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ORIGINAL ARTICLE

Reference values of whole-blood fatty acids by age and sex from European children aged 3–8 years

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OBJECTIVES: To establish reference values for fatty acids (FA) especially for n-3 and n-6 long-chain polyunsaturated FAs (LC PUFA) in whole-blood samples from apparently healthy 3–8-year-old European children. The whole-blood FA composition was analysed and the age- and sex-specific distribution of FA was determined.

DESIGN AND SUBJECTS: Blood samples for FA analysis were taken from 2661 children of the IDEFICS (identification and prevention of dietary- and lifestyle-induced health effects in children and infants) study cohort. Children with obesity ($n = 454$) and other diseases that are known to alter the FA composition ($n = 450$) were excluded leaving 1653 participants in the reference population.

MEASUREMENTS: The FA composition of whole blood was analysed from blood drops by a rapid, validated gas chromatographic method.

RESULTS: Pearson correlation coefficients showed an age-dependent increase of C18:2n-6 and a decrease of C18:1n-9 in a subsample of normal weight boys and girls. Other significant correlations with age were weak and only seen either in boys or in girls, whereas most of the FA did not show any age dependence. For age-dependent n-3 and n-6 PUFA as well as for other FA that are correlated with age (16:0, C18:0 and C18:1n-9) percentiles analysed with the general additive model for location scale and shape are presented. A higher median in boys than in girls was observed for C20:3n-6, C20:4n-6 and C22:4n-6.

CONCLUSIONS: Given the reported associations between FA status and health-related outcome, the provision of FA reference ranges may be useful for the interpretation of the FA status of children in epidemiological and clinical studies.

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INTRODUCTION

Fatty acids (FA) not only serve as major substrates for energy production but also fulfil several physiological functions. In this respect, the main focus is on polyunsaturated fatty acids (PUFA) such as alpha-linolenic acid (C18:3n-3) and linoleic acid (C18:2n-6) and, particularly, on long-chain PUFA (LC PUFA) such as eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) of the n-3 series and arachidonic acid (C20:4n-6) of the n-6 series. LC PUFA have a major role in the structure and function of cell membranes influencing their fluidity and permeability. In addition, they serve as precursors for the production of eicosanoids, such as prostaglandins, leukotrienes and thromboxanes, and docosanoids such as D-series resolvins and protectins with various effects, for example, on vasodilation, platelet aggregation, inflammation, cell growth and cell proliferation.[1] As a result LC PUFA influence normal growth, neurological and visual development as well as cognitive and immune function. Thus, an adequate status of LC PUFA is especially important in children.

The blood FA composition reflects the dietary fat intake,[2] especially LC n-3 PUFA are strongly correlated with intake.[3,4] This is why impaired PUFA availability may occur in children with unbalanced diet, malabsorption or maldigestion syndrome[5] and diseases of the liver where chain elongation and desaturation of LC PUFA are mainly located.[6,7] Beyond limited bioavailability and metabolism, monitoring of the blood FA becomes increasingly important because a vast number of studies have reported a modified blood PUFA distribution in various diseases. In particular, the most common chronic childhood diseases[8,9] in western countries, for example, asthma,[10–12] cystic fibrosis,[13,14] obesity,[15,16] diabetes,[17,18] mental health problems especially depression[19,20] and attention-deficity/hyperactivity disorders (ADHD)[21,22] are associated with modifications of the blood FA composition. Moreover, some studies have shown significant associations between the disease outcome and the LC PUFA profile, indicating beneficial effects of n-3 LC PUFA. Recently, it has also been shown that the serum cholesterylester FA profile in childhood is associated with the carotid intima media thickness in adult females[23] and with blood pressure in males and, to a lesser extent, also in females.[24] Besides dietary intake, the activity of the enzymes involved in the conversion of FA to LC PUFA, delta-9 desaturase (stearoyl-CoA desaturase1, SCD-1), delta-6 desaturase (D6D) and delta-5 desaturase (D5D), also have an important role for the FA composition because they are rate limiting in the

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biosynthesis of monounsaturated FA and LC PUFA. A high SCD-1 index has shown to be associated with obesity, whereas the D5D and D6D indices are associated with insulin sensitivity and metabolic syndrome.[15,25,26]

Whereas national data on the blood FA composition of young children stem mainly from small and a few large study populations,[27–29] international data from unselected populations are missing. Therefore, we aim to establish reference values for 3–8-year-old European children.

SUBJECTS AND METHODS

Subjects

A subsample of the IDEFICS (identification and prevention of dietary- and lifestyle-induced health effects in children and infants) cohort was included in this study. In IDEFICS, 16 228 children aged 2–9 years were examined in a population-based baseline survey in eight European countries ranging from North to South and from East to West (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden) from autumn 2007 to spring 2008. This baseline survey (T_0) was the starting point of the prospective cohort study with the largest European children's cohort established to date.[30] Additional 2517 children aged 2.0–10.9 years were newly recruited during a second survey (T_1) 2 years later; the study design has been described in detail elsewhere.[30] A subsample of the T_0 and T_1 IDEFICS cohort is included in the present analysis.

Inclusion and exclusion criteria for reference population

FA composition was analysed in a subsample of IDEFICS participants with oversampling of overweight and obese children. Therefore, weights were constructed as described in the section 'Statistical methods'. The reasons for oversampling were the focus of the IDEFICS study on overweight and obesity and a planned nested case–control study. Overall 2661 FA profiles from fasting blood samples of children aged 2.0–10.9 years were analysed. Of these, 1008 children were excluded for various reasons (Figure 1): 454 were defined as obese according to Cole and Lobstein[31] and 450 children had other diagnosed diseases (allergies, asthma, major dermatological problems, chronic rheumatic disease and diabetes mellitus) that may influence the FA composition. In addition, the upper and lower age strata included too few children for the analysis leaving 1653 participants aged 3.0–8.9 years in our analysis group.

Blood FA sampling and analysis

Blood samples were obtained by collecting a drop of blood from a fingertip or by venipuncture. A drop of native blood was then applied directly to a test strip prepared with butylated hydroxytoluene as an antioxidant. The dried strip was placed in a plastic envelope and shipped to the laboratory. FA in blood lipids were separated and determined by gas–liquid chromatography after direct derivatisation to their methyl esters without prior extraction of total lipids from the samples as previously described.[32] In brief, the strip of paper was transferred to Teflon screw-capped glass vials with 1 ml 3 N MeOH/HCl and placed in a dry bath at 90 °C for 1 h. Afterwards, 2 ml of water and 2 ml of a saturated KCl solution were added and FA methyl esters were then extracted using 2 ml of n-hexane twice. After centrifugation (1000 g for 5 min at 4–5 °C), the FA methyl esters recovered in the upper n-hexane phase were dried under nitrogen flow and vacuum by evaporating the solvent (n-hexane), then they were redissolved in about 50 µl n-hexane and taken up. Finally, 1/100th volume was injected in a gas–liquid chromatography (model GC1000, DANI Instruments SpA, Cologno Monzese, Italy) equipped with a capillary column with 30 m length, 0.32 mm inner diameter and 0.25 µm film thickness in poly-ethylene-glycol (Omegawax 320 Supelco, Bellefonte, PA, USA), a programmed temperature vaporising injector and a flame ionisation detector to quantify individual FA methyl esters, and a dedicated data system. Temperature programming went from 120 °C for 1.20 min, with an increase of 2.7 °C per min to 205 °C, a 15-min hold period, then with an increment of 5 °C per min to 220 °C and a 15-min hold period. For identification of the peaks pure reference compounds were used.

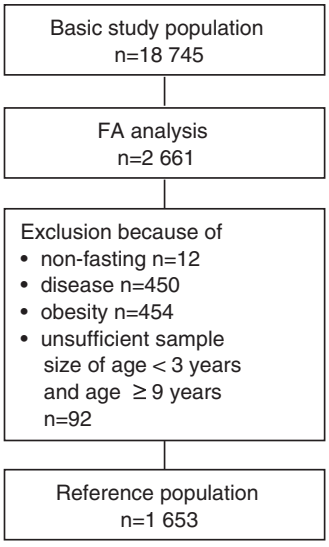

Figure 1. Flow chart of subject inclusion and exclusion.

Table 1. Basic characteristics of the analysis group

Sex	*Girls,* n *(%)*	*Boys,* n *(%)*
	836 (50.6%)	817 (49.4%)
Age categories		
3– < 4 years	128 (15.3%)	123 (15.1%)
4– < 5 years	147 (17.6%)	144 (17.6%)
5– < 6 years	103 (12.3%)	106 (13.0%)
6– < 7 years	125 (15.0%)	139 (17.0%)
7– < 8 years	197 (23.6%)	180 (22.0%)
8– < 9 years	136 (16.3%)	125 (15.3%)
	Mean (s.d.)	*Mean (s.d.)*
Age, years	6.09 (1.72)	6.05 (1.69)
Weight, kg	23.26 (6.25)	23.43 (6.28)
Height, cm	117.10 (11.99)	117.83 (12.09)
BMI, kg m^{-2}, unweighted	16.67 (1.96)	16.58 (1.89)
BMI, kg m^{-2}, weighted	16.03 (1.79)	16.11 (1.71)
BMI *z*-score, unweighted	0.57 (1.02)	0.47 (1.07)
BMI *z*-score, weighted	0.23 (0.99)	0.20 (1.02)

Abbreviation: BMI, body mass index.

Table 2. Correlations of FA proportion and age in normal weight children

FA	*Girls* (n = *523*)		*Boys* (n = *536*)	
	r	P-*value*	r	P-*value*
C16:0	−0.026	0.547	−0.129	0.003
C18:0	0.120	0.006	0.089	0.039
C24:0	0.063	0.148	0.132	0.002
C18:1n-9	−0.264	< 0.001	−0.186	< 0.001
C20:1n-9	−0.141	0.001	−0.099	0.022
C20:3n-9	−0.144	< 0.001	−0.092	0.034
C18:2n-6	0.246	< 0.001	0.171	< 0.001
C22:5n-3	−0.126	0.004	−0.025	0.567
Sum MUFA	−0.259	< 0.001	−0.179	< 0.001
Sum n-6 PUFA	0.210	< 0.001	0.200	< 0.001
Sum PUFA	0.178	< 0.001	0.167	< 0.001

Abbreviations: FA, fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; r, Pearson's correlation coefficient.

The FA composition is expressed as weight percentage of all FA detected (% wt/wt) with a C16–C24 chain length excluding *trans* FA. The following FA were measured: C16:0, C18:0, C20:0, C22:0, C24:0, C16:1, C18:1n-9, C18:1n-7, C20:1, C22:1, C24:1, C20:3n-9, C18:2n-6, C18:3n-6, C20:3n-6, C20:4n-6, C22:4n-6, C22:5n-6, C18:3n-3, C20:5n-3, C22:5n-3 and C22:6n-3. The desaturase indices were determined as follows: SCD-1—C16:1n-7/16:0 (SCD-16), C18:1n-9/C18:0 (SCD-18); D6D—C20:3n-6/C18:2n-6; D5D—C20:4n-6/C20:3n-6.

Statistical methods

Oversampling led to a high percentage of overweight children in the sample. Therefore, weights were constructed to match the distribution of

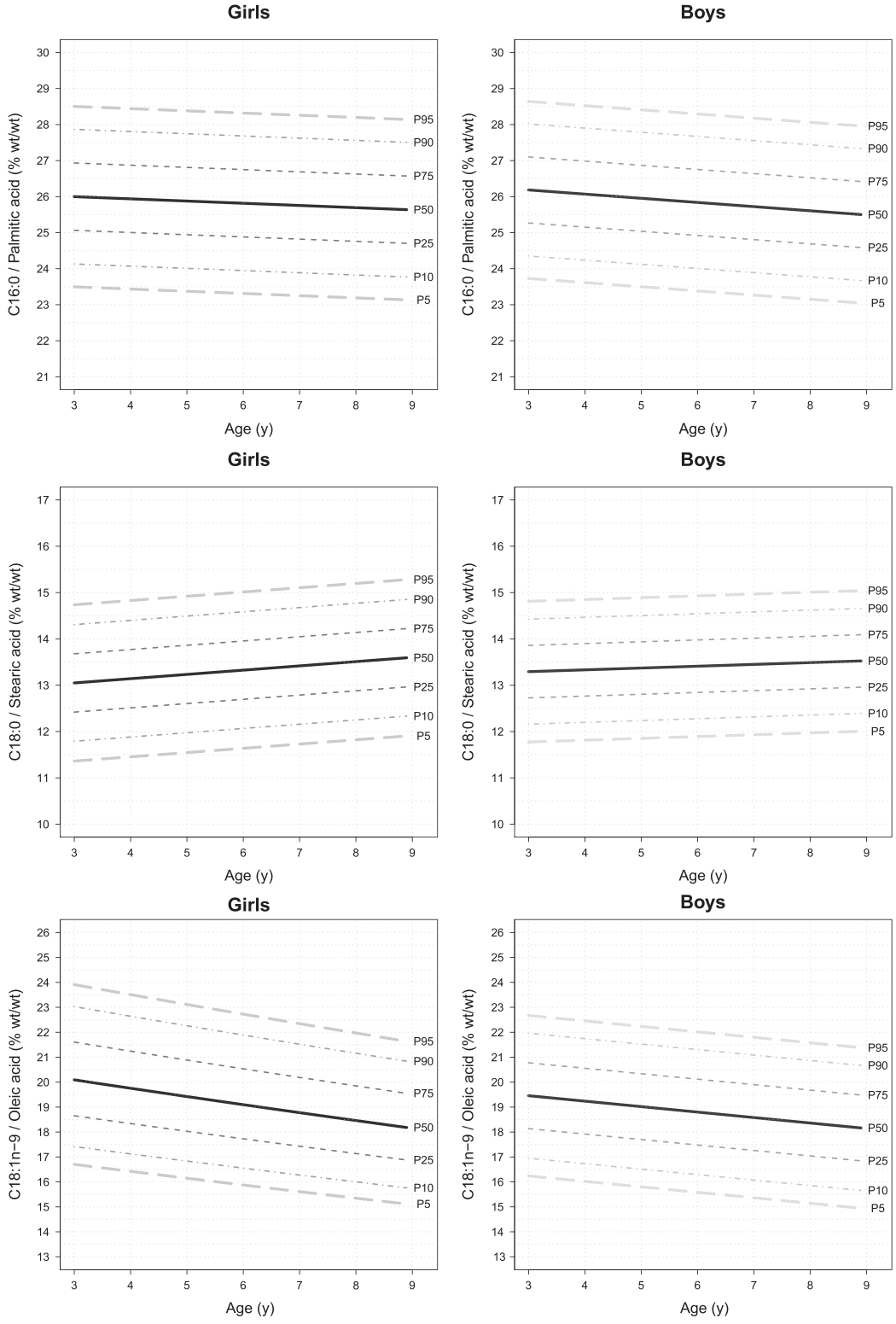

Figure 2. Percentiles (% wt/wt) of selected FA in 3–8-year-old girls and boys calculated with GAMLSS.

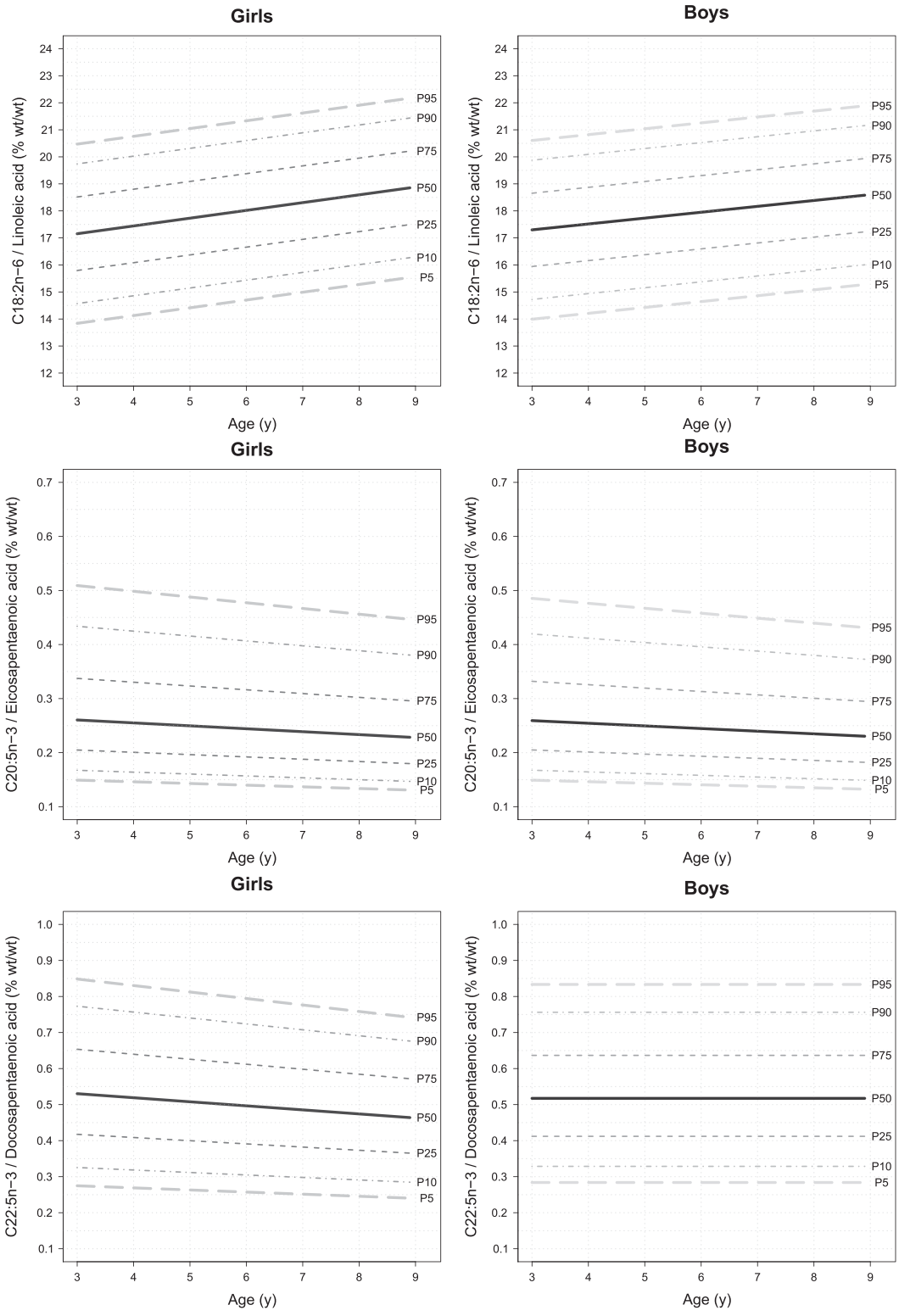

Figure 2. (Continued)

non-obese children from IDEFICS by sex and 1-year age groups. These weights are used in all analyses and for results presented in the paper except for analysis conducted in normal weight children.

Since the percentiles of most FA seemed to be similar across the age groups Pearson correlation coefficients were used to detect age dependence in a subsample of normal weight boys and girls ($n = 1059$, 536 boys, 523 girls). A cut-off of $\alpha = 0.01$ and a correlation in both, boys and girls, were used to decide which FA should be included in the further analysis with generalized additive models for location scale and shape (GAMLSS). Despite being correlated with age only in boys, C16:0 was also included because of its high proportion in blood. Moreover, the most important n-3 and n-6 PUFA and LC PUFA (except for C18:3n-3, C22:6n-3 and C20:4n-6 because no age dependence was seen) were included in analysis with GAMLSS. We calculated percentile curves as a function of age stratified by sex using the GAMLSS method as extension of the LMS method. The LMS method models three parameters: the skewness (L)

accounts for the deviation from a normal distribution using a Box–Cox transformation, the median (M) accounts for the dependence of the outcome variable on one explanatory variable and the coefficient of variation (S) accounts for the variation of data points around the mean and adjusts for non-uniform dispersion. The GAMLSS method is able to model more than one covariate and also other distributions. We used the gamlss package (version 4.2–6) of the statistical software R (version 3.0.1).[33] Different distributions, that is, the Box–Cox–Power–Exponential, Box–Cox *t*, Box–Cox Cole and Green (BCCG), gamma, inverse Gaussian, logistic, *t* family and normal distribution were fitted to the observed distribution of FA. Moreover, the influence of age on parameters of the considered distributions were modelled as a constant, as a linear function or as a cubic spline of the covariates.

Goodness-of-fit was assessed by the Bayesian information criterion and Q–Q plots to select the final model including the fitted distribution of FA and the influence of the covariates on distribution parameters. Worm plots were used as a diagnostic tool to assess whether adjustment for kurtosis was required.[34] Finally, curves for the 5th, 10th, 25th, 50th, 75th, 90th and 95th percentiles were calculated for C16:0, C18:0, C18:1n-9, C18:2n-6, C20:5n-3 and C22:5n-3 on the basis of the model that showed the best goodness-of-fit.[33,35] The best fit in GAMLSS was reached for C16:0 and C18:0 using the logistic distribution with parameters modelled as follows in both sexes: μ linearly and log(σ) as a constant; for C18:1n-9 using the gamma distribution with parameters modelled as follows: log(μ) linearly and log(σ) as constant; for C18:2n-6 using a normal distribution with μ modelled linearly and log(σ) modelled as a constant; for C20:5n-3 using the BCCG distribution with parameters modelled as follows: μ linearly and log (σ) and ν as constants. The best fit for C22:5n-3 was reached using the BCCG distribution without age dependence for boys and for girls using the BCCG distribution where the parameters were modelled as follows: μ linearly and log(σ) and ν as constants.

Percentiles of the other FA were not calculated with GAMLSS; the most interesting of them were pooled for the age groups and are presented as boxplots.

Sensitivity analysis using GAMLSS was conducted for all FA calculated with GAMLSS regarding the influence of obesity. In addition, for these FA, further GAMLSS analyses were conducted based on the data set including obese children and children with diseases.

RESULTS

The characteristics of the study population with weighted and unweighted body mass index and body mass index *z*-score are presented in Table 1.

Table 3. Percentiles of FA composition (% wt/wt) calculated with GAMLSS

FA	*Age*	*Percentile for girls*							*FA*	*Age*	*Percentile for boys*						
		5	*10*	*25*	*50*	*75*	*90*	*95*			*5*	*10*	*25*	*50*	*75*	*90*	*95*
C16:0									C16:0								
	3– < 4	23.47	24.10	25.04	25.97	26.90	27.84	28.47		3– < 4	23.67	24.30	25.21	26.13	27.04	27.96	28.58
	4– < 5	23.40	24.04	24.97	25.91	26.84	27.78	28.41		4– < 5	23.56	24.18	25.10	26.01	26.93	27.84	28.47
	5– < 6	23.34	23.98	24.91	25.85	26.78	27.72	28.35		5– < 6	23.44	24.06	24.98	25.90	26.81	27.73	28.35
	6– < 7	23.28	23.92	24.85	25.79	26.72	27.65	28.29		6– < 7	23.32	23.95	24.86	25.78	26.70	27.61	28.24
	7– < 8	23.22	23.86	24.79	25.72	26.66	27.59	28.23		7– < 8	23.21	23.83	24.75	25.66	26.58	27.50	28.12
	8– < 9	23.16	23.79	24.73	25.66	26.60	27.53	28.17		8– < 9	23.09	23.72	24.63	25.55	26.47	27.38	28.00
C18:0									C18:0								
	3– < 4	11.41	11.84	12.47	13.10	13.73	14.36	14.78		3– < 4	11.79	12.18	12.75	13.31	13.88	14.45	14.83
	4– < 5	11.50	11.93	12.56	13.19	13.82	14.45	14.88		4– < 5	11.83	12.22	12.79	13.35	13.92	14.49	14.87
	5– < 6	11.59	12.02	12.65	13.28	13.91	14.54	14.97		5– < 6	11.87	12.26	12.82	13.39	13.96	14.52	14.91
	6– < 7	11.68	12.11	12.74	13.37	14.00	14.63	15.06		6– < 7	11.91	12.30	12.86	13.43	14.00	14.56	14.95
	7– < 8	11.78	12.20	12.83	13.46	14.09	14.72	15.15		7– < 8	11.95	12.34	12.90	13.47	14.04	14.60	14.99
	8– < 9	11.87	12.30	12.93	13.56	14.19	14.82	15.24		8– < 9	11.99	12.38	12.94	13.51	14.08	14.64	15.03
C18:1n-9									C18:1n-9								
	3– < 4	16.56	17.27	18.50	19.92	21.42	22.84	23.71		3– < 4	16.13	16.84	18.03	19.35	20.67	21.85	22.56
	4– < 5	16.29	16.98	18.18	19.59	21.06	22.45	23.31		4– < 5	15.91	16.62	17.81	19.13	20.45	21.64	22.35
	5– < 6	16.01	16.69	17.88	19.26	20.71	22.08	22.92		5– < 6	15.69	16.40	17.59	18.91	20.23	21.42	22.13
	6– < 7	15.74	16.41	17.58	18.94	20.36	21.70	22.54		6– < 7	15.47	16.18	17.37	18.69	20.01	21.20	21.91
	7– < 8	15.48	16.14	17.28	18.62	20.02	21.34	22.16		7– < 8	15.25	15.96	17.15	18.47	19.79	20.98	21.69
	8– < 9	15.22	15.87	16.99	18.31	19.68	20.98	21.78		8– < 9	15.03	15.75	16.93	18.25	19.57	20.76	21.47
C18:2n-6									C18:2n-6								
	3– < 4	13.98	14.72	15.94	17.30	18.66	19.88	20.61		3– < 4	14.10	14.83	16.05	17.41	18.76	19.98	20.71
	4– < 5	14.27	15.00	16.23	17.59	18.95	20.17	20.90		4– < 5	14.32	15.05	16.27	17.62	18.98	20.20	20.93
	5– < 6	14.56	15.29	16.52	17.87	19.23	20.46	21.19		5– < 6	14.54	15.27	16.49	17.84	19.20	20.42	21.15
	6– < 7	14.85	15.58	16.80	18.16	19.52	20.75	21.48		6– < 7	14.75	15.48	16.70	18.06	19.41	20.63	21.37
	7– < 8	15.13	15.87	17.09	18.45	19.81	21.03	21.77		7– < 8	14.97	15.70	16.92	18.28	19.63	20.85	21.58
	8– < 9	15.42	16.15	17.38	18.74	20.10	21.32	22.05		8– < 9	15.19	15.92	17.14	18.49	19.85	21.07	21.80
C20:5n-3									C20:5n-3								
	3– < 4	0.15	0.17	0.20	0.26	0.33	0.43	0.50		3– < 4	0.15	0.17	0.20	0.26	0.33	0.42	0.48
	4– < 5	0.15	0.16	0.20	0.25	0.33	0.42	0.49		4– < 5	0.15	0.16	0.20	0.25	0.32	0.41	0.47
	5– < 6	0.14	0.16	0.19	0.25	0.32	0.41	0.48		5– < 6	0.14	0.16	0.20	0.25	0.32	0.40	0.46
	6– < 7	0.14	0.16	0.19	0.24	0.31	0.40	0.47		6– < 7	0.14	0.16	0.19	0.24	0.31	0.39	0.45
	7– < 8	0.14	0.15	0.19	0.24	0.31	0.39	0.46		7– < 8	0.14	0.15	0.19	0.24	0.30	0.38	0.44
	8– < 9	0.13	0.15	0.18	0.23	0.30	0.38	0.45		8– < 9	0.13	0.15	0.18	0.23	0.30	0.38	0.44
C22:5n-3									C22:5n-3								
	3– < 4	0.27	0.32	0.41	0.53	0.65	0.77	0.84		All	0.28	0.33	0.41	0.52	0.64	0.76	0.83
	4– < 5	0.27	0.32	0.40	0.51	0.63	0.75	0.82									
	5– < 6	0.26	0.31	0.40	0.50	0.62	0.73	0.80									
	6– < 7	0.25	0.30	0.39	0.49	0.61	0.72	0.79									
	7– < 8	0.25	0.29	0.38	0.48	0.59	0.70	0.77									
	8– < 9	0.24	0.29	0.37	0.47	0.58	0.68	0.75									

Abbreviations: FA, fatty acid; GAMLSS, generalized additive models for location scale and shape.

Table 4. Precentiles of FA composition (% wt/wt)

FA	*Age*	*Percentile for girls*							*FA*	*Age*	*Percentile for boys*						
		5	*10*	*25*	*50*	*75*	*90*	*95*			*5*	*10*	*25*	*50*	*75*	*90*	*95*
Saturated FA (SFA)									*Saturated FA (SFA)*								
C20:0									C20:0								
	3– < 4	0.42	0.42	0.47	0.52	0.58	0.63	0.65		3– < 4	0.42	0.43	0.47	0.52	0.57	0.61	0.65
	4– < 5	0.42	0.43	0.48	0.52	0.57	0.61	0.65		4– < 5	0.42	0.45	0.50	0.53	0.56	0.61	0.64
	5– < 6	0.41	0.44	0.47	0.51	0.58	0.62	0.68		5– < 6	0.39	0.42	0.46	0.50	0.55	0.58	0.59
	6– < 7	0.42	0.44	0.48	0.52	0.57	0.60	0.62		6– < 7	0.42	0.44	0.46	0.51	0.56	0.61	0.68
	7– < 8	0.43	0.44	0.48	0.52	0.57	0.60	0.64		7– < 8	0.41	0.43	0.45	0.51	0.56	0.61	0.63
	8– < 9	0.41	0.43	0.47	0.53	0.58	0.62	0.64		8– < 9	0.42	0.44	0.48	0.52	0.55	0.59	0.61
	All	0.42	0.43	0.48	0.52	0.57	0.61	0.65		All	0.41	0.43	0.47	0.51	0.56	0.60	0.63
C22:0									C22:0								
	3– < 4	1.37	1.49	1.65	1.80	1.95	2.09	2.23		3– < 4	1.49	1.52	1.66	1.77	1.97	2.12	2.17
	4– < 5	1.47	1.56	1.67	1.84	1.97	2.08	2.18		4– < 5	1.51	1.58	1.69	1.85	1.98	2.16	2.28
	5– < 6	1.44	1.49	1.65	1.82	2.01	2.18	2.29		5– < 6	1.49	1.56	1.62	1.74	1.90	2.04	2.26
	6– < 7	1.43	1.47	1.66	1.81	1.97	2.14	2.19		6– < 7	1.42	1.51	1.60	1.80	1.93	2.11	2.26
	7– < 8	1.52	1.56	1.63	1.78	1.96	2.08	2.19		7– < 8	1.37	1.48	1.67	1.79	1.95	2.15	2.25
	8– < 9	1.54	1.58	1.69	1.82	1.96	2.10	2.18		8– < 9	1.50	1.56	1.65	1.78	1.92	2.05	2.20
	All	1.43	1.54	1.65	1.81	1.97	2.12	2.20		All	1.47	1.53	1.65	1.79	1.94	2.11	2.24
C24:0									C24:0								
	3– < 4	2.33	2.42	2.63	2.90	3.31	3.50	3.66		3– < 4	2.50	2.65	2.81	3.04	3.22	3.49	3.58
	4– < 5	2.33	2.47	2.79	3.03	3.30	3.47	3.56		4– < 5	2.50	2.64	2.83	3.07	3.36	3.58	3.81
	5– < 6	2.37	2.51	2.78	3.02	3.38	3.59	3.76		5– < 6	2.64	2.75	2.89	3.07	3.26	3.50	3.55
	6– < 7	2.44	2.61	2.76	3.03	3.31	3.49	3.66		6– < 7	2.58	2.68	2.86	3.18	3.45	3.83	4.03
	7– < 8	2.48	2.57	2.77	3.02	3.29	3.52	3.81		7– < 8	2.53	2.63	2.86	3.13	3.40	3.64	3.84
	8– < 9	2.54	2.64	2.86	3.06	3.31	3.56	3.73		8– < 9	2.40	2.62	2.88	3.19	3.41	3.60	3.77
	All	2.37	2.53	2.76	3.03	3.31	3.51	3.68		All	2.49	2.64	2.85	3.10	3.36	3.60	3.82
Total SFA									Total SFA								
	3– < 4	41.16	41.71	43.25	44.08	45.20	46.29	46.92		3– < 4	42.52	42.73	43.78	44.80	46.24	47.08	47.35
	4– < 5	41.71	42.41	43.45	44.42	45.95	46.94	47.81		4– < 5	42.07	42.36	43.46	44.70	45.81	47.23	47.60
	5– < 6	41.73	42.02	43.26	44.51	46.13	47.48	48.57		5– < 6	42.04	42.78	43.44	44.54	45.62	47.40	47.94
	6– < 7	41.84	42.27	43.01	44.20	45.66	46.93	47.56		6– < 7	41.64	42.33	43.37	44.79	45.60	47.25	49.56
	7– < 8	41.78	42.37	43.55	44.90	46.12	47.45	48.30		7– < 8	41.90	42.50	43.48	44.75	45.83	47.18	47.78
	8– < 9	41.36	42.31	43.22	44.41	45.67	47.25	47.96		8– < 9	41.46	41.89	43.26	44.31	46.04	46.88	47.49
	All	41.55	42.19	43.26	44.42	45.85	47.14	47.82		All	41.81	42.44	43.45	44.66	45.88	47.15	47.87
MUFA									*MUFA*								
C16:1n-7									C16:1n-7								
	3– < 4	0.76	0.90	1.05	1.28	1.55	1.85	2.06		3– < 4	0.69	0.84	1.02	1.23	1.51	1.79	1.99
	4– < 5	0.77	0.86	0.99	1.18	1.46	1.79	1.93		4– < 5	0.68	0.76	0.93	1.11	1.41	1.68	1.93
	5– < 6	0.71	0.82	0.95	1.20	1.50	1.83	2.30		5– < 6	0.78	0.85	0.97	1.22	1.36	1.69	2.00
	6– < 7	0.70	0.85	0.99	1.20	1.47	1.72	1.89		6– < 7	0.71	0.78	0.92	1.13	1.35	1.58	1.99
	7– < 8	0.72	0.74	0.94	1.20	1.43	1.68	1.84		7– < 8	0.70	0.74	0.90	1.07	1.37	1.60	1.79
	8– < 9	0.69	0.79	0.95	1.18	1.38	1.61	2.06		8– < 9	0.75	0.80	1.01	1.21	1.44	1.72	1.86
	All	0.71	0.81	0.97	1.21	1.47	1.74	1.93		All	0.70	0.78	0.94	1.15	1.40	1.69	1.87
C18:1n-7									C18:1n-7								
	3– < 4	1.23	1.33	1.47	1.65	1.85	2.16	2.54		3– < 4	1.09	1.25	1.41	1.60	1.82	2.01	2.26
	4– < 5	1.26	1.30	1.40	1.58	1.74	1.97	2.32		4– < 5	1.06	1.13	1.31	1.48	1.72	1.93	2.16
	5– < 6	1.08	1.21	1.41	1.61	1.91	2.04	2.21		5– < 6	1.15	1.17	1.36	1.52	1.69	1.83	2.02
	6– < 7	1.15	1.23	1.40	1.57	1.79	2.05	2.13		6– < 7	1.06	1.13	1.30	1.52	1.68	1.98	2.12
	7– < 8	1.13	1.24	1.36	1.55	1.77	2.00	2.15		7– < 8	1.13	1.19	1.31	1.51	1.78	2.02	2.12
	8– < 9	1.04	1.17	1.34	1.55	1.73	2.06	2.20		8– < 9	1.19	1.26	1.39	1.58	1.80	2.02	2.29
	All	1.14	1.25	1.40	1.58	1.78	2.05	2.23		All	1.10	1.17	1.33	1.53	1.75	1.98	2.14
Total MUFA									Total MUFA								
	3– < 4	23.64	24.34	25.19	26.36	28.44	29.99	30.66		3– < 4	21.88	22.66	24.43	26.06	27.61	29.26	29.73
	4– < 5	21.55	22.90	23.97	25.64	27.40	29.71	30.63		4– < 5	21.73	22.15	23.34	25.40	26.78	27.85	28.34
	5– < 6	20.13	21.60	23.36	25.32	26.68	28.39	29.15		5– < 6	21.94	22.41	23.53	24.84	26.12	27.72	28.40
	6– < 7	21.27	22.28	23.39	24.60	26.23	28.02	28.93		6– < 7	21.12	22.12	22.94	24.17	25.77	27.28	27.83
	7– < 8	21.24	22.12	23.67	24.95	26.40	27.59	28.71		7– < 8	21.29	21.71	23.00	24.70	26.08	27.80	28.48
	8– < 9	21.61	22.25	23.24	24.81	26.01	27.68	28.25		8– < 9	21.01	21.93	23.49	25.11	26.37	27.32	27.78
	All	21.40	22.36	23.75	25.24	26.80	28.50	29.78		All	21.36	21.97	23.29	24.89	26.41	27.82	28.76
n-9 PUFA									*n-9 PUFA*								
C20:3n-9									C20:3n-9								
	3– < 4	0.04	0.05	0.06	0.08	0.10	0.14	0.17		3– < 4	0.04	0.05	0.07	0.08	0.11	0.14	0.17
	4– < 5	0.03	0.04	0.06	0.08	0.10	0.14	0.16		4– < 5	0.03	0.05	0.06	0.08	0.11	0.14	0.16
	5– < 6	0.04	0.05	0.06	0.08	0.10	0.14	0.17		5– < 6	0.04	0.05	0.06	0.08	0.11	0.13	0.14
	6– < 7	0.04	0.05	0.06	0.08	0.10	0.12	0.15		6– < 7	0.03	0.03	0.05	0.08	0.09	0.12	0.14
	7– < 8	0.04	0.04	0.06	0.08	0.10	0.12	0.14		7– < 8	0.04	0.05	0.06	0.07	0.10	0.12	0.14
	8– < 9	0.03	0.04	0.05	0.06	0.08	0.12	0.13		8– < 9	0.04	0.04	0.06	0.08	0.10	0.13	0.15
	all	0.03	0.04	0.06	0.08	0.10	0.13	0.15		all	0.03	0.04	0.06	0.08	0.10	0.13	0.15

Table. 4. (Continued)

FA	Age	Percentile for girls							FA	Age	Percentile for boys						
		5	10	25	50	75	90	95			5	10	25	50	75	90	95
n-6 PUFA									*n-6 PUFA*								
C18:3n-6									C18:3n-6								
	3– < 4	0.12	0.13	0.16	0.20	0.23	0.30	0.33		3– < 4	0.13	0.15	0.17	0.21	0.27	0.34	0.39
	4– < 5	0.11	0.12	0.16	0.20	0.25	0.31	0.34		4– < 5	0.11	0.14	0.18	0.21	0.28	0.34	0.39
	5– < 6	0.11	0.12	0.14	0.20	0.27	0.34	0.39		5– < 6	0.13	0.14	0.18	0.21	0.27	0.34	0.38
	6– < 7	0.12	0.14	0.17	0.22	0.30	0.34	0.38		6– < 7	0.12	0.13	0.16	0.21	0.26	0.34	0.38
	7– < 8	0.12	0.13	0.17	0.20	0.25	0.32	0.41		7– < 8	0.12	0.14	0.18	0.21	0.29	0.37	0.41
	8– < 9	0.12	0.13	0.17	0.21	0.26	0.33	0.36		8– < 9	0.11	0.13	0.16	0.22	0.25	0.33	0.37
	all	0.12	0.13	0.16	0.20	0.26	0.32	0.36		all	0.12	0.14	0.17	0.21	0.27	0.34	0.40
C20:3n-6									C20:3n-6								
	3– < 4	0.81	0.84	0.97	1.10	1.30	1.45	1.58		3– < 4	0.80	0.89	0.96	1.15	1.35	1.48	1.54
	4– < 5	0.74	0.80	0.94	1.10	1.30	1.47	1.64		4– < 5	0.84	0.90	1.02	1.18	1.42	1.55	1.67
	5– < 6	0.81	0.82	0.98	1.11	1.34	1.51	1.55		5– < 6	0.85	0.93	1.09	1.24	1.44	1.58	1.68
	6– < 7	0.87	0.94	1.07	1.18	1.30	1.48	1.57		6– < 7	0.85	0.92	1.06	1.18	1.39	1.53	1.70
	7– < 8	0.83	0.89	0.99	1.12	1.30	1.52	1.64		7– < 8	0.92	0.98	1.08	1.22	1.40	1.53	1.59
	8– < 9	0.85	0.89	1.03	1.21	1.36	1.48	1.56		8– < 9	0.88	0.95	1.08	1.26	1.43	1.60	1.77
	all	0.80	0.85	0.99	1.13	1.31	1.49	1.59		all	0.85	0.91	1.04	1.21	1.40	1.54	1.66
C20:4n-6									C20:4n-6								
	3– < 4	5.36	5.55	6.26	6.96	8.02	8.78	9.48		3– < 4	4.89	5.31	6.24	7.32	7.97	8.53	9.05
	4– < 5	5.03	5.40	6.31	6.97	8.16	8.86	9.29		4– < 5	5.43	5.87	6.56	7.37	8.44	9.07	9.38
	5– < 6	5.26	5.87	6.48	7.43	8.28	8.96	9.45		5– < 6	4.93	5.62	6.44	7.54	8.21	9.00	9.20
	6– < 7	5.28	5.71	6.60	7.54	8.35	8.89	9.29		6– < 7	5.14	5.93	6.92	7.70	8.68	9.51	10.08
	7– < 8	5.01	5.42	6.15	7.06	8.05	8.81	9.14		7– < 8	5.69	5.88	6.58	7.35	8.15	8.91	9.22
	8– < 9	4.77	5.35	6.33	7.25	8.40	8.93	9.55		8– < 9	4.87	5.67	6.61	7.57	8.43	9.29	9.65
	all	5.13	5.47	6.28	7.21	8.18	8.87	9.39		all	5.14	5.69	6.55	7.48	8.32	9.02	9.39
C22:4n-6									C22:4n-6								
	3– < 4	0.65	0.69	0.78	0.95	1.14	1.30	1.42		3– < 4	0.57	0.62	0.85	1.01	1.19	1.37	1.46
	4– < 5	0.60	0.66	0.80	0.96	1.13	1.38	1.47		4– < 5	0.60	0.72	0.84	1.02	1.21	1.35	1.55
	5– < 6	0.57	0.68	0.79	1.00	1.20	1.34	1.46		5– < 6	0.59	0.70	0.86	1.08	1.22	1.34	1.45
	6– < 7	0.66	0.73	0.85	1.06	1.23	1.44	1.50		6– < 7	0.72	0.75	0.88	1.08	1.36	1.49	1.53
	7– < 8	0.59	0.65	0.76	0.96	1.16	1.32	1.36		7– < 8	0.67	0.75	0.88	1.03	1.22	1.43	1.51
	8– < 9	0.57	0.66	0.90	1.04	1.22	1.44	1.57		8– < 9	0.56	0.68	0.87	1.07	1.28	1.46	1.62
	All	0.59	0.66	0.80	0.99	1.17	1.35	1.46		All	0.60	0.71	0.86	1.05	1.24	1.42	1.51
Total n-6 PUFA									Total n-6 PUFA								
	3– < 4	23.20	24.05	24.95	27.04	28.61	29.74	30.30		3– < 4	22.19	23.45	25.33	26.79	28.80	30.26	31.21
	4– < 5	22.70	24.17	25.71	27.61	29.26	30.69	31.52		4– < 5	24.13	25.14	26.16	27.49	29.64	31.78	32.60
	5– < 6	23.11	24.15	26.08	28.09	30.11	31.62	32.92		5– < 6	23.56	24.38	26.59	28.47	29.93	31.43	31.92
	6– < 7	24.11	25.17	26.66	28.46	30.61	31.74	32.33		6– < 7	24.41	24.98	26.68	28.70	30.33	31.89	32.63
	7– < 8	24.50	25.15	26.39	28.36	30.05	31.42	32.04		7– < 8	24.64	25.47	26.89	28.70	30.23	31.72	32.42
	8– < 9	23.70	25.13	26.94	28.83	30.42	31.52	32.15		8– < 9	24.02	25.22	27.05	28.73	30.21	31.89	32.97
	All	23.53	24.49	26.10	28.07	29.87	31.32	31.98		All	23.73	24.62	26.22	28.31	29.88	31.58	32.35
Total n-6 LC PUFA									Total n-6 LC PUFA								
	3– < 4	7.20	7.47	8.31	9.38	10.75	11.43	12.34		3– < 4	6.51	7.03	8.34	9.92	10.82	11.51	12.10
	4– < 5	6.67	7.41	8.30	9.31	10.90	11.78	12.40		4– < 5	7.17	7.82	8.66	9.95	11.28	12.26	12.75
	5– < 6	6.97	7.62	8.58	9.97	10.98	11.91	12.39		5– < 6	6.70	7.32	8.78	10.21	11.10	12.13	12.35
	6– < 7	7.27	7.81	8.70	10.03	11.00	12.09	12.39		6– < 7	7.18	7.83	9.14	10.27	11.62	12.69	13.30
	7– < 8	6.75	7.24	8.15	9.47	10.91	11.72	12.03		7– < 8	7.60	7.93	8.82	10.02	10.99	12.00	12.29
	8– < 9	6.71	7.23	8.54	9.85	11.15	12.11	12.78		8– < 9	7.01	7.96	8.93	10.30	11.59	12.36	12.90
	All	6.86	7.34	8.40	9.64	10.95	11.88	12.40		All	6.98	7.57	8.74	10.04	11.19	12.21	12.65
n-3 PUFA									*n-3 PUFA*								
C18:3n-3									C18:3n-3								
	3– < 4	0.11	0.12	0.15	0.21	0.26	0.33	0.37		3– < 4	0.10	0.11	0.14	0.19	0.24	0.31	0.36
	4– < 5	0.11	0.12	0.14	0.20	0.26	0.35	0.39		4– < 5	0.11	0.13	0.15	0.20	0.26	0.36	0.40
	5– < 6	0.11	0.12	0.13	0.19	0.26	0.31	0.33		5– < 6	0.10	0.11	0.14	0.19	0.23	0.31	0.38
	6– < 7	0.10	0.11	0.14	0.18	0.25	0.32	0.39		6– < 7	0.10	0.11	0.13	0.17	0.23	0.28	0.32
	7– < 8	0.09	0.10	0.13	0.19	0.27	0.32	0.34		7– < 8	0.10	0.11	0.13	0.18	0.25	0.31	0.35
	8– < 9	0.10	0.10	0.13	0.18	0.25	0.31	0.37		8– < 9	0.10	0.12	0.14	0.18	0.23	0.27	0.31
	All	0.10	0.11	0.14	0.19	0.26	0.32	0.38		All	0.10	0.11	0.14	0.18	0.24	0.31	0.37
C22:6n-3									C22:6n-3								
	3– < 4	0.59	0.68	0.86	1.13	1.46	1.71	1.91		3– < 4	0.58	0.69	0.84	1.08	1.46	1.66	1.85
	4– < 5	0.53	0.59	0.75	1.09	1.35	1.61	1.82		4– < 5	0.64	0.72	1.02	1.26	1.49	1.69	1.94
	5– < 6	0.63	0.76	0.95	1.18	1.47	1.79	2.19		5– < 6	0.56	0.70	0.95	1.17	1.40	1.70	1.96
	6– < 7	0.51	0.63	0.93	1.17	1.48	1.82	2.03		6– < 7	0.52	0.65	0.84	1.18	1.55	2.01	2.33
	7– < 8	0.43	0.50	0.71	1.02	1.31	1.59	1.78		7– < 8	0.56	0.63	0.80	1.02	1.31	1.59	1.75
	8– < 9	0.48	0.63	0.84	1.13	1.44	1.77	1.92		8– < 9	0.52	0.63	0.84	1.13	1.41	1.75	1.87
	All	0.50	0.59	0.82	1.11	1.40	1.71	1.92		All	0.53	0.65	0.85	1.13	1.44	1.73	1.94

Table. 4. (Continued)

FA	*Age*	*Percentile for girls*							*FA*	*Age*	*Percentile for boys*						
		5	*10*	*25*	*50*	*75*	*90*	*95*			*5*	*10*	*25*	*50*	*75*	*90*	*95*
Total n-3 PUFA									Total n-3 PUFA								
	3– < 4	1.37	1.49	1.80	2.17	2.66	3.14	3.29		3– < 4	1.34	1.52	1.70	2.01	2.48	3.03	3.18
	4– < 5	1.13	1.32	1.66	2.07	2.49	2.81	3.17		4– < 5	1.23	1.50	1.93	2.29	2.72	3.08	3.44
	5– < 6	1.45	1.53	1.81	2.11	2.63	3.01	3.67		5– < 6	1.25	1.53	1.82	2.12	2.58	3.13	3.33
	6– < 7	1.24	1.37	1.65	2.18	2.53	2.97	3.18		6– < 7	1.20	1.39	1.71	2.17	2.61	3.28	3.59
	7– < 8	1.13	1.22	1.46	1.94	2.37	2.83	3.00		7– < 8	1.26	1.36	1.65	2.03	2.40	2.68	3.02
	8– < 9	1.26	1.43	1.60	2.07	2.60	3.00	3.22		8– < 9	1.17	1.29	1.69	2.17	2.56	2.93	3.16
	All	1.19	1.34	1.62	2.09	2.52	2.97	3.21		All	1.21	1.39	1.73	2.12	2.53	3.04	3.32
Total n-3 LC PUFA									Total n-3 LC PUFA								
	3– < 4	1.22	1.32	1.59	1.94	2.52	2.85	3.05		3– < 4	1.18	1.32	1.50	1.81	2.32	2.81	2.92
	4– < 5	0.96	1.13	1.41	1.87	2.27	2.57	2.93		4– < 5	1.12	1.29	1.71	2.09	2.44	2.79	3.18
	5– < 6	1.23	1.35	1.61	1.97	2.39	2.86	3.41		5– < 6	1.06	1.28	1.67	1.94	2.39	2.89	3.21
	6– < 7	1.04	1.18	1.50	1.98	2.37	2.76	2.92		6– < 7	1.01	1.23	1.56	1.99	2.42	3.04	3.38
	7– < 8	0.91	1.02	1.24	1.80	2.15	2.55	2.86		7– < 8	1.10	1.19	1.49	1.82	2.19	2.49	2.85
	8– < 9	1.06	1.26	1.45	1.90	2.39	2.78	2.96		8– < 9	0.99	1.16	1.49	1.95	2.38	2.73	2.94
	All	0.99	1.15	1.43	1.91	2.33	2.71	2.97		All	1.04	1.21	1.54	1.94	2.35	2.80	3.04
Ratios/indices									*Ratios/indices*								
n-6/n-3									n-6/n-3								
	3– < 4	8.67	9.00	10.17	12.28	15.13	17.97	20.11		3– < 4	8.12	8.84	10.94	13.38	15.81	18.33	21.98
	4– < 5	8.79	9.59	11.03	13.35	16.71	20.15	23.35		4– < 5	8.50	8.64	10.40	12.50	14.53	18.57	22.32
	5– < 6	8.09	9.05	11.01	13.00	15.77	17.71	20.62		5– < 6	9.12	9.31	11.11	13.20	15.39	18.89	20.71
	6– < 7	9.31	9.73	11.56	13.09	16.73	20.35	21.18		6– < 7	8.27	9.30	11.15	13.22	16.71	20.78	22.03
	7– < 8	9.50	10.39	11.91	14.80	18.73	22.57	25.52		7– < 8	9.45	10.32	12.07	14.00	17.24	20.78	22.33
	8– < 9	8.83	9.40	11.63	14.09	17.07	19.74	22.94		8– < 9	9.44	10.08	11.19	13.31	17.30	21.22	23.21
	All	8.67	9.48	11.16	13.46	16.73	20.37	22.97		All	8.49	9.33	11.11	13.28	16.36	20.37	22.19
C20:4n-6/C20:3n6 (D5D)									C20:4n-6/C20:3n6 (D5D)								
	3– < 4	4.63	4.85	5.17	6.26	7.59	8.70	9.15		3– < 4	4.68	4.98	5.41	6.05	7.16	8.27	8.72
	4– < 5	4.59	4.86	5.59	6.21	7.33	8.54	9.17		4– < 5	4.16	4.65	5.46	6.31	7.31	7.85	8.44
	5– < 6	4.51	4.76	5.63	6.40	7.59	8.64	9.64		5– < 6	4.33	4.59	5.21	5.82	6.79	7.51	8.02
	6– < 7	4.31	4.80	5.69	6.27	7.20	7.96	8.40		6– < 7	4.29	4.86	5.50	6.41	7.38	8.12	8.49
	7– < 8	4.22	4.76	5.42	6.27	7.21	7.98	8.54		7– < 8	4.53	4.86	5.22	5.96	6.77	7.68	8.23
	8– < 9	4.20	4.63	5.41	6.17	6.77	7.83	8.80		8– < 9	4.21	4.60	5.19	6.11	6.75	7.59	7.87
	All	4.31	4.73	5.45	6.24	7.22	8.31	9.05		All	4.28	4.72	5.33	6.08	7.03	7.87	8.35
C20:3n-6/C18:2n-6 (D6D)									C20:3n-6/C18:2n-6 (D6D)								
	3– < 4	0.05	0.05	0.06	0.07	0.08	0.09	0.10		3– < 4	0.05	0.05	0.06	0.07	0.08	0.09	0.10
	4– < 5	0.04	0.04	0.05	0.06	0.08	0.09	0.09		4– < 5	0.05	0.05	0.06	0.07	0.08	0.09	0.10
	5– < 6	0.04	0.04	0.05	0.06	0.07	0.09	0.09		5– < 6	0.05	0.05	0.06	0.07	0.08	0.09	0.10
	6– < 7	0.05	0.05	0.06	0.06	0.07	0.08	0.10		6– < 7	0.05	0.05	0.06	0.07	0.08	0.09	0.10
	7– < 8	0.04	0.05	0.05	0.06	0.07	0.09	0.09		7– < 8	0.05	0.05	0.06	0.07	0.08	0.09	0.10
	8– < 9	0.04	0.05	0.06	0.06	0.07	0.08	0.09		8– < 9	0.05	0.05	0.06	0.07	0.08	0.10	0.11
	All	0.04	0.05	0.05	0.06	0.07	0.09	0.09		All	0.05	0.05	0.06	0.07	0.08	0.09	0.10
C16:1n-7/C16:0 (SCD-16)									C16:1n-7/C16:0 (SCD-16)								
	3– < 4	0.03	0.04	0.04	0.05	0.06	0.07	0.08		3– < 4	0.03	0.03	0.04	0.05	0.06	0.07	0.07
	4– < 5	0.03	0.03	0.04	0.05	0.06	0.07	0.07		4– < 5	0.03	0.03	0.04	0.04	0.06	0.06	0.07
	5– < 6	0.03	0.03	0.04	0.05	0.06	0.07	0.08		5– < 6	0.03	0.03	0.04	0.05	0.05	0.06	0.07
	6– < 7	0.03	0.03	0.04	0.05	0.06	0.07	0.07		6– < 7	0.03	0.03	0.04	0.04	0.05	0.06	0.07
	7– < 8	0.03	0.03	0.04	0.05	0.05	0.07	0.07		7– < 8	0.03	0.03	0.04	0.04	0.05	0.06	0.07
	8– < 9	0.03	0.03	0.04	0.05	0.05	0.06	0.07		8– < 9	0.03	0.03	0.04	0.05	0.05	0.07	0.07
	All	0.03	0.03	0.04	0.05	0.06	0.07	0.07		All	0.03	0.03	0.04	0.04	0.05	0.06	0.07
C18:1n-9/C18:0 (SCD-18)									C18:1n-9/C18:0 (SCD-18)								
	3– < 4	1.26	1.32	1.42	1.53	1.72	1.90	1.98		3– < 4	1.17	1.24	1.35	1.49	1.64	1.73	1.82
	4– < 5	1.13	1.23	1.37	1.48	1.60	1.76	1.89		4– < 5	1.16	1.22	1.32	1.41	1.57	1.68	1.80
	5– < 6	1.07	1.14	1.28	1.39	1.62	1.76	1.81		5– < 6	1.12	1.18	1.30	1.40	1.54	1.63	1.72
	6– < 7	1.11	1.18	1.28	1.39	1.55	1.71	1.78		6– < 7	1.08	1.13	1.24	1.36	1.49	1.57	1.67
	7– < 8	1.10	1.13	1.25	1.39	1.50	1.68	1.73		7– < 8	1.05	1.12	1.23	1.37	1.49	1.61	1.68
	8– < 9	1.08	1.18	1.26	1.39	1.51	1.61	1.72		8– < 9	1.12	1.16	1.28	1.39	1.53	1.65	1.72
	All	1.10	1.18	1.30	1.44	1.57	1.74	1.85		All	1.10	1.16	1.28	1.40	1.53	1.66	1.74

Abbreviations: D5D, delta-5-desaturase index; D6D, delta-6-desaturase index; FA, fatty acid; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; n-6/n-3, ratio of sums of all n-6 PUFA/n-3 PUFA; SCD, stearoyl-CoA desaturase; SCD-16, C16:1n-7/C16:0 ratio; SCD-18, C18:1n-9/C18:0 ratio; SFA, saturated FA.

Significant but weak correlations of the FA proportion with age were seen for C18:1n-9, C18:2n-6 and sums of monounsaturated FA, PUFA and n-6 PUFA in both, girls and boys. Some age dependencies were only seen in either boys or in girls (Table 2).

C18:0 and C18:2n-6 showed a positive trend with age, whereas this trend was negative for C16:0, C18:1n-9 and C20:5n-3 in both boys and girls (Figure 2). C22:5n-3 decreased in girls but not in boys. The corresponding percentiles based on GAMLSS are shown in Table 3. Only pooled percentiles are shown for C22:5n-3 in boys because no age-dependence was detected. Percentiles of the other FA, total saturated, unsaturated, polyunsaturated FA, n-6/n-3 ratio and important indices of enzyme activity stratified by sex and age as well as pooled for age groups are shown in Table 4. As most of those FA are not or only weakly correlated with age,

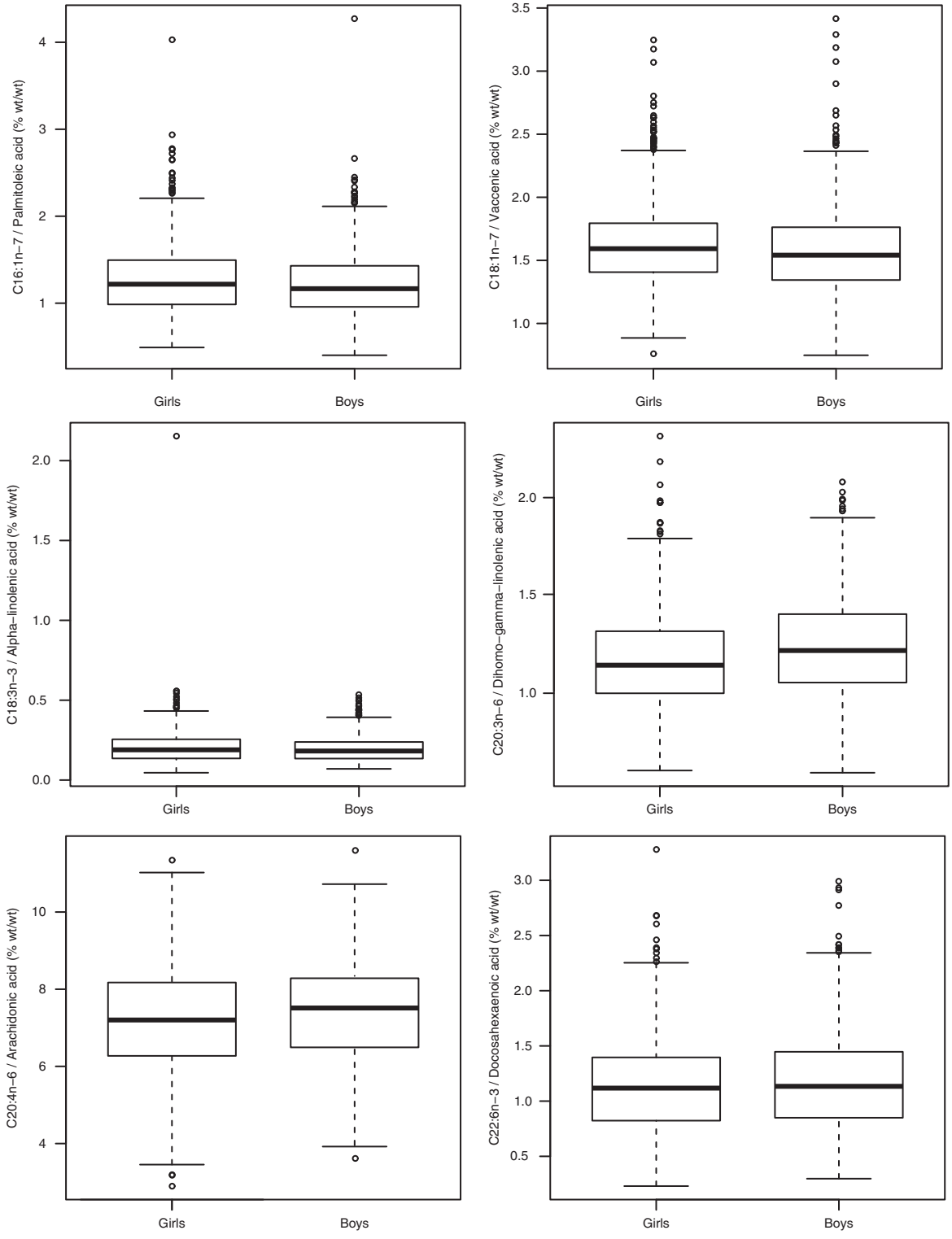

Figure 3. Proportion (% wt/wt) of selected FA pooled for 3–8-year-old girls and boys.

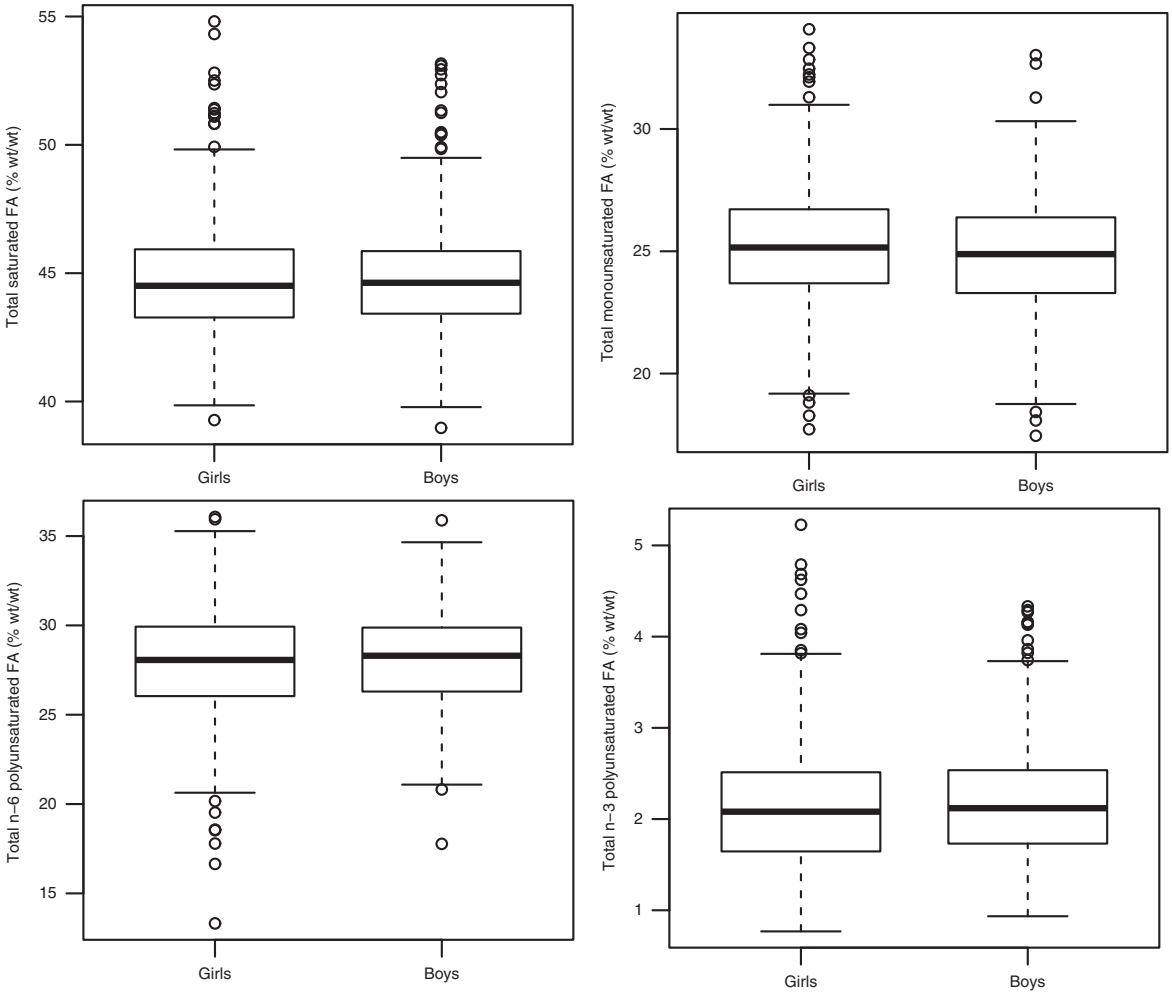

Figure 3. (Continued)

Figure 3 shows boxplots for the pooled percentage of selected FA in 3–8-year-old boys and girls.

Most FA levels were pretty similar in boys and girls but the median FA proportions of C20:3n-6, C20:4n-6 and C22:4n-6 seemed to be slightly higher in boys than in girls (Table 4 and Figure 3).

Sensitivity analysis regarding the influence of obesity according to common definitions from the World Health Organization,[36,37] Centers for Disease Control,[38] and Cole and Lobstein[31] showed only slightly deviating percentile curves when obese children were included (Supplementary Figure A). For the sake of completeness and to allow comparisons with other studies, percentiles of selected FA for the total study sample (including obese children and children with diseases) are shown in Supplementary Figure B and Supplementary Table A. They hardly differ from the percentiles in Figure 2 with the exception that the positive trend of C18:0 with age disappeared.

DISCUSSION

We have presented the whole-blood FA distributions for apparently healthy 3–8-year-old European girls and boys, and important indices as marker of enzyme activity that can be used as reference values in clinical and epidemiological studies. To our knowledge, the present study is the first to provide data on the FA composition of a large sample of European children.

Whole blood as the source of FA analysis

We used whole blood for FA analysis as that can easily be obtained from a drop of blood from the fingertip or venipuncture. A rapid and simple analysis method was used that allows FA measurement in large study populations and that was validated by several laboratories.[32,39,40] Conventionally, FA were measured in plasma or serum reflecting short-term fat intake or in red blood cells (RBC) reflecting long-term fat intake. Whole-blood FA derive from both, plasma (54% by volume) and circulating cells (46% by volume), the latter predominantely consisting of RBC.[41] As cell membranes are comprised mainly of phospholipids that are particularly rich in LC PUFA such as C20:5n-3 and C22:6n-3 whole blood contains higher LC PUFA and lower PUFA percentages than serum.[32] This is in line with data showing that the C20:5n-3 and C22:6n-3 content of the whole blood is highly correlated with that of RBC.[42] Whole blood includes FA from all lipid classes with phospholipids of plasma and RBC being the main contributors of the FA amount and profile. Therefore, whole blood provides a balanced picture of the FA status and is representative for the total FA pool.[39] Several studies confirmed that whole blood reflects the dietary FA intake and is a suitable marker for long-term essential FA intake. This is also true for n-3 FA that are reasonably

stable in fasting whole blood and can therefore be used to determine n-3 FA status.[4,32,43]

Montgomery *et al*.[44] also used whole blood samples for FA measurement in 493 school children aged 7–9 years from Oxfordshire, UK, and found similar percentages of n-6 FA but markedly higher n-3 FA than in our sample. Whole blood FA have also been measured in a nested case–ontrol study of the Physicians' Health Study showing an 72% and 81% lower risk of sudden death in men with n-3 LC PUFA levels in the upper third and fourth quartiles, respectively, compared with the lowest quartile.[45] The values of n-3 and n-6 PUFA reported are considerably higher than in our study. Different values compared with our study may be explainable by differences in the analytical method and the range of FA measured. In addition, in the latter study they can also result from the higher age of the participants and an additional extraction step in the analysis compared with our method.[45] Furthermore, the dietary intake may have been higher in both more homogenous study populations,[44,45] whereas our sample is pretty heterogeneous and represents the FA composition of children with various dietary habits from throughout Europe.

Age dependence of FA

In the subsample of normal weight children, only a few FA were (weakly) associated with age. The analysis using GAMLSS revealed a negative association of C18:1n-9 and a positive association of C18:2n-6 and C18:0 with age.

Accordingly, in a large German sample of 2- and 6-year-old boys and girls, no major age dependencies were observed in the FA composition of serum glycerophospholipids. In line with the trend across the age groups in our sample, C18:2n-6 was slightly higher and C18:1n-9 slightly lower in 6- compared with 2-year-old children.[29]

In the sample of 493 school children aged 7–9 years, children of the lowest age group (7 years) had higher C22:5n-3, C20:4n-6 and C18:2n-6 than older children.[44] Our data confirmed a decreasing trend of C22:5n-3 with age in girls but we found C18:2n-6 to increase with age and no age dependence for C20:4n-6.

Harris *et al*.[4] measured erythrocyte FA in 160 000 patients aged from 10 to 99 years and reported an increase of C22:6n-3 and C20:5n-3 and a decrease of C18:2n-6 over the first seven decades. Total saturated FA, total PUFA, C20:4n-6 and C18:3n-3 remained almost the same across all ages. A positive age trend of C18:2n-6 or a negative trend of C20:5n-3, as observed in our sample, was not reported by Harris *et al*., but their sample may not be comparable to ours because of older age groups and the selection of patients whose blood samples were analysed for diagnostic purposes.

In a Hungarian sample of 188 healthy subjects including newborns, infants, children and young adults C18:2n-6 was positively associated with age[28] confirming our results and those from others,[29] whereas C18:0, C20:4n-6 and C18:3n-3 were higher in children than in young adults.[28] In contrast to our findings, C16:0, C20:5n-3, C22:6n-3, n-3 PUFA and n-3 LC PUFA showed a positive trend with age, whereas C20:4n-6, n-6 PUFA and n-6 LC PUFA showed a negative trend.[28]

Recently, data of whole-blood FA composition of 2–9-year-old children of the Italian IDEFICS cohort were compared with neonates, adults and elderly from other Italian samples.[40] C22:6n-3 levels of the 2–9-year-old children were found to be much lower as compared with neonates, adults and elderly. The authors concluded that this resulted either from lower intakes or from the fact that the rates of utilisation and resulting physiological requirements are higher than in adults. C18:3n-3, C18:2n-6, n-6 PUFA and total PUFA were lowest in neonates and increased progressively in children and adults, whereas mono-unsaturated FA in children were significantly lower than in adults and neonates. A positive trend of C18:2n-6, n-6 PUFA and total PUFA with age is in line with our data but we observed an inverse association of monounsaturated FA with age.

Whereas the Italian IDEFICS subsample included a high proportion of overweight and obese children,[40] we constructed and applied weights to correct for the oversampling because studies showed an altered FA composition in obese children,[46] for example, C22:6n-3 levels being inversely associated with the body mass index.[16] However, our sensitivity analysis regarding the influence of obesity showed only minor differences in the percentile curves when obese children were included.

Sex differences of FA

In our sample, C20:3n-6, C20:4n-6 and C22:4n-6 seemed to be slightly higher in boys than in girls. In British school children, boys not only had higher mean C20:3n-6 and C20:4n-6 but also higher C22:5n-3 and C22:6n-3 than girls.[44] In a large German cohort—in line with our results—6-year-old boys had higher C20:3n-6 and C22:4n-6 than girls.[29]

In Italian samples, among the main n-6 and n-3 PUFA only C18:2n-6 was significantly different in all groups (neonates, children, adults and elderly) being higher in females than in males.[40]

Ratio and indices of FA percentages

We also provide the n-6/n-3 ratio and indices of enzyme activity that can be used as reference values for further studies. Ratios of n-6/n-3 or n-3/n-6 FA have been used in several studies to assess associations between the FA composition and health outcome or corresponding surrogate markers.[47–50] Enzyme activity indices estimated from product/precursor ratios for SCD-1 (SCD-16, SCD-18), the rate-limiting enzymes in the biosynthesis of C16:1n-7 and C18:1n-9, and for D6D and D5D, required in the production of eicosanoids from C18:2n-6 and C18:3n-3, are important factors of the blood FA composition besides dietary intake. Studies have shown that obesity and metabolic syndrome are associated with high SCD-1 and D6D indices, and low D5D index.[15,16,26] However, it is important to keep in mind that these activity indices were only estimated and not directly measured. Thus, our results need to be interpreted with caution because they reflect not only the influence of genetics including single-nucleotide polymorphisms and haplotypes but also the environmental and dietetic factors, such as the FA and carbohydrate intake, that can alter the FA composition and the desaturase activities.

Strengths and limitations

This is the first study that shows FA reference values for a large European sample of 3.0–8.9-year-old children. We have also reported the estimated enzyme activities that have not been provided before. However, as the dietary FA intakes were not analysed, we do not know whether the indicated reference ranges for PUFA correspond to dietary intakes that are in line with the recommendations.

CONCLUSION

Reported associations and causal relationship between the LC PUFA status and health-related outcomes increase the interest in FA determination. Therefore, the presented FA reference ranges of European children may be useful for the interpretation of the whole-blood FA composition. They provide a basis for comparison in epidemiological and clinical studies, and may also be used as an orientation for clinical practice in future. As the dietary intake does not meet the recommended daily amount for total PUFA and particularly not for n-3 LC PUFA in children and adolescents,[51] the presented reference values for PUFA do not allow conclusions with regard to health outcomes. Prospective studies relating FA

level to health outcomes are needed to determine target values of blood PUFA and particularly n-3 LC PUFA composition for primary and secondary prevention.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

The information in this document reflects the author's view and is provided as is.

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