Desaturase Activity Is Associated With Weight Status and Metabolic Risk Markers in Young Children

Maike Wolters, Heike Schlenz, Claudia Börnhorst, Patrizia Risé, Claudio Galli, Luis A. Moreno, Valeria Pala, Alfonso Siani, Toomas Veidebaum, Michael Tornaritis, Arno Fraterman, Stefaan de Henauw, Staffan Marild, Denes Molnar, and Wolfgang Ahrens on behalf of the IDEFICS consortium*

Context: Activity of delta-9, delta-6, and delta-5 desaturases (D9D, D6D, D5D) are associated with obesity, insulin resistance, and dyslipidemia.

Objective: To investigate the association of estimated desaturase activities with weight status, insulin resistance, and dyslipidemia in children, cross-sectionally and longitudinally.

Design: The IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants) cohort study was used, with examinations at baseline (T0) and after 2 years (T1).

Setting and Participants: Children aged 2 to less than 10 years from eight European countries were recruited in kindergartens/primary schools. Children with available data on fatty acids, outcome, and covariate information were included in the analyses.

Methods: Whole blood fatty acids were analyzed in 2600 children at baseline. D9D (16:1n-7/16:0), D6D (20:3n-6/18:2n-6), and D5D (20:4n-6/20:3n-6) activities were estimated from product-precursor fatty acids ratios. Body mass index (BMI), Homeostatic Model Assessment index, and high-density lipoprotein cholesterol (HDL), and triglycerides (TG) served as outcomes for weight status, insulin resistance, and dyslipidemia, respectively. Linear and logistic regression and repeated measures models were used to assess the cross-sectional and longitudinal associations between desaturase activity and outcomes.

Results: In the cross-sectional analysis, D9D and D6D were positively associated with BMI and TG z-scores and inversely with HDL z-scores. D5D was inversely associated with BMI and TG z-scores (ie, a D5D increase of 1 unit is associated with a BMI z-score decrease of 0.07 and a 28% lower odds ratio for TG $\geq$75th percentile). Longitudinally, similar associations were found for T0 desaturase activities with BMI and for T0 D6D with HDL at follow-up (T1). Baseline D6D and D5D were positively associated with the change of HDL z-score from T0 to T1, and D6D with the change of Homeostatic Model Assessment index z-score.

Conclusion: Desaturase activities are associated with metabolic risk markers already in young children and appear to predict the metabolic risk. **(*J Clin Endocrinol Metab* 100: 3760–3769, 2015)**

Associations between the fatty acid (FA) composition in blood or serum with childhood obesity (1, 2), insulin resistance (3), and dyslipidemia (4) have been reported in several studies. Besides dietary intake of FA, the activity of the desaturase enzymes involved in the conversion of saturated FA to monounsaturated FA, δ-9 desaturase (D9D; also, stearoyl-coenzyme A [CoA] desaturase), and of polyunsaturated FA (PUFA) to long-chain PUFA, δ-6 desaturase (D6D) and δ-5 desaturase (D5D), plays an important role in modulating the FA composition of body

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* Author affiliations are shown on the next page.

Abbreviations: BMI, body mass index; CI, confidence interval; CoA, coenzyme A; D5D, delta-5 desaturase; D6D, delta-6 desaturase; D9D, delta-9 desaturase; FA, fatty acid; HDL, high-density lipoprotein; HOMA, Homeostatic Model Assessment index; IDEFICS, Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants; ISCED, International Standard Classification of Education; OR, odds ratio; PUFA, polyunsaturated fatty acid; T0, baseline examination; T1, examination after 2 years; TG, triglyceride.

tissues. Although direct measurement of the enzyme activity is difficult, it can be estimated from the product-precursor ratio as confirmed by studies indicating that genetic variability in the stearoyl-CoA desaturase and FADS gene cluster affect the blood FA profile as well as estimated desaturase activity (5, 6). Studies in adults showed that the estimated activity of D9D and D6D is increased and of D5D is decreased in obese subjects (7) and in subjects with related metabolic disturbances such as insulin resistance (8) and dyslipidemia (9).

Particular interest has been on D9D that catalyzes the desaturation of 12–19 carbon saturated fatty acids to monounsaturated fatty acids (MUFA). D9D has been shown to be involved in obesity and insulin sensitivity (10–12). The enzyme plays a central role in the de novo lipogenesis with the genes of lipid synthesis being downregulated and those of lipid oxidation being upregulated when D9D is lacking. Thus, D9D-deficient mice were resistant to carbohydrate and fat-induced obesity and hepatic steatosis as well as to hypertriglyceridemia and insulin resistance (10, 11, 13). Therefore, low levels of D9D seem to protect against obesity, insulin resistance, and dyslipidemia, whereas high D9D expression is associated with these metabolic disturbances. The preferred D9D substrates are palmitoyl-CoA and stearoyl-CoA, leading to palmitoleoyl- and oleoyl-CoA, respectively. Several studies in adults confirmed the association between estimated D9D activity and body mass index (BMI) (7, 14), obesity (15), triglyceride (TG) (16, 17), high-density lipoprotein cholesterol (HDL) (17), incident diabetes (18), and cancer (19, 20).

D6D and D5D catalyze the rate-limiting steps in the conversion of the n-6 PUFA linoleic acid (18:2n-6) and the n-3 PUFA α-linolenic acid (18:3n-3) to their long-chain metabolites arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3). While D6D is positively associated with obesity and metabolic risk factors such as dyslipidemia and insulin resistance, D5D is negatively related (21, 22). The underlying mechanisms are still not well understood. The relation of D6D and D5D with dyslipidemia may result from variations in the D6D- and D5D-encoding FADS1 and FADS2 gene cluster that has shown to be strongly associated with serum HDL and TG (23–26). Elevated TG and low HDL in turn can lead to insulin resistance (27). Additionally, D6D and D5D influence the long-chain PUFA profile. Because long-chain PUFAs also act as ligands for transcription factors (eg, the peroxisome proliferators activating receptors), they may have an impact on the regulation of the expression of genes potentially linked to obesity, insulin resistance, and dyslipidemia (28). Furthermore, insulin receptor binding and affinity as well as translocation of glucose transporters are affected by the FA composition of cell membranes and have been suggested as another possible mechanism of how D6D and D5D can influence insulin resistance (29). Studies in adults showed that D5D was negatively and D6D was positively related to BMI (7, 14, 17), TG (17), and insulin resistance measured as Homeostatic Model Assessment index (HOMA) (14), whereas the association was reversed for HDL (17). Prospective cohort studies in adults also indicated an increased disease risk with elevated D6D activity for incident diabetes mellitus type 2 (29), total and cardiovascular mortality (30), and a reduced risk with elevated D5D activity for diabetes (29), coronary heart disease (31), and total and cardiovascular mortality (30).

Similar associations reported so far in adults on the role of D9D, D6D, and D5D in obesity and related metabolic disturbances have been confirmed in school children (1, 2, 22). However, data in younger children and longitudinal data from a large sample are missing. Therefore, this explorative study aims to investigate the association of estimated D9D, D6D, and D5D activity with markers of obesity (BMI z-scores), insulin resistance (HOMA z-scores as the surrogate marker for screening purposes (32)), and dyslipidemia (HDL z-scores and TGs) in children aged 2 to less than 10 years of the European Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infant (IDEFICS) cohort.

Materials and Methods

Subjects

In the IDEFICS baseline survey (T0), 16 228 girls and boys (50.8%) aged 2 to less than 10 years from eight European countries were examined (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain, Sweden) in 2007–2008. Of these children, 11 041 were reexamined during a second survey (T1) 2 years later. Because in IDEFICS, community- and setting-oriented intervention programs were implemented to promote the adoption

Leibniz Institute for Prevention Research and Epidemiology - BIPS (M.W., H.S., C.B., W.A.), Bremen, Germany; Department of Pharmacological and Biomolecular Sciences (P.R., C.G.), DiSFeB, University of Milan, Milan, Italy; Growth, Exercise, Nutrition and Development (GENUD) Research Group (L.A.M.), University of Zaragoza, Zaragoza, Spain; Epidemiology and Prevention Unit (V.P.), Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale Dei Tumori, Milan, Italy; Epidemiology and Population Genetics (A.S.), Institute of Food Sciences, National Research Council, Avellino, Italy; National Institute for Health Development (T.V.), Tallinn, Estonia; Research and Education Institute of Child Health (M.T.), Strovolos, Cyprus; Medizinisches Versorgungszentrum Dr. Eberhard und Partner Dortmund (A.F.), Laboratoriumsmedizin, Dortmund, Germany; Department of Public Health, Faculty of Medicine and Health Sciences (S.H.), Ghent University, Ghent, Belgium; Department of Public Health and Community Medicine (S.M.), Queen Silvia Children's Hospital, Gothenburg, Sweden; National Institute of Health Promotion (D.M.), University of Pécs, Pécs, Hungary; and Institute of Statistics (W.A.), Faculty of Mathematics and Computer Science, University of Bremen, Bremen, Germany

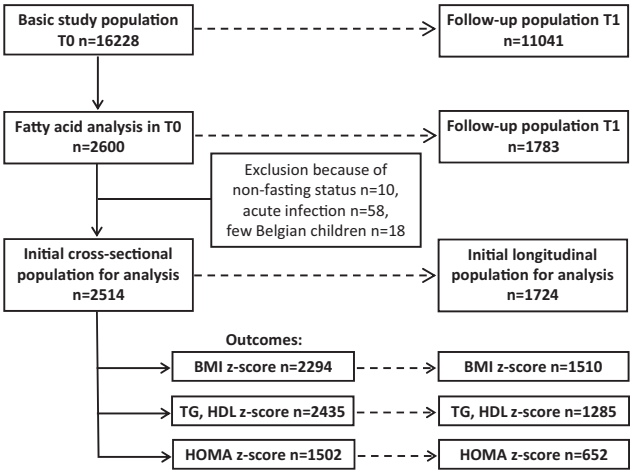

Figure 1. Flow chart of the inclusion and exclusion of IDEFICS participants. Only subjects with complete outcome and covariate data were included in the three analysis samples.

of a healthy obesity-preventing lifestyle to implement an intervention program, in each country, eight mutually comparable areas were allocated to either intervention or control status (33). The study and all procedures were approved by the ethics committees of the involved study centers. Written informed consent was provided by the parents of the participating children. The study design has been described in detail elsewhere (34). FA composition was analyzed in a subsample of the T0 IDEFICS participants with oversampling of overweight and obese children. Overall, 2600 FA profiles from T0 fasting blood samples of children aged 2 to less than 10 years were analyzed. In the final analyses, only children with information on the outcome measure under investigation, FA data, and complete covariate information for this analysis were considered. Reasons for exclusion of children were 1) non-fasting blood drawing, 2) acute infections, and 3) Belgian children as the sample consisted only of 18 children (Figure 1).

Anthropometric measurements, blood sampling, and analysis

Height of the children was measured to the nearest 0.1 cm with a calibrated stadiometer (Seca 225 stadiometer, Birmingham, UK). Body weight was measured in fasting state in light underwear on a calibrated scale accurate to 0.1 kg (Tanita BC 420 SMA, Tanita Europe GmbH, Sindelfingen, Germany). BMI was calculated as weight (kg) divided by height (m) squared. Fasting blood samples were obtained by collecting a drop of blood from a fingertip or by venipuncture. FAs in whole blood were separated and determined by a validated gas-liquid chromatography method after direct derivatization to their methyl esters without prior extraction of total lipids from the samples, as described previously (35–38). The estimated desaturase activities were determined from the product-precursor ratios as follows: D9D: 16:1n-7/16:0; D6D: 20:3n-6/18:2n-6; and D5D: 20:4n-6/20:3n-6. For statistical analysis, D9D and D6D were multiplied by 100 (ie, the scale was converted to receive meaningful effect estimates in later models).

A point-of-care analyzer was used to assess fasting blood glucose, HDL and TG in one drop of native capillary or venous blood on the spot using the Cholestech LDX analyzer (Cholestech, Hayward, CA). Insulin was determined by an electrochemiluminescence immunoassay (ECLIA, Roche Modular System, Mannheim, Germany) and HOMA was calculated as follows: HOMA = (serum insulin (μlU/ml) $\times$ fasting glucose (mmol/L)/22.5.

Medical history and lifestyle factors

The examination program included a personal interview on health conditions and on breastfeeding of the participating child. Questionnaires were used to collect parental information such as the educational level of both parents. The dietary intake of the previous 24 hours was assessed with a computer-assisted 24-hour dietary recall as described in detail elsewhere (39, 40).

Objective measurement of physical activity was conducted in a subset of the children using the uniaxial Actigraph accelerometer (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton Beach, FL) and the ActiTrainer (http://www.actitrainer.com), technology based on the Actigraph.

Outcome variables

Age- and sex-specific z-scores were used for all outcomes except TG to account for the age and sex dependencies of the biomarkers and anthropometric measures. The z-scores of HDL, TG, HOMA, and BMI were calculated using the recently published reference values for blood lipids (41) and HOMA (42) and using the BMI reference values from Cole and Lobstein (43). For TG, only sex-specific z-scores were used because no age dependence was found in a previous study (41). The detection limit for TG was 0.509 mmol/l. Because 55.9% of the boys and 46.8% of the girls of the reference population had TG values below or equal to the detection limit, the true percentile value of these children was unknown. For this reason, the value 55.9/2 = 27.95 was assigned to all boys and the percentile value of 46.8/2 = 23.40 to all girls of the reference population, with values falling below the detection limit as described previously (41). In our cross-sectional study population, 45.8% of children had TG values below or equal to the detection limit at T0; in the longitudinal population, this was true for 45.4% at T0 and 41.9% at T1. Hence, we decided to account for the resulting left-truncated distribution by using two categories built on TG values above versus below the 75th sex-specific percentile based on the recently published reference values (41).

Covariates

Potential confounders such as age (continuous), sex, country (seven categories), and maximum International Standard Classification of Education (ISCED) level of parents (aggregated to three categories) were considered. A binary variable indicating control versus intervention regions was used to adjust for potential differences resulting from the implemented intervention effects (33). Because energy intake is a determinant of BMI in children (40, 44), BMI z-score as an outcome was also adjusted for energy intake (kcal/d) measured based on a single 24-hour dietary recall. To control for misreporting variables indicating under- and overreports derived based on adapted Goldberg cutoffs (44) were used. HOMA, TG, and HDL z-scores as outcomes were adjusted for BMI z-scores (continuous).

Information on breastfeeding used in sensitivity analyses was classified in four categories, namely "not breastfed," "0–3 months," "3–6 months," and "more than 6 months." As an

indicator of physical activity in sensitivity analyses, we used time spent in moderate and vigorous physical activity determined from the accelerometer data.

Statistical methods

Linear regression and repeated measures models were used to assess the cross-sectional and longitudinal associations between desaturase activity and BMI, HOMA, and HDL.

In the cross-sectional analysis, the metabolic outcome (BMI z-score, HOMA z-score, or HDL z-score) at T0 was regressed on the exposure of interest (D9D, D6D, or D5D, respectively) at T0 adjusting for age, sex, country, and the maximum ISCED level of parents. Models for BMI z-score were additionally adjusted for energy intake and misreporting, whereas models for HOMA and HDL z-scores were additionally adjusted for BMI z-score.

For the longitudinal analysis, first, the change in the outcomes between T0 and T1 (Δ outcome = T1 outcome − T0 outcome) was regressed on the exposures (D9D, D5D, or D6D, respectively) at T0 including the same covariates as above and additionally including the baseline value of the outcome and a binary indicator for control versus intervention regions. This model assesses whether the desaturase activity in T0 is related to the change in the outcome variable between T0 and T1 independently of the value of the variable at T0.

In a second step, a repeated measures model (see, for example, Singer and Willett (45)) was run to assess the effects of the exposures at T0 on the outcomes at T0 versus T1; for example, whether the effects of the exposures at T0 on the outcomes differ between T0 and T1 (rate of change; reference category: T1). The general model was built as follows:

$$Outcome_{i,j} = \beta_0 + u_i + \beta_1 T0\_exposure_i + \beta_2 time_j + \beta_3 T0\_exposure_i \times time_{i,j} + \beta_{covars} T0\_covariates_i + \varepsilon_{i,j}$$

$Outcomes_{ij}$, denotes the outcome value of individual i measured at time j ($i = 1,\ldots N$ where N denotes the number of study subjects; $j = 0,1$ denotes the T0 and T1 assessment), β_0 denotes the intercept, u_i is a random subject-specific intercept, β_1 is the effect of the T0 exposure on the outcome measured in T1, β_2 gives the difference in the outcome between T0 and T1 (reference: T1), β_3 describes the difference in average effects of the exposure between T0 and T1 (reference: T1; rate of change), β_{covars} was used to indicate all effect estimates of the covariates included in the different models (eg, if age and sex were included as covariates, β_{covars} would indicate the two effect estimates β_{age} and β_{sex} for age and sex), and $\varepsilon_{i,j}$ is the error term for individual i at time j.

This model was run for all outcomes and exposures using SAS Proc Mixed. The same covariates as in the cross-sectional analysis were included. To account at least partially for multiple testing, we used a P value of .01.

Because of the left-truncated distribution of TG (because of the large amount of values below the detection limit), logistic regression models were used to assess the associations between desaturase activity and TG. A binary variable was built indicating TG values above versus below the 75th sex-specific percentile using recently published reference values (41). In the cross-sectional analysis, the T0 TG category was related to desaturase activities at T0 adjusting for age, sex, country, BMI z-score, and maximum ISCED level of parents at T0. In the longitudinal model, the T1 TG category was related to desaturase activities at T0 adjusting for T0 values of TG (sex-specific z-score), age, sex, country, BMI z-score, and maximum ISCED level of parents. Results are presented as odds ratios and 99% confidence intervals (99% CI).

All analyses were performed using SAS statistical software, version 9.3 (SAS Institute, Inc., Cary, NC).

Results

Study subjects

Table 1 shows the characteristics of the analysis groups at T0 and 2 years later at T1, including the mean z-scores for the outcome variables. The number of children in the present analyses vary because of differences in available exposure and outcome measurements and because of dropouts in the follow-up survey (Figure 1).

Compared to the total IDEFICS population, the children included in our study had higher BMI z-scores because of the oversampling of overweight and obese children in the subsample with FA analyses. Accordingly, the socioeconomic status of the parents of the included children is lower than in the IDEFICS population. This was expected because of the inverse association of overweight/obesity and the parental educational attainment shown in the IDEFICS population and in other studies (46).

Cross-sectional and longitudinal analyses

Table 2 shows the results of the cross-sectional analyses. Results of the longitudinal analyses are given in Table 3, showing the associations between BMI, HOMA, HDL and estimated desaturase activity at T0

1) with Δ outcome (ie, the change in the outcome variable between T0 and T1 adjusting for the baseline value) and
2) with the T1 outcome and the rate of change of the association between T0 and T1 (reference: T1) that describes whether the effect of the T0 desaturase activity on the outcome differs between T0 and T1 (results of the repeated measures models).

Weight status

In the cross-sectional analysis, positive associations were found between D9D and D6D with BMI z-score and a negative association for D5D (Table 2). This means for D9D that an increase of 1 unit (eg, from 0.05 to 0.06 because of the conversion of the scale) is associated with a BMI z-score increase of 0.23. For D5D, an increase of 1 unit is associated with a BMI z-score decrease of 0.07.

In the longitudinal analysis, none of the desaturases was found to be associated with the change in BMI z-score between T0 and T1 (Table 3). D9D was positively related

Table 1. Characteristics of the Cross-Sectional and Longitudinal Analysis Samples at Baseline and at Follow-Up

	Cross-Sectional Analysis Baseline			Longitudinal Analysis Follow-Up		
	BMI n = 2294 Mean (SD)	TG, HDL n = 2435 Mean (SD)	HOMA n = 1502 Mean (SD)	BMI n = 1510 Mean (SD)	TG, HDL n = 1285 Mean (SD)	HOMA n = 652 Mean (SD)
Age (years)	6.2 (1.7)	6.2 (1.8)	6.4 (1.7)	8.2 (1.8)	8.2 (1.7)	8.5 (1.6)
Age (minimum–maximum), years	2.1–9.7	2.0–9.7	3.0–9.7	3.9–11.7	3.9–10.9	4.9–10.9
Male sex, n (%)	1157 (50.4)	1233 (50.6)	748 (49.8)	759 (50.3)	654 (50.9)	326 (50)
ISCED, n (%)[a]						
Levels 0, 1, and 2	387 (16.9)	397 (16.3)	246 (16.4)	249 (16.5)	206 (16.0)	98 (15.0)
Levels 3 and 4	1300 (56.7)	1395 (57.3)	864 (57.5)	847 (56.1)	728 (56.7)	364 (55.8)
Levels 5 and 6	607 (26.5)	643 (26.4)	392 (26.1)	414 (27.4)	351 (27.3)	190 (29.1)
BMI categories, T0, n (%)						
Thin	146 (6.4)	153 (6.3)	96 (6.4)	102 (6.8)	84 (6.5)	41 (6.3)
Normal	1148 (50.0)	1217 (50.0)	775 (51.6)	763 (50.5)	643 (50.0)	330 (50.6)
Overweight	536 (23.4)	560 (23.0)	340 (22.6)	344 (22.8)	293 (22.8)	148 (22.7)
Obese	464 (20.2)	505 (20.7)	291 (19.4)	301 (19.9)	265 (20.7)	133 (20.4)
BMI categories, T1, n (%)						
Thin				94 (6.2)	81 (6.3)	44 (6.7)
Normal				715 (47.4)	600 (46.7)	308 (47.2)
Overweight				384 (25.4)	327 (25.4)	167 (25.6)
Obese				317 (21.0)	277 (21.6)	133 (20.4)
BMI, kg/m^2, T0	17.95 (3.30)	17.97 (3.30)	17.83 (3.30)	17.89 (3.30)	17.96 (3.30)	17.92 (3.30)
BMI z-score, T0	0.99 (1.34)	1.00 (1.34)	0.93 (1.34)	0.96 (1.33)	1.01 (1.33)	0.97 (1.32)
BMI, kg/m^2, T1				19.05 (4.00)	19.08 (4.00)	19.10 (3.97)
BMI z-score, T1				1.06 (1.28)	1.07 (1.28)	1.04 (1.26)
HOMA, T0			1.17 (1.05)			1.11 (1.09)
HOMA z-score, T0			0.39 (1.12)			0.29 (1.11)
HOMA, T1						1.76 (1.41)
HOMA z-score, T1						0.59 (1.16)
HDL, mg/dl, T0		51.5 (13.8)			51.7 (13.6)	
mmol/liter		1.33 (0.36)			1.34 (0.35)	
HDL z-score, T0		−0.05 (0.99)			−0.04 (0.98)	
HDL, mg/dl, T1					52.4 (13.0)	
mmol/liter					1.36 (0.34)	
HDL z-score, T1					−0.17 (0.96)	
TG, mg/dl, T0[b,c]		48.0 (69.0–118.0)			48.0 (67.0–115.0)	
mmol/liter		0.54 (0.78–1.33)			0.54 (0.76–1.30)	
TG z-score, T0[b,c]		0.20 (0.86–1.70)			0.20 (0.81–1.37)	
TG, mg/dl, T1[b,d]					51.0 (74.0–128.0)	
mmol/liter					0.58 (0.84–1.45)	
TG z-score, T1[b,d]					0.29 (0.95–1.87)	
Estimated desaturase activity, T0						
D9D	0.050 (0.016)	0.050 (0.016)	0.051 (0.016)	0.050 (0.016)	0.050 (0.016)	0.050 (0.016)
D6D	0.069 (0.018)	0.069 (0.017)	0.068 (0.017)	0.070 (0.018)	0.070 (0.018)	0.069 (0.017)
D5D	6.204 (1.362)	6.218 (1.364)	6.208 (1.351)	6.212 (1.343)	6.238 (1.340)	6.223 (1.329)

SD, standard deviation.

[a] Maximum of both parents.

[b] Data presented as median (75th–95th percentile) because of left-truncated distribution.

[c] Children with TG values below or equal to the detection limit: cross-sectional population 45.8% at T0, longitudinal population 45.5% at T0.

[d] Children with TG values below or equal to the detection limit: 41.9% at T1.

to the BMI z-score in T1 ($\beta = 0.19$, $P < .001$), ie, an increase of 1 unit is related to a BMI z-score increase of 0.19 2 years later. However, the relation was significantly smaller compared to the relation with the BMI z-score at T0 (rate of change: 0.06, $P < .0001$; ie, the effect on the T0 outcome was 0.06 units higher compared to the T1 outcome). D6D was also positively and D5D negatively associated with T1 BMI z-score where the strength of the effects did not significantly differ from the effect on T0 BMI z-score (nonsignificant rates of change).

Insulin resistance

In the cross-sectional analysis, no significant associations with HOMA were seen. The longitudinal analysis revealed a positive association of D6D with the change in HOMA z-score between T0 and T1 ($\beta = 0.07$, $P = .007$) (ie, 1-unit D6D increase was associated with an increase of 0.07of Δ HOMA).

Dyslipidemia

D9D and D6D were inversely related to the HDL z-score in our cross-sectional analysis. D6D and D5D were

Table 2. Cross-Sectional Association of Desaturase Activities With Weight Status and Metabolic Outcomes

	Effect on T0 Outcome[a] β	*P* Value
Outcome: BMI z-score (n = 2294)		
D9D[b]	0.23	<.0001
D6D[b]	0.12	<.0001
D5D	−0.07	.000
Outcome: HOMA z-score (n = 1502)		
D9D[b]	0.02	.231
D6D[b]	−0.02	.269
D5D	−0.03	.096
Outcome: HDL z-score (n = 2435)		
D9D[b]	−0.07	<.0001
D6D[b]	−0.06	<.0001
D5D	−0.01	.606
Outcome: TG T1 ≥ P75 (Ref < P75) (n = 2435)	**OR**	**99% CI**
D9D[b]	1.61	1.47–1.76
D6D[b]	1.11	1.02–1.19
D5D	0.72	0.65–0.79

[a] BMI z-score, HOMA z-score, TG category, and HDL z-score were regressed on the T0 exposure of interest (D9D, D6D, or D5D, respectively) adjusting for age, sex, country, and maximum ISCED level of parents. Models for BMI z-score were additionally adjusted for energy intake and misreporting; models for HOMA, TG, and HDL z-scores were additionally adjusted for BMI z-score.

[b] For statistical analysis, D9D and D6D were multiplied by 100 (ie, the scale was converted to receive meaningful effect estimates in the models).

positively related to the change in HDL between T0 and T1, whereas D6D showed a negative association with the T1 HDL z-scores.

Because the two categories of TG values above versus below the 75th sex-specific percentile (P75) were based on reference values, proportions of children assigned to these groups differ in our study population. In our cross-sectional study population, 1676 (68.8%) had TG values below and 759 (31.2%) had values equal to or greater than P75, in the longitudinal population 828 (64.4%) had TG values below and 457 (35.6%) had values equal to or greater than P75. D9D and D6D were positively and D5D was negatively associated with TG in our cross-sectional analysis. An increase of 1 unit of D9D or D6D (eg, from 0.05 to 0.06 because of the conversion of the scale) was associated with an odds ratio ([99% CI]) of 1.61 [1.47–1.76] or 1.11 [1.02–1.19]), indicating a 61% or 11% higher risk of TG equal to or greater than P75. In contrast, a D5D increase of 1 unit was associated with a 28% lower odds ratio.

Birth weight, breastfeeding, and physical activity may also have influenced the outcomes, but were not included in the main models to avoid a further reduction of the analyses samples. Results remained almost unchanged in a sensitivity analysis of the subgroups when all models were run again adjusting for birth weight, objectively measured physical activity, and breastfeeding (data not shown).

Discussion

This study analyzed the cross-sectional and longitudinal associations of estimated desaturase activities with weight status, insulin resistance, and blood lipids in a large sample of 2- to younger than 10-year-old children of the IDEFICS cohort. To our knowledge, this is the first study that investigated the desaturase activity in relation to metabolic outcomes in a large multinational cohort of young children.

Desaturase activity and weight status

In accordance with the data from studies in adults (7, 14, 15, 17), our cross-sectional analysis showed a positive association of D9D with the BMI z-score as also reported from two studies in Japanese school children (aged approximately 12 years) using FA from plasma phospholipids or total plasma lipids (1, 2). To our knowledge, only one other study investigated longitudinal relations of D9D with the weight status in children: Abe et al. (21) compared the changes of the weight status with changes in D9D between baseline and a second measurement 3 years later and found no association. Because high D9D activity seems to play a crucial role in obesity development, a positive association of D9D with the T0 and T1 BMI was expected and confirmed in our study.

As expected, our cross-sectional analysis showed a positive association of D6D and a negative association of D5D, with the BMI z-score confirming results from Japanese studies in school children in which such associations were also found for weight status (21) and waist-to-height ratio (21, 22). Also, in 264 adolescents (mean age 15 years), D6D was positively associated with obesity (47). D6D and D5D at T0 were not related to the change in the BMI from T0 to T1 in our study, whereas in Japanese school children, increases in relative weight between baseline and 3 years later were associated with increases in D6D and decreases in D5D in boys (21).

Desaturase activity and insulin resistance

In line with our results, no relation of D9D activity with HOMA was observed in 112 Japanese school children (2), whereas in 32 obese Japanese children, D9D was correlated with fasting glucose and fasting insulin (1). Also in a study with 437 Japanese adults, D9D was

Table 3. Longitudinal Association of Desaturase Activities With Weight Status and Metabolic Outcomes

	Linear Regression Effect on Δ Outcome[a]		Repeated Measures Model: Effect on T1 Outcome[b]		Repeated Measures Model: Rate of Change[c] (Ref.: T1)	
	β	*P* Value	*β*	*P* Value	*β*	*P* Value
Outcome: BMI z-score (n = 1510)						
D9D[d]	−0.93	.399	0.19	<.0001	0.06	<.0001
D6D[d]	0.01	.210	0.12	<.0001	−0.01	.177
D5D	0.00	.904	−0.06	.005	−0.02	.132
Outcome: HOMA z-score (n = 652)						
D9D[d]	0.02	.488	0.02	.534	0.00	.912
D6D[d]	0.07	.007	0.06	.021	−0.08	.004
D5D	−0.02	.568	−0.02	.491	0.00	.925
Outcome: HDL z-score (n = 1285)						
D9D[d]	0.00	.994	−0.04	.025	0.00	.854
D6D[d]	0.01	<.0001	−0.07	<.0001	0.02	.133
D5D	0.04	.008	0.04	.030	−0.06	.002
			OR		**99% CI**	
Logistic regression						
Outcome: TG T1 ≥ P75 (Ref < P75) (n = 1285)						
D9D[d]			1.01		0.89–1.15	
D6D[d]			1.04		0.93–1.17	
D5D			0.90		0.79–1.03	

[a] Δ outcome: change in the outcome variable between T0 (baseline examination) and T1 calculated subtracting the T0 value from the T1 value (eg, Δ BMI z-score = T1 BMI z-score − T0 BMI z-score). Δ outcome was regressed on the T0 exposures (D9, D6, or D5, respectively) at T0 adjusting for age, sex, country, and maximum ISCED level of parents, including a binary variable for control versus intervention regions. Models for BMI z-score were additionally adjusted for energy intake and misreporting; models for TG category, HOMA and HDL z-scores were additionally adjusted for BMI z-score.

[b] The change in the outcomes between T0 and T1 (Δ outcome = T1 outcome − T0 outcome) was regressed on the T0 exposures (D9D, D6D, or D5D, respectively) including the same covariates as in the first footnote and additionally the baseline value of the outcome. This model assesses whether the desaturase activity in T0 is related to the change in the outcome between T0 and T1 independently of the T0 outcome level.

[c] The rate of change of the effect between T0 and T1 describes whether the effect of the T0 exposure on the outcome differs between T0 and T1. A significant *P* value for the rate of change indicates that the effect of the T0 exposure on the outcome was significantly larger/smaller (positive/negative; significant *P* value) in T0 compared to T1 (reference category).

[d] For statistical analysis, D9D and D6D were multiplied by 100 (ie, the scale was converted to receive meaningful effect estimates in the models).

associated with insulin resistance measured as C-peptide concentrations (8).

Somewhat surprisingly, our cross-sectional analysis showed no association between D6D and HOMA, whereas a positive association of D6D with the change in HOMA z-score between T0 and T1 was observed. In 237 Japanese school children, D6D was positively associated, whereas D5D was negatively associated with insulin and HOMA in a cross-sectional analysis (22), confirming similar results in Japanese adults (8). Another Japanese study found a positive correlation between D6D with HOMA and, after 3 years, also between D6D changes and HOMA changes in school girls, but not in boys (21). Our longitudinal analysis indicated D6D to be positively associated with a change in insulin resistance. Thus, D6D may be related to the diabetes risk as concluded by a recent review (29).

Studies (48, 49) indicate that insulin resistance and diabetes mellitus type 2 result from an interplay between genetic and environmental factors. These factors may differ between geographic regions. Therefore, we ran additional stratified analyses to check whether the association between desaturase activity and HOMA z-scores differs between children from northern, eastern, and southern Europe. Results of cross-sectional analysis remained almost unchanged. The longitudinal effect of D6D on the change in HOMA could only be confirmed in children from southern Europe (n = 351), whereas no significant association was seen in children from northern (n = 161) or eastern European countries (n = 140), which may at least partly result from the smaller study samples (data not shown). Further research is needed to investigate potential differences in determinants of insulin resistance between European regions.

Desaturase activity and dyslipidemia

As expected, D9D and D6D were inversely related to the T0 HDL z-score and D6D to the T1 HDL z-score in our study. A cross-sectional relation with D6D was also observed in Japanese school children (22) and in US adolescents (47). In the latter study, agreeing with our results, no

association of D5D with HDL z-score was observed, whereas two Japanese studies found a positive association in boys (22) or in all children (1). Longitudinally, we found a positive association of D5D with the change between T0 and T1 HDL z-score, although no cross-sectional relation and no relation with T1 was seen. All desaturase activities were associated with TG, cross-sectionally indicating an increased risk for elevated TG with higher D9D and D6D and a lower risk with higher D5D. Also in 32 obese Japanese children, D9D was positively and D5D was negatively correlated with serum TG (1). Our results confirm other studies in Japanese school children (22) and in adolescents (47), where D6D was positively associated, whereas D5D was inversely associated with TG independent of the BMI.

Interconnection between estimated desaturase activities and metabolic outcomes

To a large extent, our results confirm cross-sectionally the positive association of D9D and D6D and the inverse association of D5D with the metabolic risk profile already in young children and show longitudinal associations of desaturase activity with some of the metabolic risk markers. We investigated the role of desaturase activities as exposures because they have been shown to be important determinants of metabolic risk. However, desaturase activities are interconnected with metabolic risk markers, particularly with weight status and insulin resistance, and they influence each other. Body fatness is a determinant of FA composition of blood and several studies investigated the association of metabolic outcomes and desaturase activities analyzing overweight and metabolic outcomes as potential predictors for desaturase activities (1, 2, 21). Therefore, cross-sectional results need to be interpreted carefully because reverse causation cannot be precluded. On the other hand, studies on the genetic variation in the FADS1 and FADS2 genes suggest that D6D and D5D are involved in diabetes genesis (29) and are associated with serum HDL (23, 25). This is in agreement with our longitudinal data indicating that baseline D6D may play a role for the change in insulin resistance from T0 to T1 and for HDL at T1. Accordingly, data from large cohort studies in adults indicate effects of desaturase activity on type 2 diabetes (29) and cardiovascular and total mortality (30).

Limitations and strengths

A limitation of our study is that the enzyme activities were only estimated from the product-precursor ratios that are commonly used as surrogates because direct measurement is hardly possible for ethical reasons. Thus, our results need to be interpreted with caution because they reflect not only the influence of genetics, but also environmental and dietetic factors that can alter the FA composition and therefore also the estimated desaturase activities.

Another limitation is that FA concentrations were not measured after 2 years. Therefore, desaturase activities could not be estimated for T1 and longitudinal relations of changes in desaturase activity to changes in weight status, insulin resistance, and dyslipidemia could not be analyzed.

We were not able to adjust for all covariates because certain data (eg, on genetics and single dietary FA) were not available or respective data included too many missing values (eg, income and profession as part of the socioeconomic status). However, an advantage is that we controlled for the effects of birth weight, breastfeeding, and objectively measured physical activity in sensitivity analyses.

The longitudinal analyses are important strengths of our study. Most previous studies in children applied single regression models to analyze cross-sectional (1, 2, 21) and longitudinal associations (21) of desaturase activities and metabolic outcomes. We additionally applied repeated measures modeling in our study to explicitly consider correlations between the repeated measurements in the same subject and to gain knowledge on the rates of change of the association of desaturase activity with metabolic outcomes over time. Additionally, all models applied were adjusted for several important covariates.

Other strengths of our study include the large sample of young children from several European countries with standardized assessments of exposure and outcome measures as well as covariates but also the application of previously lacking reference data for the calculation of the z-scores for blood lipids and HOMA.

We used whole blood for FA analysis that can easily be obtained from a drop of blood from the fingertip. It includes FA from all lipid classes and is representative for the total FA pool (37). A rapid and simple analysis method was used that allows FA measurement in large study populations and that was validated by several laboratories (36–38). Because most other studies discussed here used plasma for FA analysis, which differs in composition from whole blood (36), study results regarding associations of desaturase activities and outcomes may also be influenced by the source of analyzed samples, in particular, 16:1 seems to be higher in plasma than in whole blood (37).

We decided to use only the 16:1/16:0 but not the 18:1/18:0 ratio for D9D activity because circulating TG in whole blood contains almost 70% of 18:1 (37). Therefore, increments of TG result in an elevation of 18:1/18:0, which is in this context not related to a change in the enzyme activity. Further, the 18:1/18:0 ratio is less suit-

able for the estimation of desaturase activity because dietary intake of 18:1 is usually high compared to 16:1 and may mask the association of desaturase activity with weight status and metabolic risk factors (51).

Conclusion

Desaturase activities are associated with metabolic risk markers already in young children and appear to predict the metabolic risk at an early stage. Hence, desaturase activities may help to identify subjects for whom preventive measures are required. In this context, the recently defined reference values for the estimated desaturase activity in children provide a basis for evaluation (35).

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Address all correspondence and requests for reprints to: Maike Wolters, Leibniz Institute for Prevention Research and Epidemiology - BIPS, Department Epidemiological Methods and Etiological Research, Achterstr. 30, 28359 Bremen, Germany. E-mail: wolters@bips.uni-bremen.de.

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