Application of LC-MS and CE-MS based metabolomics to study type 2 diabetes development in lean, overweight and obese humans

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INTRODUCTION

The risk of type 2 diabetes mellitus (T2DM) development is related to BMI, therefore this disease mainly occurs among overweight (OW) and obese (OB) people. However lean (L) individuals may also suffer from T2DM. The evolution of T2DM is a multistep process and starts with insulin resistance (IR), which may evolve into pre-diabetic state i.e.: impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). In up to 70% of patients pre-diabetic state evolves into T2DM. In the present study metabolic fingerprinting methodology was used to find metabolites changing with the T2DM evolution. Metabolic differences between healthy, IR and pre-diabetic individuals were evaluated in different BMI groups (L, OW or OB).

MATERIALS & METHODS:

Study was performed on serum samples obtained from 53 L, 59 OW and 55 OB individuals. Mean age: 50 years) and sex (60% female) were matched between the groups. Samples were analyzed by the two platforms commonly used in metabolomics studies (LC-MS and CE-MS). Health status (control, IR or pre-diabetic) was defined based on fasting glucose level, HOMA-IR, and 2-h 75-g OGTT. Statistical analysis was used to find differences between controls, IR and pre-diabetics in each BMI group.

CE-TOF-MS

LC-QTOF-MS

CE-MS parameters: capillary: 50 µm of diameter and 96 cm of length; samples inj.: for 35 s at 50 mbar pressure; separation conditions: 25 mbar pressure and 30 kV voltage, the current observed: 25 µA; scan range: 50 - 1000 m/z, capillary voltage: 3500 V, Ion mode: ESI+.

LC-MS parameters: column: Discovery HS C_{18} 2 cm × 2.1 mm, 3 µm, column temp.: 40°C, flow rate: 0.6 ml/min, inj. vol.: 10 µl, scan range: 50 - 1000 m/z, capillary voltage: 3000 V, Ion mode: ESI+.

- **Data analysis:** Feature finding - Mass Hunter Qualitative Analysis B.06.00 (Agilent)
- Alignment and data filtering – Mass Profiler Professional 12.1 (Agilent)
- Univariate analysis (t-test) - Excel (Microsoft) - p-value≤ 0.05.
- Multivariate analysis (S-plot based on OPLS-DA model) – Simca 13.0 (Umetrics).

Identification

Identification of significant features was performed based on MS/MS fragments (QTOF) or by analysis of authentic standards (QTOF and TOF).

Fig. 1. Prediction of IR individuals by the OPLS-DA model discriminating (lean) healthy from pre-diabetes (LC-MS data). The number close to red dot is HOMA-IR.

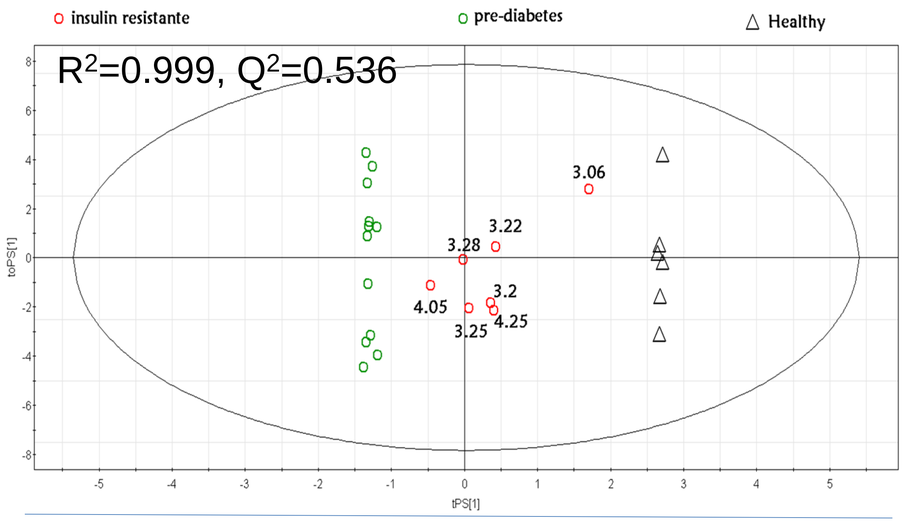
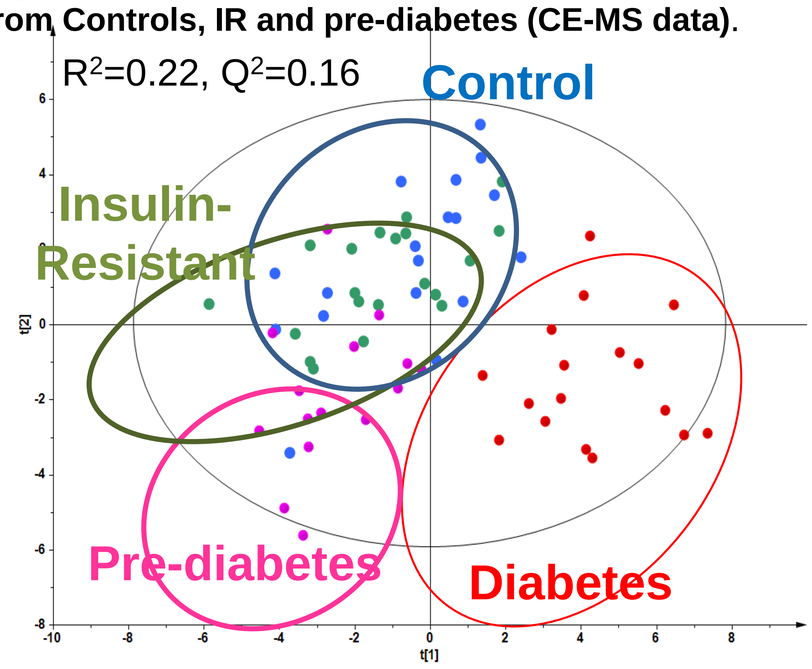


Fig. 2. PLS-DA model discriminating diabetic patients from Controls, IR and pre-diabetes (CE-MS data).



RESULTS

Table 1. Significant metabolites with % of change between healthy and pre-diabetes in different BMI groups.

Metabolite	Lean	Owerweight	Obese
	Change [%]		
Stearamide (18:0)	96*S	8	16
Oleamide (18:1)	17*S	-15	1
Palmitic amide	33*S	-14	-11
Hexacosanoyl carnitine	17*S	26*S	34*S
Dodecanoyl carnitine	-36*S	-12*S	-11*S
Lyso PC (22:4)	-2	70*S	-7
Lyso PC (22:6)	-17*S	-26**S	-20*S
Lyso PC (20:5)	-48*S	-42*S	12
Lyso PC (18:0)	42*S	21*S	1
Lyso PC (18:2)	49*S	28*S	6
Lyso PC (14:0)	30*S	65*S	36*S
Lyso PE (20:4)	14*S	26*S	2
Lyso PE (18:0)	49*S	41*S	3
Lyso PE (18:1)	80*S	19*S	-6
Sphingosine-1-phosphate	45***S	5	5
Valine	16*S	18*S	10*S
cortisol	99**S	20*S	32*S
Phe-Phe	52*S	4	59**S
Hydrohydecadienediynoic acid	9	-43*S	-12
creatine	32*	-6	33*
Leucine	27*	15*	-32*

S – S-plot, *p<0.05, **p<0.01, ***p<0.001

CONCLUSIONS

- Branched-chain amino acids change with the T2DM evolution independently on BMI.
- Acylcarnitines discriminate controls from pre-diabetes in each BMI group, however a percentage of change positively correlated with BMI.
- Fatty acid amides, cortisol, and sphingosine-1-phoshate increase in pre-diabetes belonging to L group.
- The changes in the level of lysophospholipids between healthy and pre-diabetic individuals were higher in L and OW, than in OB.
- Metabolites changing with T2DM evolution are dependent on BMI.