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Three-Year Tracking of Fatty Acid Composition of Plasma Phospholipids in Healthy Children

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Key Words
Phospholipid fatty acids · Dietary fat · Tracking of fatty acid composition · Plasma phospholipid fatty acid composition, children

Abstract
Objectives: The fatty acid composition of plasma phospholipids reflects the dietary fatty acid intake as well as endogenous turnover. We aimed at investigating the potential tracking of plasma phospholipid fatty acid composition in children that participated in a prospective cohort study. ***Methods:*** 26 healthy children participated in a longitudinal study on health risks and had been enrolled after birth. All children were born at term with birth weights appropriate for gestational age. Follow-up took place at ages 24, 36 and 60 months. At each time point a 24-hour dietary recall was obtained, anthropometric parameters were measured and a blood sample for phospholipid fatty acid analysis was taken. ***Results:*** Dietary intake of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids at the three time points were not correlated. We found lower values for plasma MUFA and the MUFA/SFA ratio at 60 months compared to 24 months. In contrast, total PUFA, total *n*–6 and *n*–6 long-chain polyunsaturated fatty acids (LC-PUFA) were higher at 60 months. Significant averaged correlation coefficients (average of Pearson's R for 24 versus 36 months and 36 versus 60 months) were found for *n*–6 LC-PUFA (r = 0.67), *n*–6/*n*–3 LC-PUFA ratio (r = 0.59) and arachidonic acid/linoleic acid ratio (r = 0.64). Partial tracking was found for the docosahexaenoic acid/α-linolenic acid ratio (r = 0.33). Body mass index and sum of skinfolds Z-scores were similar in the three evaluations. ***Conclusions:*** A significant tracking of *n*–6 LC-PUFA, *n*–6 LC-PUFA/*n*–3 LC-PUFA ratio, arachidonic acid/linoleic acid ratio and docosahexaenoic acid/α-linolenic acid ratio may reflect an influence of individual endogenous fatty acid metabolism on plasma concentrations of some, but not all, fatty acids.

Introduction

Cardiovascular disease is the main cause of morbidity and mortality among adults in industrialized countries [1]. It is unanimously recognized that atherosclerosis originates in childhood although clinical expressions of cardiovascular disease only appear during adulthood [2–7]. Quality and quantity of dietary fat are an important environmental factor influencing serum lipid profile and hence susceptibility to atherosclerosis. Fatty acid composition of plasma lipid classes reflects food habits and is associated with the risk of coronary heart disease [8]. Plasma phospholipid fatty acid composition is accepted as a reliable biomarker of dietary fatty acid intake in observational and interventional studies [9–11]. Due to a

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slower turnover of plasma phospholipids than plasma triacylglycerols, day-to-day variation is attenuated, but on the other hand, almost instant incorporation of dietary fatty acids has been demonstrated in tracer studies [12]. However, in addition to dietary fat intake, endogenous fatty acid and phospholipid metabolism modulates plasma and membrane fatty acid composition [13–15].

Plasma fatty acid composition of children was determined at three time points during pre-school age to assess the extension of fatty acid tracking over time, with the aim to distinguish fatty acids or fatty acid classes according to the degree of metabolic control of their respective plasma concentrations.

Subjects and Methods

Twenty-six children (13 male, 13 female) participated in a longitudinal investigation on health risks and had been enrolled after birth. All children were born at term with birth weights appropriate for gestational age. Blood samples were obtained at age 24, 36 and 60 months in combination with scheduled preventive health checks.

The study was approved by the local ethical committees, and informed parental consent was obtained for each subject before enrolment.

Venous blood samples were obtained from an antecubital vein with sodium ethylene diamine tetraacetate (1 mg/ml) as anticoagulant. After centrifugation, plasma was immediately frozen in plastic vials with a snap-on lid and stored at –18°C until analysis. Plasma lipids were extracted into chloroform/methanol (2/1) [16]. The phospholipid fraction was isolated by thin-layer chromatography. Fatty acid methyl esters were prepared by transesterification with methanolic HCl and analyzed by high-resolution capillary gas chromatography as described previously [17]. Results were expressed as percentage (wt/wt) of all FA detected with a chain length between 12 and 24 carbon atoms.

Weight, height, tricipital and subscapular skin folds were measured [18] and body mass index was calculated [19]. Data from the National Center of Health Statistics was used as reference values [20]. Food habits were obtained via 24-hour recalls [21]. Eating models were used to enable easier collection of information and to quantify results [21]. The McCance and Widdowson's tables were used to convert food intake into energy and nutrient intake [22].

Z-scores of age-specific fatty acid levels were calculated based on mean levels and corresponding standard deviations (SD). Pearson correlation coefficients of age-specific Z-scores were estimated to assess level of tracking. The corresponding test of significance for Pearson correlation coefficients was not used due to its unhelpful null hypothesis (H0: $\rho = 0$). Overall tracking was assumed if Pearson correlation coefficients for 24 versus 60 and 36 versus 60 months exceeded 0.4. Partial (or later) tracking was assumed for Pearson correlation coefficients ≤ 0.4 between 24 and 36 months, but Pearson correlation coefficients >0.4 between 36 and 60 months. Additionally, time period-weighted averages were calculated from correlation coefficients from 24 to 36 and 36 to 60 months. Differences of mean levels at time points were tested by analysis of variance.

All calculations were carried out with the software package SAS version 9.1 (SAS Institute Inc., Cary, N.C., USA) and with the statistical software package R2.0.1 (http://www.r-project.org/).

Table 1. Mean daily intake (SD) of fatty acid classes (% energy of total fat-derived energy for those food items with available information for individual fatty acid classes) at age 24, 36 and 60 months (n = 26)

	24 months	36 months	60 months
SFA, %*	32.1 (15.9)	46.3 (5.2)	47.5 (4.5)
MUFA, %*	56.9 (14)	46.2 (3.8)	44.2 (3.3)
PUFA, %	11 (10.2)	7.6 (2.3)	8.3 (2.2)

SFA = Saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

* Significant difference between time points ($p < 0.05$), identified by ANOVA.

Table 2. Mean levels (SD) of Z-scores of body mass index and sum of tricipital and subscapular skinfolds at age 24, 36 and 60 months (n = 26)

Z-scores	24 months	36 months	60 months
Body mass index	0.5 (1.1)	0.23 (1.2)	0.64 (1.3)
Sum of skinfolds	0.1 (0.66)	0.28 (0.89)	0.12 (0.83)

No significant differences ($p < 0.05$) between time points identified by ANOVA.

Results

Dietary Fat Intake

As expected, total daily energy intake of the infants increased with advancing age from 891 ± 168 kcal at 24 months to 1,331 ± 309 kcal at 36 months and to 1,572 ± 358 kcal at 60 months of age. Food habits changed from infancy to school age with an increasing percentage of energy from total fat (29.6 ± 6.4% at 24 months, 36.9 ± 5.2% at 36 months, 38.9 ± 5.6 at 60 months). The contribution of saturated fatty acids (SFA) increased with advancing age, while monounsaturated fatty acids (MUFA) and PUFA decreased (table 1). Intake of SFA, MUFA and polyunsaturated fatty acids (PUFA, ≥18 C-atoms, ≥2 double bonds) was not significantly correlated between the three time points.

Table 3. Mean weight percentages (SD) of plasma phospholipid fatty acids at age 24, 36 and 60 months (n = 26)

	24 months	36 months	60 months
SFA*	50.2 (2.1)	47.3 (1.2)	48.5 (1.3)
MUFA*	13.6 (2.1)	12.5 (1.5)	11.1 (1.5)
PUFA*	35.6 (2.9)	39.7 (1.6)	39.9 (1.8)
n–6 LC-PUFA*	11.8 (1.7)	14.2 (1.6)	13.8 (1.7)
n–3 LC-PUFA*	3.4 (0.9)	4.2 (0.8)	4.1 (1.0)
PUFA/SFA*	0.71 (0.1)	0.84 (0.1)	0.82 (0.1)
MUFA/SFA*	0.27 (0.04)	0.27 (0.04)	0.23 (0.03)
n–6 LC-PUFA/*n*–3 LC-PUFA	3.7 ± 1.1	3.5 ± 0.9	3.5 ± 0.8
AA/LA	0.39 (0.1)	0.47 (0.1)	0.44 (0.1)
DHA/ALN	24.7 (12.2)	29.8 (17.8)	30.6 (9.5)

* ANOVA $p < 0.05$.

LC-PUFA = Long-chain polyunsaturated fatty acids; PUFA = polyunsaturated fatty acids; MUFA = monounsaturated fatty acids; SFA = saturated fatty acids; AA = arachidonic acid; LA = linoleic acid; DHA = docosahexaenoic acid; ALN = α-linolenic acid.

Table 4. Correlation between plasma fatty acids and dietary PUFA intake (% of total energy intake and weight percent of fat intake) at age 24, 36 and 60 months

	PUFA	*n*–6 LC-PUFA	*n*–3 LC PUFA	*n*–6 LC-PUFA/*n*–3 LC-PUFA	PUFA/SFA
Energy intake					
24 months	0.52**	0.11	0.23	–0.24	0.5*
36 months	–0.1	–0.1	–0.41	0.36	–0.16
60 months	0.02	0.35	–0.01	0.2	0.1
Fat intake					
24 months	0.53**	0.06	0.15	–0.21	0.53**
36 months	–0.05	–0.2	–0.43*	0.35	–0.15
60 months	0.23	0.52*	0.1	0.1	0.26

* $p < 0.05$; ** $p < 0.01$.

Nutritional Status

Body mass index and sum of tricipital and subscapular skinfolds of the children observed at the three time points were similar, indicating no change with time of the nutritional status (table 2).

Fatty Acid Composition of Plasma Phospholipids

Differences were found between plasma phospholipid fatty acids from the different sampling points. MUFA and SFA were lower at 60 months compared to 24 months, while total PUFA were higher at age 60 months (table 3).

A significant correlation between PUFA intake and plasma fatty acids was particularly observed at 24 months (table 4). Expressing PUFA intake as percentage of daily energy intake (kcal) or as percentage of daily fat intake (g) did not significantly influence the results.

Correlations of plasma phospholipid fatty acids between the three time points are shown in table 5. The highest weighted average correlations between time points were observed for *n*–6 long-chain polyunsaturated fatty acids (LC-PUFA, ≥20 C-atoms, ≥2 double bonds), for the *n*–6/*n*–3 LC-PUFA ratio (fig. 1) and for the arachidonic acid/linoleic acid ratio. Partial tracking was found for the docosahexaenoic acid/α-linolenic acid ratio (table 5). No tracking was observed for SFA, MUFA or total PUFA.

Discussion

In the children studied, dietary intake changed from 2 years to school age, with an increasing percentage of energy from total fat which was similarly contributed by

Table 5. Pearson correlation coefficients between age-specific Z-scores of plasma phospholipid fatty acids at different time points

Fatty acid	Pearson correlation coefficient			
	24 vs. 36 months	24 vs. 60 months	36 vs. 60 months	weighted average
SFA	0.00	–0.02	0.13	0.09
MUFA	0.40	0.25	0.71	0.61
PUFA	0.43	0.14	0.47	0.46
n–6 LC-PUFA	0.68	0.63	0.67	0.67
n–3 LC-PUFA	0.21	0.31	0.38	0.32
PUFA/SFA	0.30	0.04	0.23	0.25
MUFA/SFA	0.28	0.20	0.67	0.54
n–6 LC-PUFA/*n*–3 LC-PUFA	0.48	0.58	0.64	0.59
AA/LA	0.77	0.63	0.57	0.64
DHA/ALN	0.13	0.47	0.43	0.33

LC-PUFA = Long-chain polyunsaturated fatty acids; PUFA = polyunsaturated fatty acids; MUFA = monounsaturated fatty acids; SFA = saturated fatty acids; AA = arachidonic acid; LA = linoleic acid; DHA = docosahexaenoic acid; ALN = α-linolenic acid.

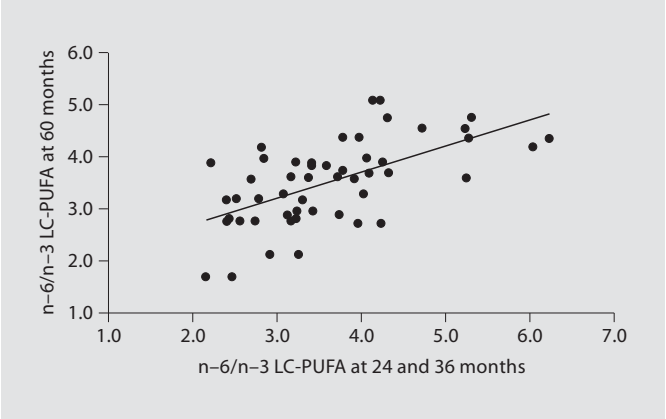

Fig. 1. Overall tracking (24 vs. 60 and 36 vs. 60 months) for plasma phospholipid *n*–6/*n*–3 long-chain polyunsaturated fatty acids ratio (r = 0.59).

all major groups of fatty acids (table 1). The increasing percentage of energy from SFA might be due to an increasing consumption of animal fat (milk, butter and lard) at age 60 months. Intake of SFA, MUFA and PUFA were not correlated between the three time points agreeing with intraindividual changes in food preferences but also with large day-to-day variation.

There is an ongoing discussion about the most suitable compartment to sample for the analysis of a biomarker for fatty acid intake [23]. Plasma triacylglycerols, cholesterol esters and phospholipids have been shown to be indicative for dietary compliance in clinical studies, although compositional changes are generally not proportional to dietary changes [24]. As for none of these lipid fractions the period for which they indicate the dietary fatty acid composition can exactly be specified and as we were mainly interested in arachidonic and docosahexaenoic acids, we decided to analyze plasma phospholipids, which show relatively high percentages of these fatty acids. Furthermore, a very good correlation for PUFA percentages in muscle phospholipids and serum phospholipids has been observed (r = 0.8 for both arachidonic and docosahexaenoic acid) in study participants on controlled diets for 3 months [25]. Thus, plasma phospholipids can be indicative of whole-body PUFA status under steady-state conditions.

In our subjects a correlation between PUFA from diet and plasma PUFA and PUFA/SFA could be observed at age 24 months but not at the older ages (table 4). SFA and MUFA from diet did not correlate with plasma fatty acids at any of the three time points. Similar results were observed in other studies in children older than 9 years and in adults [26].

This might be explained by a more stable food pattern at age 2 years and a greater day-to-day variability of food intake in older children. Furthermore, 24-hour recall is not a very accurate method for the assessment of dietary intake [27, 28]. High variability in nutrient intakes requires prolonged dietary recording of food habits in order to obtain precise and reliable information [29]. Unfortunately, under the given study conditions, this was not possible as compliance with 3-day food records was very poor. Nevertheless, the absence of a correlation be-

tween the observed dietary intakes at the different infantile ages studied and no correlation between PUFA intake and plasma phospholipid PUFA content is in agreement with an important further influencing factor on plasma PUFA, which might be endogenous metabolism.

The food recall was performed on the day preceding the collection of blood samples. Changes in phospholipid fatty acid composition occur only within 1 or 2 weeks [30], thus a high correlation of the results cannot be expected. Although metabolism seems to influence fatty acid composition of plasma phospholipids and cholesterol esters in addition to dietary intake, they might reflect medium-term intake of fatty acids better than many of the questionnaire based methods applied for dietary evaluation [31].

Although dietary PUFA were not correlated at the three time points, *n*–6 LC-PUFA and *n*–6/*n*–3 LC-PUFA ratio in plasma phospholipids were correlated between the three observations with weighted average correlation coefficients of 0.67 and 0.59 respectively, while *n*–3-LC-PUFA did not track between time points (table 5). A low and irregular intake of fish in the studied population may explain these results. However, the *n*–6/*n*–3 LC-PUFA ratio, which seems important for a balanced synthesis of eicosanoids, correlated between the three time points. Although less pronounced compared to the arachidonic acid/linoleic acid ratio, a tracking of the docosahexaenoic acid/α-linolenic acid ratio was also observed (table 5; fig. 1).

The tracking of the plasma fatty acid percentages over time observed in our study agrees in principle with observations in groups of Finnish children and adolescents in respect to LC-PUFA, as higher tracking was described for arachidonic acid (r = 0.54) than for docosahexaenoic acid (r = 0.38) over 6 years [32]. The fact that in this study additionally lower but significant tracking for individual saturated and monounsaturated fatty acids was observed, might be explained by the higher number of subjects studied and the observation that tracking of individual fatty acids is higher than tracking of groups of fatty acids [33].

The tracking of *n*–6 LC-PUFA percentages is in agreement with a significant influence of individual endogenous PUFA metabolism on plasma phospholipid composition in children of this age group [34]. This corresponds to the demonstration of a clear influence of genetic variants of the desaturation system on the plasma phospholipid *n*–6 LC-PUFA percentage in adults, while *n*–3 LC-PUFA were much less affected by these genes [35]. As LC-PUFA are relevant modulators of membranes properties [36], are required as precursors for the synthesis of biologically active substances, e.g. eicosanoids [37, 38], and are involved in the regulation of gene expression [39], this might be of considerable importance.

We did not find significant tracking of plasma phospholipid SFA and MUFA between time points, which indicates less importance of the endogenous metabolism.

In conclusion, the present study shows that plasma phospholipid fatty acid composition has a overall tracking for *n*–6 LC-PUFA, *n*–6/*n*–3 LC-PUFA ratio and arachidonic acid/linoleic acid ratio, and a partial (or later) tracking for docosahexaenoic acid/α-linolenic acid ratio which might be related to interindividual differences of the activity of the desaturation/elongation pathway. This points towards the importance of personalized dietary recommendations.

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