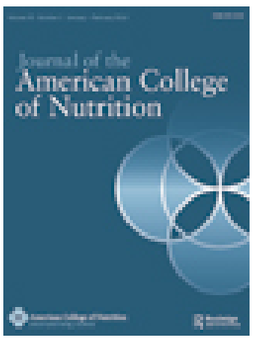

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Associations Among Fatty Acids, Desaturase and Elongase, and Insulin Resistance in Children

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ABSTRACT

Objectives: Fatty acid profiles and desaturase (SCD-16, SCD018, D5D, D6D) and elongase (ELOVL6) enzyme activity have been associated with adiposity and metabolic disease. While this has been studied in adults, few studies have included children. The objective of this study was to evaluate these markers in children and identify relationships with markers of metabolic health. It was hypothesized that these lipid markers would be correlated to adiposity and metabolic disease.

Methods: This study was a cross-sectional analysis of fourth- and fifth-grade children (n = 86, aged 9–12) participating in a comprehensive nutrition program. Any student enrolled in the program was eligible for inclusion in this study. Fasting plasma was collected and analyzed for total fatty acids, glucose, insulin, and full lipid panels. Insulin resistance was estimated using calculated homeostatic model assessment for insulin resistance (HOMA-IR) values.

Results: There were no differences in lipid markers, glucose, insulin, or HOMA-IR among children classified as normal weight, overweight, or obese. SCD-16, D5D, and ELOVL6 activity was significantly correlated to HOMA-IR values ($r = 0.39$, $p = 0.001$; $r = -0.33$, $p = 0.006$; $r = -0.37$, $p = 0.005$, respectively). In regression analysis, body mass index for age percentile, D6D activity, ELOVL6 activity, and systolic blood pressure were the most significant predictors of HOMA-IR values (adjusted $r^2 = 0.39$, $p \leq 0.001$).

Conclusions: There was no relationship between these lipid markers and adiposity in this population; however, there were correlations with HOMA-IR. Regardless of adiposity, there may be underlying changes in fatty acid and lipid metabolism associated with the development of metabolic diseases.

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Childhood obesity; desaturase activity; elongase activity; insulin resistance; lipid biomarker

Introduction

Despite an increasing effort to combat childhood obesity, obesity prevalence among U.S. youth aged 2 to 11 years remains high at 17 %[1]. Excess adiposity early in life is especially alarming because longitudinal studies have demonstrated that childhood overweight and obesity can be predictive of metabolic disease development later in life [2,3]. Common biomarkers used to determine metabolic risk include anthropometrics, blood cholesterol, triglycerides (TGs), blood pressure, and markers of impaired glucose tolerance [4,5]. However, such biomarkers do not reflect acute health status and are more reflective of long-term behaviors. Following these measurements over time is useful but can miss the mark when attempting to reduce risk of chronic disease development in children. Thus, gaining insight into subclinical changes occurring before the onset or during the early progression of risk factors will be beneficial in identifying those who are most at risk.

Concentrations of specific fatty acids (FAs) and desaturase and elongase enzyme activities have been used to characterize the role of lipids in obesity-related diseases such as type 2 diabetes (T2D), dyslipidemia, and metabolic syndrome. Plasma FA composition reflects both dietary fat intake over several weeks [6,7] and endogenous FA metabolism [8,9]. Studies demonstrate high relative percentages of palmitic, palmitoleic, and dihomo-γ-linolenic acid and low percentages of linoleic acid and docosahexaenoic acid in the plasma and serum of children with insulin resistance and increased adiposity [10–13]. Many of these studies have investigated FA composition in older age groups (11–15 years), with few studies in younger children.

Desaturase enzymes modulate FA composition in the body by inserting double bonds into specific positions of the saturated FA chain, and elongase enzymes catalyze the elongation of saturated and monounsaturated FAs (MUFAs) to form long-chain FAs. Stearoyl-CoA desaturase (SCD; also known as Δ9 Desaturase) is the rate-limiting enzyme in the biosynthesis of MUFAs, whereas Δ5 Desaturase (D5D) and Δ6 Desaturase (D6D) catalyze the conversion of polyunsaturated FAs (PUFAs) to long-chain PUFAs. Elongase of long-chain FAs' family member 6 (ELOVL6) is responsible for the endogenous conversion of FAs with 12, 14, and 16 carbons into longer-chain FAs. Because measuring enzyme activity is difficult in humans, activity estimates using product-to-precursor ratios

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have been developed (e.g., SCD-16 = 16:1n7/16:0; SCD-18 = 18:1n9/18:0; D6D = 20:3n6/18:2n6; D5D = 20:4n6/20:3n6; ELOVL6 = 18:0/16:0) [14,15].

Many studies have reported that excess adiposity, particularly in the abdominal region, is associated with increased D6D and SCD activity [12,16–19]. There are some reports that the relationship between adiposity and SCD is only observed in overweight or obese, but not normal-weight, children [10,18]. In general, D6D and SCD are positively associated with increased fasting insulin, TG, and insulin resistance measured using the homeostatic model assessment for insulin resistance (HOMA-IR), and D5D is often negatively associated with these measurements [12,16–19]. However, the mechanisms underlying this association are not well understood and the relationships between the desaturase activities and these variables are not consistent across studies. In mouse studies, targeted disruption of the ELOVL6 genes rendered mice resistant to diet-induced insulin resistance [20,21]. However, a study in adult humans reported that deletion of ELOVL6 does not prevent the development of insulin resistance [15]. Further, a study of Japanese children (aged 11–12 years, n = 112) by Okada et al. observed no significant correlation between elongase and fasting insulin or HOMA-IR [13].

More studies evaluating children may help clarify these relationships and provide more insight into the detection and prevention of these health problems early in life. To further elucidate these associations, we investigated the cross-sectional relationships between plasma FA composition, desaturase and elongase enzyme activities, anthropometric measures, features of dyslipidemia, and insulin resistance in fourth- and fifth-grade children (9–12 years). It was hypothesized that FA composition and desaturase and elongase enzyme activity were associated with obesity and markers of metabolic disease.

Materials and methods

Participants

Participants were recruited from fourth- and fifth-grade classrooms in one Northern California elementary school participating in the Shaping Healthy Choices Program (SHCP) [22]. Children volunteered to participate in the blood draw and provided oral assent and parents provided written informed consent. Participants were provided a gift card as an incentive after the blood draw. The University of California, Davis Institutional Review Board approved the study protocol.

Measurement of anthropometrics and dietary data

Height and weight were measured at the school site. The methods followed guidelines published by the Centers for Disease Control and Prevention (CDC). Height was measured using a transportable stadiometer (Seca 213, Chino, CA, USA) and body weight was measured using an electronic scale (Seca 803b, Chino, CA, USA). Body mass index (BMI; kg/m^2) was calculated and age- and sex-specific BMI percentiles were derived. Classifications for adiposity were based on those determined by the CDC and the Expert Committee on Childhood and Adolescent Overweight and Obesity using the 2000 CDC growth charts [23–25]. Under these classifications, overweight was defined as at or above the 85th percentile and obese was defined as at or above the 95th percentile of the sex-specific BMI-for-age growth chart [25]. Dietary intake was self-reported by students with the help of their parents at home using the 2004 Block Food Frequency Questionnaire (FFQ) for ages 8 through 17. Students were provided detailed classroom instruction on completing the FFQ and were given standardized bowls and a plate consistent with the diagram provided with the FFQ to use as a reference [22]. Completed FFQs were analyzed by NutritionQuest (Berkeley, CA, USA).

Measurement of plasma FA composition and estimation of desaturase enzyme activities

After participants fasted overnight, blood samples were collected by registered nurses at a health fair or health screening event at the school site organized by the SHCP researchers. Plasma total FAs were quantified as fatty acid methyl esters (FAMEs) by gas chromatography with mass spectrometry as previously described [26,27]. Briefly, plasma aliquots (50 μL) were enriched with 5 μL 0.2 mg/mL butylated hydroxytoluene/EDTA in 1:1 methanol:water and extraction surrogates, including deuterated-tri-palmitoyl glycerol (CDN Isotopes, Pointe-Claire, Quebec, Canada), deuterated distearoylphosphotidylcholine (Avanti Polar Lipids, Alabaster, AL, USA), dodeca-(9E)-enoyl cholesterylesters (NuChek Prep, Elysian, MN, USA), and dodecatrienoic acid (NuChek Prep). Lipids were extracted twice with cyclohexane:2-propanol:ammonium acetate (10:8:11) and combined organic extracts were dried under vacuum and reconstituted in 1:1 methanol:toluene. Extract sub-aliquots were diluted to 4.5:1 methanol:toluene, enriched with 15:1n5 free FA surrogate to track methylation efficiency, and incubated with 0.5 M anhydrous sodium methoxide at (1 hour at 60°C), followed by 0.5 M methanolic HCl (30 minutes at 60°C) to form FAMEs. Solutions were then neutralized with potassium carbonate, diluted with saturated saline, and extracted with hexane. Hexane aliquots were enriched tricosanoate methyl ester internal standard (23:0; NuChek Prep) and analyzed by GC-MS (Agilent 6890+ GC/5973 N MSD, Agilent Technologies, San Jose, CA, USA), with electron impact ionization and in simultaneous selected ion monitoring/full scan mode. Residues were separated by temperature and flow ramps on a 30 mx 0.25 mm id, 0.25 μm DB-225 ms column (Agilent Technologies) and quantified with ChemStation vE.02.02.1421 (Agilent Technologies) using internal standard methodologies against 5- to 8-point calibration curves.

Measurement of biomarkers

Blood samples were sent to the University of California, Davis Medical Center for analysis of plasma glucose and total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol with a UniCel DxC 800 hemotology analyzer (Beckman Coulter, West Sacramento, CA, USA) and insulin with Chemiluminescent Microparticle ImmunoAssay on Abbott Architect i1000 System (Abbott Laboratories, Chicago, IL, USA). HOMA-IR was calculated with the equation (fasting glucose [mg/dL] / fasting insulin [μU/mL]) / 405.

Statistical methods

Means and standard deviations were calculated for continuous variables and distributions were examined for normality. Variables that did not follow a nearly normal distribution were log-transformed. Analysis of variance (ANOVA) was used to determine differences in anthropometric measures, dietary intake of fat and FAs, blood biomarkers, plasma FA concentration, and desaturase activity among normal-weight, overweight, and obese participants. Student's *t*-tests were used to determine differences between sexes. ANOVA was also used to determine differences in the aforementioned variables among quartiles of HOMA-IR values. A Bonferroni correction for multiple comparisons was used to compare differences when the global F test was significant ($p < 0.05$). Pearson's correlation coefficients were used to determine the relationship among desaturase activities and all anthropometric and biomarker measurements and dietary FAs. A multivariate linear regression analysis with blood biomarkers, FA concentration, dietary intake, and desaturase activities was conducted to determine potential predictors of HOMA-IR value. Variance inflation factors were examined to determine collinearity between variables, and those with a variance inflation factor value >10 were omitted from the model. Akaike information criterion was used to determine the model with the best fit. Stata 13 (StataCorp, College Station, TX, USA) was used for all statistical analyses. Statistical significance was determined using $\alpha = 0.05$.

Results

Of the 249 students enrolled in the overall SHCP study who were eligible to participate in this study, 86 (35%) consented. Of these 86 students, 42 were classified as normal weight, 26 were overweight, and 18 were considered obese. There were significant differences in BMI among all groups, while the BMI-for-age percentile was only different between the normal-weight group compared to overweight and obese groups (Table 1). There were significantly more females in the normal-weight category compared to overweight and obese; however, there were no differences between the number of males in each group (Table 1). There were no differences in age, blood pressure, cholesterol, TG, or glucose measurements; however, those in the obese group had greater insulin and HOMA-IR values compared to the normal-weight group (Table 1). No differences were observed in reported dietary intake of total fat, saturated

Table 1. Anthropometric and cardiometabolic risk factors and total fatty acid concentration of normal weight, overweight, and obese participants.

Variable	Normal Weight (n = 42)	Overweight (n = 26)	Obese (n = 18)
		Mean ± SD	
Age (years)	10.3 ± 0.7	10.7 ± 0.7	10.5 ± 0.7
Sex (n [%])			
Male	10 (24%)	13 (50%)	11 (61%)
Female	32 (76%)^{a}	13 (50%)^{b}	7 (39%)^{c}
BMI (kg/m^2)	17.3 ± 1.3^{a}	21.0 ± 0.9^{b}	26.2 ± 3.1^{c}
BMI-for-age percentile	54.8 ± 19.0^{a}	89.7 ± 3.1^{b}	97.6 ± 1.3^{b}
Waist circumference (cm)	66.1 ± 8.7^{a}	62.5 ± 10.2^{ab}	79.5 ± 15.4^{bc}
Waist-to-height ratio	37.2 ± 18.1	36.5 ± 22.4	44.6 ± 24.7
Systolic blood pressure (mm Hg)	99.2 ± 9.7	101.9 ± 10.6	98.9 ± 9.8
Diastolic blood pressure (mm Hg)	67.2 ± 18.7	66.0 ± 9.7	67.4 ± 9.4
Total cholesterol (mg/dL)	155.8 ± 23.7	165.6 ± 24.2	156.4 ± 23.1
LDL cholesterol (mg/dL)	87.2 ± 20.6	96.6 ± 23.1	88.9 ± 18.8
HDL cholesterol (mg/dL)	57.2 ± 15.1	54.5 ± 8.23	51.3 ± 11.3
Triglycerides (mg/dL)	69.4 ± 42.2	69.2 ± 30.0	73.3 ± 52.8
Glucose (mg/dL)	86.2 ± 13.1	81.3 ± 14.6	77.2 ± 13.5
Insulin (μU/mL)	11.9 ± 4.9^{a}	20.2 ± 12.1^{b}	20.9 ± 8.6^{bc}
HOMA-IR	2.6 ± 1.2^{a}	4.3 ± 2.9^{b}	4.1 ± 2.1^{b}
Total NEFA (μM)	8856.7 ± 1949.4	8800.5 ± 2503.2	8038.4 ± 1733.1
Saturated FA (μM)	2792.2 ± 664.4	2564.1 ± 635.7	2893.1 ± 852.8
14:0 myristic acid	83.6 ± 26.4	96.1 ± 67.7	86.2 ± 41.1
15:0 pentadecanoic acid	17.0 ± 4.8	18.1 ± 7.1	15.9 ± 7.6
16:0 palmitic acid	1938.3 ± 470.7	2044.3 ± 618.0	1804.5 ± 461.7
17:0 heptadecanoic acid	23.5 ± 7.5	24.2 ± 7.9	21.5 ± 6.9
18:0 stearic acid	704.1 ± 161.4	692.2 ± 176.3	617.8 ± 133.0
Monounsaturated FA (μM)	1767.1 ± 503.8	1855.9 ± 644.5	1733.1 ± 508.1
16:1n7 palmitoleic acid	116.3 ± 51.7	150.1 ± 82.5	115.6 ± 50.5
18:1n9 oleic acid	1539.3 ± 445.7	1584.9 ± 525.8	1512.3 ± 453.8
n-6 Polyunsaturated FA (μM)	4061.8 ± 868.9	3829.4 ± 1026.7	3537.9 ± 659.4
18:2n6 linoleic acid	3132.2 ± 757.0	2916.5 ± 824.2	2746.5 ± 547.0
18:3n6 γ-linolenic acid	33.8 ± 14.6	34.4 ± 18.2	30.7 ± 11.8
20:3n6 dihomo-γ-linolenic acid (DGLA)	112.8 ± 32.8	125.7 ± 50.4	105.6 ± 20.1
20:4n6 arachidonic acid	717.6 ± 166.7	685.1 ± 188.3	597.3 ± 173.4
n-3 Polyunsaturated FA (μM)	235.6 ± 106.7	222.0 ± 84.1	203.3 ± 78.8
18:3 n3 α-linolenic acid	56.7 ± 23.4	56.9 ± 26.0	52.9 ± 20.8
20:5 n3 eicosapentaenoic acid (EPA)	34.6 ± 29.3	27.5 ± 14.2	27.5 ± 24.6
22:6 n3 Docosahexaenoic acid (DHA)	112.1 ± 56.6	103.5 ± 49.0	93.5 ± 42.0
Ratios			
Omega 6/Omega 3	19.03 ± 5.30	18.03 ± 3.30	18.64 ± 4.50

Different superscripts are given to values that are statistically different than one another using $p \leq 0.05$.*

BMI = body mass index, LDL = low-density lipoprotein, HDL = high-density lipoprotein, HOMA-IR = homeostatic model assessment for insulin resistance, NEFA = non-esterified fatty acid, FA = fatty acid.

Table 2. Anthropometric and cardiometabolic risk factors and total fatty acid concentration in male and female participants.

	Males (n = 33)	Females (n = 53)
	Mean ± SD	
Age (years)	10.5 ± 0.1	10.3 ± 0.1
BMI (kg/m^2)	21.4 ± 3.9^a	19.4 ± 3.7^b
BMI-for-age percentile	83.9 ± 19.8^a	68.1 ± 23.8^b
Waist circumference (cm)	67.0 ± 15.7	68.1 ± 9.5
Waist-to-height ratio	35.0 ± 24.9	40.8 ± 17.8
Systolic blood pressure (mm Hg)	98.8 ± 10.4	100.0 ± 9.6
Diastolic blood pressure (mm Hg)	68.2 ± 20.2	65.7 ± 8.5
Total cholesterol (mg/dL)	160.4 ± 21.2	157.3 ± 25.0
LDL cholesterol (mg/dL)	92.9 ± 19.5	87.4 ± 21.8
HDL cholesterol (mg/dL)	55.4 ± 11.3	54.8 ± 13.8
Triglycerides (mg/dL)	70.9 ± 47.0	70.2 ± 35.8
Glucose (mg/dL)	83.5 ± 12.3	82.8 ± 14.6
Insulin (μU/mL)	15.8 ± 10.0	16.0 ± 8.6
HOMA-IR	3.3 ± 2.3	3.3 ± 2.0
Total NEFA (μM)	8644.9 ± 1783.0	8869.2 ± 2279.3
Saturated FA (μM)	2754.8 ± 605.8	2858.5 ± 795.2
14:0 myristic acid	84.8 ± 35.1	94.1 ± 57.6
15:0 pentadecanoic acid	16.5 ± 5.4	18.2 ± 6.9
16:0 palmitic acid	1925.8 ± 459.6	2000.8 ± 558.1
17:0 heptadecanoic acid	22.4 ± 5.9	24.6 ± 8.3
18:0 stearic acid	686.1 ± 135.2	696.1 ± 178.6
Monounsaturated FA (μM)	1787.2 ± 489.7	1837.8 ± 604.1
16:1n7 palmitoleic acid	128.0 ± 65.6	133.3 ± 68.9
18:1n9 oleic acid	1553.0 ± 421.4	1584.8 ± 511.5
n-6 Polyunsaturated FA (μM)	3880.3 ± 782.8	3944.4 ± 946.3
18:2n6 linoleic acid	3003.9 ± 654.6	3019.9 ± 792.9
18:3n6 γ-linolenic acid	33.6 ± 11.9	35.2 ± 19.3
20:3n6 dihomo-γ-linolenic acid (DGLA)	113.1 ± 33.1	121.7 ± 43.5
20:4n6 arachidonic acid	668.2 ± 175.3	699.9 ± 180.3
n-3 Polyunsaturated FA (μM)	222.6 ± 82.5	228.5 ± 99.5
18:3 n3 α-linolenic acid	56.8 ± 20.5	58.0 ± 27.1
20:5 n3 eicosapentaenoic acid (EPA)	29.3 ± 20.9	32.6 ± 26.1
22:6 n3 Docosahexaenoic acid (DHA)	106.0 ± 48.5	103.5 ± 52.0
Ratios		
Omega 6/Omega 3	18.7 ± 4.6	18.7 ± 4.6

Different superscripts are given to values that are statistically different than one another using $p \leq 0.05$.*

BMI = body mass index, LDL = low-density lipoprotein, HDL = high-density lipoprotein, HOMA-IR = homeostatic model assessment for insulin resistance, NEFA = nonesterified fatty acid, FA = fatty acid.

FA, MUFA, or PUFA among groups (data not shown). No differences were observed in any anthropometric, lipid, or FA markers; glucose; insulin; or HOMA-IR by sex (Table 2). BMI and BMI-for-age were significantly greater in males compared to females (Table 2).

There were no differences in FA composition, desaturase enzyme activities, or elongase enzyme activities among the three groups (Table 1, Table 3). In examining correlations between desaturase enzyme activities and blood biomarkers, no relationships were observed in lipid markers or glucose; however, there were significant correlations among SCD-16, SCD-18, and D5D activity with fasting insulin ($r = 0.36$, $p = 0.001$; $r = 0.23$, $p = 0.030$; $r = -0.35$, $p = 0.001$, respectively; data not shown). ELOV6 was negatively correlated to fasting insulin ($r = -0.42$, $p = 0.0001$; data not shown). SCD-16, D5D, and ELOVL6 activity was also significantly correlated with HOMA-IR values ($r = 0.39$, $p = 0.001$; $r = -0.33$, $p = 0.006$; $r = -0.37$, $p = 0.005$, respectively; data not shown).

Based on the regression analysis, BMI-for-age percentile, D6D activity, ELOVL6 activity, and systolic blood pressure were the most significant predictors of HOMA-IR values (adjusted $r^2 = 0.39$, $p \leq 0.001$; Table 4). When evaluating differences among HOMA-IR values by quartiles, those in the lowest quartile had a significantly higher ELOVL6 activity and lower SCD-16 activity compared to those in the second, third, and fourth quartiles ($p = 0.04$, $p = 0.05$, $p = 0.001$, respectively, for ELOVL6 and $p = 0.05$, $p = 0.01$, $p < 0.001$, respectively, for SCD-16; data not shown). Participants in the lowest quartile also had greater D5D activity compared to participants the third and fourth quartile ($p = 0.03$, $p = 0.01$, respectively; data not shown).

Table 3. Plasma desaturase and elongase enzyme activity in normal weight, overweight, and obese participants.

	Normal Weight (n = 42)	Overweight (n = 26)	Obese (n = 18)
Desaturase activity		Mean ± SD	
SCD-16 (16:1n7/16:0)	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.02
SCD-18 (18:1n9/18:0)	2.19 ± 0.43	2.27 ± 0.39	2.43 ± 0.40
D6D (18:3n6/18:2n6)	0.01 + 0.01	0.01 ± 0.00	0.01 ± 0.00
D5D (20:4n6/20:3n6)	6.74 ± 2.04	5.94 ± 1.86	5.70 ± 1.40
Elongase activity			
ELOVL6 (18:0/16:0)	0.37 ± 0.04	0.35 ± 0.05	0.35 ± 0.04

No statistical differences in estimated desaturase and elongase enzyme activity were observed among the groups.

SCD-16 = Stearoyl CoA Desaturase-16, SCD-18 = Stearoyl CoA Desaturase-18, D6D = Delta-6-Desaturase, D5D = Delta-5-Desaturase, ELOVL6 = Elongase of long-chain fatty acids' family member 6.

Discussion

Childhood insulin resistance is implicated in the development of not only cardiovascular disease but also other metabolic disorders later in life. For instance, insulin resistance in childhood is a modifying factor for the development of hypertension, T2D, and metabolic syndrome in adulthood [28,29]. Further, studies demonstrate that the presence of these metabolic disorders varies among obese individuals depending on the degree of insulin sensitivity [30,31]. Children with increased insulin sensitivity tend to have more favorable metabolic profiles compared to their insulin-resistant counterparts [32].

Previous studies have shown strong associations between adiposity and lipid biomarkers, yet no relationships with adiposity were present in this sample. In an evaluation of cardiovascular disease risk in children (aged 3–6 years), Messiah et al. also reported a lack of relationship between BMI and LDL and total cholesterol [33]. This could be potentially explained by the phenomenon known as the metabolically healthy obese, which suggests that not all overweight and obese individuals

Table 4. Multivariate regression analysis with predictors in the model and the outcome variable, homeostatic model assessment for insulin resistance.

Predictors	B_x	p Value
BMI-for-age percentile	0.01	0.001
D6D	17.76	0.001
ELOVL6	−2.93	0.027
Systolic blood pressure	0.01	0.030
	$r^2 = 0.43$	
	Adjusted $r^2 = 0.39$	
	$p < 0.001$	

B_x represents the standardized regression coefficient.

BMI = body mass index, D6D = Delta-6-Desaturase, ELOVL6 = Elongase of long-chain fatty acids' family member 6.

have cardiometabolic abnormalities [34–36]. While this has been studied mostly in adults, there are some studies that have observed this phenomenon in children [37,38], suggesting that the relationship between adiposity and blood lipids can be variable. In addition, it has been reported that children and adolescents at this age are subject to natural fluctuations in their blood lipids as they are growing [39–41]. There may also be differences in genetics, activity level, ethnicity, and diet that can help explain the variability in blood lipids observed in this study, but due to limited sample size we are unable to explore those relationships.

Considering the relationship between insulin resistance and chronic disease, exploring adiposity in this context is important. Based on BMI-percentile classifications, no differences in plasma lipids, FA composition, or desaturase enzyme activities were observed. However, children in the obese category did have higher waist circumference measurements compared to normal-weight and overweight children. The observed variability in lipid markers suggests that while there are physical changes occurring as children's BMI percentile moves up, they may not yet be experiencing aberrations in lipid metabolism. This might be a critical window of time to intervene and help children develop healthy habits and therefore maintain healthy adipose levels as they get older to prevent the development of abnormal lipid metabolism.

While the children in this study did not exhibit any relationships between lipid markers and adiposity, elevated fasting insulin and HOMA-IR values in the overweight and obese groups were observed. It has been suggested that insulin plays a regulatory role in SCD-16 and SCD-18 activity, where insulin increases SCD-16 activity [42]. Consistent with this observation, a positive relationship between both SCD-16 and SCD-18 and fasting insulin was observed. There also was a positive relationship between SCD-16 and HOMA-IR, suggesting a potential relationship between alterations in FA metabolism and insulin action. The observed negative correlation between D5D and HOMA-IR is also consistent with other studies [12,16–18]. In evaluating the desaturase enzyme activities by HOMA-IR quartile, those in the higher quartiles tended to have greater SCD-16 and lower D5D activities, indicating that magnitude of insulin resistance can influence the degree of enzyme activity. Although the children in this study are healthy according to the traditional cardiometabolic risk factor cutoff, the behavior of lipid metabolism markers indicates that the children in the highest quartile of insulin resistance tended to have different enzyme activities compared to those in lower quartiles, which may indicate early disease progression.

Results from the multiple regression analysis suggest that D6D activity and ELOVL6 activity were the strongest predictors of HOMA-IR in this population. These results are consistent with those of Choi et al., who found that D6D was a major predictor of HOMA-IR and metabolic risk in Korean boys (10.5 ± 0.4 years, n = 131) [17] and Zhou et al., who found that D6D was correlated with HOMA-IR in healthy adolescent girls (15.3 ± 0.7 years, n = 178) [19]. Although Okada et al. found no relationship between elongase activity and HOMA-IR in children (aged 11–12 years, n = 112) [13], Venäläinen et al. reported that lower elongase activity was associated with higher cardiometabolic risk scores in Finnish children (6–8 years, n = 736) [43], which is consistent with the results of this current study. Research in adults has demonstrated that insulin resistance is characterized by increased proportions of individual FAs, such as palmitic, palmitoleic, and dihomo-γ-linolenic acid [12,44–46]. While these individual FAs did not contribute significantly to the regression model, the ratio of 18:0/16:0 was a negative predictor of HOMA-IR. This means that decreased ELOVL6 activity reflects increasing insulin resistance. Dyslipidemia has been reported to be involved in the development of T2D [47] and may link desaturase activity to T2D risk [48]. Notably, in the absence of obvious dyslipidemia symptoms such as hypertriglyceridemia, hypercholesterolemia, high LDL cholesterol, or low HDL cholesterol, the children in this study exhibited a similar relationship between desaturase enzyme activities and HOMA-IR as observed in adults with insulin resistance and T2D, suggesting that these shifts in lipid metabolism may be early adaptive responses to the changing energy balance and fuel partitioning associated with this disorder.

At present, there is not a well-established cutoff for HOMA-IR in children. One study in obese prepubertal and pubertal children and adolescents (aged 5–18 years) determined that HOMA-IR cutoffs for boys ranged from 2.67 to 5.22 and for girls ranged from 2.22 to 3.82 [49]. Another study in 691 apparently healthy Indian adolescents (aged 10–17 years) established a HOMA-IR cutoff of 2.5 [50]. Notably, in the current study HOMA-IR values were higher than values reported in the literature regarding children, with an average of 2.6 in normal-weight children and 4.1 to 4.3 in overweight and obese children. The mechanism(s) underlying these elevated values is unknown. It is possible that children in this age group (9–10 years) have higher HOMA-IR scores, perhaps due to the onset of puberty, where insulin levels have been reported to increase [51,52]. While children were asked to fast for 8 hours before participating in a blood draw, this information was self-reported by the child and his or her parent, which may be a source of error. However, the values are consistent within this population and the relationships between insulin metabolism and lipid metabolism in this population are similar to those reported elsewhere in both children and adults.

One limitation of the current study is that all associations are based on cross-sectional analyses of adiposity, lipid, and dietary data; therefore, causal relationships cannot be determined. In addition, other factors that influence plasma FA profiles and desaturase enzyme activity, such as genetic, hormonal, and other metabolic factors, were not evaluated in this study. The participants were recruited from a nutrition intervention in a school setting, so direct measurement of enzyme activities would be too difficult to carry out in this large community-based study. Last, Tanner stages were not evaluated in this population, so evaluation of the potential effects puberty may have on the relationships between lipid and insulin metabolism markers was not possible.

Conclusions

While traditional biomarkers of metabolic disease risk are not always associated with increasing adiposity in children, other underlying changes in FA and lipid metabolism associated with

the development of metabolic diseases, such as insulin resistance, likely exist. These relationships might be unobserved when comparing children by BMI percentile or other measures of body fat. Therefore, evaluating markers such as desaturase enzyme activities during early childhood could help predict the development of insulin resistance in the future. Having this information, combined with other markers of metabolic health, a more complete picture of early disease risk, development, and progression can be used to guide development of interventions that aim to improve health outcomes in young children.

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