

# Adipose tissue signatures related to weight changes in response to calorie restriction and subsequent weight maintenance using lipidome and gene profiling network analysis

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## 1. Introduction

Maintenance of weight loss remains an obstacle in successful treatment of obese individuals. The long term impact of calorie restriction on adipose tissue biology is poorly characterized. Molecular and lipid markers of these processes may provide wider understanding in the mechanisms underlying weight control. We aimed at identifying relevant relationship between clinical traits, clusters of genes and adipose tissue (AT) fatty acids (FAs) with respect to low calorie diet (LCD) and subsequent weight maintenance.

## 2. Methods

### 2.1 Subjects

Data presented here are part of the DiOGenes study, a Pan-European randomized DI trial investigating the effects of diets with different content of protein and glycemic index on weight-loss maintenance and metabolic and cardiovascular risk factors after a phase of calorie restriction, in obese/overweight individuals ([www.diogenes-eu.org](http://www.diogenes-eu.org)). AT FAs and 221 genes expression profiling were analyzed in 135 obese women at baseline, after an 8-week LCD and after 6 months of *ad libitum* weight maintenance diet. After LCD, individuals were stratified into 3 groups according to weight change during the second phase of maintenance.

### 2.2 Network analysis

A sparse Graphical Gaussian Model (GGM) was used to estimate partial correlations in each set of variables (bio-clinical, FAs and mRNA level) and regularized canonical correlation analysis (CCA) was used to assess links between paired sets of variables. Adding a regularization parameter to the estimated covariance matrices, both in GGM and in CCA, allowed us to obtain sparse relationship estimations that is only the most important links between pairs of variables. To stress out the macro-structure of the network, a spin-glass model and simulated annealing were used to obtain a vertex clustering <sup>24</sup>. Graphs were laid out using force-based algorithms in Gephi0.8.2 software ([gephi.org](http://gephi.org), 25, 26). Nodes' colors indicate betweenness centrality. Betweenness centrality is represented by a diverging color gradient in which the red nodes have the highest betweenness and the green nodes the lowest. The variables are connected by an edge only if they have been selected by the sparse estimation. For valid comparison between groups of elements that have very different scale of correlations, the sparsity degree used to estimate each subgraph (within variables of the same sets or between variables belonging to two different sets) was set to give an equal number of edges and nodes. Edge thickness is proportional to the strength of the correlation (CCA) or of the partial correlation (GGM) but should only be compared for a given set of estimation. The biological functions represented by mRNAs from each cluster were searched using Ingenuity Pathways Analysis (IPA) software. The

significance of canonical pathways was tested using Fisher Exact test with the set of 221 genes as reference.

### 3. Results

Whatever the nutritional stages, waist circumference was correlated with metabolic syndrome transcripts independently of weight change (Fig1). After LCD, a strong positive relationship between AT myristoleic acid content and *de novo* lipogenesis (DNL) mRNAs was found (Fig 2). This relationship was also observed after weight maintenance, in individuals that continued to lose weight (Fig 3). By contrast, women regaining weight showed an increase in growth factors, angiogenesis and proliferation signaling (Fig 4).

### 4. Figures

Fig 1

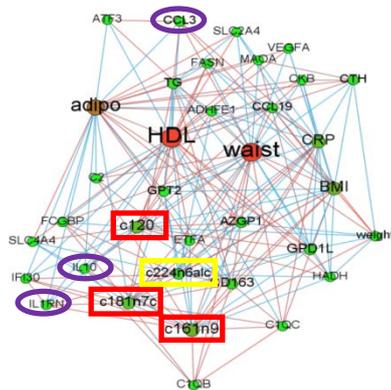


Fig 2

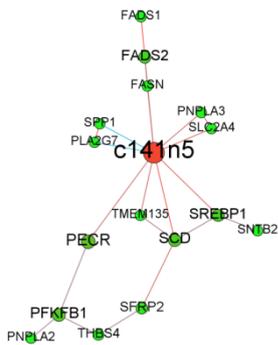
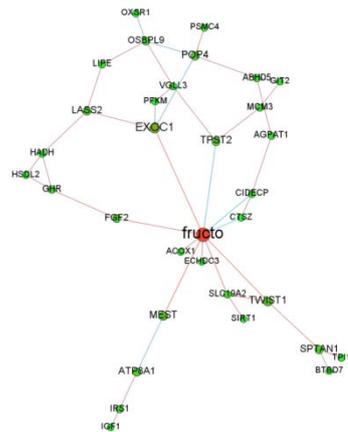


Fig 4

Fig 3



### 5. Conclusion

A combination of network inference and node clustering using gene expression, lipidome and bio-clinical data has linked a characteristic structure of AT network to a slimmed phenotype thereby identifying myristoleic acid as main lipidic biomarkers for DNL and stearoyl CoA desaturase activity. The anabolic signature unique to individuals with unsuccessful weight control suggests detrimental cell proliferation. Here, we show that AT FAs and genes function as network hubs that form different focal nodes involved in various cellular processes for information on weight control phases.

### 6. Acknowledgments

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