

Fatty acids in serum lipid fractions as indicators of fat intake in 5-year-old children in the STRIP project

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Abstract

The objective of the present study was to investigate the association between dietary fat composition and the proportion of fatty acids in serum lipid fractions as biomarkers of previous dietary fat quality in 5-year-old children. Using 4-consecutive-day food records, the food consumption of half (n=50) of a group of randomly selected children who are participating in a prospective randomized coronary risk factor intervention project (STRIP; n=1062 at recruitment at 7 months of age) was estimated immediately prior to a blood sampling for analysis of fatty acids in serum lipid fractions, whereas in the other half of the group (n=52), food consumption was measured 5 to 15 days before the sampling. Nutrient intakes were analyzed using the Micro-Nutrica program. The fatty acid composition of serum triglycerides (TG), cholesterol esters (CE) and phospholipids (PL) was analyzed using gas chromatography.

When intake was recorded immediately prior to measurement of serum fatty acids, SAFA intake was significantly correlated to TG-SAFA, but did not correlate with CE-SAFA or PL-SAFA. PUFA intake was significantly correlated to serum TG-PUFA and CE-PUFA. When intake had been recorded 5 to 15 days prior to measurement of serum fatty acids, SAFA intake was significantly correlated to TG-SAFA. Significant correlation was also obtained between dietary and serum PUFA in serum TG fraction. The correlations between MUFA intake and serum MUFA concentrations were always poor irrespective of when intake had been recorded.

In conclusion, serum TG-SAFAs reflect short-term SAFA intake and serum TG-PUFAs reflect short-term PUFA intake in 5-year-old children.

Introduction

Fatty acid compositions of adipose tissue, erythrocyte and platelet membranes and serum lipid fractions have been used to estimate previous fat intake and proportions of fat types consumed (1,2). Dietary fat intake over a relatively long period is assumed to be reflected in adipose tissue fatty acids, whereas medium-term dietary intake is reflected by fatty acids in serum cholesterol esters (CE) and phospholipids (PL) (1). As biomarkers of immediately preceding intake of dietary fatty acids, serum CE fatty acids have been widely used (3-6), although fatty acids in serum triglycerides (TG) may reflect short-term intake even better (2,7).

Accurate estimation of food consumption is difficult in children as dietary assessment methods have been constructed

mainly for adult populations and may function inappropriately in young age groups. The fatty acid profile of several serum lipid fractions has been determined in healthy children (8-15), but only occasionally has the data also included information about fatty acid intake. Some data is available about the connection between type of fat ingested and the fatty acid composition of serum cholesterol esters and phospholipids in children (13,15,16), but the link between short-term dietary fat intake of children and the fatty acid composition of serum TGs has remained poorly characterized.

Basing our hypothesis on accumulated knowledge of the intermediary metabolism of lipids in serum lipid fractions, we speculated that the fatty acid composition of serum TGs might be the best reflector of immediately preceding dietary intake of fatty acids. To test this hypothesis, we evaluated the connections between fatty acid compositions of the three major serum lipid fractions, triglycerides, cholesterol esters and phospholipids, and the quality of recent and previous fat intake in a cohort of 5-year-old children.

Subjects and methods

The children in this study were participants in the STRIP project (Special Turku Coronary Risk Factor Intervention Project

for Children), which is a prospective randomized trial aimed at decreasing the exposure of children to the known environmental risk factors of coronary heart disease. The trial, described in detail elsewhere (17,18), included 1062 children in 1054 families, who were randomized to an intervention (n=540) or a control group (n=522) at the child's age of 6 months. Frequently repeated counseling of the intervention families aimed at reducing children's intake of saturated fat and cholesterol and correspondingly increasing the intake of monounsaturated and polyunsaturated fat. The control families received only general dietary information as currently delivered at Finnish well-baby clinics.

The children were recruited for the STRIP trial between March 1990 and June 1992. This study was carried out between May 1994 and June 1995. One hundred and two 5-year-old children were randomly selected for this substudy among participants in the main project. They were divided into children whose 4-consecutive-day food consumption was recorded for the four days prior to the blood sampling (n=50), and children whose 4-consecutive-day food recording was completed 5 to 15 days before the blood sampling (n=52). Study groups comprising equal numbers of intervention

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Table 1. Fatty acid intake in the diet (4-day mean) and proportions of fatty acids in serum triglycerides, cholesterol esters and phospholipids in 5-year-old children. Values are means \pm SD.

Fatty acid	Dietary intake as % total fat			Serum triglycerides§			Serum cholesterol esters§			Serum phospholipids§		
	Group I n=50	Group II n=52	p*	Group I n=50	Group II n=52	p	Group I n=50	Group II n=52	p	Group I n=49	Group II n=52	p
SAFA	42.5 \pm 5.0	40.5 \pm 4.7	0.04	34.6 \pm 4.7	35.6 \pm 5.3	0.04	12.7 \pm 1.1	13.5 \pm 1.2	0.001	44.7 \pm 1.9	45.3 \pm 2.5	0.20
C16:0	20.0 \pm 2.2	19.2 \pm 2.1	0.06	27.3 \pm 3.4	27.9 \pm 3.7	0.12	10.7 \pm 0.9	11.3 \pm 1.0	0.002	27.7 \pm 1.9	28.0 \pm 1.8	0.34
C18:0	8.4 \pm 1.1	8.2 \pm 1.2	0.43	5.3 \pm 1.2	5.6 \pm 1.4	0.23	1.2 \pm 0.2	1.4 \pm 0.3	0.004	16.6 \pm 1.6	16.9 \pm 1.3	0.37
MUFA	33.0 \pm 2.1	33.9 \pm 2.2	0.03	49.8 \pm 3.4	49.6 \pm 3.3	0.93	24.3 \pm 4.1	24.9 \pm 4.0	0.35	14.7 \pm 1.5	15.0 \pm 1.5	0.17
PUFA	15.0 \pm 3.6	15.8 \pm 2.8	0.20	15.5 \pm 3.7	14.7 \pm 4.7	0.09	63.0 \pm 4.0	61.6 \pm 4.4	0.06	40.6 \pm 2.4	39.7 \pm 3.3	0.09
C18:2n-6	11.5 \pm 3.0	11.7 \pm 2.7	0.66	11.7 \pm 3.2	11.0 \pm 3.8	0.18	53.3 \pm 3.8	52.7 \pm 3.9	0.35	23.1 \pm 2.2	23.0 \pm 2.4	0.98
C18:3n-3	2.0 \pm 0.6	2.1 \pm 0.8	0.35	1.2 \pm 0.4	1.1 \pm 0.6	0.10	1.0 \pm 0.2	0.9 \pm 0.2	0.06	0.4 \pm 0.1	0.3 \pm 0.1	0.006
P/S ratio	0.37 \pm 0.13	0.40 \pm 0.11	0.16	0.47 \pm 0.17	0.44 \pm 0.19	0.07	4.98 \pm 0.57	4.60 \pm 0.64	0.001	0.91 \pm 0.08	0.88 \pm 0.12	0.15

Group I: Food intake was recorded during the four days before the blood sampling; Group II: Food intake recording was finished 5 to 15 days before blood sampling; § Fatty acid proportions are expressed as % of all fatty acids in each serum lipid fraction; * Two-sample t-test

(n=53) and control (n=49) children and also boys (n=50) and girls (n=52).

The project has been approved by the Joint Ethics Committee of Turku University and the Turku University Central Hospital. Informed consent was obtained from all parents/guardians.

Food consumption and blood collection

Food consumption was evaluated using food records kept by the parents or day-care personnel on four consecutive days, which had been chosen freely by the parents. Nutrient intakes were calculated using the Micro Nutrica® program developed at the Research Centre of the Social Insurance Institution, Turku, Finland.

This program uses the Food and Nutrient Data Base of the Social Insurance Institution and calculates 62 nutrients of the most commonly used foods and dishes in Finland and includes data on all foods commonly consumed by infants and children. The program is continuously updated.

After an overnight fast, a blood sample was drawn from the antecubital vein using minimal stasis. Cutaneous analgesia was attained with lidocaine and prilocaine creme (EMLA®, Astra; Södertälje, Sweden). After clotting of the venous blood sample at room temperature for 30 to 60 min and low-speed centrifugation (3400 \times g for 12 min), serum was separated and stored at -25°C for a period of less than a month and then stored at -80°C until analyzed.

Analysis of fatty acids in serum lipid fractions

The fatty acid composition of serum triglycerides, cholesterol esters and phospholipids was analyzed using gas chromatography. Lipids were extracted from 0.5 ml serum aliquots with 6 ml chloroform-methanol (1:1, v/v). The extract was purified by the two phase system, and the lipid fractions were separated by thin-layer chromatography (Silica Gel 60 G, Merck, Darmstadt, Germany) using petroleum ether/ diethyl ether/acetic acid (85:15:1, v/v/v) as the developing solvent. Fractions corresponding to triglycerides, cholesterol esters and phospholipids were located in UV light, scraped off and extracted five times with 4 ml chloroform-

Table 2. Correlation coefficients between the proportions of fatty acids in the diet and in serum triglycerides, cholesterol esters or phospholipids. P-values for the correlation coefficients are in parenthesis.

Fatty acid	Triglycerides			Cholesterol esters			Phospholipids		
	Group I n=50	Group II n=52	p*	Group I n=50	Group II n=52	p	Group I n=49	Group II n=52	p
SAFA	0.48	0.36	0.48	0.21	-0.008	0.28	0.10	-0.00	0.62
C16:0	<0.001	(0.009)		(0.15)	(0.69)		(0.49)	(0.99)	
C18:0	0.36	0.23	0.49	0.13	-0.12	0.22	0.28	0.07	0.29
	(0.01)	(0.10)		(0.38)	(0.39)		(0.06)	(0.64)	
C18:0	0.23	-0.02	0.22	0.05	-0.06	0.59	-0.27	-0.02	0.21
	(0.10)	(0.91)		(0.71)	(0.67)		(0.06)	(0.89)	
MUFA	-0.29	0.08	0.07	-0.16	-0.06	0.62	-0.27	-0.04	0.25
	(0.04)	(0.55)		(0.25)	(0.68)		(0.07)	(0.76)	
PUFA	0.69	0.27	0.01	0.35	0.06	0.14	0.34	0.03	0.12
	(<0.001)	(0.05)							
C18:2n-6	0.69	0.27	0.01	0.30	0.11	0.33	0.11	-0.15	0.21
	(<0.001)	(0.05)		(0.03)	(0.44)		(0.43)	(0.29)	
C18:3n-3	0.30	0.13	0.38	-0.13	-0.11	0.92	0.09	-0.17	0.20
	(0.003)	(0.34)		(0.36)	(0.42)		(0.55)	(0.23)	
P/S ratio	0.74	0.26	0.001	0.43	-0.01	0.02	0.27	-0.02	0.15
	(<0.001)	(0.07)		(0.002)	(0.96)		(0.06)	(0.91)	

Group I: Food intake was recorded during the four days before the blood sampling; Group II: Food intake recording was finished 5 to 15 days before blood sampling; * p for differences in correlation coefficients between Group I and Group II

methanol (2:1, v/v). The fractions were dried in N_2 stream and saponified with 0.5 N NaOH in methanol at 100°C for 90 min. The fatty acids were esterified with 20% BF_3 in methanol at 85°C for 5 min. After addition of 0.5 ml of water saturated with NaCl, the methyl esters were extracted three times with 1 ml of n-hexane and analyzed using a Varian 3700 gas chromatograph (capillary column, length 30 m, diameter 0.25 mm, filled with Durabond DB-225, thickness 1.15 μ m). The temperature was programmed from 140°C to 220°C. Fatty acids from 14:0 to 22:6 were quantified with a flame-ionization detector and a Spectra-Physics 4270 integrator. The amounts of the individual fatty acids (C14:0 to C22:6) are expressed as percentages of the total area of all fatty acids peak detected.

Statistical methods

The results are expressed as means and standard deviations (SD). The two sample t-test was used to analyze differences in food records between the groups. Analysis of covariance with food record values as covariate were used to test the differences in serum fatty acid proportions. Associations between numerical measurements were evaluated using Pearson's correlation coefficient. The test of equality of two correlations was performed using normal distribution after Fisher's Z-transformation. Statistical computing was performed using the SAS release 6.08 program package (SAS Institute, Cary, NC). Differences were considered significant at $P < 0.05$.

Results

Fatty acids in children's diet and in serum lipid fractions

The fatty acid compositions of the diets of children whose food consumption was recorded for the four days prior to blood sampling and that of children whose four-day food consumption was recorded 5 to 15 days prior to blood sampling resembled each other closely (Table 1). In serum triglycerides, SAFAs, MUFAs and PUFAs comprised $\approx 35\%$, $\approx 50\%$ and $\approx 15\%$ of the fatty acids, respectively, in both groups of children. In serum cholesterol esters, SAFAs, MUFAs and PUFAs represented $\approx 13\%$, $\approx 24\%$ and $\approx 62\%$ of all fatty acids, respectively, in children whose food consumption was recorded just before the blood sampling as well as in children whose food consumption was recorded 5 to 15 days prior to the blood sampling. In serum phospholipids, SAFAs, MUFAs and PUFAs comprised $\approx 45\%$, $\approx 15\%$ and $\approx 40\%$ of all fatty acids, respectively, in both groups (Table 1). There were no

differences in dietary fatty acid intake or serum fatty acid composition between girls and boys (*data not shown*).

Dietary fat – fatty acid composition of serum lipid fractions

In these calculations dietary fatty acid proportion was expressed as percentages of total fat intake and due to the glycerol component, the sum of SAFAs, MUFAs and PUFAs is below 100%. On the other hand, fatty acid composition of serum lipid fractions was expressed as percentages of all fatty acids in each fraction. Thus, direct comparison of exact figures representing fatty acid intake and fatty acids in serum lipid fractions is not possible.

The proportions of SAFAs in serum triglycerides, cholesterol esters and phospholipids were slightly lower, markedly lower and almost similar to the respective proportions in the diet. The proportion of MUFAs in serum triglycerides was considerably higher than that in the diet, whereas the proportions of MUFA in serum cholesterol esters and phospholipids were lower than those in the diet (Table 1).

The proportion of PUFAs in serum triglycerides was very close to that of the diet measured just before the recording of food intake or earlier (Table 1). The proportion of PUFAs was 4 times higher in serum cholesterol esters than in the diet, PUFAs representing over 60% of all fatty acids in this lipid fraction in both groups of children (Table 1). The proportion of PUFAs in serum phospholipids was almost 3 times higher than their share in the diet.

Serum lipid fractions and dietary intake of fatty acids

When dietary data were collected just before blood sampling, dietary intake of SAFAs correlated rather well with serum triglyceride SAFA (Figure 1) and moderately well with palmitic acid (Table 2). Dietary intake of PUFAs and serum triglyceride PUFAs correlated well, as did serum triglyceride linoleic acid and the P/S ratio when the dietary data were collected just before blood sampling. In the group of children whose food consumption was recorded 5 to 15 days prior to the blood sampling only the proportion of SAFAs correlated moderately well, while that of PUFAs and linoleic acid correlated weakly in serum triglycerides with the same dietary fatty acids. The proportion of MUFAs in serum triglycerides did not reflect the intake of MUFAs at all in either group (Figure 1). The differences in the correlations between the two groups of

children with respect to triglycerides were significant for PUFA ($p=0.01$), linoleic acid ($p=0.01$) and the P/S ratio ($p=0.001$) (Table 2).

When dietary data were collected just before blood sampling, the correlations between the dietary intake of PUFAs and the PUFA content of serum cholesterol esters or phospholipids were markedly poorer than those between dietary PUFA and PUFA in serum triglycerides. Both dietary intakes of PUFA and linoleic acid and the P/S ratio of the diet correlated moderately well with the respective values in the serum cholesterol esters when food consumption was recorded immediately prior to the blood sampling (Table 2). In these children, the content of PUFAs in serum phospholipids also correlated reasonably well ($r=0.43$) with the respective dietary fatty acids (Table 2). The proportion of fatty acids in serum cholesterol esters or serum phospholipids did not reflect the fatty acid composition of diet at all when the dietary data were collected 5 to 15 days before the blood sampling (Table 2).

The differences in the correlations between triglycerides and cholesterol esters were significant with respect to PUFA ($p=0.02$), linoleic acid ($p=0.02$), α -linolenic acid ($p=0.04$) and with respect to the P/S ratio ($p=0.02$) in the group where the dietary data were collected just before blood sampling. Also in this group of children, the correlations were stronger in triglycerides than in phospholipids: the differences were significant with respect to SAFA ($p=0.04$), PUFA ($p=0.02$), linoleic acid ($p<0.001$) and P/S ratio ($p=0.002$). When food consumption was recorded 5 to 15 days before the blood sampling, no differences in the level of correlation were seen, either between triglycerides and cholesterol esters or between triglycerides and phospholipids.

The intake or serum fatty acid proportions in a few subjects differed from the corresponding mean values by more than 3 SD (Figures 1-3). All analyses were repeated without such outliers, but the results did not change essentially.

Discussion

In this study, saturated and polyunsaturated fatty acids in serum triglycerides correlated statistically significantly with the quality of fat in the diet of the 5-year-old children. The relatively fast turnover of fatty acids in serum triglycerides (1,19) matched our results well. Previously, cholesterol ester fatty acids have been widely used as a measure of the short-term supply of dietary fatty acids (20), whereas fatty acids in serum phospholipids are believed to reflect poorly the immediately

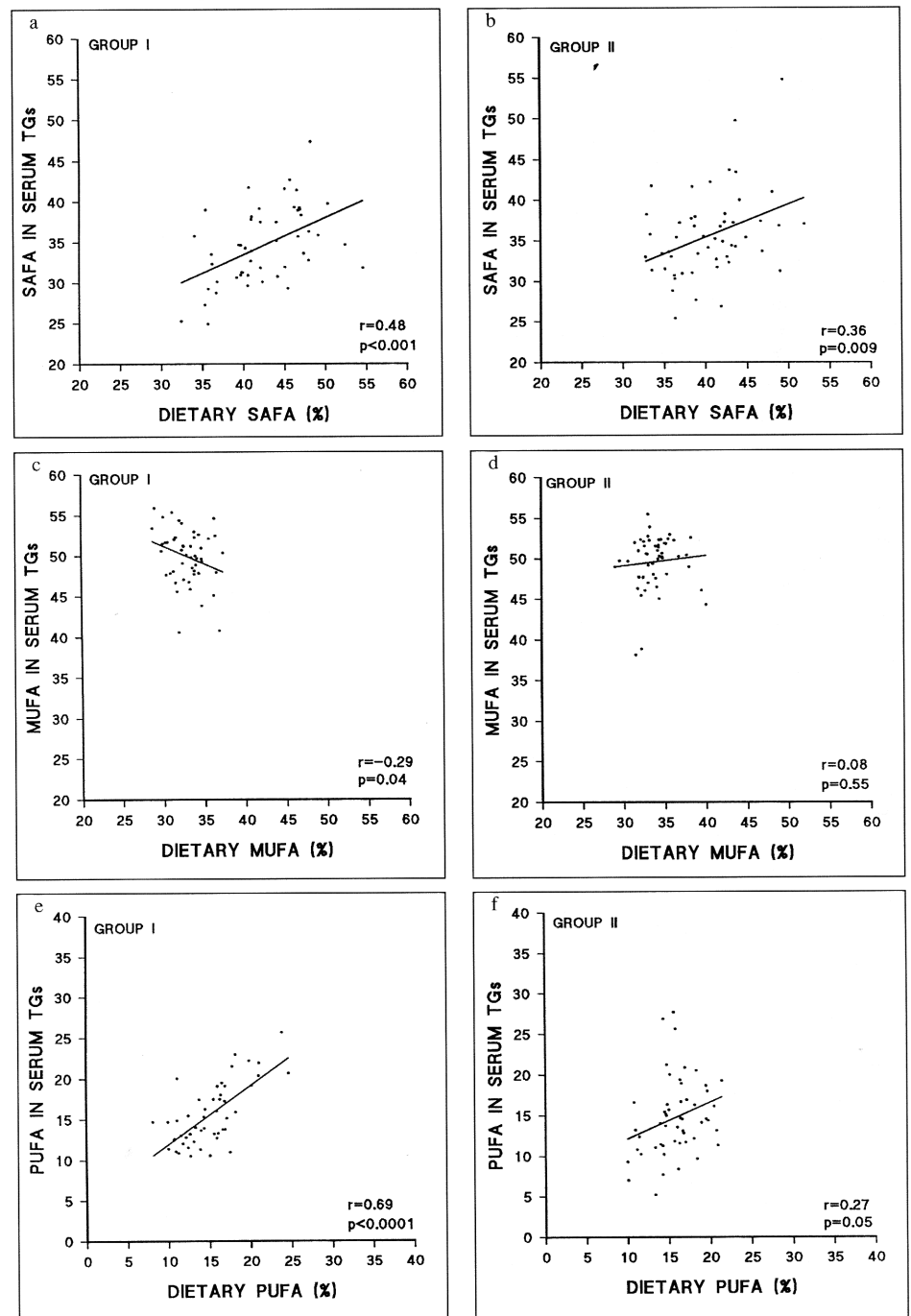
preceding dietary intake. On the other hand, phospholipid fatty acids may reflect more closely endogenously produced fatty acids as a result of intermediary fatty acid metabolism (7).

When the dietary intake data were collected by 4-day food records kept on the days immediately prior to the blood sampling, dietary PUFA correlated best with serum triglyceride PUFA and less well with cholesterol ester PUFA and phospholipid PUFA. The correlation between SAFA intake and SAFA in serum triglycerides in these samples was also statistically significant. Compared with the present study, almost similar correlations for all lipid fractions and diet have been reported in previous studies in adults (5,21) and in Finnish children and young adults between dietary and cholesterol ester PUFAs (12) and between dietary and phospholipid PUFAs (16). On the other hand, dietary MUFA intake correlated inversely with MUFA in all three serum lipid fractions in previous studies (12,15) as well as in our study.

The best biomarkers, which rapidly show changes in quality of fat intake, are probably the fatty acids that are not synthesized endogenously typically essential n-3 and n-6 polyunsaturated and trans fatty acids (2). The serum fatty acid composition of serum lipid fractions depends not only on the diet but also on digestion, absorption, *de novo* synthesis, usage and catabolism, excretion and transfer to other body pools of the individual fatty acids (22). The metabolism of fatty acids by chain elongation, desaturation and retro-conversion is regulated partly by a complex feedback network, where increased availability of one fatty acid may inhibit or induce elongation or desaturation of others. In this study, the proportion of MUFAs in serum triglycerides considerably exceeded that in the diet, probably because the first step in the desaturation of SAFAs produces MUFAs of the n-9 and n-7 series (23,24). MUFA production via SAFA desaturation varies markedly, and this may explain why dietary intake so poorly predicts serum triglyceride MUFA.

The affinities of the fatty acid desaturation enzymes for individual fatty acids also vary markedly (23-25), and fatty acids of the three main series of unsaturated fatty acids (n-3, n-6 and n-9) compete for the same elongation and desaturation enzymes. A consequence of such complex metabolic interaction is that an increase in the intake of one series of fatty acids may, as a secondary effect, change serum concentrations in fatty acids of the other series as well, thus hampering the estimation of dietary intake (2). This

Figure 1. Scatter plots and regression lines of the proportion of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in serum triglycerides vs. dietary intake of SAFA, MUFA and PUFA, respectively. The intake values are expressed as percentages of dietary fat. The data are from 5-year-old children whose 4-day food consumption was recorded just before the blood sampling (n=50, Group I) and from other children whose food consumption was estimated 5 to 15 days before the blood sampling (n=52), GROUP II).



study clearly shows that the best serum lipid biomarkers of current fatty acid intake are total PUFAs and linoleic acid (n-6) in serum triglycerides when the dietary data were collected just before the blood sampling. Previously, dietary linoleic acid intake and proportion of linoleic acid in serum triglycerides have correlated well in adults (21,26), and in this study, the correlation was excellent also in children. The fact that linoleic acid is an essential fatty acid probably explains why the

correlations between intake and proportions in the three serum lipid fractions are so good, and why the linoleic acid proportion of various tissues also closely reflects dietary intake (26).

Assuming that 4-day food records measure average intake of fatty acids accurately, our study suggests that fatty acids in serum triglycerides indicate reasonably well the quality of fat intake in children. However, a significant correlation was only obtained if dietary intake was

assessed just before the blood sampling. Compared with the fatty acid composition of serum CE and PL, the overall fatty acid composition of serum TGs was closest to that of the subjects' diet. The advantage of measuring fatty acids in plasma lipids is that this method is not susceptible to errors in dietary reporting and it can thus be regarded as an objective qualitative estimate of dietary fat intake. However, the disadvantage of measurements from serum is that they do not provide information about the absolute intake of fatty acids. Clearly, serum triglyceride fatty acids are good biomarkers for short-term intake of SAFAs and PUFAs, whereas MUFAs in all serum lipid fractions reflect MUFA intake poorly. Serum triglyceride fatty acid composition can be used when assessing short-term changes in children's fat intake both at group and individual level, for example in dietary intervention studies to monitor the success of intervention.

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Figure 2. Scatter plots and regression lines of the proportion of polyunsaturated fatty acids (PUFA) in serum cholesterol esters vs. dietary intake of PUFA. The intake values are expressed as percentages of dietary fat. The data are from 5-year-old children whose 4-day food consumption was recorded just before the blood sampling (n=50, Group I) and from other children whose food consumption was estimated 5 to 15 days before the blood sampling (n=52, Group II).

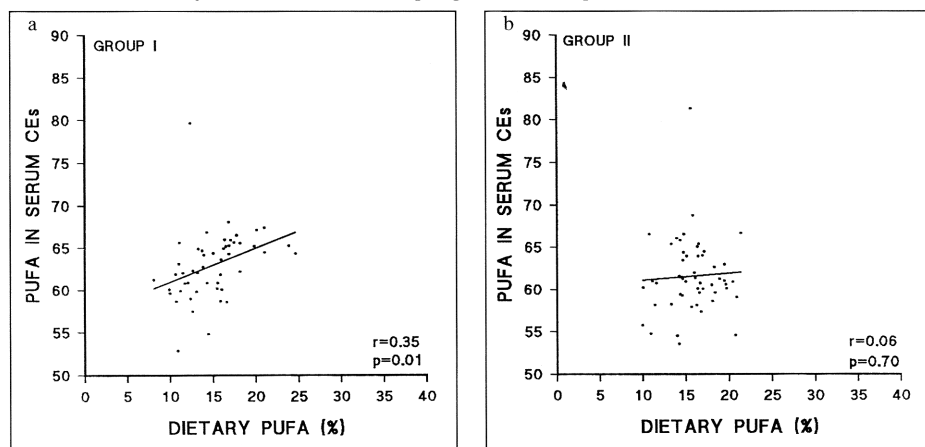


Figure 3. Scatter plots and regression lines of the proportion of polyunsaturated fatty acids (PUFA) in serum phospholipids vs. dietary intake of PUFA. The intake values are expressed as percentages of dietary fat. The data are from 5-year-old children whose 4-day food consumption was recorded just before the blood sampling (n=50, Group I) and from other children whose food consumption was estimated 5 to 15 days before the blood sampling (n=52, Group II).

