Effect of a 2-y dietary and physical activity intervention on plasma fatty acid composition and estimated desaturase and elongase activities in children: the Physical Activity and Nutrition in Children Study¹

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ABSTRACT

Background: Evidence on the effects of lifestyle interventions on plasma fatty acid composition in children is limited.

Objective: We investigated the effects of a dietary and physical activity intervention on plasma fatty acid composition of cholesteryl esters (CEs) and phospholipids and estimated desaturase and elongase activities in children.

Design: We conducted a 2-y controlled dietary and physical activity intervention based on Finnish nutrition and physical activity recommendations in a population sample of 506 children aged 6–8 y. We assessed plasma fatty acid composition by gas chromatography and estimated desaturase and elongase activities as the ratio of product fatty acids to precursor fatty acids. We analyzed data by using linear mixed models adjusted for age and sex.

Results: The proportion of total polyunsaturated fatty acids (PUFAs) in CEs tended to increase in the intervention group compared with the control group (P = 0.007 for group \times time interaction). The proportion of total PUFAs in phospholipids (P = 0.019 for group \times time interaction) and the proportion of linoleic acid in CEs (P = 0.038 for group \times time interaction) decreased in the control group. The proportion of α -linolenic acid in CEs (P < 0.001 for group \times time interaction) increased and in phospholipids (P = 0.015 for group \times time interaction) tended to increase in the intervention group. The proportion of stearic acid in CEs decreased in the intervention group $(P = 0.001 \text{ for group} \times \text{time interaction})$. The proportion of oleic acid in CEs (P = 0.002 for group \times time interaction) increased and in phospholipids (P = 0.023 for group \times time interaction) tended to increase in the control group. Estimated elongase activity in CEs decreased in the control group (P = 0.050 for group \times time interaction). Intervention had no effect on estimated desaturase activities.

Conclusions: Dietary and physical activity intervention had a beneficial effect on plasma fatty acid composition in children by preventing the decrease in the proportion of total PUFAs and linoleic acid and by increasing the proportion of α -linolenic acid. This study was registered at clinicaltrials.gov as NCT01803776. *Am J Clin Nutr* 2016;104:964–72.

Keywords: fatty acid, cholesteryl ester, phospholipid, desaturase, elongase, intervention, diet, physical activity, children

INTRODUCTION

Plasma fatty acid composition reflects the dietary intake of saturated and polyunsaturated fat during the preceding weeks (1–5) and is also influenced by several other factors such as adiposity, physical activity, and genetic factors (6–9). Plasma fatty acid composition may predict the risk of developing type 2 diabetes and cardiovascular diseases in adults (10–13) and has also been linked to increased cardiometabolic risk in children (14).

Eating a healthy diet and increasing physical activity starting in childhood are key factors in the prevention of type 2 diabetes and cardiovascular disease (15, 16). One of the reasons for the beneficial effects of these lifestyle changes could be their influence on plasma fatty acid composition. A dietary intervention beginning in infancy to decrease dietary saturated fatty acid and cholesterol intake resulted in lower proportions of SFAs and higher proportions of PUFAs in serum triglyceride fractions after 5 y of follow-up (17). Other studies on the effects of dietary interventions on plasma fatty acid composition in children are scarce. Moreover, evidence on the effects of physical activity or

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combined dietary and physical activity interventions on plasma fatty acid composition among children is limited.

Increased stearoyl-CoA-desaturase (SCD)⁹ and Δ 6-desaturase (D6D) activities and decreased Δ 5-desaturase (D5D) activity in plasma are associated with a weakened insulin sensitivity and an increased risk of type 2 diabetes in adults (18, 19). Moreover, high SCD and D6D activity, as well as low elongase activity in plasma cholesteryl esters (CEs) and phospholipids, estimated by the ratios of the proportions of individual fatty acids (20), are linked to increased cardiometabolic risk already in childhood (14). To our knowledge, there are no studies on the effects of dietary and physical activity interventions on the desaturase and elongase activities among children.

In many cases, it is still unclear whether associations found between certain fatty acids and some disease risks indicate harmful or beneficial effects of the fatty acids themselves or metabolic changes affecting the concentration of these fatty acids. In addition, the metabolism and fatty acid composition of plasma CE and phospholipid fractions vary greatly, and there are also differences in how the individual fatty acids reflect dietary intake or liver fatty acid metabolism (14). Therefore, separate fatty acid analyses of plasma CEs and phospholipids provide more information than the total plasma fatty acid analysis in which, for example, changes in triacylglycerol concentrations could mask the changes in other fractions.

We investigated the effects of a 2-y individualized and familybased dietary and physical activity intervention on plasma fatty acid composition, as well as estimated desaturase and elongase activities in a population sample of children. We hypothesized that a lifestyle intervention aimed at enhancing diet quality, increasing physical activity, and decreasing sedentary behavior according to the Finnish nutrition and physical activity recommendations increases the proportion of PUFAs and decreases the proportion of MUFAs and SFAs in plasma CEs and phospholipids among children.

METHODS

Study design and study population

The PANIC (Physical Activity and Nutrition in Children) Study is a controlled dietary and physical activity intervention study in a population sample of children from the city of Kuopio, Finland (registered at clinicaltrials.gov as NCT01803776). The study was primarily carried out at the Institute of Biomedicine at the Kuopio campus of the University of Eastern Finland. Altogether, 736 children 6-8 y of age who were registered for the first grade in 16 primary schools of Kuopio were invited to participate in the baseline study in 2007-2009 (Figure 1). Invitation letters were sent by mail to the principal custodians of the children, who were asked to contact the research secretary for participation. Of the 736 invited children, 512 (70%) participated in the baseline study. Based on the comprehensive school health examination data, the participants did not differ in age, sex distribution, or BMI-SD score (SDS) from all children who started the first grade in Kuopio during the years 2007-2009. In total, 6 children were excluded from the intervention study

because of severe physical disability or withdrawal during baseline examinations.

The 506 eligible children were then allocated to the intervention group (306 children, 60%) or the control group (200 children, 40%) by matching them according to the location (urban compared with rural) and size (large compared with small) of the schools to minimize differences in baseline characteristics between the groups. Dividing the children in the intervention or control groups according to schools made it possible to organize after-school exercise clubs conducted at schools only for the intervention group and to avoid nonintentional intervention in the control group. More children were included in the intervention group than in the control group because of a larger number of dropouts expected in the intervention group and to have sufficient statistical power for comparison between the groups. Therefore we ended up with 9 intervention schools with only intervention subjects and 7 control schools with only control subjects.

Of the 506 children who participated in the baseline study, 440 (87%) attended the 2-y follow-up study (Figure 1). The median (IQR) of the intervention period was 2.1 y (2.1–2.2 y) in both groups. No interim measurements were taken during the 2-y follow-up. Altogether, 45 (15%) children in the intervention group and 21 (11%) children in the control group dropped out during the 2-y follow-up. Data on plasma fatty acid composition were available for 464 children (290 children in the intervention group and 174 children in the control group) at baseline and for 386 children (236 children in the intervention group, 150 children in the control group) at 2-y follow-up. The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo. Both the children and their parents gave their written informed consent.

Dietary and physical activity intervention

The children and their parents allocated to the intervention group had 6 dietary counseling sessions of 30-45 min and 6 physical activity counseling sessions of 30-45 min during the 2-y intervention period. The dietary and physical activity counseling sessions occurred at 0.5, 1.5, 3, 6, 12, and 18 mo after baseline. Authorized clinical nutritionists and specialists in exercise medicine of the PANIC study gave detailed and individualized advice on how to enhance diet quality, increase physical activity, and decrease sedentary behavior. The children and their families also received fact sheets on diet quality, physical activity, and sedentary behavior; verbal and written information on opportunities to exercise in Kuopio; and some financial support for physical activity, such as exercise equipment and admission for indoor sports. The children were also encouraged to participate in after-school exercise clubs organized by the PANIC study and supervised by trained exercise instructors. In the exercise clubs, the children had the opportunity to engage in different kinds of physical activities, such as indoor ball games, cross-country skiing, and skating.

The goals of the intervention were I) to decrease the consumption of foods high in saturated fat, particularly high-fat dairy and meat products; 2) to increase the consumption of foods high in unsaturated fat, particularly high-fat vegetable oil-based margarines, vegetable oils, and fish; 3) to increase the consumption of vegetables, fruit, and berries; 4) to increase the consumption of whole-grain products and other foods high in

 $^{^{9}}$ Abbreviations used: CE, cholesteryl ester; D5D, Δ 5-desaturase; D6D, Δ 6-desaturase; PANIC, Physical Activity and Nutrition in Children; SCD, stearoyl-CoA-desaturase; SDS, SD score.

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FIGURE 1 Flowchart of the PANIC Study in Finnish children. PANIC, Physical Activity and Nutrition in Children.

dietary fiber; 5) to decrease the consumption of foods high in sugar, particularly sugar-sweetened beverages, sugar-sweetened dairy products, and candies; 6) to decrease the consumption of foods high in salt and the use of salt in cooking; 7) to avoid excessive energy intake; 8) to increase total physical activity by emphasizing its diversity; and 9) to decrease total and particularly screen-based sedentary behavior. These goals were based on the Finnish Nutrition Recommendations (21) and the Finnish Recommendations for Physical Activity of School-aged Children (22). The children and their parents in the control group received verbal and written advice on a health-improving diet and physical activity according to the Finnish recommendations (21, 22) at baseline but no active intervention.

Assessment of body size and composition

Body height and weight were assessed by trained research personnel. Body height was measured to accuracy of 0.1 cm by a wall-mounted stadiometer in the Frankfurt plane without shoes. Body weight was measured to accuracy of 0.1 kg after overnight fasting, with an empty bladder, and standing in light underwear by use of a calibrated InBody 720 device (Biospace). BMI was calculated by dividing body weight (kg) by body height (m) squared. BMI-SDS was calculated by use of Finnish references (23).

Assessment of plasma fatty acid composition and calculation of estimated desaturase and elongase activities

Blood sampling was conducted after a 12-h overnight fast. Plasma fatty acid composition was analyzed by gas chromatography, as described previously (5, 24). Plasma samples were extracted with chloroform-methanol (2:1), and the lipid fractions were separated by solid-phase extraction with an aminopropyl column. Fatty acids in plasma CEs and phospholipids were transmethylated with 14% boron trifluoride in methanol and were analyzed by the 7890A gas chromatograph (Agilent Technologies Inc.) equipped with a 25-m FFAP column. Cholesteryl nonadecanoate (Nu Chek Prep Inc.), trinonadecanoin, and

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phosphatidylcholine dinonadecanoyl (Larodan Fine Chemicals) served as internal standards. The relative amount of fatty acids was expressed as a percentage of the total amount of fatty acids reported (25). The amounts of some fatty acids present in plasma phospholipids were under the detection limit in plasma CEs.

The desaturase and elongase activities in plasma CEs and phospholipids were estimated as the ratio of the single product fatty acid divided by the single precursor fatty acid. In terms of fatty acids in CEs, the formulas were set as follows: SCD is 16:1n-7/16:0, D6D is 18:3n-6/18:2n-6, and D5D is 20:4n-6/20:3n-6 (26). In terms of phospholipids, the same ratios were used except for the D6D that equaled 20:3n-6/18:2n-6 (26). The proportion of dihomo- γ -linolenic acid was used in phospholipids because the proportion of γ -linolenic acid was too low for detect in phospholipids, and this ratio is a valid estimate for activity of D6D (26). The ratio for estimation of the elongase activity both in CEs and phospholipids was 18:1n-7/16:1n-7 (27).

Sample size calculations

The original power calculations were performed according to the primary outcomes of the PANIC Study, which were BMI-SDS, waist circumference, fasting serum insulin, fasting plasma glucose, HOMA-IR, fasting plasma triglycerides, HDL cholesterol, LDL cholesterol, systolic and diastolic blood pressure, and the cardiometabolic risk score. The plasma fatty compositions of CEs and phospholipids were secondary outcomes. We determined the number of children required at 2-y follow-up to detect a ≥ 0.30 -SD difference in the primary outcomes between the intervention group (60% of children) and the control group (40% of children) with a power of 80% and a 2-sided P value for the difference between the groups of 0.05, allowing for a 20% loss to follow-up or missing data. According to these power calculations, we would need a sample size of ≥ 275 children in the intervention group and ≥ 183 children in the control group at baseline and \geq 220 children in the intervention group and \geq 147 children in the control group at 2-y followup for the whole PANIC study. In this secondary outcome study, the fatty acid data were available for 236 children in the intervention group and 150 children in the control group at 2-y follow-up, which is in the range of our original power calculation.

Statistical methods

Statistical analyses were performed by using the IBM SPSS Statistics, version 21 (IBM Corp.). Differences in basic characteristics between the intervention group and the control group at baseline were tested by the *t* test for independent samples except for the difference in sex distribution that was tested by the χ^2 test. The effects of the 2-y intervention on plasma fatty acid composition were analyzed by use of the linear mixed models. Study group (intervention group, control group), time (baseline, 2-y follow-up), and group × time interaction were included as fixed factors in the model. Participant and school were included as random effects in the model to account for intraparticipant and intraschool correlations between the repeated measures for each participant. The interaction between study group and time was analyzed after adjustment for age and sex. We also made further adjustments for BMI-SDS. Therefore, the linear mixed models we used were as follows: (study group, time, age, sex, group \times time interaction) and (study group, time, age, sex, BMI-SDS, group \times time interaction). Differences and interactions with 2-sided *P* values <0.05 were considered statistically significant.

RESULTS

Baseline and 2-y follow-up characteristics

The basic characteristics of the children at baseline and 2-y follow-up are presented in **Table 1**. Children in the intervention group were 129.0 cm tall and weighed 27.0 kg, and the BMI-SDS was -0.16 at baseline, whereas at 2-y follow-up they were 140.8 cm tall and weighed 34.4 kg, and the BMI-SDS was -0.14. There were no differences in sex distribution, age, body weight, body height, or BMI-SDS between the intervention group and the control group at baseline (data not shown). During the 2-y follow-up, 15% of the intervention group and 10% of the control group dropped out (Figure 1).

Effect of intervention on fatty acid composition of plasma cholesteryl esters

The proportion of stearic acid decreased in the intervention group but not in the control group after adjustment for age and sex $(P = 0.001 \text{ for group } \times \text{ time interaction; Table 2})$. The proportion of total MUFAs did not change significantly in the intervention group but increased in the control group (P = 0.007for group \times time interaction). The proportion of oleic acid tended to decrease in the intervention group but increased in the control group (P = 0.002 for group \times time interaction). The proportion of total PUFAs tended to increase in the intervention group but decreased in the control group (P = 0.007 for group \times time interaction). The proportion of linoleic acid did not change significantly in the intervention group but decreased in the control group (P = 0.038 for group \times time interaction). The proportion of α -linolenic acid increased in the intervention group but decreased in the control group (P < 0.001 for group \times time interaction). Estimated elongase activity did not change significantly in the intervention group but decreased in the control group (P = 0.050 for group \times time interaction). The intervention had no effects on estimated desaturase activities in plasma CEs. Additional adjustment for BMI-SDS did not alter the results, except that it weakened the effect of intervention on the proportion of linoleic acid (P = 0.054 for group \times time interaction) (data not shown).

Effect of intervention on fatty acid composition of plasma phospholipids

The proportion of total MUFAs tended to decrease in the intervention group but did not change significantly in the control group after adjustment for age and sex (P = 0.021 for group \times time interaction; **Table 3**). The proportion of oleic acid did not change significantly in the intervention group but tended to increase in the control group (P = 0.023 for group \times time interaction). The proportion of total PUFA did not change significantly in the intervention group but decreased in the

TABLE	1
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Basic characteristics of the children with complete data on plasma fatty acid composition at baseline and at 2-y follow-up¹

	Intervent	ion group	Contro	l group
	Baseline $(n = 290)$	2-y follow-up (<i>n</i> = 236)	Baseline $(n = 174)$	$\begin{array}{l} 2-y \text{ follow-up} \\ (n = 150) \end{array}$
Sex				
Girls, n (%)	133 (46)	112 (47)	90 (52)	80 (53)
Boys, <i>n</i> (%)	157 (54)	124 (53)	84 (48)	70 (47)
Age, y	7.6 ± 0.02	9.8 ± 0.02	7.62 ± 0.03	9.7 ± 0.04
Body height, cm	129.0 ± 0.32	140.8 ± 0.39	128.4 ± 0.45	140.3 ± 0.54
Body weight, kg	27.0 ± 0.28	34.4 ± 0.46	26.8 ± 0.39	34.3 ± 0.60
BMI-SDS ²	-0.16 ± 0.06	-0.14 ± 0.07	-0.19 ± 0.08	-0.12 ± 0.08

¹Values are means \pm SEMs or *n* (%) of the total sample.

²SDS, SD score, based on Finnish reference values (22).

control group (P = 0.019 for group \times time interaction). The proportion of α -linolenic acid tended to increase in the intervention group but tended to decrease in the control group (P = 0.015 for group \times time interaction). The proportion of docosapentaenoic acid did not change significantly in the intervention group but decreased in the control group (P = 0.003 for group \times time interaction). The intervention had no effects on estimated desaturase or elongase activities in plasma phospholipids. Further adjustment for BMI-SDS had no effect on the results regarding the fatty acid composition of phospholipids.

DISCUSSION

To our knowledge, this is the first study to explore the effects of combined dietary and physical activity intervention on plasma fatty acid composition in children. The results from the present study demonstrate that a 2-y individualized and family-based dietary and physical activity intervention in school-aged children attenuated the decrease in PUFAs and the increase in MUFAs in plasma CEs and phospholipids relative to the control group. The proportion of α -linolenic acid increased, and the proportion of oleic acid decreased in the intervention group compared with the control group. Moreover, the proportion of stearic acid in plasma CEs decreased in the intervention group relative to the control group.

We found a decrease in the proportion of oleic acid and an increase in the proportion of α -linolenic acid in CEs in the intervention group compared with the control group. In addition, the proportion of linoleic acid decreased in the control group. These findings may be caused by the substantial increase in the consumption of vegetable oil-based margarine (fat 60-80%) and increase in the intake of PUFAs in the intervention group, as previously reported (28). We have reported earlier that the consumption of vegetable oil-based margarine is inversely associated with all plasma SFAs and MUFAs and directly associated with the proportion of linoleic and α -linolenic acid in CEs at baseline (5). These results and our previous findings together may result in the higher intake of linoleic acid and α -linolenic acid in the intervention group and may explain the differences in their proportions in CEs. However, because the margarines in Finland are rich in rapeseed oil, the intake of MUFAs also increased in the intervention group (28), and yet the proportion of MUFAs and oleic acid was found to decrease in CEs and

phospholipids compared with the control group. This could be a consequence of more efficient incorporation of linoleic acid and α -linolenic acid into plasma lipid fractions replacing MUFAs. However, it is also possible that higher intake of PUFAs had a beneficial effect on liver fatty acid metabolism because PUFAs have been shown to reduce liver fat content, de novo lipogenesis, and SCD activity (29, 30). The pattern with increased proportion of oleic acid and decreased proportions of linoleic acid and α -linoleic acid seen in the control group may suggest increased de novo lipogenesis and SCD activity, whereas this development may have been prevented by the better diet quality in the intervention group. This view is also supported, despite the lack of significant interaction between group and time, by the increased proportion of palmitoleic acid and increased estimated SCD activity in CEs in the control group.

We also found that the proportion of stearic acid in CEs decreased among children in the intervention group but not in the control group. In a previous study with adults, a decrease in the proportion of stearic acid in plasma CEs was reported during a 6-wk Healthy Nordic Diet intervention (31). We have previously shown that the intervention slightly decreased the consumption of butter-based spreads (28), which may contribute to this finding. However, the intake of butter and butter-oil mixture was not associated with stearic acid in CEs at baseline (5). In addition, a dietary intervention to decrease dietary SFA and cholesterol intake resulted in a lower proportion of palmitic acid in serum triglycerides but a higher proportion of stearic acid in serum CEs among children 5 y of age (17). Therefore the more probable explanation for the decreased proportion of stearic acid in CEs is, as in the case of oleic acid, the increased consumption of vegetable oil-based margarine in the intervention group. In line with this, a decrease in the proportion of stearic acid in CEs but not in PLs has been found after a rapeseed oil diet (26).

The results of the present study showed no effect of lifestyle intervention on the estimated desaturase activities in children. This is in accordance with an exercise and dietary counseling study among adults showing no changes in desaturase estimates after 1 y (18). Previous short-term dietary intervention studies have resulted in a decrease in the estimated SCD activity and an increase in estimated D5D activity (26, 31) and also a decrease in estimated D6D activity (26). This difference might be explained by the greater modification of dietary fatty acid composition

TABLE 2 Fatty acide

Fatty acids (mol%) and estimated desaturase and elongase activities in plasma cholesteryl esters in the intervention group and in the control group with complete data on plasma fatty acid composition at baseline and after 2-y follow-up¹

		Intervention group				Control group			
Fatty acids	Baseline $(n = 290)$	2-y follow-up $(n = 236)$	2-y change in mean	P for 2-y change	Baseline $(n = 174)$	2-y follow-up $(n = 150)$	2-y change in mean	P for 2-y change	P-group $ imes$ time interaction
Total SFAs	12.8 (12.6, 13.0)	12.7 (12.5, 12.8)	-0.1	0.027*	12.9 (12.7, 13.1)	12.8 (12.6, 13.1)	-0.1	0.809	0.233
14:0 myristic acid	0.82(0.79, 0.86)	0.82 (0.78, 0.86)	-0.004	0.845	$0.84 \ (0.80, \ 0.89)$	0.85 (0.80, 0.90)	0.01	0.864	0.789
16:0 palmitic acid	11.4 (11.2, 11.5)	11.2 (11.1, 11.4)	-0.2	0.024*	11.4 (11.3, 11.6)	11.4 (11.2, 11.5)	-0.04	0.463	0.401
18:0 stearic acid	0.65(0.61, 0.70)	$0.62 \ (0.57, 0.66)$	-0.03	0.001*	0.61 (0.56, 0.67)	0.63 (0.58, 0.69)	0.02	0.129	0.001*
Total MUFAs	24.1 (23.7, 24.4)	23.9 (23.5, 24.3)	-0.2	0.260	23.4 (23.0, 23.9)	24.0 (23.5, 24.4)	0.6	0.010*	0.007*
16:1n-7 palmitoleic acid	2.59 (2.49, 2.69)	2.64 (2.54, 2.74)	0.05	0.360	2.42 (2.29, 2.55)	2.56 (2.43, 2.69)	0.14	0.030*	0.259
18:1n-9 oleic acid	20.5 (20.1, 20.7)	20.2 (19.9, 20.5)	-0.3	0.086	20.0 (19.6, 20.3)	20.4 (20.0, 20.7)	0.4	0.012*	0.002*
18:1n-7 cis-vaccenic acid	1.08 (1.05, 1.10)	1.06 (1.04, 1.09)	-0.02	0.099	1.06 (1.03, 1.09)	1.04 (1.01, 1.07)	-0.02	0.062	0.662
Total PUFAs	63.1 (62.7, 63.5)	63.5 (63.1, 63.9)	0.4	0.086	63.7 (63.2, 64.2)	63.2 (62.7, 63.7)	-0.5	0.034*	0.007*
18:2n–6 linoleic acid	52.4 (51.9, 52.9)	52.6 (52.0, 53.1)	0.2	0.586	53.2 (52.6, 53.7)	52.6 (51.9, 53.2)	-0.6	0.027*	0.038*
18:3n-3 α -linolenic acid	1.05 (1.02, 1.09)	1.08 (1.05, 1.12)	0.03	0.044*	1.07 (1.03, 1.12)	1.01 (0.96, 1.05)	-0.06	0.001*	$< 0.001^{*}$
18:3n–6 γ -linolenic acid	0.96(0.91, 1.01)	0.99 (0.94, 1.05)	0.03	0.351	0.90(0.83, 0.97)	0.97 (0.90, 1.04)	0.07	0.086	0.445
$20.3n-6$ dihomo- γ -linolenic acid	$0.71 \ (0.69, \ 0.73)$	0.73 (0.71, 0.74)	0.02	0.015*	$0.68 \ (0.66, \ 0.70)$	0.70(0.68, 0.72)	0.02	0.025*	0.816
20:4n–6 arachidonic acid	5.91 (5.72, 6.09)	6.00(5.82, 6.19)	0.09	0.074	5.85(5.63, 6.06)	5.92 (5.70, 6.14)	0.07	0.286	0.780
20:5n-3 EPA	1.26 (1.17, 1.34)	1.29 (1.20, 1.39)	0.03	0.436	1.27 (1.16, 1.39)	1.29 (1.17, 1.40)	0.02	0.795	0.778
22:6n-3 DHA	$0.82 \ (0.78, \ 0.86)$	$0.81 \ (0.77, 0.85)$	-0.01	0.618	0.79 (0.75, 0.84)	$0.78\ (0.73,\ 0.83)$	-0.01	0.432	0.761
Stearoyl-CoA-desaturase (16:1n-7/16:0)	0.23 (0.22, 0.24)	0.24 (0.23, 0.25)	0.01	0.173	0.21 (0.20, 0.22)	0.23 (0.21, 0.24)	0.02	0.017*	0.304
A6-Desaturase (18:3n-6/18:2n-6)	0.019 (0.017, 0.020)	0.019 (0.018, 0.020)	0.001	0.361	0.017 (0.016, 0.019)	0.019 (0.017, 0.020)	0.002	0.068	0.390
Δ5-Desaturase (20:4n–6/20:3n–6)	8.50 (8.15, 8.84)	8.45 (8.10, 8.80)	-0.05	0.659	8.87 (8.46, 9.27)	8.73 (8.32, 9.15)	-0.14	0.336	0.633
Elongase (18:1n-7/16:1n-7)	$0.45 \ (0.44, \ 0.47)$	$0.45 \ (0.43, \ 0.46)$	-0.01	0.318	$0.48 \ (0.46, \ 0.50)$	$0.44 \ (0.42, \ 0.46)$	-0.04	0.001*	0.050
¹ Values are means (95% CIs) of prop	ortion of fatty acids and	l estimated desaturase an	d elongase act	ivities in pla	sma cholesteryl esters a	t baseline and after 2-y	follow-up, the	2-y changes	in the means and

P values for the differences between the means at baseline, and 2-y follow-up from the linear mixed models adjusted for age and sex. Study group, time, and their interactions are included as fixed factors in the model, and participant and school are included as random effects in the model. *P < 0.05. mol%, molar percentage.

Fatty acids (mol%) and estimated desaturase and elongase activities in plasma phospholipids in the intervention group and in the control group with complete data on plasma fatty acid composition at baseline and after 2-y follow-up¹

	Intervent	ion group			Control	group			
	Baseline	2-y follow-up	2-y change	P for 2-y	Baseline	2-y follow-up	2-y change	P for 2-y	P -group \times time
Fatty acids	(n = 290)	(n = 236)	in mean	change	(n = 174)	(n = 150)	in mean	change	interaction
Total SFAs	45.4 (45.2, 45.6)	45.5 (45.3, 45.7)	0.1	0.231	45.4 (45.2, 45.7)	45.6 (45.4, 45.9)	0.2	0.027*	0.320
14:0 myristic acid	0.46(0.43, 0.49)	$0.48 \ (0.45, \ 0.51)$	0.02	0.111	0.45(0.41, 0.49)	$0.46\ (0.42,\ 0.50)$	0.01	0.467	0.673
15:0 pentadecanoic acid	0.19 (0.18, 0.20)	$0.19 \ (0.18, \ 0.20)$	-0.003	0.493	$0.19\ (0.18,\ 0.21)$	0.20(0.19, 0.22)	0.01	0.087	0.077
16:0 palmitic acid	29.1 (28.9, 29.4)	29.0 (28.7, 29.2)	-0.1	0.014^{*}	29.3 (29.0, 29.6)	29.1 (28.8, 29.4)	-0.2	0.058	0.964
17:0 margaric acid	$0.34 \ (0.32, \ 0.35)$	0.33 $(0.32, 0.34)$	-0.01	0.024*	$0.34 \ (0.33, \ 0.36)$	$0.34 \ (0.33, \ 0.36)$	-0.001	0.739	0.252
18:0 stearic acid	13.4 (13.3, 13.6)	13.7 (13.5, 13.8)	0.3	<0.001*	13.4 (13.2, 13.5)	13.6 (13.5, 13.8)	0.2	$< 0.001^{*}$	0.563
20:0 arachidic acid	$0.44 \ (0.42, \ 0.45)$	0.43 (0.41, 0.45)	-0.01	0.106	0.42 (0.40 , 0.44)	$0.42 \ (0.41, \ 0.45)$	0.001	0.227	0.051
22:0 behenic acid	0.80(0.77, 0.84)	$0.81 \ (0.77, 0.84)$	0.01	0.503	0.77 (0.73, 0.82)	$0.80\ (0.76,\ 0.84)$	0.03	0.038*	0.227
24:0 lignoceric acid	$0.65 \ (0.62, \ 0.68)$	$0.66\ (0.63,\ 0.69)$	0.01	0.365	$0.65\ (0.61,\ 0.69)$	0.66(0.63, 0.70)	0.01	0.132	0.537
Total MUFAs	14.3 (14.1, 14.6)	14.1 (13.9, 14.4)	-0.2	0.052	14.0 (13.8, 14.3)	14.2 (13.9, 14.5)	0.2	0.158	0.021^{*}
16:1n-7 palmitoleic acid	$0.58 \ (0.55, \ 0.61)$	$0.58 \ (0.56, \ 0.61)$	0.001	0.937	$0.55\ (0.51,\ 0.58)$	$0.56\ (0.53,\ 0.59)$	0.01	0.393	0.536
18:1n-9 oleic acid	10.5 (10.3, 10.7)	10.4 (10.2, 10.6)	-0.1	0.160	$10.4 \ (10.1, \ 10.6)$	10.6 (10.3, 10.8)	0.2	0.074	0.023^{*}
18:1n-7 cis-vaccenic acid	1.39 (1.36, 1.42)	1.38 (1.35, 1.41)	-0.01	0.512	1.38 (1.34, 1.41)	1.34 (1.30, 1.37)	-0.04	0.010*	0.106
20:1n-9 eicosenoic acid	0.28 (0.28, 0.29)	0.28 (0.27, 0.29)	-0.004	0.205	$0.29\ (0.28,\ 0.30)$	0.28 (0.27, 0.29)	-0.01	0.003*	0.119
24:1n-9 nervonic acid	1.53 (1.44, 1.63)	1.49 (1.40, 1.59)	-0.04	0.046^{*}	1.46 (1.34, 1.57)	1.45 (1.33, 1.56)	-0.01	0.784	0.303
Total PUFAs	40.2 (40.0, 40.5)	40.3 $(40.1, 40.6)$	0.1	0.399	40.5 (40.2, 40.9)	40.2 (39.8, 40.5)	-0.3	0.020*	0.019*
18:2n-6 linoleic acid	20.9 (20.6, 21.2)	21.1 (20.8, 21.4)	0.2	0.066	21.2 (20.8, 21.6)	21.1 (20.7, 21.5)	-0.1	0.554	0.108
18:3n-3 α -linolenic acid	$0.41 \ (0.39, \ 0.43)$	0.42 (0.40 , 0.44)	0.01	0.068	$0.41 \ (0.38, \ 0.43)$	$0.39\ (0.37,\ 0.41)$	-0.02	0.094	0.015*
$20:3n-6$ dihomo- γ -linolenic acid	2.86 (2.79, 2.93)	2.89 (2.82, 2.96)	0.03	0.357	2.78 (2.70, 2.86)	2.82 (2.74, 2.91)	0.04	0.308	0.822
20:4n-6 arachidonic acid	8.35 (8.11, 8.58)	8.30 (8.06, 8.54)	-0.05	0.522	8.40 (8.12, 8.68)	8.27 (7.99, 8.55)	-0.13	0.159	0.481
20:5n-3 EPA	1.27 (1.19, 1.36)	1.29 (1.20, 1.38)	0.02	0.695	1.30 (1.20, 1.41)	1.36 (1.25, 1.46)	0.06	0.289	0.558
22:4n-6 adrenic acid	0.33 (0.32, 0.34)	0.32 (0.31, 0.32)	-0.01	0.002*	$0.31 \ (0.30, \ 0.32)$	0.31 (0.29, 0.32)	-0.001	0.138	0.461
22:5n-6 osbond acid	0.22 (0.21, 0.23)	0.22 (0.21, 0.22)	-0.005	0.073	0.21 (0.20, 0.22)	0.21 (0.20, 0.22)	-0.004	0.230	0.858
22:5n-3 docosapentaenoic acid	1.28 (1.23, 1.33)	1.29 (1.24, 1.34)	0.01	0.805	1.26 (1.20, 1.32)	1.20 (1.14, 1.26)	-0.06	$< 0.001^{*}$	0.003*
22:6n-3 DHA	4.68(4.48, 4.88)	4.50 (4.30, 4.70)	-0.18	0.008*	4.69 (4.45, 4.93)	4.56 (4.32, 4.80)	-0.13	0.106	0.692
Stearoyl-CoA-desaturase (16:1n-7/16:0)	0.020 (0.019, 0.021)	0.020 (0.019, 0.021)	0.0002	0.725	0.019 (0.018, 0.020)	0.019 (0.018, 0.020)	0.001	0.277	0.526
A6-Desaturase (20:3n-6/18:2n-6)	$0.139\ (0.135,\ 0.143)$	0.139 $(0.135, 0.143)$	0.00004	0.984	0.133 (0.128, 0.138)	$0.136\ (0.131,\ 0.141)$	0.003	0.315	0.438
Δ5-Desaturase (20:4n-6/20:3n-6)	3.01 (2.88, 3.13)	2.97 (2.84, 3.09)	-0.04	0.385	3.13 (2.98, 3.28)	3.03 (2.88, 3.18)	-0.1	0.086	0.421
Elongase (18:1n-7/16:1n-7)	2.60 (2.49, 2.71)	2.59 (2.48, 2.70)	-0.01	0.796	2.69 (2.55, 2.82)	2.55 (2.41, 2.68)	-0.14	0.043*	0.153
¹ Values are means (95% CIs) of prof	portion of fatty acids an	d estimated desaturase a	ind elongase ad	ctivities in p	lasma phospholipids at	baseline and after 2-y f	ollow-up, the	2-y changes i	n the means and

P values for the differences between the means at baseline, and 2-y follow-up from the linear mixed models adjusted for age and sex. Study group, time, and their interactions were included as fixed factors in the model, and participant and school were included as random effects in the model. *P < 0.05. mol%, molar percentage. in these studies. However, it is also possible that rapid substantial changes in the intake of fatty acids used in the calculation of estimates, for example palmitoleic acid and linoleic acid, may affect their amounts in plasma and thus desaturase estimates without or in addition to actual change in enzyme activity.

We found decreased estimated elongase activity in CEs in the control group. Because this estimate was calculated from the ratio of *cis*-vaccenic acid to palmitoleic acid, it may represent the activity of elongase 5 or 6 or both (32). The proportion of docosapentaenoic acid in phospholipids decreased in the control group, which may also indicate decreased elongase 5 activity. A high proportion of palmitoleic acid and low elongase activity estimates in erythrocyte membranes have been associated with worsening of hyperglycemia (27) and also with higher plasma concentration of C-reactive protein (33), which indicates that low elongase activity and accumulation of palmitoleic acid may be biomarkers for unfavorable metabolic changes.

To our knowledge, there are no previous intervention studies about the effects of physical activity on plasma fatty acid composition in children, and there are only a few such studies in adults (7, 8). Aerobic training has been reported to increase the proportion of oleic acid and to decrease the proportion of arachidonic acid in muscle phospholipids and the proportion of di-homo- γ linolenic acid in serum CEs but not in muscle triglycerides or other fatty acids in serum phospholipids or CEs compared with the control group (34). Nevertheless, enhancing dietary fat quality is a more important way of improving plasma fatty acid composition than physical activity.

The strengths of our study are a relatively large population sample of children and a carefully conducted, individualized, and family-based dietary and physical activity intervention with a long follow-up and detailed fatty acid analysis. Because the sensitivity of individual fatty acid to dietary or metabolic changes may be different in plasma CEs and phospholipids, separate analyses can reveal changes not seen in all fractions or whole plasma. A weakness of the study is that we did not randomly allocate the children in the intervention and control group. However, we divided the children into groups by matching them according to the location and size of the schools. This allowed us to organize after-school exercise clubs at schools that minimized the nonintentional intervention in the control group. This is why we controlled for the possible random effect of the schools in the mixed-model analyses. Another weakness is that we were not able to assess the true enzyme activities, because liver biopsy would be needed for that purpose (19). We used ratios calculated from the proportions of individual fatty acids for the estimation of desaturase and elongase activities in plasma lipid fractions. This approach has been widely used (35, 36).

In conclusion, the 2-y individualized and family-based lifestyle intervention aimed at enhancing overall diet quality, increasing physical activity, and decreasing sedentary behavior prevented the decrease in the proportion of total PUFAs and linoleic acid, as well as increased the proportion of α -linolenic acid relative to the control group. These findings on the beneficial effects of dietary and physical activity intervention on plasma fatty acid composition in children may be useful in developing lifestyle counseling strategies to prevent metabolic syndrome, type 2 diabetes, and cardiovascular diseases since childhood. We thank Sirkku Karhunen for her expertise in the assessment of plasma fatty acid.

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REFERENCES

- Vessby B, Gustafsson IB, Boberg J, Karlström B, Lithell H, Werner I. Substituting polyunsaturated for saturated fat as a single change in a Swedish diet: effects on serum lipoprotein metabolism and glucose tolerance in patients with hyperlipoproteinaemia. Eur J Clin Invest 1980;10:193–202.
- Riboli E, Ronnholm H, Saracci R. Biological markers of diet. Cancer Surv 1987;6:685–718.
- Nikkari T, Luukkainen P, Pietinen P, Puska P. Fatty acid composition of serum lipid fractions in relation to gender and quality of dietary fat. Ann Med 1995;27:491–8.
- Zock PL, Mensink RP, Harryvan J, Vries JHM, Katan MB. Fatty acids in serum cholesteryl esters as quantitative biomarkers of dietary intake in humans. Am J Epidemiol 1997;145:1114–22.
- Venäläinen T, Schwab U, Ågren J, de Mello V, Lindi V, Eloranta AM, Kiiskinen S, Laaksonen DE, Lakka TA. Cross-sectional associations of food consumption with plasma fatty acid composition and estimated desaturase activities in Finnish children. Lipids 2014;49:467–79.
- Pickens CA, Sordillo LM, Comstock SS, Harris WS, Hortos K, Kovan B, Fenton JI. Plasma phospholipids, non-esterified plasma polyunsaturated fatty acids and oxylipids are associated with BMI. Prostaglandins Leukot Essent Fatty Acids 2015;95:31–40.
- Allard C, Alteresco M, Ferguson RJ, Chaniotis L, Choquette G, Skinner J. Changes in adipose tissue and increased serum cholesterol of coronary patients following training. Can Med Assoc J 1973;109: 194–7.
- Hurter R, Peyman MA, Swale J, Barnett CW. Some immediate and long-term effects of exercise on the plasma-lipids. Lancet 1972;2: 671–4.
- Kunesová M, Hainer V, Tvrzicka E, Phinney SD, Stich V, Parizkova J, Zak A, Stunkard AJ. Assessment of dietary and genetic factors influencing serum and adipose fatty acid composition in obese female identical twins. Lipids 2002;37:27–32.
- Vessby B, Tengblad S, Lithell H. Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. Diabetologia 1994;37:1044–50.
- Laaksonen DE, Lakka TA, Lakka HM, Nyyssönen K, Rissanen T, Niskanen LK, Salonen JT. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabet Med 2002;19:456–64.
- Laaksonen DE, Nyyssönen K, Niskanen L, Rissanen TH, Salonen JT. Prediction of cardiovascular mortality in middle-aged men by dietary and serum linoleic and polyunsaturated fatty acids. Arch Intern Med 2005;165:193–9.
- Schwab U, Lauritzen L, Tholstrup T, Haldorsson TI, Riserus U, Uusitupa M, Becker W. Effect of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: a systematic review. Food Nutr Res 2014;2014:58.
- 14. Venäläinen T, Ågren J, Schwab U, de Mello VD, Eloranta AM, Laaksonen DE, Lindi V, Lakka TA. Cross-sectional associations of plasma fatty acid composition and estimated desaturase and elongase activities with cardiometabolic risk in Finnish children: the PANIC study. J Clin Lipidol 2016;10:82–91.
- Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren WM, Albus C, Benlian P, Boysen G, Cifkova R, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). Int J Behav Med 2012;19:403–88.

- Paulweber B, Valensi P, Lindström J, Lalic NM, Greaves CJ, McKee M, Kissimova-Skarbek K, Liatis S, Cosson E, Szendroedi J, et al. A European evidence-based guideline for the prevention of type 2 diabetes. Horm Metab Res 2010;42:S3–36.
- Salo P, Viikari J, Rask-Nissilä L, Hämäläinen M, Rönnemaa T, Seppänen R, Simell O. Effect of low-saturated fat, low-cholesterol dietary intervention on fatty acid compositions in serum lipid fractions in 5-year-old children: the STRIP project. Eur J Clin Nutr 1999;53:927–32.
- Corpeleijn E, Feskens EJM, Jansen EHJM, Mensink M, Saris WH, de Bruin TW, Blaak EE. Improvements in glucose tolerance and insulin sensitivity after lifestyle intervention are related to changes in serum fatty acid profile and desaturase activities: the SLIM study. Diabetologia 2006;49:2392–401.
- Kröger J, Schulze MB. Recent insights into the relation of delta 5 desaturase and delta 6 desaturase activity to the development of type 2 diabetes. Curr Opin Lipidol 2012;23:4–10.
- Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of fatty acids and insulin action. Ann N Y Acad Sci 2002;967:183–95.
- National Nutrition Council. Suomalaiset ra-vitsemussuositukset. Ravinto ja liikunta tasapainoon. [Finnish Nutrition Recommendations]. Helsinki (Finland): Edita Prima Oy; 2005 (in Finnish).
- 22. Ministry of Social Affairs and Health. Recommendations for physical activity in early childhood education. Helsinki (Finland): Handbooks of the Ministry of Social Affairs and Health; 2005 (in Finnish).
- 23. Saari A, Sankilampi U, Hannila ML, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: length/height-for-age, weight-for-length/height, and body mass index-for-age. Ann Med 2011;43:235–48.
- Agren JJ, Julkunen A, Penttilä I. Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. J Lipid Res 1992;33:1871–6.
- Vessby B, Gustafsson IB, Tengblad S, Berglund L. Indices of fatty acid desaturase activity in healthy human subjects: effects of different types of dietary fat. Br J Nutr 2013;110:871–9.
- Warensjö E, Riserus U, Gustafsson IB, Mohsen R, Cederholm T, Vessby B. Effects of saturated and unsaturated fatty acids on estimated desaturase activities during a controlled dietary intervention. Nutr Metab Cardiovasc Dis 2008;18:683–90.

- Mahendran Y, Ågren J, Uusitupa M, Cederberg H, Vangipurapu J, Stančáková A, Schwab U, Kuusisto J, Laakso M. Association of erythrocyte membrane fatty acids with changes in glycemia and risk of type 2 diabetes. Am J Clin Nutr 2014;99:79–85.
- 28. Viitasalo A, Eloranta AM, Lintu N, Väistö J, Venäläinen T, Kiiskinen S, Karjalainen P, Peltola J, Lampinen EK, Haapala EA, et al. The effects of a 2-year individualized and family-based lifestyle intervention on physical activity, sedentary behavior and diet in children. Prev Med 2016;87: 81–8.
- Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L, Berglund J, Pulkki K, Basu S, Uusitupa M, et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. Am J Clin Nutr 2012; 95:1003–12.
- Hodson L, Fielding BA. Stearoyl-CoA desaturase: rogue or innocent bystander? Prog Lipid Res 2013;52:15–42.
- Adamsson V, Cederholm T, Vessby B, Risérus U. Influence of a healthy Nordic diet on serum fatty acid composition and associations with blood lipoproteins: results from the NORDIET study. Food Nutr Res 2014;58:24114.
- Green CD, Ozguden-Akkoc C, Wang Y, Jump DB, Olson LK. Role of fatty acid elongases in determination of de novo synthesized monounsaturated fatty acid species. J Lipid Res 2010;51:1871–7.
- 33. Takkunen MJ, de Mello VD, Schwab US, Ågren JJ, Kuusisto J, Uusitupa MI. Associations of erythrocyte membrane fatty acids with the concentrations of C-reactive protein, interleukin 1 receptor antagonist and adiponectin in 1373 men. Prostaglandins Leukot Essent Fatty Acids 2014;91:169–74.
- Andersson A, Sjödin A, Olsson R, Vessby B. Effects of physical exercise on phospholipid fatty acid composition in skeletal muscle. Am J Physiol 1998;274:E432–8.
- Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fattyacid composition of skeletal-muscle phospholipids. N Engl J Med 1993;328:238–44.
- Vessby B, Ahrén B, Warensjö E, Lindgärde F. Plasma lipid fatty acid composition, desaturase activities and insulin sensitivity in Amerindian women. Nutr Metab Cardiovasc Dis 2012;22:176–81.