

The association of desaturase 9 and plasma fatty acid composition with insulin resistance–associated factors in female adolescents

Yuan E. Zhou^a, Grace M. Egeland^a, Sara J. Meltzer^b, Stan Kubow^{a,*}

^a*School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec, Canada H9X 3V9*

^b*Division of Endocrinology and Metabolism, Royal Victoria Hospital, Montreal, Quebec, Canada H3A 1A1*

Received 31 January 2008; accepted 8 September 2008

Abstract

Desaturase 9 ($\Delta 9$), which converts saturated fatty acids (SFAs) into monounsaturated fatty acids, is an important component in leptin-mediated energy homeostasis in rodent models. Few human studies, however, have been performed regarding the clinical relevance of $\Delta 9$, particularly whether $\Delta 9$ is involved in the relationship between blood fatty acid profiles and insulin resistance–associated factors. The aim of the present study was to examine fatty acid data from 178 apparently healthy female adolescents and determine whether (a) $\Delta 9$ has independent associations with adiposity, insulin resistance level, and fasting plasma polyunsaturated fatty acids (PUFAs); (b) $\Delta 9$ is a predictor of fasting blood lipid profile; and (c) the associations between fasting plasma fatty acid component and insulin resistance level are independent of abdominal obesity level. Desaturase 9-16 (surrogate of $\Delta 9$ as calculated by plasma ratio C16:1 n-7/C16:0) correlated with waist girth ($r = .160, P < .05$), homeostasis model assessment of insulin resistance (HOMA-IR) ($r = .201, P < .01$), plasma PUFAs (eg, C20:4 n-6 [$r = -.269, P < .001$], C22:6 n-3 [$r = -.274, P < .001$]). After adjustment for dietary SFAs, $\Delta 9$ -16 had stronger correlation with waist ($r = .227, P < .01$) and significant correlation with PUFAs, whereas it had a nonsignificant correlation with HOMA-IR. The same pattern was observed with $\Delta 9$ -18 (surrogate of $\Delta 9$ as calculated by plasma ratio C18:1 n-9/C18:0). After adjustment for dietary SFAs, waist, and HOMA-IR, $\Delta 9$ -16 and $\Delta 9$ -18 were still positive predictors of triglyceride (both $P < .001$) and apolipoprotein B ($\Delta 9$ -18, $P < .001$; $\Delta 9$ -16, $P = .052$). After adjustment for waist, HOMA-IR only remained a positive determinant of medium-chain SFAs (C14:0, $P < .001$; C16:0, $P < .05$); but it emerged to be inversely related to C20:4 n-6 ($P < 0.1$). The positive and independent associations of medium-chain SFAs with insulin resistance level suggest their vital roles in diabetes pathogenesis, whereas certain PUFAs such as C20:4 n-6 appear to be protective. The observed associations of $\Delta 9$ with adiposity and plasma lipid profile in these apparently healthy female adolescents support the concept derived from rodent models that $\Delta 9$ activity is independently reflective of higher body mass index and higher circulatory triglyceride levels. © 2009 Elsevier Inc. All rights reserved.

1. Introduction

Disturbed lipid metabolism is an important component of the insulin resistance syndrome [1]. Dyslipidemia is detectable even before the appearance of fasting or postprandial hyperglycemia, suggesting that the altered lipid metabolism occurs long before type 2 diabetes mellitus fully develops [2].

Fatty acids' role in modulation of insulin resistance is supported by animal models [3]. In addition, there is now evidence that biosynthesis of fatty acids is also related to insulin resistance and adiposity. Fatty acid profile of plasma,

serum, and erythrocytes reflects both dietary intake and modulation by endogenous synthesis. The activity of desaturase 9 ($\Delta 9$) has been positively associated with insulin action [4]. In cross-sectional studies, serum medium-chain saturated fatty acids (SFAs) were positive determinants of insulin resistance measures in both males and females over a wide range of glucose tolerance [5,6]. In longitudinal studies, where a middle-aged male population was followed for 10 years and another elderly male population was followed for 4 years, serum SFAs were predictors of the development of impaired fasting glycemia and type 2 diabetes mellitus [7,8]. To date, however, previous human studies have not examined whether insulin action and associated factors predict fatty acid composition in blood.

In addition to the insulin-inducing action on $\Delta 9$ well demonstrated in various rodent models [4], mouse models

* Corresponding author.

E-mail address: stan.kubow@mcgill.ca (S. Kubow).

with gene mutation or knockout have also demonstrated that $\Delta 9$ is closely under the regulation of leptin [9,10], indicating that the role of adiposity is not negligible. Moreover, dietary polyunsaturated fatty acid (PUFA) supplement inhibited the gene expression of murine liver stearoyl-coenzyme A desaturase 1 (*SCD1*) that is equivalent to $\Delta 9$ in human [11]; and this repression effect was independent of insulin [12]. High carbohydrate intake induced the elevated level of C16:1 n-7 and C18:1 n-9 in both mice [13] and human [14]. The aforementioned evidence suggests that, besides insulin, other regulatory factors also exist, which modify the activity of the desaturases to influence the plasma fatty acid composition. Furthermore, evidence from mouse studies have demonstrated that *SCD1* knockout mouse showed impaired biosynthesis of hepatic cholesterol esters and triglycerides (TG) [15], suggesting that $\Delta 9$ could exert an important influence on lipid metabolism. Despite abundant animal studies indicating an important role of $\Delta 9$ in affecting insulin, energy, and lipid metabolism, human studies are limited. A positive association between adiposity and $\Delta 9$ was reported in several recent studies on elderly people [16], middle-aged population [17], and children [18]. A key limitation of the latter studies was that the observed association was not adjusted by dietary SFA intake that could affect the ratios C16:1 n-7/C16:0 ($\Delta 9$ -16) and C18:1 n-9 /C18:0 ($\Delta 9$ -18), the surrogate measures of $\Delta 9$ used in these studies. Moreover, no previous human studies have examined the associations of $\Delta 9$ with either dietary PUFA intake or plasma PUFAs.

In the present study, we investigated the cross-sectional associations of fasting plasma fatty acid profile and $\Delta 9$ with insulin resistance indices, blood lipids, adiposity, and dietary SFA intake in apparently healthy teenage girls. Young adolescents were chosen to potentially provide new insights regarding the early mechanisms that underlie the development of metabolic disorders in later life and because adolescents are relatively free of confounders compared with the previously studied elderly population. The tested hypotheses were that, in apparently healthy adolescent girls, (1) a positive association of plasma medium SFAs and homeostasis model assessment of insulin resistance (HOMA-IR) exists independent of abdominal adiposity; (2) $\Delta 9$ and abdominal adiposity are positively associated independently of insulin resistance and dietary SFAs intake; and (3) $\Delta 9$ is positively associated with blood lipids independent of the insulin resistance, abdominal adiposity, and dietary SFA intake.

2. Subjects and methods

2.1. Subjects

The study population consists of adolescent girls ($n = 189$) from the Montreal mother-daughter gestational diabetes mellitus (GDM) case-control cohort (Appendix A, Egeland and Meltzer, submitted). Mother-daughter pairs affected or

unaffected by GDM that resulted in singleton term (37–42 weeks) deliveries at the Royal Victoria Hospital (Montreal, Quebec, Canada) in 1989 to 1991 were recruited for a 15-year follow-up evaluation. Pregnancies with preexisting or pregnancy-related medical complications were excluded to ensure no confounding influence of other diseases. Eligible mother-daughter pairs were identified from patient records and the McGill Obstetrics and Neonatal Database. Controls were frequently matched by 4 maternal age groups and 4 socioeconomic strata based on postal code census income at the time of delivery. Among the case-control cohort of 189 mother-daughter pairs, fatty acid data were available for 179 adolescent subjects. One of these 179 subjects was excluded because of her diabetic status; thus, there were 178 adolescents' fatty acid data used for data analysis. The study was approved by the ethical review board of the McGill University Health Center, and informed consent was obtained from all participants.

2.2. Anthropometric and biochemical measures

Subjects came to the Royal Victoria Hospital in the morning after an overnight fast. Height was measured without shoes to the nearest centimeter using a stadiometer. Waist girth was assessed at the end of exhalation by using a standard tape measuring the level midway between the costal margin and the iliac crest when the subjects were standing. Circumference was measured to the nearest 0.1 cm. For each subject, the measurements were repeated 3 times; the average of values were presented and used in analyses. Weight and the percentage of body fat were evaluated by bioelectrical impedance (Tanita TBF-310; Tanita, Tokyo, Japan). Body mass index (BMI) was calculated as weight (in kilograms)/height² (in meters). Fasting plasma was used for the fatty acid analysis and fasting serum for biochemical tests on TG, apolipoprotein B (apo B), low-density lipoprotein cholesterol, high-density lipoprotein (HDL) cholesterol, and hemoglobin A_{1c}.

Glucose was analyzed on the Beckman-Coulter LX20 analyzer (Beckman Instruments, Fullerton, CA) using the glucose oxidase technique, insulin was analyzed by an immunometric assay with chemiluminescent detection on a DPC Immulyte Immunoanalyzer (Diagnostic Products, Los Angeles, CA), and hemoglobin A_{1c} was measured using a Roche Cobas Mira analyzer (Roche, Laval, Quebec, Canada) with the reagent Roche Unimate 3 hemoglobin A_{1c}. Lipid profiles were performed using the Beckman-Coulter LX20 analyzer. Apolipoprotein B was measured using an immunonephelometric technique on a Beckman-Coulter Image nephelometer.

2.3. Fatty acid analysis

A 1-step extraction and transesterification method was applied instead of a traditional fatty acid analysis procedure that requires multiple steps [19,20]. Briefly speaking, 50- μ L plasma samples were added into 10-mL screw-cap tubes containing 1.9 mL run solution that consisted of 1.7 mL

butylated hydroxytoluene (BHT)-methanol, 100 μ L acetyl chloride, and 100 μ L internal standard. After waterbath heating at 100°C for 1 hour, 1.0 mL hexane was added to each tube. The tubes were then vortexed for 30 seconds, and the upper organic phase was collected. The extract was evaporated under nitrogen, redissolved in hexane, and transferred to gas chromatography vial inserts for the test on a 100-m \times 0.25-mm-internal diameter \times 0.25- μ m Varian CP-select CB for fatty acid methyl ester fused-silica capillary column in a Varian 3400 CX gas chromatograph equipped with a flame ionization (Varian, Palo Alto, CA). The temperature program was as follows: initial, 80°C with a 1-minute hold; ramp: 30°C/min to 180°C, 1°C/min to 196°C, 20°C to 230°C with a 15-minute hold, and 30°C/min to 270°C with an 8-minute hold. The detector temperature was set at 275°C, and injector temperature was at 250°C. The carrier gas was helium set at a flow rate of 2 mL/min. The signal to noise ratio was set at 3, allowing a method detection limit of 8.6×10^{-4} mg/mL. The fatty acids were identified by comparing each peak's retention time with those of methyl ester standards (GLC-6923 containing 31 FAMES plus added C12:0 and C18:3 FAMES, Nu-Check Prep, Elysian, MN). Seronorm lipid (SERO AS, Billingstad, Norway) containing 23 fatty acids of animal origin was used as an additional external control. Data were collected in a Varian Star 3400 CX gas chromatograph (Varian, Montreal, Quebec, Canada) and Saturn W/S version 5.4.1 (Integration) software (Varian, Palo Alto, CA). The relative amount of each fatty acid was quantified by integrating the area under the peak and dividing the results by the total area for all fatty acids. The C18:1 *trans*-isomers appeared as a series of overlapping peaks that the Saturn program was unable to separate to quantify; thus, the C18:1 *trans*-isomers (t6, t9, and t11) were identified and integrated as 1 broad peak (C18:1t total).

Interassay coefficients of variation (CV) percentages were assessed by analyzing duplicate standard references during each run of fatty acid analysis. The overall CV percentages of the most abundant SFAs and monounsaturated fatty acids (MUFAs) in plasma were less than or around 10%: C14:0, 5.51%; C16:0, 9.26%; C18:0, 10.40%; C16:1 n-7, 8.92%; C18:1 n-9, 10.70%. Interassay CV percentages of primary PUFAs are as follows: C18:2 n-6, 10.27%; C20:4 n-6, 18.00%; C20:5 n-3, 12.19%; C22:6 n-3, 18.53%.

2.4. Statistical analysis

Data are presented as mean (SD) if normally distributed and median (interquartile range) if not normally distributed. Fatty acid in plasma was expressed as a percentage of total fatty acids in plasma. Saturated fatty acids, MUFAs, omega 3 fatty acids (n-3 PUFAs), omega 6 fatty acids (n-6 PUFAs), and *trans*-fatty acids (TFAs) were calculated by summing the concentrations of individual fatty acids if they were detectable.

The use of the C16:1 n-7/C16:0 and C18:1 n-9/C18:0 ratios as crude surrogate measures of the activity of $\Delta 9$ is well established in human studies because direct measure of

enzyme activity is not normally feasible. As expected, in the current study, C16:1 n-7/C16:0 and C18:1 n-9/C18:0 are related to the fasting plasma MUFA profile because the Spearman correlation coefficients between the surrogate markers of $\Delta 9$ and MUFAs were highly positively correlated (data not shown). A major limitation to the use of C16:1 n-7/C16:0 and C18:1 n-9/C18:0 ratios as indices of metabolic activity of $\Delta 9$ is the inherent bias of medium-chain SFA intake. To circumvent this latter confounding influence, endogenous medium-chain SFAs were adjusted according to levels of C15:0. This latter odd-number carbon atom fatty acid is not synthesized by humans and is obtained from dairy fat and ruminant meat products [21]. In the linear regression models, C15:0 was a predictor for the predicted variables of C16:1 n-7/C16:0 and C18:1 n-9/C18:0 ratios. One may regard the residuals of these ratio as that which cannot be explained by dietary intake, that is, the remaining endogenous product. Similar statistical approach is widely applied in other epidemiologic studies [22] to assess the importance of other predictors after adjustment for 1 major predictor. Although it is only able to partly remove the effect of dietary intake because of a variety of dietary sources of C16:0 and C16:1 and the variability of fatty acid composition in foods, this attempt allows for a closer estimate to the true endogenous levels of C16:0 and C16:1. Both $\Delta 9$ -16 and $\Delta 9$ -18 were calculated. Although C18:1 n-9 is important in terms of maintenance of normal physiologic functioning such as membrane fluidity and lipoprotein metabolism, its abundant existence in food makes it more difficult to estimate its endogenous levels. In contrast, $\Delta 9$ -16 represents potentially a more accurate measure of $\Delta 9$ because C16:1 n-7 is mainly derived from endogenous pathways.

Insulin resistance was estimated by the HOMA-IR, calculated as follows: $\text{HOMA-IR} = [\text{fasting insulin (in microunits per milliliter)} \times \text{fasting plasma glucose (in millimoles per liter)}] / 22.5$. The HOMA-IR was used because this measure is suggested to be applicable for a broad range of possible coefficients of insulin resistance and β -cell function and across ethnic groups [23]. Interactions of mother's gestational diabetes status on the associations of $\Delta 9$ with anthropometric measures and glucose homeostasis indices were tested with general linear models. Because there were no observed interactions, the results of associations of $\Delta 9$ and fasting plasma fatty acid profile with measures of interest are presented for all daughters pooled into 1 group. Spearman correlations were conducted for the correlations of $\Delta 9$ with adiposity measures, HOMA-IR, and PUFAs. Student *t* test was used to examine the significance of Spearman correlation coefficients before and after adjustment [24,25]. Multi-variable linear regression was used to evaluate whether the associations of the 2 unadjusted $\Delta 9$ ratios and the 2 $\Delta 9$ ratios adjusted for C15:0 with TG and lipoprotein would remain when adiposity level and HOMA-IR were entered as covariates. Because of their skewed distribution, HOMA-IR, fasting insulin, and TG were log transformed before correlation and regression analysis. All *P* values were

obtained from 2-sided tests. Data were analyzed with the SAS software (version 9.1; SAS Institute, Cary, NC).

3. Results

3.1. Subjects' characteristics

The mean age of the female adolescents was 15.3 (SD = 0.7) years, their mean BMI was 22.9 (SD = 4.4) kg/m², and their mean waist was 77.9 (SD = 10.5) cm. Most of participants were white (77.8%). Other anthropometric and biologic characteristics are presented elsewhere (Appendix A, Egeland and Meltzer, submitted). All the subjects had at least 1 menses to be eligible to participate into the study.

Desaturase 9 and fatty acid profiles of fasting plasma of the 2 groups were unrelated to mothers' previous GDM status (Table 1). Significant differences were only found for C16:0 and C18:1t (*P* < .05); however, the differences were less than 1% and were of no clinical significance.

3.2. Fatty acid profile of fasting plasma, glucose homeostasis measures, and adiposity

After adjustment for waist girth, fasting insulin and HOMA-IR were significantly and positively associated with

Table 1

Mean proportion (percentage of total fatty acids) of fatty acids in fasting plasma of the study population

	Control (n = 96)	Case (n = 82)	<i>P</i>
SFAs	36.78 (1.79)	36.02 (2.10)	<.05
C12:0 ^{a,b}	0.07 (0.08)	0.06 (0.05)	<.1
C14:0 ^a	1.02 (0.61)	0.94 (0.65)	NS
C15:0 ^a	0.27 (0.07)	0.26 (0.08)	NS
C16:0 ^a	24.11 (2.03)	23.33 (1.64)	<.05
C17:0	0.39 (0.05)	0.37 (0.06)	.05
C18:0	8.27 (0.78)	8.21 (0.86)	NS
MUFAs	24.66 (2.53)	24.70 (2.29)	NS
C16:1 n-7 ^a	1.82 (0.85)	1.84 (0.63)	NS
C18:1 n-9	19.59 (2.15)	19.53 (1.97)	NS
TFAs ^a	1.00 (0.58)	0.90 (0.40)	<.1
C18:1t ^{a,c,e}	0.82 (0.44)	0.74 (0.33)	<.05
C18:2t ^{d,e}	0.21 (0.11)	0.20 (0.07)	NS
n-6 PUFAs ^a	32.15 (3.54)	32.64 (3.08)	NS
C18:2 n-6	23.57 (2.69)	24.07 (2.56)	NS
C20:4 n-6	7.70 (1.56)	7.70 (1.38)	NS
n-3 PUFAs ^a	5.37 (1.22)	5.53 (0.82)	NS
C20:3 n-3 ^a	1.65 (0.43)	1.82 (0.50)	<.05
C20:5 n-3 ^a	0.53 (0.32)	0.53 (0.27)	NS
C22:6 n-3 ^a	1.64 (0.80)	1.72 (0.81)	NS
Δ9-16 (C16:1 n-7/C16:0) ^a	0.08 (0.03)	0.08 (0.02)	NS
Δ9-18 (C18:1 n-9/C18:0) ^a	2.34 (0.43)	2.35 (0.47)	NS

All values are presented as mean (SD) unless otherwise indicated. NS indicates not significant.

^a Data are not normally distributed; values are median (interquartile range).

^b Control, n = 89; case, n = 72.

^c Control, n = 95; case, n = 82.

^d Control, n = 90; case, n = 78.

^e C18:1t is the sum of all C18 TFAs with 1 double bond; C18:2t is the sum of C18:2ct, C18:2tc, and C18:2tt.

Table 2

Glucose homeostasis indices as predictor of plasma fatty acids after adjustment for waist girth

	Fasting glucose			Fasting insulin			HOMA-IR		
	β	±	SE	β	±	SE	β	±	SE
SFAs	0.028		0.409	1.973	0.715 [‡]		1.823	0.690 [‡]	
C12:0	-0.004		0.018	0.035	0.032		0.034	0.031	
C14:0	-0.016		0.096	0.571	0.166 [§]		0.527	0.160 [‡]	
C15:0	5.0 × 10 ⁻⁴		0.014	0.079	0.025 [‡]		0.074	0.024 [‡]	
C16:0	0.005		0.332	1.226	0.589 [†]		1.123	0.568 [†]	
C18:0	0.137		0.170	0.148	0.310		0.173	0.299	
MUFAs	0.044		0.496	0.555	0.872		0.526	0.840	
C16:1 n-7	0.009		0.117	0.299	0.204		0.279	0.196	
C18:1 n-9	0.105		0.430	0.547	0.759		0.535	0.730	
TFAs	0.058		0.090	0.051	0.145		0.059	0.139	
C18:1t	0.068		0.077	0.024	0.123		0.037	0.119	
C18:2t	-0.010		0.021	0.027	0.037		0.022	0.036	
n-6 PUFAs	0.429		0.635	-2.459	1.097 [†]		-2.166	1.058 [†]	
C18:2 n-6	0.203		0.541	-1.144	0.945		-0.996	0.910	
C20:4 n-6	0.301		0.308	-1.176	0.542 [†]		-1.015	0.523 [*]	
n-3 PUFAs	-0.492		0.204 [†]	-0.169	0.371		-0.268	0.357	
C20:3 n-3	-0.057		0.072	0.161	0.130		0.135	0.125	
C20:5 n-3	-0.189		0.067 [‡]	-0.091	0.122		-0.131	0.117	
C22:6 n-3	-0.183		0.117	-0.116	0.212		-0.143	0.203	

All indices (except fasting glucose) are tested with the log-transformed values. C18:1t is the sum of TFAs with C18 and 1 double bond; C18:2t is the sum of C18:2ct and C18:2tc.

* .05 ≤ *P* < .10.

† *P* < .05.

‡ *P* < .01.

§ *P* < .001.

median-chain SFAs C14:0, C15:0, and C16:0 (Table 2). Conversely, the significant positive association of fasting insulin and HOMA-IR with C16:1 n-7 disappeared after adjustment for waist girth. For n-3 PUFAs, fasting glucose was a negative predictor of C20:5 n-3 (*P* < .01) and total n-3 FAs (*P* < .01).

For n-6 PUFAs, fasting insulin and HOMA-IR inversely predicted total n-6 FAs and C20:4 n-6 (Table 2) after adjustment for waist girth. Fasting insulin and HOMA-IR were inversely predictive of C18:2 n-6; however, after adjustment for waist girth, the associations were not significant (*P* > .05). In contrast, the associations of fasting insulin and HOMA-IR with C20:4 n-6 were only significant (for fasting insulin, *P* < .05; for HOMA-IR, .05 ≤ *P* ≤ .10) after adjustment for waist girth.

3.3. Δ9, dietary saturated fat intake, adiposity, insulin resistance level and PUFAs, lipids, and lipoprotein in fasting blood

Both unadjusted Δ9-16 and Δ9-16 adjusted for C15:0 were positively correlated with adiposity measures; but adjusted Δ9-16 was more strongly correlated with BMI, percentage of body fat, and waist girth than unadjusted Δ9-16 (*P* < .05). On the other hand, the significant (*P* < .01) correlation between Δ9-16 and HOMA-IR disappeared after adjustment for dietary SFAs intake (Table 3). The Δ9-18 showed a similar pattern of changes of the correlations with

Table 3

Spearman correlation coefficients of $\Delta 9$ ratios with indices of adiposity and insulin resistance and PUFAs before and after adjustment of dietary SFAs intake

	$\Delta 9-16$ (adjusted)	$\Delta 9-16$	P^c	$\Delta 9-18$ (adjusted)	$\Delta 9-18$	P
BMI	0.268 ^{§,d}	0.204 [‡]	<.05	0.185 [†]	0.147*	<.1
% Body fat	0.251 [§]	0.197 [‡]	<.10	0.093	0.071	NS
Waist girth	0.227 [‡]	0.160 [†]	<.05	0.138*	0.106	NS
HOMA-IR ^{a,b}	0.109	0.210 [‡]	<.01	0.050	0.096	<.05
Fasting glucose	0.059	0.068	NS	-0.032	-0.030	NS
Fasting insulin ^{a,b}	0.093	0.195*	<.01	0.051	0.099	<.05
PUFAs	-0.500 [§]	-0.682 [§]	<.001	-0.450 [§]	-0.568 [§]	<.001
C18:2 n-6	-0.495 [§]	-0.593 [§]	<.001	-0.259 [§]	-0.321 [§]	<.01
C20:4 n-6	-0.127*	-0.269 [§]	<.001	-0.348 [§]	-0.445 [§]	<.001
C20:5 n-3	-0.075	-0.046	NS	-0.231 [‡]	-0.212 [‡]	NS
C22:6 n-3	-0.187 [†]	-0.274 [§]	<.01	-0.118	-0.166 [†]	<.05

n = 178 unless otherwise indicated. $\Delta 9-16$ (adjusted) and $\Delta 9-18$ (adjusted) are residuals of $\Delta 9-16$ and $\Delta 9-18$ corrected for C15:0.^a n = 170.^b Data are logistic transformed.^c Significant difference between the unadjusted and adjusted correlation coefficients.^d Actual significances of spearman correlations of $\Delta 9$ with the parameters on the left hand side of the table.* .05 $\leq P < .10$.† $P < .05$.‡ $P < .01$.§ $P < .001$.

adiposity measures and HOMA-IR with and without adjustments (Table 3). Both $\Delta 9-16$ and $\Delta 9-18$ were strongly negatively correlated ($P < .001$) with the primary plasma PUFAs both before and after adjustment for C15:0, particularly for C18:2 n-6, but not for the relationship between $\Delta 9-16$ and C20:5 n-3.

The medium-chain SFA C15:0 was highly correlated with other medium-chain fatty acids such as C16:0 ($r = .501$, $P < .001$) and C16:1 ($r = .424$, $P < .001$), which can be explained by the common food source from which they originate. For example, as shown in Table 4, before adjustment, in the regression model with $\Delta 9$ as a predictor of TG, the $\beta_{\Delta 9-16}$ was 4.222 and the $\beta_{\Delta 9-18}$ was 0.254. As $\Delta 9-16$ and $\Delta 9-18$ represent activity of the same desaturase enzyme, their predictive values should be similar. As expected, after adjustment for C15:0, the β -coefficients for $\Delta 9-16$ and $\Delta 9-18$ were similar (0.066 vs 0.096, respectively). Similar patterns were also observed in the regression model for the other plasma lipids (Table 4). Also as was expected,

β -coefficients of C15:0 for fasting insulin and HOMA-IR were weaker relative to other SFAs (Table 2), given that C15:0 is an unlikely factor in the causal pathway between $\Delta 9$ and either insulin resistance or adiposity. In addition, the associations of C15:0 with adiposity measures and glucose homeostasis index were consistent with those of other median-chain SFAs. As expected, no significant correlations were observed between C15:0 and adiposity measures (eg, BMI [$r = -.081$, $P = .283$], waist girth [$r = -.070$, $P = .355$], body fat percentage [$r = -.045$, $P = .554$]). Furthermore, weak correlations of C15:0 with fasting insulin ($r = 0.214$, $P = .005$) and HOMA-IR ($r = 0.200$, $P = .009$) were observed; and there was no significant correlation between C15:0 and fasting glucose ($r = -0.032$, $P = .670$).

Adjustment for saturated fat intake did not change the significance or magnitude of the associations of $\Delta 9-16$ and $\Delta 9-18$ with plasma lipoprotein profiles (Table 4). Both adjusted and unadjusted $\Delta 9-16$ and $\Delta 9-18$ were strong predictors of TG ($P < .001$), independent of waist

Table 4

Desaturase 9 ratios as predictor of fasting plasma TG and main lipoprotein and apo B

	$\Delta 9-16$ (adjusted)			R^2	$\Delta 9-16$			R^2	$\Delta 9-18$ (adjusted)			R^2	$\Delta 9-18$			R^2
	β	\pm	SE		β	\pm	SE		β	\pm	SE		β	\pm	SE	
TG ^a	0.066	0.014 [§]	0.148	4.222	0.656 [§]	0.233	0.096	0.012 [§]	0.308	0.254	0.027 [§]	0.377				
Apo B ^b	0.022	0.013*	0.033	1.242	0.634*	0.039	0.047	0.012 [§]	0.104	0.115	0.028 [§]	0.110				
LDL	0.075	0.049	NA	3.913	2.342*	0.021	0.108	0.045 [†]	0.039	0.266	0.105 [†]	0.042				
HDL	-0.006	0.021	NA	-0.790	1.00	NA	-0.070	0.019 [§]	0.066	-0.173	0.044 [§]	0.073				
Cholesterol	0.121	0.054 [†]	0.031	6.480	2.572 [†]	0.038	0.118	0.050 [†]	0.035	0.302	0.117 [†]	0.040				

n = 170. All values are β estimates \pm SEs adjusted for waist girth and HOMA-IR. LDL indicates low-density lipoprotein. NA indicates not applicable.* $P < .01$.^a Data are logistic transformed.^b n = 167.* .05 $\leq P < .10$.† $P < .05$.§ $P < .001$.

girth and HOMA-IR. Likewise, both adjusted $\Delta 9$ -18 and unadjusted $\Delta 9$ -18 were pronounced predictors of apo B and HDL ($P < .001$).

4. Discussion

The present study is the first study conducted in a human context to examine and demonstrate important relationships between $\Delta 9$ and certain key metabolic indices, which previously have only been noted in rodent models [10,26]. In that regard, a positive relationship was shown between $\Delta 9$ and adiposity and a negative association of $\Delta 9$ to plasma PUFAs was observed, which occurred independently of dietary saturated fat intake. The present work also demonstrated novel associations between $\Delta 9$ and blood lipid profiles, which remained significant after the adjustment of adiposity, dietary saturated fat intake, and insulin resistance level. The present findings showing important metabolic associations with $\Delta 9$ thus indicate that this enzyme activity could also be clinically relevant in the adolescent female population.

As hypothesized, positive associations were shown between $\Delta 9$ and all adiposity measures, including waist girth, BMI, and body fat percentage. The above associations became even stronger after adjustment of dietary saturated fat intake, suggesting that these associations were not spurious because of confounding effects of saturated fat intake. In contrast to the strong positive association between adiposity and $\Delta 9$, the weak positive associations between $\Delta 9$ and HOMA-IR became nonsignificant after the adjustment for C15:0, suggesting that the latter association was largely mediated by saturated fat intake.

It is conceivable that the present findings might reflect a regulatory role of adipose tissue–derived hormones such as leptin in suppressing $\Delta 9$. In rodent models, the gene of SCD-1, 1 of the 4 $\Delta 9$ gene isoforms predominantly existing in mice liver and highly homologous to human $\Delta 9$, was ranked on the top of the list of genes uniquely repressed by leptin [10]. Mice lacking *SCD1* gene expression showed the lipodystrophy syndrome and were hypermetabolic [9]. Insulin is considered to be capable to induce SCD-1 through sterol regulatory element–binding protein-1c (SREBP-1c)–mediated pathway [27]. The effect of leptin on SCD-1 in mice was apparently independent of insulin and SREBP-1c because leptin inhibited the transcription, translation, and activity of hepatic SCD-1 regardless of blood insulin levels and expression levels of SREBP-1c [28]. This latter study indicates that insulin is subordinate to leptin in terms of regulating SCD-1, which is supported by the data of the present work that show a strong relationship of $\Delta 9$ with adiposity relative to a nonsignificant association of $\Delta 9$ with HOMA-IR.

To our knowledge, the present study is the first study done in a human context to report negative associations of $\Delta 9$ -16 and $\Delta 9$ -18 with plasma PUFAs, especially C18:2 n-6, C20:4

n-6, and C22:6 n-3. Polyunsaturated fatty acids have been suggested to be involved in inhibiting expression of genes related to lipogenesis [29]. Therefore, fasting plasma PUFAs could be indicative of down-regulation of the expression of genes related to $\Delta 9$. Because C18:2 n-6 is an essential fatty acid and C22:6 n-3 is largely exogenously derived, these fatty acids reflect dietary intake levels and could indicate the capability of PUFA to down-regulate $\Delta 9$ -16 and $\Delta 9$ -18. In support of this concept, previous mouse studies have shown that PUFA supplementation down-regulates expression of hepatic SCD-1 [11]. Similarly, diabetic mice supplemented with either (3% wt/vol) linolenic acid or (1% wt/vol) arachidonic acid exhibited repression of insulin-induced hepatic SCD-1 messenger RNA by 97% and 99%, respectively [12]. In the present study, the association of PUFAs with $\Delta 9$ -16 and $\Delta 9$ -18 remained significant after adjustment with HOMA-IR, also suggesting an insulin-independent effect of PUFAs on $\Delta 9$. The repression of PUFAs on lipogenesis may not only be hepatic specific, as inhibition of expression and activity of rat lipogenic enzymes in adipose tissue by corn oil has also been reported in adipose tissue, although the effects were less pronounced than those observed in the liver [30].

The strong and positive associations of $\Delta 9$ -16 and $\Delta 9$ -18 with plasma TG and apo B that were independent of adiposity, insulin resistance level, and dietary saturated fat are strongly supported by previous rodent studies showing that $\Delta 9$ is critical in the synthesis of hepatic cholesterol ester and TG, which plays a key role in the hepatic very low-density lipoprotein synthesis [10,26]. Moreover, the transcript level of $\Delta 9$ in human adipose tissue has been reported to be positively associated with plasma TG [14]. The role of $\Delta 9$ in hepatic lipid synthesis is due to the role of C18:1 n-9, the major product of this enzyme, as the preferred substrate of acyl-coenzyme A:cholesterol acyltransferase and diacylglycerol acyltransferase, responsible for the synthesis of cholesterol ester and TG, respectively [10,26]. In that regard, SCD-1 knockout mice or mice lacking of SCD-1 expression showed deficient hepatic concentrations of TG and cholesterol and reduced hepatic production of very low-density lipoprotein [9,13,15]. Conversely, in hyperlipidemic mice, a 4-fold increase in hepatic SCD activity was accompanied by a 2-fold increase in plasma TG [31]. In addition, the expression and activity of $\Delta 9$ in primary human skeletal myocytes exhibited a strong negative correlation with fatty acid oxidation and a strong positive correlation with intramuscular TG synthesis [32]. Similarly, a 2-fold increase in the ratio of human plasma C18:1 n-9 to C18:0 was shown to be accompanied by a 4-fold increase in plasma TG concentrations [31].

In this study, the fatty acid profiles in healthy adolescents are consistent with previous studies involving either obese children or middle-aged and older adults showing that plasma medium-chain SFAs are positively associated with insulin resistance level and that MUFA C16:1 n-7 is positively associated with adiposity level [16–18]. In line

with previous human studies [33,34], positive association was observed between plasma medium-chain SFAs and HOMA-IR in the present work. As compared with PUFA, SFA is less likely to be oxidized and more likely to accumulate in cells [35,36]. Hence, the insulin-resistant state could result in preferential oxidation of PUFA, leading to the observed altered plasma profile of decreased PUFA and increased medium SFA. The association of dietary SFA intake with elevated insulin resistance is reported to be due to an altered membrane saturation degree, leading to altered biomembrane fluidity and associated transmembrane signaling pathways [36]. In the current study, the association of $\Delta 9$ with HOMA-IR was lost after the adjustment of SFA intake using C15:0, further supporting the concept that dietary SFA is an important modifier of insulin resistance.

In this study, no significant association was observed between specific plasma n-6 fatty acids and insulin resistance indices such as HOMA-IR. On the other hand, a negative association between C18:2 n-6 and insulin resistance indices was observed originally that was no longer seen after adjustment for waist girth (Table 2), which was likely due to a strong inverse correlation between C18:2 n-6 and waist girth (data not shown). The strong and negative association seen between the C18:2 n-6 and adiposity in the present work is consistent with previously reported studies [16–18]. Increasing HOMA-IR accompanied by lower blood C18:2 n-6 levels has been reported by other studies [16–18,34]; however, no mechanistic explanation has been given regarding this association. A possible explanation might be due to the indications that C18:2 n-6 is an indicator of total fat consumption as seen from clinical trials comparing low-fat vs median-fat diets [37]. Low C18:2 n-6 intake has been related to relatively low total fat intake, which would lead to increased synthesis of endogenous medium-chain SFAs, such as C16:0 [37]. Therefore, the apparent negative association between low plasma C18:2 n-6 and insulin resistance indices may be driven by the positive association of these indices with medium-chain SFAs.

In contrast to C18:2 n-6, the originally nonsignificant negative associations of C20:4 n-6 with both HOMA-IR and fasting insulin became marginally significant and significant, respectively, after adjustment of waist girth. These latter observations are consistent with previous human studies that have reported a negative association of fasting insulin or HOMA-IR with C20:4 n-6 levels in fasting serum, erythrocyte membrane, and skeletal muscle membrane [33,38–40]. As a critically important physiologic PUFA fatty acid, C20:4 n-6 has many unique physiologic functions including its role as an eicosanoid precursor and the most abundant fatty acid in biomembranes [41]. Besides dietary intake, it can be derived in *de novo* synthesis from C20:3 n-6 via desaturase 5, whose activity is positively associated with insulin sensitivity and negatively associated with insulin resistance [4]. In the present study, desaturase 5 could not be computed by the ratio C20:4 n-6/C20:3 n-6 because C20:3 n-6 was nondetectable. The observed

negative associations of C20:4 n-6 with both HOMA-IR and fasting insulin, however, indicate a direct association between C20:4 n-6 and insulin action.

The n-3 fatty acids did not show significant associations with HOMA-IR or adiposity measures in this study population, apart from a positive association of C20:3 n-3 with HOMA-IR, which was not observed after adjustment for adiposity. On the other hand, a negative association was observed between fasting glucose and n-3 PUFAs, which was largely based upon an inverse association between fasting glucose and C20:5 n-3 (Table 2). The n-3 PUFA supplementation trials by fish oil did not show significant fasting plasma glucose-lowering effects [42,43]. More studies are warranted to determine whether the observed association in the present study came by chance or it indicated that lower fasting glucose is a physiologic condition that is modulated by altered endogenous n-3 PUFA metabolism.

A limitation of the present study was that the causal relationships between the variables of interest cannot be determined based on the cross-sectional data. Besides adiposity, insulin resistance, and dietary factors, there could be other hormonal, genetic, or metabolic factors that could impact upon fatty acid profiles and the activity of $\Delta 9$. Thus, there are a variety of other endogenous factors that could account for the observed positive association between C20:3 n-3 and adiposity. In addition, as the current study was conducted in adolescent girls, more studies are needed to confirm whether conclusions from the present study could be generalized to other populations. Furthermore, the impact of mother's gestational diabetes status on the daughter's fatty acid metabolism or on the fatty acid metabolism among mothers themselves is worthwhile for future study, as available data from the current study were insufficient to detect such effects. Measures that could be improved for future studies include (1) the use of glucose clamp or frequently sampled intravenous glucose tolerance tests, which are more accurate than HOMA-IR to detect insulin resistance level and insulin sensitivity, and (2) application of dual-energy x-ray scanning, which is a more precise measure of body composition. In addition, the state of puberty could be considered to be examined more closely because it is an important determinant of insulin action during growth spurt. Another option for future studies would be the use of the fatty acid profiles of erythrocyte membranes as a surrogate marker of relatively long-term dietary intake modified by *de novo* synthesis [44].

In summary, the results of the current study suggest that, independent of insulin resistance level and dietary medium-chain SFA intake, increased activity of $\Delta 9$ leading to higher endogenous synthesis of MUFAs is reflective of higher BMI, whereas dietary PUFAs appear to inhibit $\Delta 9$ activity. On the other hand, high intake of saturated fat as assessed by plasma 15:0 content is associated with increased insulin resistance. In addition, $\Delta 9$ is positively and strongly associated with circulatory TG levels, independent of adiposity, insulin resistance, and dietary saturated fat intake.

Overall, the present findings indicate the importance of maintaining a healthy body weight and balanced fat intake as early as adolescence in the maintenance of a healthy metabolic profile related to insulin activity and lipoprotein and fatty acid metabolism.

Acknowledgment

This study was supported by a Canadian Institute of Health Research grant. We thank Sina Gallo and Donna Leggee for their contribution in fatty acid analysis. The participation of our subjects and the cooperation of their parents are also greatly appreciated.

References

- [1] McGarry JD. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 2002;51:7-18.
- [2] Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 2002;23:201-29.
- [3] Kusunoki J, Kanatani A, Moller DE. Modulation of fatty acid metabolism as a potential approach to the treatment of obesity and the metabolic syndrome. *Endocrine* 2006;29:91-100.
- [4] Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of fatty acids and insulin action. *Ann NY Acad Sci* 2002;967:183-95.
- [5] Folsom AR, Ma J, McGovern PG, Eckfeldt H. Relation between plasma phospholipid saturated fatty acids and hyperinsulinemia. *Metabolism* 1996;45:223-8.
- [6] Lovejoy JC, Champagne CM, Smith SR, et al. Relationship of dietary fat and serum cholesterol ester and phospholipid fatty acids to markers of insulin resistance in men and women with a range of glucose tolerance. *Metabolism* 2001;50:86-92.
- [7] Laaksonen DE, Lakka TA, Lakka HM, et al. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. *Diabet Med* 2002;19:456-64.
- [8] Vessby B, Tengblad S, Lithell H. Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. *Diabetologia* 1994;37:1044-50.
- [9] Cohen P, Miyazaki M, Socci ND, et al. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science* 2002;297:240-3.
- [10] Cohen P, Friedman JM. Leptin and the control of metabolism: role for stearoyl-CoA desaturase-1 (SCD-1). *J Nutr* 2004;134:2455S-63S.
- [11] Ntambi JM. Dietary regulation of stearoyl-CoA desaturase 1 gene expression in mouse liver. *J Biol Chem* 1992;267:10925-30.
- [12] Waters KM, Ntambi JM. Polyunsaturated fatty acids inhibit hepatic stearoyl-CoA desaturase-1 gene in diabetic mice. *Lipids* 1996;31 (Suppl):S33-6.
- [13] Miyazaki M, Kim YC, Ntambi JM. A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase 1 gene reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis. *J Lipid Res* 2001;42:1018-24.
- [14] Mangravite LM, Dawson K, Davis RR, Gregg JP, Krauss RM. Fatty acid desaturase regulation in adipose tissue by dietary composition is independent of weight loss and is correlated with the plasma triacylglycerol response. *Am J Clin Nutr* 2007;86:759-67.
- [15] Miyazaki M, Kim YC, Gray-Keller MP, Attie AD, Ntambi JM. The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. *J Biol Chem* 2000;275:30132-8.
- [16] Warensjö E, Risérus U, Vessby B. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. *Diabetologia* 2005;48:1999-2005.
- [17] Warensjö E, Öhrvall M, Vessby B. Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women. *Nutrition, Metab Cardiovasc Dis* 2006;16:128-36.
- [18] Okada T, Furuhashi N, Kuromori Y, Miyashita M, Iwata F, Harada K. Plasma palmitoleic acid content and obesity in children. *Am J Clin Nutr* 2005;82:747-50.
- [19] Masood A, Stark KD, Salem Jr N. A simplified and efficient method for the analysis of fatty acid methyl esters suitable for large clinical studies. *J Lipid Res* 2005;46:2299-305.
- [20] Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res* 1986;27:114-20.
- [21] Smedman AEM, Gustafsson IB, Berglund LGT, Vessby BOH. Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors. *Am J Clin Nutr* 1999;69:22-9.
- [22] Baylin A, Kim MK, Donovan-Palmer A, et al. Fasting whole blood as a biomarker of essential fatty acid intake in epidemiologic studies: comparison with adipose tissue and plasma. *Am J Epidemiol* 2005;162:373-81.
- [23] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004;27:1487-95.
- [24] Kutner MH, Nachtsheim CJ, Neter J, Li W. *Applied linear statistical models*. 5th ed. New York: McGraw-Hill; 2005.
- [25] Hittner JB, May K. DEPCORR: a SAS program for comparing dependent correlations. *Appl Psychol Meas* 1998;22:93-4.
- [26] Ntambi JM, Miyazaki M. Regulation of stearoyl-CoA desaturases and role in metabolism. *Prog Lipid Res* 2004;43:91-104.
- [27] Heinemann FS, Ozols J. Stearoyl-CoA desaturase, a short-lived protein of endoplasmic reticulum with multiple control mechanisms. *Prostaglandins Leukot Essent Fatty Acids* 2003;68:123-33.
- [28] Biddinger SB, Miyazaki M, Boucher J, Ntambi JM, Kahn CR. Leptin suppresses stearoyl-CoA desaturase 1 by mechanisms independent of insulin and sterol regulatory element-binding protein-1c. *Diabetes* 2006;55:2032-41.
- [29] Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of gene expression. *Nutr Rev* 2004;62:333-9.
- [30] Fougère F, Perdereau D, Gouhot B, Ferre P, Girard J. Effect of diets rich in medium-chain and long-chain triglycerides on lipogenic-enzyme gene expression in liver and adipose tissue of the weaned rat. *Eur J Biochem* 1992;208:381-7.
- [31] Attie AD, Krauss RM, Gray-Keller MP, et al. Relationship between stearoyl-CoA desaturase activity and plasma triglycerides in human and mouse hypertriglyceridemia. *J Lipid Res* 2002;43:1899-907.
- [32] Hulver MW, Berggren JR, Carper MJ, et al. Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. *Cell Metabolism* 2005;2:251-61.
- [33] Clifton PM, Nestel PJ. Relationship between plasma insulin and erythrocyte fatty acid composition. *Prostaglandins Leukot Essent Fatty Acids* 1998;59:191-4.
- [34] Wang L, Folsom AR, Zheng ZJ, Pankow JS, Eckfeldt JH. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr* 2003;78:91-8.
- [35] Clarke SD, Gasperikova D, Nelson C, Lapillonne A, Heird WC. Fatty acid regulation of gene expression: a genomic explanation for the benefits of the Mediterranean diet. *Ann NY Acad Sci* 2002;967:283-98.
- [36] Manco M, Calvani M, Mingrone G. Effects of dietary fatty acids on insulin sensitivity and secretion. *Diabetes Obes Metab* 2004;6:402-13.
- [37] King IB, Lemaitre RN, Kestin M. Effect of a low-fat diet on fatty acid composition in red cells, plasma phospholipids, and cholesterol esters: investigation of a biomarker of total fat intake. *Am J Clin Nutr* 2006;83:227-36.
- [38] Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-

- acid composition of skeletal-muscle phospholipids. *N Engl J Med* 1993;328:238-44.
- [39] Samuelson G, Bratteby LE, Mohsen R, Vessby B. Dietary fat intake in healthy adolescents: inverse relationships between the estimated intake of saturated fatty acids and serum cholesterol. *Br J Nutr* 2001;85:333-41.
- [40] Rodríguez Y, Christophe AB. Long-chain [omega]6 polyunsaturated fatty acids in erythrocyte phospholipids are associated with insulin resistance in non-obese type 2 diabetics. *Clin Chim Acta* 2005;354:195-9.
- [41] Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ. *Modern nutrition in health and disease*. 10th ed. Philadelphia: Lippincott William & Wilkins; 2005.
- [42] Farmer A, Montori V, Dinneen S, Clar C. Fish oil in people with type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2001:CD003205.
- [43] Nettleton JA, Katz R. n-3 long-chain polyunsaturated fatty acids in type 2 diabetes: a review. *J Am Diet Assoc* 2005;105:428-40.
- [44] Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. *Am J Clin Nutr* 2007;86:74-81.