PUFA. We have previously shown that LC-PUFA supplementation in 36 children with PKU has enhanced information processing in the central nervous system, as assessed by visual evoked potential latencies.\textsuperscript{21} Here, we report the effect of LC-PUFA supplementation on fatty acid status and motor skills in the same study group.

**METHODS**

**Trial Design**

This open clinical trial included patients with classic PKU 1 to 11 years of age and was performed in accordance with the Declaration of Helsinki/Somerset West and ICH-GCP guidelines. The protocol was reviewed by the ethics committee of the Bavarian Board of Physicians, Munich, Germany. Information of patients and/or parents and acquisition of their consent was carried out in writing. Inclusion was restricted to patients with a documented average blood phenylalanine level, determined at least monthly over the previous 6 months, <360 μmol/L. Patients with additional diseases or abnormal signs in the general or neurological examination were excluded.

At baseline, patients were examined clinically, blood was taken for plasma phospholipid fatty acid status, routine blood tests, and phenylalanine concentration. The Rostock-Oseretzky Scale (ROS) was performed as described below.

Patients were then supplied with fish oil capsules (Ameu®, Omega Pharma, Berlin, Germany) providing 500 mg fish oil per capsule (35% omega-3 fatty acids including 18% eicosapentaenoic acid [EPA] and 12% DHA); the coating (gelatin) contained 3 mg phenylalanine. To ensure a dose of >15 mg DHA/kg body weight, patients received one capsule per day for each 4 kg body weight, rounding up to the next full capsule. Thus, patients received 2 to 10 capsules per day in two divided doses. All other aspects of dietary treatment remained unchanged. After 90 days of fish oil supplementation, a follow-up examination was performed in the same way as the baseline examination.

Healthy, age-matched children without special diets volunteered as controls. Written informed consent was obtained from the guardians. Controls were examined clinically, the motometric scale (ROS) was assessed at baseline, and again after 90 days in a subgroup. For ethical reasons, no blood sample was taken in controls, and they did not receive fish oil.

**Subjects**

Sixty-five patients, 1 to 11 years of age, were screened. Thirty-eight patients were enrolled, and 36 completed the protocol (6.3 ± 0.6 years of age, 19 females, two dropouts). The diagnosis of PKU had been established by newborn screening and confirmed by further testing and, in most patients, by molecular genetics. Dietary treatment had been initiated within the first 3 weeks of life. All patients were regularly seen in our clinic and followed a strict protein-restricted diet. The mean daily phenylalanine intake was 13.9 ± 0.6 mg/kg and did not change during the study. The dosage of synthetic amino acids followed current recommendations for protein intake in children\textsuperscript{22} with an added surplus of 20% to account for possible differences in biological value of synthetic amino acid mixtures relative to natural protein.

Controls were 30 healthy, age-matched children (6.6 ± 0.5 years of age, 15 females).

**Fatty Acid Analysis**

Venous blood with sodium-ethylene-diamine-tetraacetate as anticoagulant (1 mg/mL) was taken at least 4 hours after the last meal, at baseline, and after 90 days of fish oil supplementation. Plasma was separated immediately and frozen at −80°C until further analysis. Total lipids were extracted from 0.25 mL plasma with chloroform/methanol after addition of internal standard (C15:0), according to the method of Folch. Lipid classes were separated by one-dimensional thin layer chromatography on silica gel plates (Merck, Darmstadt, Germany) with development in n-heptane:diisopropylether:acetic acid (60:40:3). The band containing phospholipids (PL) was identified by comparison to standards, removed by scraping, and transesterified with methanolic hydrochloric acid. Fatty acids were analysed by high-resolution capillary gas liquid chromatography with a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Boeblingen, Germany), equipped with a 50 m × 0.32 mm (inside diameter) polar cyanopropyl silicone-coated column (SGE, Weiterstedt, Germany). Fatty acid determination was performed at a column-head pressure of 1.3 bar and an initial temperature of 130°C increasing by 3°C per minute to 180°C and then by 4°C per minute to 220°C. Peak identification was performed by comparison with authentic standards (Nu-Chek-Prep, Elysian, Minnesota).

**Motometric Rostock-Oseretzky Scale by Kurth (ROS)**

The ROS is a reliable test battery of body coordination and fine motor skills in children 5 to 11 years of age.\textsuperscript{23} The test was performed in the subgroup of 24 children with PKU and 11 controls within this age range.

The test battery contains five subtests of specific motor functions (static balance with and without optic control, dynamic balance, fine motor ability with consideration of velocity and accuracy, and motoric-rhythmic coordination). Every
subtest is demonstrated and explained until the proband has a complete understanding of the task, but there is no training phase. The subtests are first evaluated separately to yield test-specific C-values, based on the child's age. The C-values are added to a total C-score, which serves to determine the motor-development index (MQ). Similar to the IQ, the MQ is normalized to a median score of 100, derived from the normative population. The minimal total C-score of 1 represents a MQ of 28, the highest achievable C-score is 50 and represents an MQ of 149.

Subtests:

1. Coins: Twenty coins are placed in two lines at the edge of a table. The proband picks up the coins using the dominant hand one at a time and puts them into a small box placed behind the coin line. The number of coins put into the box within 15 seconds is counted.

2. Maze: With a soft pencil, the proband follows a small maze printed on paper. Evaluation criteria are touching, crossing, or interruption of the predicted line (maze mistakes) and the time required to finish the task (maze time).

3. Dynamic balance: The proband balances heel-to-toe over wooden ledges of different width (8, 7, 6, 5, 4, 3, 2 cm), starting with the widest. The end of the test is determined by either stepping aside, or by finishing all ledges without stepping aside. The number of balanced ledges is recorded.

4. Static balance: Standing on heels or toes on both feet (arranged in tandem, with eyes open and closed), standing on the toes (feet arranged next to each other, with eyes open or closed), standing on one foot (right and left, with eyes open and closed), and standing on one foot while crossing the hands behind the back. Evaluation criterion is the number of fulfilled tasks without losing equilibrium.

5. Motoric-rhythmic coordination: Clapping and stamping of six different rhythms. Evaluation criterion is the accuracy of the clapped rhythm for four measures.

The test was always performed under the same circumstances. One investigator (H.R.) examined all tested children (PKU and controls) at baseline and follow-up, each time in the same room of the hospital. Only the proband, the investigator, and the guardian were in the room. The guardian was allowed to watch the procedure but not to speak or gesture to proband or investigator. The raw data (raw scores, mistakes, time) were recorded on a standardized worksheet. Calculation of the C-value and MQ test were performed by a different investigator (S.B.) who was masked with respect to the identity of the person tested and the test date (baseline/follow-up).

Data Analysis

Comparisons between patients and controls were performed by analysis of variance (ANOVA) and Student's t test. To test treatment effects, MANOVA for repeated measures was used, with “group” as a between-subjects factor with two levels (patients and controls) and “time” as within-subjects factor with two levels (baseline and follow-up). After verification for normal distribution in all data sets, cases were weighted by group where appropriate. T test for paired samples was applied in addition for comparisons of baseline and follow-up evaluations. The Statistical Package for the Social Sciences for Windows 11.0 (SPSS Inc., Chicago, Ill.) was used for all statistical operations. Significance was accepted for P < .05. Data are given as mean ± SEM unless otherwise mentioned.

RESULTS

Plasma Phospholipid Fatty Acid Profiles

Compared with data of healthy children of the same ethnicity and from the same region,24 children with PKU showed a similar fatty acid composition of plasma phospholipids. Somewhat lower values were found in patients for total n-3 fatty acids and especially DHA (2.37 ± 0.1%) than in the reference population (2.62 ± 0.99%), whereas there were higher levels for total n-6 fatty acids (36.19 ± 0.49% vs 32.82 ± 2.68% in healthy children) and linoleic acid. Therefore, the n3 to n6 ratio tended to lower values in the patients. However, mean fatty acid levels in the patients were within the range of values observed in healthy omnivorous children in Europe.24

Fish oil supplementation increased the DHA concentration almost threefold and the EPA concentration about eightfold. This pronounced increase of DHA and EPA over time indicates a good compliance with the fish oil capsules. Total concentration of the n-6 fatty acids decreased by approximately 20% because of a decrease of especially arachidonic acid (AA) (by about 25%) and of its precursors 18:2n6 and 20:3n6. Mead acid (20:3n9) decreased by almost 50%, consistent with an increasing n-3 pool (Table I).

Coordination and Fine Motor Skills

At baseline, ROS was performed in 24 patients and 22 controls. Although all patients achieved results within the normal range, they showed poorer mean motor skills than controls with a significantly lower overall MQ (Table II). The children with PKU performed worse in all but one subtest, with particular marked differences in coin sorting and maze mistakes (testing finger–hand dexterity, hand–wrist speed, and eye–hand coordination) as well as in dynamic balance (Table II).

All 24 patients and 11 controls were retested after 3 months. During this interval, only the patients received fish oil supplements. At follow-up, the children with PKU showed a marked improvement in overall MQ (Table III) and in the subtests coin sorting and dynamic balance (Table IV), and there was no change in controls.

Relationship between Fatty Acid Status and Motometric Test Results

Using linear regression or bivariate correlation, no significant correlations were found between the plasma concentrations of the fatty acids analyzed (DHA, EPA, AA; total n3, total n6, n3 to n6 ratio) and the motometric performance (MQ and subtest C-values). Changes in the fatty acid concentrations during the supplementation period did not correlate to changes in the MQ or the ROS subtests. Plasma
phenylalanine levels did not correlate to motometric test results at baseline (P = .45) or at follow-up (P = .69).

**Tolerance**

Fish oil supplementation for 90 days showed no serious adverse effects. Even though the fish oil capsules contained small amounts of phenylalanine (3 mg/capsule), plasma phenylalanine concentrations did not change during the intervention period (Table I). Patients judged the overall tolerance as necessary in any case.

**Table I.** Plasma concentration of phenylalanine (μmol/L) and major fatty acids in plasma phospholipids (% weight/weight, mean ± SEM) in children with PKU before and after 3 months of fish oil supplementation

<table>
<thead>
<tr>
<th></th>
<th>PKU (N = 24)</th>
<th>Controls (N = 11)</th>
<th>MANOVA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MQ</td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>107 ± 3.25</td>
<td>115 ± 3.54</td>
<td>116 ± 3.96</td>
</tr>
<tr>
<td></td>
<td>P = .027</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>P = .011</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

*Repeated measures by group, with between-subject factor "group" and within-subject factor "time."
†Baseline vs follow-up within the same group (PKU or controls).

**Table II.** Results of the Rostock-Oseretzky Scale subtests (C-values) at baseline and after 3 months in children with PKU and controls

<table>
<thead>
<tr>
<th>C-values</th>
<th>PKU (N = 24)</th>
<th>Controls (N = 22)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coins</td>
<td>6.2 ± 0.4</td>
<td>7.4 ± 0.5</td>
<td>.061</td>
</tr>
<tr>
<td>Maze time</td>
<td>8.1 ± 0.5</td>
<td>7.4 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Maze mistakes</td>
<td>4.7 ± 0.4</td>
<td>6.1 ± 0.4</td>
<td>.030</td>
</tr>
<tr>
<td>Dynamic balance</td>
<td>4.1 ± 0.5</td>
<td>5.5 ± 0.5</td>
<td>.042</td>
</tr>
<tr>
<td>Static balance</td>
<td>6.0 ± 0.5</td>
<td>6.9 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Motor-rhythm</td>
<td>3.9 ± 0.5</td>
<td>4.9 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>C-score</td>
<td>33.0 ± 1.3</td>
<td>38.2 ± 1.4</td>
<td>.009</td>
</tr>
<tr>
<td>MQ</td>
<td>107 ± 3</td>
<td>119 ± 3</td>
<td>.010</td>
</tr>
</tbody>
</table>

*For repeated measures by group, with between-subject factor “group” and within-subject factor “time.”
DISCUSSION

Significant deficits in body coordination and fine motor skills were found in this group of children with PKU under good metabolic control with treatment since the newborn period following current standards. A supply of fish oil with high amounts of DHA and EPA for 3 months significantly increased both the plasma phospholipid n-3 fatty acids and results of the motometric test. The change of the MQ is not likely to constitute a normal developmental process or a training effect because there was no change in the age-matched control group after the same follow-up period. Thus, our data indicate that LC-PUFA supply may exert a benefit on body coordination and fine motor skills in patients with PKU.

The prerequisite for the normal development of proper hand motor function is the maturation of corticomotorneu- ronal connections. In healthy children, the development of motor function is variable and probably not complete before entering puberty. The various components of motor control appear to mature by 8 to 12 years of age. The reduced MQ values in our patients may reflect either a structural deficit or a developmental delay, but the latter seems more likely given the response to fish oil. A study in adult patients with early treated PKU, with completed psychomotor development, may help clarify this question.

Chronically elevated phenylalanine concentrations are known to have an impact on brain function, but the concurrent phenylalanine level seems to contribute as well. Therefore, we chose to include only patients with good metabolic control, that is, an average phenylalanine level <360 μmol/L, over 6 months. Plasma phenylalanine concentration remained stable over the study period, and there was no correlation between current phenylalanine concentrations and the motometric test results. Thus, the improved motor function is not a result of changes in current metabolic control.

Dietary treatment of patients with PKU may contribute to the observed deficits. Depending on the individual phenylalanine tolerance, the PKU diet provides roughly one-sixth of the usual intake of natural proteins in healthy children. Concomitantly, all other components of protein rich foods are avoided. Clinically relevant deficiencies have been recognized for calcium, iron, zinc, and vitamin B12, which are currently supplemented with amino acid substitutes for PKU. More recently, LC-PUFA depletion of plasma lipids has been reported in patients with PKU. DHA is considered the functionally most relevant LC-PUFA because it has decisive functions in the assembly, maintenance, and proper function of brain cell membrane lipids. The endogenous synthesis of DHA from the precursor α-linoleic acid found in vegetable oil is rather inefficient in humans. Several studies in healthy newborn infants have recognized the role of DHA supply as an important factor in postnatal psychomotor development.

The observed significant improvement of the MQ with fish oil supply supports a beneficial role of DHA. Abnormalities in the brain white matter are found in untreated and even early treated patients with PKU on magnetic resonance imaging. Electrophysiological studies revealed prolonged latencies of visual and somatosensory evoked potentials in varying proportions, which responded to LC-PUFA supply in two recent studies. High-dose DHA may directly or indirectly revert these abnormalities. In generalized peroxisomal disorders, DHA supplementation reduced MRI signs of dysmyelination, which supports this notion. The lack of significant correlations between the increase of LC-PUFA concentrations and improvement of motor function only partially argues against a biological relationship because plasma concentrations do not necessarily represent fatty acid availability in relevant tissues. Further, the assumed effect involves several steps in the uptake and metabolism of fatty acids by the brain, a process probably too complex to be represented by a linear relationship with plasma levels.

Given the functional consequences, we consider n-3 LC-PUFA conditionally essential substrates that should be supplied with the diet even beyond infancy. Since early and continuously treated patients with PKU benefit from dietary supplementation with n-3 LC-PUFA, their addition to the synthetic amino acid mixtures appears advisable. The dosage used in this study resulted in supranormal plasma n-3 LC-PUFA levels and was associated with a reduction of arachidonic acid values, hence a lower dosage of supplementation appears to be preferable and should be evaluated.

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REFERENCES


