METABOLOMIC PROFILE OF PLASMA FROM PATIENTS WITH POLYCYSTIC OVARY SYNDROME AND NON-HYPERANDROGENIC WOMEN

Escobar-Morreale, Héctor F.[1,2], Insenser, María[1,2], Vinaixa, María[2,3], Correig, Xavier[2,3], San Millán, José L.[2,4]

[1]Endocrinology, Hospital Ramón y Cajal, Madrid; [2]CIBER de Diabetes y Enfermedades Metabólicas (CIBERDEM); [3]Metabolomics Platform, Universitat Rovira i Virgili, Tarragona; [4]Molecular Genetics, Hospital Ramón y Cajal, Madrid.

1. BACKGROUND

- Polycystic ovary syndrome (PCOS), an androgen excess disorder of premenopausal women, frequently associates obesity and insulin resistance, and is a risk factor for type 2 diabetes.
- Obesity plays a major role on the development of metabolic abnormalities associated ith PCOS
- However, androgen excess by itself might also contribute to adipose tissue dysfunction and metabolic disturbances

2. GOALS

- We aimed to study the metabolomic profile of plasma from obese and non-obese PCOS patients as compared with non-hyperandrogenic control women.

3. METHODS

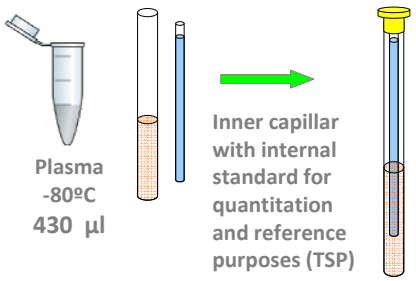
3.1 EXPERIMENTAL DESIGN

N=80

Matched for age and BMI

- NON-HYPERANDROGENIC
  - Obese (OC), N=20
  - Non-Obese (NOC), N=20
- PCOS
  - Obese (OP), N=20
  - Non-Obese (NOP), N=20

3.2 SAMPLE CONDITIONING

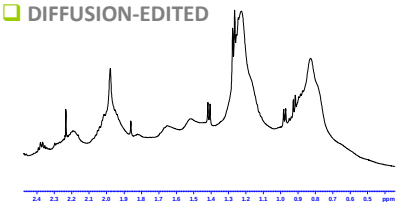


3.3 ^{1}H-NMR PLASMA PROFILING

T=310K, Bruker AVIII 600 with cryoprobe

^{1}H-NMR experiments

- 1D-NOESYGPPR
- CPMG
- DIFFUSION-EDITED



4. RESULTS

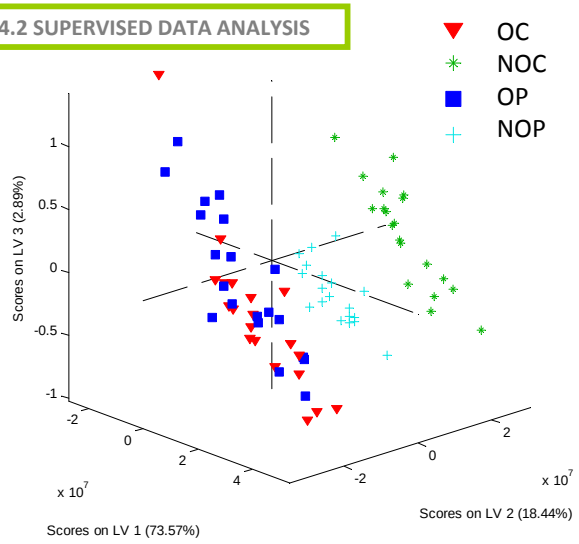
4.1 EXPLORATORY DATA ANALYSIS

A previous exploratory PCA model based on 1D-NOESY was performed in order to explore similarities and dissimilarities between the four groups (data not shown).

PCA preliminary model was rather influenced by obesity- non-obesity differences and did not reveal any clear trend clustering according to PCOS diagnosis.

There is lot of confunding variance within the dataset due to strong signals from CaEDTA2- and MgEDTA2- complexes as well as free EDTA highly that highly interfere the ^{1}H NMR spectra overlapping with endogenous metabolites. Thus spectra suffer from serious misalignments from sample to sample that might compromise further analysis.

4.2 SUPERVISED DATA ANALYSIS



A supervised PLS-DA (a regression extension of PCA) shows that there is a clear the discrimination of PCOS and control groups in non-obese women, whereas such a discrimination was not possible in obese women.

A PLS-DA was performed in non-obese women in order to explore whether this model was able to discriminate PCOS and Non-Hyperdandrogenic women

Permutation test was subsequently used to validate the success rate of this PLS-DA model

4.3 PLS-DA model validation

Classification problems in metabolomics data analysis are complex due to the many variables few samples issue. This makes that many solutions can be found to separate the classes. Recently Westerhuis et al [1] have pointed out the idea behind permutation test in order to overcome PLS-DA overfitting and validation issues

A permutation test can evaluate whether the specific classification of the non-obese individuals (in the two designed groups, either PCOS or non-PCOS) is significantly better than any other random classification in two arbitrary groups. As the groups are formed in a random way, the assumption is that no difference exists between them. By repeating the permutation test many times, a H_0 distribution of classifications that are expected not to be significant is formed.

"Balanced success rates percentage" reported as an average of false positives and false neg. rates	PCOS vs Control Non-Obese women 5 LV's PLS-DA model with leave one out cross validation N=20 iterations	
	PLS-DA model of real data	Mean of 20 random permutations of classes
	59.36 %	48.20 %
	p= 6.2342e-006, then H_0 is rejected and we can conclude that there are significant differences between groups despite the low grade of success rate	

8. CONCLUSIONS

Our preliminary results show that non-obese PCOS women present with a lipoprotein profile similar to that of obese women (either with or without PCOS) that is significantly different than that of non-obese controls.

Particularly a PLS-DA model is able to significantly discriminate between PCOS and control women in non-obese case

Ongoing studies using variable selection algorithms will determine the precise lipoprotein profile causing these differences.

REFERENCES

Westerhuis et al , Metabolomics (2008) 4:81–89

ACKNOWLEDGEMENTS

Grant FIS PI 080944

CIBER de Diabetes y Enfermedades Metabólicas (CIBERDEM) is an initiative of ISCIII (Ministerio de Ciencia e Innovación).