

The effect of FADS2 gene rs174583 polymorphism on desaturase activities, fatty acid profile, insulin resistance, biochemical indices, and incidence of type 2 diabetes

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Background: In this study, we investigated the associations of erythrocytes fatty acid composition, activities of delta-5 desaturase (D5D) and delta-6 desaturase (D6D), and other metabolic risk factors, with type 2 diabetes (T2D) risk to determine if rs174583 polymorphism of FADS2 gene had any effect on these associations. **Materials and Methods:** Fatty acid profile of erythrocytes was determined using gas chromatography-mass spectrometry in 95 T2D patients and 95 apparently healthy participants. The genotypes of single-nucleotide polymorphism (SNP) of FADS2 gene were determined using the polymerase chain reaction-restriction fragment length polymorphism technique. Other biochemical parameters were measured in the serum using standard analytical procedures. **Results:** D6D activity was increased ($P < 0.001$) and D5D activity was decreased in T2D patients ($P < 0.001$) compared to controls. Homeostatic model assessment insulin resistance (HOMA-IR) index was positively correlated with D6D ($r = 0.34$, $P < 0.001$) and negatively correlated with D5D ($r = -0.19$, $P = 0.02$). Palmitic acid ($P < 0.001$) and dihomo-gamma-linolenic acid ($P = 0.03$) were higher and linoleic acid ($P < 0.001$) and arachidonic acid (AA) ($P < 0.001$) were lower in T2D patients. The distribution of rs174583 genotypes which includes C/T, C/C, and T/T was not different in the two groups ($P = 0.63$). **Conclusion:** In the population studied, there was a strong association in the erythrocytes fatty acid composition, D5D and D6D activities and other metabolic risk factors between non-T2D and T2D patients. In addition, there was a strong association in erythrocytes DGLA and AA contents and D5D activities between rs174583 genotypes in all participants. However, the distribution of rs174583 genotypes did not differ significantly between T2D patient and controls, and it did not appear to be an association between rs174583 SNP and incident of T2D.

Key words: Fatty acid desaturase, fatty acid profile, single-nucleotide polymorphism, type 2 diabetes

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INTRODUCTION

Type 2 diabetes (T2D) is considered a multifactorial disease.^[1] Recently, there increasing evidence to support the hypothesis that a considerable inflammatory component can be as result of T2D and its development toward cardiovascular diseases. There is correlation with abnormal metabolism of fatty acids.^[2] Considering the broad roles that fatty acids have in various cellular functions, from membrane fluidity to gene expression regulation and cell signaling, there may

be an effective role in the prevention or progression of these diseases.^[3,4]

Delta-5- (D5D) and delta-6-desaturases (D6D) are two key enzymes regarded as the rate-limiting enzymes in the pathway of long-chain polyunsaturated fatty acids (LC-PUFA) biosynthesis [Figure 1]. D5D and D6D are encoded by Fatty acid desaturase1 (FADS1) and fatty acid desaturase 2 (FADS2) genes, respectively. These gene clusters are located on the chromosom11 (11q12-13.1) with a head-to-head direction.^[5]

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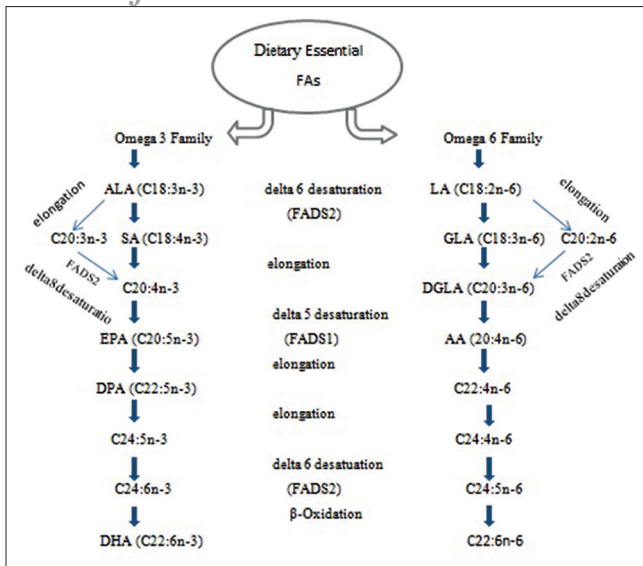


Figure 1: Pathways of LC-PUFA synthesis from omega-6 and omega-3 essential PUFAs. ALA = α -Linoleic acid; AA = arachidonic acid; DGLA = dihomo-gamma-linoleic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; GLA = gamma-linoleic acid; LA = linoleic acid; SA = stearidonic acid; LC-PUFA = Long-chain polyunsaturated fatty acids

Recently, several studies have demonstrated strong relationships between single-nucleotide polymorphisms (SNPs) in FADS1 and FADS2 genes with alteration in the activity of D5D and D6D enzymes which lead to changes in the profile of endogenous fatty acids. This may play an important role in the pathogenesis of diabetes and predispose a person to heart attacks.^[6,7]

In this study, we aim to investigate the associations between rs174583 polymorphism of FADS2 gene with T2D risk in an Iranian population. We also investigated if this polymorphism had any effect on erythrocytes fatty acid composition, activities of D5D and D6D enzymes, and other metabolic risk factors in this population of non-T2D and T2D patients.

MATERIALS AND METHODS

Study population

In this case-control study, two groups of men between 40 and 60 years of age were recruited from health centers associated to Isfahan University of Medical Sciences: The study subjects consisted of, the patients group, 95 men with T2D and fasting plasma glucose (FPG) ≥ 126 mg/dl, and the control group, 95 healthy men with no history of diabetes and no other chronic and acute illnesses, FPG < 100 mg/dl and triglycerides (TG) < 200 mg/dl who were checked regularly at health centers. All participants entered the study voluntarily after giving written informed consent and filling out a clinical questionnaire. Participants were excluded from the study if they were receiving insulin or were smoking. Participants were questioned on the usage of fish oil supplements, and

those who were taking fish oil supplements were excluded from the study. Likewise, patients with any unusual or special diet were excluded from the study.

Collection and preparation of blood samples

Venous blood samples were obtained after at least 10–12 h overnight fasting from participating individuals by venipuncture and collected into two ethylenediaminetetraacetic acid (EDTA) tubes. One of the blood tubes was immediately frozen at -80°C for DNA extraction and molecular studies. The second tube was centrifuged at 2000 rpm for 10 min at room temperature, and plasma was removed and used for the biochemical assays.

After removal of the plasma, the remaining content of the tube which contains white blood cells, platelets, and red blood cells (RBCs) were washed three times with an equal volume of isotonic saline (9%) to remove the residual plasma and buffy coat. The final pellet was centrifuged at 2000 rpm for 5 min and the washed packed RBCs (hematocrit = 98%) was added in aliquots of 1 ml to a microtube containing 0.1 mg butylated hydroxytoluene (BHT), as an anti-oxidant, and were immediately stored at -80°C , until the analysis of fatty acid profile.

Biochemical assays

FPG, TG, high-density lipoprotein cholesterol (HDL-C) were assayed by enzymatic methods using commercial kits (Pars Azmun, Iran) using a BT4500 Autoanalyzer (biotecinca, Italy). Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedwald formula for specimens with plasma TG levels < 400 mg/dl. For specimens with TG levels > 400 mg/dl, LDL-C level was measured using a commercial enzymatic assay. Fasting plasma insulin was measured by immunoenzymometric assay using Monobind kit (USA). Insulin resistance index (IR) was computed with the homeostatic model assessment (HOMA) using the following formula: $\text{IR} = (\text{fasting insulin } [\mu\text{IU/ml}] \times \text{fasting glucose } [\text{mmol/l}]) / 22.5$.^[8]

Analysis of fatty acids composition in erythrocyte

In this study, fatty acids of RBCs were extracted and methylated using a one-step procedure according to Raquel *et al.* with modification.^[9] Briefly, 150 μL of RBCs sample was added to a screw-cap glass vial containing 25 μg of internal standard heptadecanoic acid (C17:0) and 100 μg of BHT and mixed with 1 ml of methanolic NaOH solution (1N). Saponification was performed at 85°C for 10 min. Transmethylation was then performed by adding 2 ml H_2SO_4 (1N) in methanol, flushing with N_2 and heating at 85°C for 40 min. After cooling, 0.5 ml of saturated solution of NaCl and 1 ml of hexane were added and tubes were vigorously shaken for 1 min. The upper hexane layer containing fatty acid methyl esters (FAMES)

was collected, washed with 1 ml of HPLC grade water and dried with 1 g sodium sulfate anhydrous (Na_2SO_4). The volume of the extract was then reduced to about 100 μl under N_2 gas and samples were transferred to GC vials for further analysis.

FAMES were analyzed by GC-MS (HP6890, Agilent technologies) equipped with a SP-2560 column (100 m \times 0.25 mm ID \times 0.2 μm film thickness) (Supelco, Bellefonte, PA, USA) and Detector (MSD HP 6890) as described elsewhere.^[10]

To assess the reproducibility of the erythrocytes fatty acid analysis, the intra-assay coefficient of variation (CVs) was determined by the analysis of 5 aliquots of a pooled blood sample in the same day and was found to be <5%. The interassay reproducibility was assessed by analysis of 10 aliquots of the same sample on different days spread over 2 months. The interassay imprecision (CV) for all the fatty acids measurement was <6%, except for EPA which had a CV of 9.3%.

Measurement of desaturases activity

The activity of desaturase enzymes was estimated from the concentration ratio of the enzyme product to its substrate. Thus, D6D activity was estimated using the ratio of dihomo-gamma-linoleic acid (DGLA, C20:3n-6) to linoleic acid (LA, C18:2n-6), and D5D activity was estimated from the ratio of arachidonic acid (AA, C20:4n-6) to DGLA (C20:3n-6).^[11]

Genotyping

In this study, SNP rs174583 that is located in the position chr11:61842278 in the intron region of FADS2 gene, was investigated, based on minor allele frequency obtained from previous studies.^[12] Genomic DNAs was extracted from whole blood using a DNA isolation kit according to the manufacturer protocol (Genet Bio, Korea). SNP rs174583 was genotyped using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method previously described.^[13]

Primers of forward and reverse were designed with DNASIS software (version 3, Hitachi Software Engineering, Japan) and their specific binding to the desired area was confirmed using BLAST software (<http://primerBLAST>). The sequences of forward and reverse primers used were respectively as follows: (5'AGGAAGCAGACCACAGAGTC3') and (5'ATCTGGAAGCAGGACACAGC3').

Using a program at (http://endonuclease_mapper) to find the restriction sites on the sequence of the amplified DNA, *TauI* was selected as the restriction enzyme since its digestion site could be introduced by mutation at SNP rs174583.

Statistical methods

Statistical analyses were performed using SPSS (version 20.0, IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test was used to assess normality of the variables. Normal quantitative variables were reported as mean \pm SD. Non-normal quantitative variables were shown as median and interquartile range. Log transformation was performed on some of the skewed variables before analysis to reach a normal distribution. To compare the parameters including, clinical characteristics, RBCs fatty acid composition, and desaturase activity, student t-test and K-independent non-parametric analysis (Mann-Whitney U test) were used for the normal and non-normal distribution, respectively. The Pearson's chi-square test was performed for comparing allele frequencies among two groups of control and T2D. Pearson coefficient was used to associate some of normal variables with each other in all subjects. One way ANOVA and Bonferroni correction tests were used to compare the variables between genotypes in each group. $P < 0.05$ was considered as statistically significance.

RESULTS

Clinical characteristics, erythrocyte fatty acid composition, desaturase activity

Clinical characteristics, erythrocyte fatty acid composition, and desaturase activity of the two groups are presented in Table 1. All the characteristics studied, except age and diastolic blood pressure were different between the two groups ($P < 0.05$). The composition of fatty acids in erythrocytes was different among T2D patients compared to controls, in that, palmitic acid (PA) (C16:0) and dihomo-gamma-linolenic acid (DGLA) (C20:3n-6) were increased ($P < 0.001$ and $P = 0.03$, respectively), and LA (C18:2n-6) and AA (C20:4n-6) were decreased ($P < 0.001$ and $P < 0.001$ respectively), in T2D patients compared to controls. Eicosapentaenoic acid (EPA) (C20:5n-3) was slightly, but nonsignificantly, decreased in T2D patients. There was no differences in the contents of other fatty acids measured (i.e., stearic acid [SA] (C18:0), oleic acid (C18:1n-9), and docosahexaenoic acid (DHA) (C22:6n-3) in the two groups [Table 1].

Consequently, D6D activity was increased ($P < 0.001$) and D5D activity ($P < 0.001$) was decreased in T2D patients compared to controls [Table 1].

Correlation between homeostatic model assessment-insulin resistance and body mass index with desaturase activity and erythrocyte fatty acid composition in all subjects

HOMA-IR and insulin were significantly higher in T2D patients compared to controls, as expected. Using the Pearson correlation coefficient, in all participants, HOMA-IR had a significant positive correlation with D6D activity ($r = 0.34$, $P < 0.001$), PA content ($r = 0.4$, $P < 0.001$),

Table 1: Clinical and biochemical characteristics of the study participants

Characteristics	Controls (n=95)	T2D (n=95)	P
Age (year)	53.23±10.87	53.67±8.47	0.33
BMI (kg/m ²)	26.24±3.71	27.87±3.50	0.002
Waist (cm)	92.72±8.57	97.67±13.25	0.002*
Systolic BP (mmHg)	116.11±17.12	124.60±13.93	<0.001*
Diastolic BP (mmHg)	77.37±12.80	77.58±5.61	0.28*
TC (mg/dl)	194.83±31.87	171.64±33.96	<0.001
TG (mg/dl) [†]	123.36±46.83	183.82±113.96	<0.001
HDL-C (mg/dl)	50.97±7.99	47.29±7.97	<0.001
LDL-C (mg/dl)	118.65±28.49	89.38±30.88	<0.001
FPG (mg/dl) [‡]	97.36 (93.27, 99.25)	145.34 (132.46, 178.52)	<0.001*
Insulin (μlu/ml) [‡]	2.61 (1.95, 3.46)	4.63 (3.60, 11.82)	<0.001*
HOMA-IR [†]	1.08±0.16	4.51±0.63	<0.001
FAs (percentage total of FAs)			
PA (C16:0) [‡]	26.45 (25.59, 27.66)	30.70 (26.78, 34.43)	<0.001*
SA (C18:0) [†]	23.89±1.89	23.99±2.70	0.86
OA (C18:1n-9)	12.61±2.11	12.87±1.51	0.57*
LA (C18:2n-6) [†]	11.31±1.76	8.85±2.10	<0.001
DGLA (C20:3n-6) [†]	1.27±0.33	1.37±0.36	0.03
AA (C20:4n-6) [†]	16.81±2.40	15.64±2.60	<0.001
EPA (C20:5n-3) [†]	0.15±0.05	0.13±0.11	0.1
DHA (C22:6n-3) [†]	3.01±0.82	2.95±1.11	0.5
Estimated desaturase activity			
C20:4n-6/C20:3n-6 (D5D)	14.11±4.19	12.05±3.23	<0.001
C20:3n-6/C18:2n-6 (D6D)	0.11±0.02	0.16±0.03	<0.001

Data are presented as mean±SD except those with nonnormal distribution: [‡]median (25 percentiles, 75 percentiles) *P*<0.05 was considered statistically significant. [†]mean±SD the data were logarithmically transformed. *Mann-Whitney U-test were used. T2D= Type II diabetes; BP= Blood pressure; BMI= Body mass index; TC= Total-cholesterol; TG= Triglyceride; HDL-C= High-density lipoprotein cholesterol; LDL-C= Low density lipoprotein cholesterol; FPG= Fasting plasma glucose; HOMA-IR= Homeostatic model assessment-insulin resistance; SD= Standard deviation; D5D= Delta 5 desaturase; D6D= Delta 6 desaturase; DHA= Docosahexaenoic acid, PA: Palmitic acid; SA= Stearic acid; OA= Oleic acid; LA= Linoleic acid; AA= Arachidonic acid; FAs= Fatty acids; EPA= Eicosapentaenoic acid; DGLA=Dihomo-gamma-linolenic acid

and body mass index (BMI) (*r* = 0.17, *P* = 0.04). Whereas, it was negatively associated with D5D activity (*r* = -0.19, *P* = 0.02) and erythrocytes LA content (*r* = -0.39, *P* < 0.001). Furthermore, BMI was positively correlated with D6D activity (*r* = 0.27, *P* < 0.001) and PA (*r* = 0.24, *P* < 0.001) content and negatively associated with D5D activity (*r* = -0.16, *P* = 0.03) and LA content (*r* = -0.24, *P* < 0.001) [Table 1].

Distribution of rs174583 genotypes

The observed genotypes of rs174583 polymorphism included C/T, C/C, and T/T. No significant difference between frequency distributions of each genotype in the control and T2D groups was observed (*P* = 0.63). Highest and lowest percentage of genotypes frequencies were for CT with 81.6%, CC 5.8%, and TT with 12.6% in total participants [Table 2].

The effect of rs174583 genotypes on plasma lipids, erythrocyte fatty acid composition, and desaturase activity

No significant difference was observed in plasma lipid levels (i.e. TC, HDL-C, and LDL-C), with the exception of TG, between different genotypes in each group. In T2D patients, TG was significantly higher in TT genotype (minor allele homozygote), and lower in CC (major allele homozygote) compared to CT groups. In

Table 2: Genotypic frequency of “rs 174583” in type II diabetes patients and control group

Genotype	T2D group (n=95), n (%)	Control group (n=95), n (%)	P
CT	80 (84.0)	75 (79.0)	0.63
CC	5 (5.0)	6 (6.3)	
TT	10 (11.0)	14 (14.7)	

χ²=0.9, df=2. T2D=Type II diabetes

control group, HOMA-IR was higher in TT genotype and lower in CC compared to CT, although the difference was not statistically significant. DGLA was significantly higher in TT group and lower in CC compared to CT in both T2D patients and control group. AA was significantly lower in TT genotype and higher in CC compared to CT in both the study groups. DHA was also lower in TT genotype and higher in CC compared to CT in both the study groups. Although, the difference was statistically significant only between TT and CC genotypes in T2D patients. D5D activity was significantly lower in TT genotype and higher in CC compared to CT in both study groups, whereas, D6D activity was higher in TT group and lower in CC compared to CT in both the study groups. Although the difference was statistically significant only in control group [Table 3].

Table 3: The relationship between fatty acid desaturase 2 rs174583 genotypes and biochemical parameters

Characteristics	Controls			P	T2D			P
	CC (5)	CT (80)	TT (10)		CC (6)	CT (75)	TT (14)	
TC (mg/dl)	204.3±39.31	202±32.73	197.64±24.31	0.70	149.61±32.12	171.57±34.85	183.22±22.62	0.20
TG (mg/dl)	130.24±24.52	119.73±49.63	139.85±35.32	0.33	105.25±31.24 ^{†a}	153.33±72.43 ^{†, a, b}	276.54±68.42 ^{†, b}	0.01*
HDL-C (mg/dl)	52.35±10.91	49.91±712.1	51.64±5.95	0.61	48.33±6.41	47.81±7.72	47.83±7.50	0.73
LDL-C (mg/dl)	126.51±33.52	12731±28.15	118.52±24.41	0.82	80.62±32.53	90.43±32.22	85.15±20.12	0.70
FPG (mg/dl)	93.25±6.22	96.33±4.76	95.54±6.23	0.28	140.22±20.53 [†]	161.34±35.43 [†]	144.93±18.42 [†]	0.16
HOMA-IR	0.56±0.11 [†]	1.10±0.18 [†]	1.51±0.61 [†]	0.40	5.32±3.51 [†]	4.24±0.61 [†]	6.75±2.35 [†]	0.44
PA (C16:0)	25.61±0.60 [†]	27.02±2.22 [†]	26.80±1.53 [†]	0.17	31.11±6.52	30.60±4.53	32.44±3.30	0.41
SA (C18:0)	23.92±0.93 [†]	23.71±1.82 [†]	24.81±2.12 [†]	0.07	24.32±1.33	23.72±2.81	25.73±1.82	0.09
OA (C18:1n-9)	12.90±0.72 [†]	12.71±1.93 [†]	11.70±3.13 [†]	0.40	12.22±1.73	12.93±1.41	12.72±1.90	0.57
LA (C18:2n-6)	10.33±1.51 ^a	11.05±1.64 ^a	12.85±1.80 ^b	0.001*	7.53±2.60	8.91±2.06	9.12±1.11	0.32
DGLA (C20:3n-6)	0.99±0.14 ^{†, a}	1.22±0.21 ^{†, a}	1.71±0.30 ^{†, b}	<0.001*	1.08±0.11 ^a	1.35±0.32 ^{a, b}	1.64±0.42 ^b	0.02*
AA (C20:4n-6)	18.42±1.12 ^{†, a}	16.93±2.53 ^{†, a}	15.40±1.32 ^{†, b}	0.001*	17.45±1.92 ^{†, a}	15.73±2.44 ^{†, a}	13.63±3.15 ^{†, b}	0.01*
EPA (C20:5n-3)	0.15±0.04	0.15±0.05	0.14±0.04	0.22	0.15±0.15 [†]	0.14±0.11 [†]	0.12±0.07 [†]	0.16
DHA (C22:6n-3)	3.51±0.23 [†]	3.02±0.80 [†]	2.70±0.52 [†]	0.11	3.24±1.11 ^a	3.04±1.12 ^{a, b}	2.12±0.80 ^b	0.04*
AA/DGLA (D5D)	18.83±2.90 ^a	14.52±3.81 ^c	9.41±2.10 ^b	<0.001*	16.42±0.63 ^a	12.24±3.05 ^c	8.82±2.80 ^b	<0.001*
DGLA/LA (D6D)	0.09±0.02 ^a	0.11±0.02 ^a	0.13±0.02 ^b	0.005*	0.14±0.04	0.15±0.03	0.18±0.04	0.13

Data are presented as mean±SD. FAs are presented as percentage of total FAs. A number of participants in each group are given in parenthesis. [†]The data were logarithmically transformed. **P*<0.05 by one-way ANOVA was considered statistically significant. ^{a,b,c}Letters represent a significant difference at *P*<0.05 by the Bonferroni correction. T2D= Type II diabetes; TC= Total-cholesterol; TG= Triglyceride; HDL-C= High-density lipoprotein cholesterol; LDL-C= Low density lipoprotein cholesterol; FPG= Fasting plasma glucose; HOMA-IR= Homeostatic model assessment-insulin resistance; AA=Arachidonic acid; LA= Linoleic acid; DGLA=Dihomo-gamma-linolenic acid; D5D= Delta 5 desaturase; D6D= Delta 6 desaturase; DHA= Docosahexaenoic acid, PA= Palmitic acid; SA= Stearic acid; OA= Oleic acid; LA= Linoleic acid; FAs=Fatty acids; EPA= Eicosapentaenoic acid; DGLA=Dihomo-gamma-linolenic acid

DISCUSSION

In the present study, the association of FADS2 gene rs174583 SNP with erythrocytes fatty acids composition and serum lipids profile was investigated in male T2DM patients and controls. There seems to be sex and gender differences in risk, pathophysiology, and complications of T2DM attributable to biological, lifestyle, and psychosocial factors. To eliminate these compounding effects, only male participants were investigated in the study.

The D5D and D6D are key enzymes involved in the metabolism of PUFAs. In this study, we investigated the associations of erythrocytes fatty acid composition and activities of D5D and D6D, with T2D risk to determine if rs174583 polymorphism of FADS2 gene had any effect on these associations.

The findings of this study showed that control group had higher levels of erythrocytes n-6 PUFA, AA, LA, and D5D activity compared to T2D patients. Conversely, T2D patients had higher levels of erythrocytes DGLA and D6D activity compared to controls. These findings are consistent with a recent paper by Yary *et al.*^[14] who reported that higher serum total n-6 PUFA, LA, and AA levels and D5D activity were associated with a lower risk of incident T2D, and higher GLA and DGLA levels and D6D activity were associated with a higher risk.

Other prospective studies that investigated desaturase activity estimated from fatty acid profile in blood also reported a clear

direct relation of D6D activity and a strong inverse relation of D5D activity with T2D incidence.^[15] D6D activity is increased by insulin and the DGLA concentration is regulated by D6D activity.^[16] Hyperinsulinemia may increase the expression of D6D leading to increased DGLA concentration in T2D patients. Therefore, factors affecting the activity of these desaturases are may have public health implications.

PA was significantly higher in our T2D patients (*P* < 0.001) which in is in line with several previous studies demonstrating its relationship with insulin resistance and T2D development.^[17] We observed that PA had a positive strong correlation with HOMA-IR. The exact mechanism of induced insulin resistance by PA is still unknown, but it seems that it acts through inhibition of insulin receptor substrate and kinase cascade.^[18] Furthermore, PA was directly correlated with BMI. Studies in mice have shown that PA-rich diets may lead to alteration in the gut microbiotic and stimulation of weight gain relative to non-saturated fatty acids.^[19]

In addition, LA content was significantly lower in our T2D patients compared with control group, and there was a clear negative association with HOMA-IR and BMI. Recently,

Fujii *et al.* reported that low levels of plasma LA were associated with an increase in visceral fat, FPG, HbA1c, systolic BP, and HOMA-IR in Japanese participants.^[20] This is presumably because that the increase in adipose tissue

mass leads to induced insulin resistance and impaired glucose and lipid metabolism and that has a significant role in predisposing individuals to T2D.^[21] It may be relevant to note that several studies have demonstrated that a lower serum LA and increased PA and DGLA levels and increased D6D activity can increase the risk of cardiovascular diseases.^[22-25]

Several studies have shown that T2D is associated with disturbances in plasma lipids and lipoproteins metabolisms such as TG, TC, HDL-C, and LDL-C.^[26] In our study, T2D patients had decreased levels of TC and LDL-C in comparison to the control group. The main reason for this is the use of cholesterol-lowering drugs in these participants. These drugs are competitive inhibitors of HMG-COA reductase, inhibiting the endogenous pathway of cholesterol synthesis, and finally reducing the serum cholesterol.

In this study, we observed that participants with increased BMI had higher estimated D6D and lower D5D activities. Furthermore, HOMA-IR was correlated positively with D6D and negatively with D5D activities. Warensjö *et al.* reported that obesity was directly correlated to D6D and inversely associated with D5D activities.^[27] Other studies have also reported increased activities of D6D associated with obesity, diabetes, and metabolic syndrome in adult.^[28,29] Choi *et al.* also found that D6D activity had a positive correlation with HOMA-IR. They concluded that increase in DGLA and D6D leads to lower expression in D5D and the development of insulin resistance in obese individuals and predispose them to the metabolic disease.^[28] Similarly, Murff and Edwards reported that D5D and D6D activities have a potential correlation with insulin resistance and T2D; D5D activity with a lower risk and D6D activity with a higher risk of incident T2D.^[30]

Sone *et al.*^[31] in Japan have investigated the association between a number of variants of D5D and D6D gene FAD1 and FAD2 including rs174583 and fatty acid composition and found no correlation between erythrocytes fatty acids composition and D5D and D6D activities between the three genotypes in controls. However, they reported higher levels of TG in TT genotypes and lower levels in CC compared to CT group. In contrast, Schaeffer *et al.*^[32] in Germany showed a strong association between fatty acid composition of serum phospholipids and the FADS1 FADS2 gene clusters. They showed that individuals carrying the minor T alleles have increased levels of the fatty acids LA, DGLA, and α -LA and decreased levels of GLA, AA, and EPA, a finding similar to the present study.

We carried out genotyping of SNP rs174583 of FADS2 gene cluster by PCR-RFLP method for 190 participants. From the data presented here, it seems that persons with TT

genotype have higher levels of plasma TG, higher content of erythrocytes LA and DGLA and D6D activity, and lower content of AA and DHA and D5D activity.

CONCLUSIONS

There was a strong association in the erythrocytes fatty acid composition, D5D and D6D activities and other metabolic risk factors between non-T2D and T2D patients in the population studied. Furthermore, there was a strong association in erythrocytes DGLA and AA contents and D5D activities between rs174583 genotypes in all participants. However, the distribution of rs174583 genotypes did not differ significantly between T2D patient and controls, and it does not appear that having the minor TT genotype alone would significantly increase the risk of incidence T2D, as suggested by some studies.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Hansen T. Type 2 diabetes mellitus – A multifactorial disease. *Ann Univ Mariae Curie Sklodowska Med* 2002;57:544-9.
- Glass CK, Olefsky JM. Inflammation and lipid signaling in the etiology of insulin resistance. *Cell Metab* 2012;15:635-45.
- Das UN. Essential fatty acids: Biochemistry, physiology and pathology. *Biotechnol J* 2006;1:420-39.
- Ibarguren M, López DJ, Escribá PV. The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health. *Biochim Biophys Acta* 2014;1838:1518-28.
- Marquardt A, Stöhr H, White K, Weber BH. cDNA cloning, genomic structure, and chromosomal localization of three members of the human fatty acid desaturase family. *Genomics* 2000;66:175-83.
- Rzehak P, Heinrich J, Klopp N, Schaeffer L, Hoff S, Wolfram G, *et al.* Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. *Br J Nutr* 2009;101:20-6
- Li SW, Wang J, Yang Y, Liu ZJ, Cheng L, Liu HY, *et al.* Polymorphisms in FADS1 and FADS2 alter plasma fatty acids and desaturase levels in type 2 diabetic patients with coronary artery disease. *J Transl Med* 2016;14:79.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC, *et al.* Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin

- concentrations in man. *Diabetologia* 1985;28:412-9.
9. Rodrigues RO, Costa H, Lima R, Amaral JS. Simple methodology for the quantitative analysis of fatty acids in human red blood cells. *Chromatographia* 2015;78:1271-81.
 10. Namazi G, Asa P, Movahedian A, Sarrafzadegan N, Sadeghi M, Pourfarzam M, *et al.* Investigation of membrane fatty acid profiles in erythrocytes of patients with stable coronary artery disease. *J Clin Lipidol* 2016;10:930-6.
 11. Bokor S, Dumont J, Spinneker A, Gonzalez-Gross M, Nova E, Widhalm K, *et al.* Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-6 desaturase activities estimated by serum fatty acid ratios. *J Lipid Res* 2010;51:2325-33.
 12. Malerba G, Schaeffer L, Xumerle L, Klopp N, Trabetti E, Biscuola M, *et al.* SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. *Lipids* 2008;43:289-99.
 13. Ota M, Fukushima H, Kulski JK, Inoko H. Single nucleotide polymorphism detection by polymerase chain reaction-restriction fragment length polymorphism. *Nat Protoc* 2007;2:2857-64.
 14. Yary T, Voutilainen S, Tuomainen TP, Ruusunen A, Nurmi T, Virtanen JK, *et al.* Serum n-6 polyunsaturated fatty acids, Δ5- and Δ6-desaturase activities, and risk of incident type 2 diabetes in men: The Kuopio Ischaemic heart disease risk factor study. *Am J Clin Nutr* 2016;103:1337-43.
 15. Kröger J, Schulze MB. Recent insights into the relation of Δ5 desaturase and Δ6 desaturase activity to the development of type 2 diabetes. *Curr Opin Lipidol* 2012;23:4-10.
 16. Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of fatty acids and insulin action. *Ann N Y Acad Sci* 2002;967:183-95.
 17. Hirabara SM, Curi R, Maechler P. Saturated fatty acid-induced insulin resistance is associated with mitochondrial dysfunction in skeletal muscle cells. *J Cell Physiol* 2010;222:187-94.
 18. Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, *et al.* Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem* 2002;277:50230-6.
 19. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI, *et al.* Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A* 2005;102:11070-5.
 20. Fujii C, Kawai T, Azuma K, Oguma Y, Katsukawa F, Hirose H, *et al.* Relationships between composition of major fatty acids and fat distribution and insulin resistance in Japanese. *J Diabetes Res* 2017;2017:1567467.
 21. Fujioka S, Matsuzawa Y, Tokunaga K, Tarui S. Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. *Metabolism* 1987;36:54-9.
 22. Warensjö E, Sundström J, Vessby B, Cederholm T, Risérus U. Markers of dietary fat quality and fatty acid desaturation as predictors of total and cardiovascular mortality: A population-based prospective study. *Am J Clin Nutr* 2008;88:203-9.
 23. Petersson H, Basu S, Cederholm T, Risérus U. Serum fatty acid composition and indices of stearoyl-CoA desaturase activity are associated with systemic inflammation: Longitudinal analyses in middle-aged men. *Br J Nutr* 2008;99:1186-9.
 24. Kim OY, Lim HH, Lee MJ, Kim JY, Lee JH. Association of fatty acid composition in serum phospholipids with metabolic syndrome and arterial stiffness. *Nutr Metab Cardiovasc Dis* 2013;23:366-74.
 25. Kim SR, Jeon SY, Lee SM. The association of cardiovascular risk factors with saturated fatty acids and fatty acid desaturase indices in erythrocyte in middle-aged Korean adults. *Lipids Health Dis* 2015;14:133.
 26. Krauss RM. Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care* 2004;27:1496-504.
 27. Warensjö E, Ohrvall M, Vessby B. Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women. *Nutr Metab Cardiovasc Dis* 2006;16:128-36.
 28. Choi YS, Jang HB, Park JY, Lee HJ, Kang JH, Park KH, *et al.* Associations between estimated desaturase activity and insulin resistance in Korean boys. *Osong Public Health Res Perspect* 2014;5:251-7.
 29. 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.
 30. Murff HJ, Edwards TL. Endogenous production of long-chain polyunsaturated fatty acids and metabolic disease risk. *Curr Cardiovasc Risk Rep* 2014;8. pii: 418.
 31. Sone Y, Kido T, Ainuki T, Sonoda M, Ichi I, Kodama S, *et al.* Genetic variants of the fatty acid desaturase gene cluster are associated with plasma LDL cholesterol levels in Japanese males. *J Nutr Sci Vitaminol (Tokyo)* 2013;59:325-35.
 32. Schaeffer L, Gohlke H, Müller M, Heid IM, Palmer LJ, Kompauer I, *et al.* Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum Mol Genet* 2006;15:1745-56.