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 with PCOS.
- However, androgen excess by itself might also contribute to adipose tissue dysfunction and metabolic disturbances.

2. GOALS

- We aimed to study the metabolic 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
- PCOS

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

3.2 SAMPLE CONDITIONING

Plasma
-80ºC 430 µl

Inner capillary with internal standard for quantitation and reference purposes (TSP)

3.3 ¹H-NMR PLASMA PROFILING

T=310K, Bruker Avii 600 with cryoprobe
¹H-NMR experiments
- 1D-NOEYPGRPP
- 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 confounding variance within the dataset due to strong signals from CaEDTA- and MgEDTA2- complexes as well as free EDTA highly that highly interfere the ¹H 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 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-Hyperandrogenic women.

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

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 H₂ distribution of classifications that are expected not to be significant is formed.

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.

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


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