NEW APPROACHES IN THE DIAGNOSTICS OF GESTATIONAL DIABETES MELLITUS (GDM)- RESULTS OF THE STUDY

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INTRODUCTION

The gestational diabetes mellitus (GDM) is glucose intolerance that begins or is first recognized during pregnancy. It is currently a growing health problem worldwide affecting up to 14% of all pregnant women depending on the racial and ethnic group as well as the diagnostic and screening criteria. Both newborns and GDM mothers are susceptible to the development of type 2 diabetes.
In recent years, membrane fatty acid-based functional lipidomics has become a convenient and relevant molecular tool for examining the nutritional-metabolic status of patients compared to healthy controls, thus allowing lipidomic phenotypes to be identified in healthy and sick subjects. Membrane fatty acid-based functional lipidomics is a useful tool in molecular diagnostics that examines the levels of SFA and monounsaturated fatty acids (MUFA), either those synthesized endogenously or those obtained from the diet, as well as polyunsaturated fatty acids (PUFA) whose intake is essential for humans, with the assumption of precursors from the diet. Since all fatty acids are vital structural components of membrane phospholipids, they are necessary for proper fetus development [1]. Fatty acids content in plasma correlates with the ongoing diet, whereas their membrane lipid content occurs as a result of membrane structure and remodeling as the effect of physiological and pathological changes towards the homeostatic balance. In particular, MUFAs are biologically and pathophysiological significant molecules, especially for metabolic diseases. They are responsible for membrane fluidity, cell proliferation, lipid-mediated cytotoxicity, pathogenesis of obesity and cancer, programmed cell death and the unfolded protein response [2-4]. The main MUFAs are oleic, palmitoleic and vaccenic acids (VA). A recent study suggested an inverse association between the synthesis of VA in obese mice and the gluconeogenesis. Moreover, a higher concentration of VA in red blood cells is connected with a reduced risk of T2DM, lower fasting glucose and better insulin sensitivity. Taking into account that GDM is a metabolic disease that occurs quite suddenly in an otherwise healthy woman, early lipidomic evaluation could evidence the initial pathway of molecular imbalance, which is of particular significance when examining the membranes and their composition.

AIM

The aim of the present study, therefore, is to investigate the erythrocyte membranes fatty acid profile in GDM versus pregnant women without carbohydrate disturbance, and by doing so, envisage the presence of a specific fatty acid pathway as a biomarker of the molecular transformations involved in the onset of GDM.

MATERIALS AND METHODS

The blood of GDM patients was obtained from Department of Diabetology and Metabolic Diseases of MUL and the blood of NGT patients was obtained from Clinic of Obstetrics and Gynecology, M. Pirogow Hospital in Lodz. The criteria were as follows: elevated one or more plasma glucose level after a 2h/75g glucose tolerance test (OGGT); Caucasian ethnic background; age 25-35; no GDM in previous pregnancy; no family history of diabetes in first degree relatives; absence of concomitant diseases (chronic or acute infections); no taking insulin or other hypoglycemic medications.
Fatty acid-containing lipids from erythrocyte membranes were extracted with chloroform/methanol (2:1,v/v), converted to methyl esters, and analyzed with the use of gas chromatography with flame ionization detector (GC-FID) [5, 6].
Statistical analysis
Multivariate statistical analyses were performed. To explore the correlations between NGT and GDM groups, unsupervised Principle Component Analysis (PCA) were first applied.

RESULTS & CONCLUSIONS

The analysis of fatty acids profiles from erythrocytes revealed several differences between GDM and NGT women in the third trimester, and the results were correlated with biochemical data. Among the 14 measured FA representing the membrane lipidomic profile, the concentrations of three saturated FA (myristic, palmitic, and stearic acids) tended to decrease in GDM patients, with the percentage content of stearic acid significantly changed. Additionally, the relative content of monounsaturated fatty acids (MUFAs) tended to increase, in particular, the oleic acid and *cis*-vaccenic acid contents were significantly increased in erythrocyte membranes of the GDM group in comparison with the NGT group. Of special interest is the 14% increase of concentration of cis-vaccenic acid. This is the first time that such an association between impaired *cis*-vaccenic acid content in the erythrocyte membrane and GDM development has been evidenced. It is important to note that 11- *cis* octadecenoic acid, also known as *cis*-vaccenic acid (VA), is synthesized in humans from palmitic acid (hexadecanoic acid) which is converted to palmitoleic acid (9-*cis* hexadecenoic acid) by stearoyl-CoA-desaturase (Δ9-desaturase) (SCD; EC 1.14.19.1) and then to vaccenic acid by elongase ((ELOVL5/6 EC), scheme).

Table 1. Characteristics of the study population

	GDM*	NGT*	p
Age [years]	31.0 (28.0-35.0)	29.0 (28.0-30.0)	0.1144
BMI [kg/m^2]	23.7 (21.4-26.3)	20.9 (20.4-21.3)	0.0258
FPG [mg/dL]	85.0 (79.0-92.0)	77.5 (75.1-84.0)	0.0616
OGTT 120' [mg/dL]	160.0 (153.0-172.0)	99.6 (88.8-100.9)	<0.0001
CRP [mg/L]	3.5 (2.3-8.3)	1.5 (1.1-2.5)	0.0119
Insulin [μU/mL]	10.7 (6.6-19.8)	13.9 (7-15.5)	0.7746
HOMA-IR	2.0 (1.5-3.8)	2.3 (1.3-3.2)	0.5899
HOMA-B	203.3 (119.5-241.9)	287.3 (208.5-344.0)	0.0573
QUICKI	0.34 (-.032-0.37)	0.34 (0.31-0.36)	0.5899
Total cholesterol [mg/dL]	259.9 (233.3-283.4)	219.5 (191.5-242.0)	0.0070
HDL [mg/dL]	74.1 (57.0-86.0)	61.4 (53.9-67.1)	0.0802
LDL [mg/dL]	141.0 (116.0-173)	119.0 (105.0-139.0)	0.1567
TG [mg/dL]	215.0 (165.5-251.5)	157.6 (116.5-203.9)	0.0398

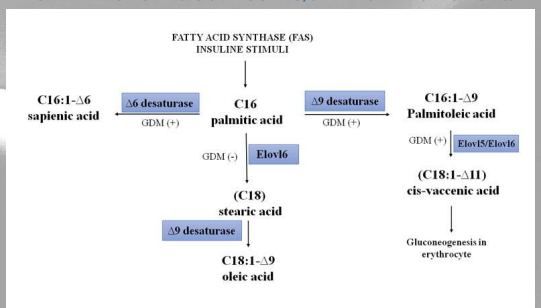
*P<0.05; the median is significantly different from corresponding control values.

Table 2. Erythrocyte membrane lipidomic profile using 14 fatty acids as a representative cluster.

Fatty acid$	GDM*	NGT*	Δ(%) **	p
	Saturated fatty acids (SFAs)			
Myristic (C14:0)	0.16 (0.13-0.21)	0.17 (0.14-0.21)	-6%	0.5735
Palmitic (C16:0)	16.87 (16.34-17.39)	17.35 (16.08-17.47)	-3%	0.4496
Stearic (C18:0)	11.75 (10.99-12.31)	12.13 (11.94-12.87)	-3%	0.0487
	Monounsaturated fatty acids (MUFAs)			
Sapienic (C16:1)	0.21 (0.11-0.34)	0.15 (0.13-0.17)	+29%	0.1041
Palmitoleic (C16:1(9cis))	0.24 (0.19-0.48)	0.23 (0.12-0.48)	+4%	0.4663
Oleic (C18:1 (9cis))	18.25 (17.72-19.22)	18.65 (17.78-19.47)	-2%	0.6507
Vaccenic (C18:1 (11cis))	1.28 (1.15-1.49)	1.10 (0.96-1.18)	+14%	0.0071
	Polyunsaturated fatty acids (PUFAs)			
Linoleic (C18:2 (9,12))	13.04 (11.79-14.03)	13.47 (12.80-14.78)	-3%	0.2521
Alpha-linolenic (C18:2 (9,12,15))	0.49 (0.40-0.61)	0.41 (0.35-0.47)	+16%	0.1307
Gamma-linolenic (C18:3 (6,9,12))	2.51 (2.27-2.92)	2.13 (1.94-2.96)	+15%	0.1986
Arachidonic (C20:4 (5,8,11,14))	22.33 (21.18-23.66)	21.25 (20.33-23.11)	+5%	0.2885
Eicosapentanoic –EPA (C20:5 (5,8,11,14,17)).	1.02 (0.75-1.22)	1.12 (0.85-1.22)	-10%	0.9454
Docosapentaenoic –DPA C22:5(7,10,13,16,19)	3.60 (3.24-4.00)	3.70 (3.25-4.06)	-3%	0.5365
Docosahexaenoic –DHA (C22:6 (4,7,10,13,16,19))	7.71 (6.29-8.50)	7.52 (4.67-9.62)	+2%	0.9672

$ Fatty acids are reported as relative percentages of fatty acid methyl esters (% rel.) obtained by gas chromatographic analysis and calibration procedure, after erythrocyte membrane isolation and work-up as previously reported.
* - median with IQR
** - Differences between GDM and NGT groups

POSSIBLE INFLUENCE OF GDM ON PATHWAY OF METABOLIC TRANSFORMATION OF PALMITIC ACID TO *CIS*-VACCENIC, SAPIENIC AND OLEIC ACIDS.



On the presented scheme, this metabolic transformation is showed, starting from palmitic acid and hypothetic pathway of metabolic transformation of palmitic acid to cis-vaccenic, sapienic and oleic acids in GDM. In contrast, the 11-trans vaccenic acid it is widely present in ruminants and is acquired by humans in abundance from consumed dairy products [7].

In our studies, the 11-cis VA was the only isomer detected in erythrocyte membranes [8]. We postulate, basing on the differences between the GDM and NGT lipidomic profiles, that stearic and *cis*-vaccenic acids can be considered as dual biomarkers of specific SFA-MUFAs conversion. Our results show SFA-MUFA families are more involved in metabolic diseases like GDM. The GDM group revealed also 29% higher sapienic acid levels contents, but this change was not statistically significant. The effect of sapienic acid in GDM pathogenesis has not been studied so far, therefore, this research area looks promising and requires further study. No significant changes of polyunsaturated fatty acids (PUFAs) were observed in GDM and NGT erythrocytes. In GDM, it seems that a crucial metabolic shift occurs in the biotransformation of palmitic acid between the two pathways depicted on scheme. The study requires validation due to its pilot / exploratory nature.

Literature:
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