Poster Note 64592

Adding Information to Metabolic Pathway Understanding of Rat Liver Tissue Changes on High Fat Diets

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Introduction

High fat intake is known to increase triglycerides (TGs) in liver tissue and may lead to detrimental health events such as diabetes, liver disease and stroke. Here we study liver samples from rats subjected to high fat (HF) controlled diets by performing proteomic analyses. We augment knowledge from lipidomic and metabolomic analyses on matched samples where we previously saw modulation of molecules, and postulated their association with specific pathways. Using protein member enrichment statistics and unlabeled quantitation here we find pathways related to lipogenesis, TG synthesis, and endogenous oxidative stress. This proteomic analysis with the Thermo Scientific™ ProteinCenter™ software corroborates our small molecule observations of pathway regulation in livers of rats subject to high-fat diets.

Methods

Sample Preparation

Twenty male Wistar rats were fed HF , high fat/ high carbohydrate mix (Mix), low fat (LF) or standard chow (SD) for 4 weeks (n= 4 per diet group). Liver tissue samples matching samples previously subjected to lipidomic, metabolomic, and fatty acid synthesis rate determination were prepared for proteomic analysis by solubilized in 2% SDS followed by FASP cleanup and trypsin digestion.

Liquid Chromatography and Mass Spectrometry

LC/MS/MS were collected with 400ng of protein separated using a 2% to 35% acetonitrile (2hr) gradient on a C18 column. Mass spectrometry analysis was performed with a Thermo Scientific™ LTQ Velos™ MS using positive mode electrospray, and data dependent acquisition.

Protein Identification and Quantification

Peptide identification was carried out with Thermo Scientific™ Proteome Discoverer™ 2.0 software and SEQUEST® HT against a Rattus norvegicus (SwissProt TaxID=10116) protein sequence database. Alterations to the default processing workflow LTQ Orbi Precursor Area Quantitation (CID;SequestHT;Percolator) include precursor mass tolerance of 0.8 Da, and dynamic rather than static carbamidomethylation of Cys residues.

Data Normalization

Unlabeled precursor quantitation values were loaded into Perseus. A median experiment was computed from 5 Standard Chow samples. Missing values from HF, LF, Mix and Standard Chow were imputed with 1.8 SD at the left tail; using 0.3 SD distribution (Figure 3). Ratios of HF, LF, and Mix were computed relative to the median Standard Chow experiment. Data were Z-normalized.

Differential Analysis

Ratio data were loaded into ProteinCenter software where protein profiling by soft clustering was performed using a group count of 2 for the pairwise compared quantitative ratio groups. Default was kept for alfa core.

Pairwise two sided t-tests were performed among the HF, LF, and Mix quantitative ratio groups. Permutation FDR calculations with Perseus using a local FDR p=0.05 threshold for significance between groups matched the ProteinCenter results shown in Figures 6 and 7.

Pathway Analysis

Annotation enrichment for KEGG pathways was performed using the Thermo Scientific Cloud Pathway Over-representation module, backed by ProteinCenter (Figures 9 and 10).

FIGURE 1. Schematic of wet lab work and raw data processing: grey box indicates new unlabeled protein data described in this analysis.

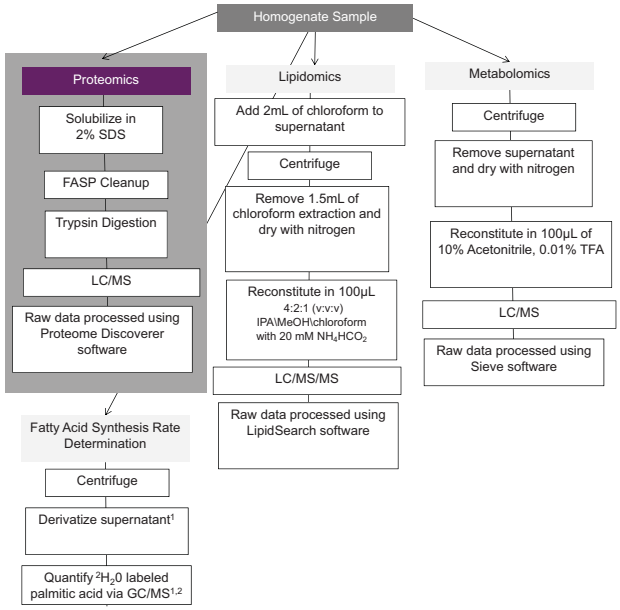

FIGURE 2. Standard Proteome Discoverer 2.0 software workflows used to process data for protein identification and quantification.

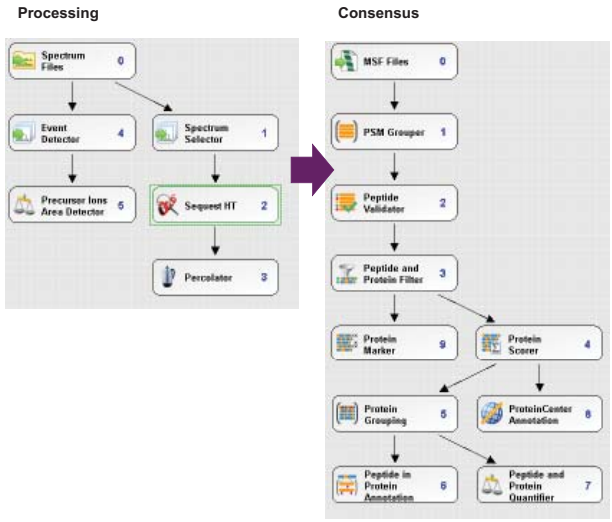

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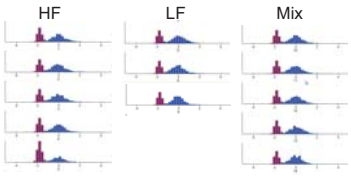
FIGURE 3. Data distributions and imputed values after ratio to standard diet and Z-normalization.



Results

FIGURE 4. Box plots showing HF, LF, and Mix diets following normalization.

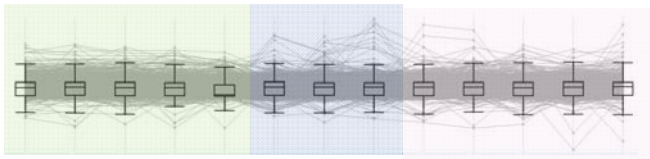


FIGURE 5. PCA showing unsupervised separation of HF (Green), Mix (Pink), and LF (Blue) diet groups.

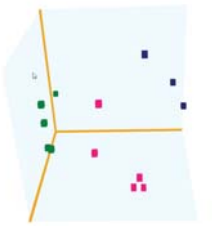


FIGURE 6. Proteins over-expressed in high-fat diet group.

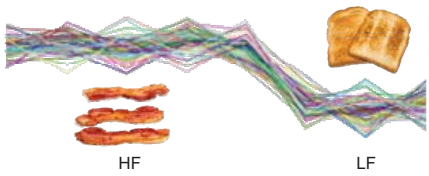


FIGURE 7. Proteins over-expressed in low-fat diet group.

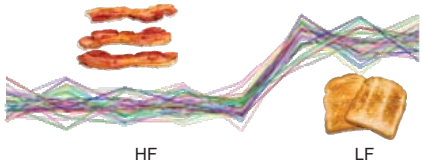


FIGURE 8. Venn Diagram showing overlap of proteins following 5% permutation FDR cutoff for pair-wise T-tests.

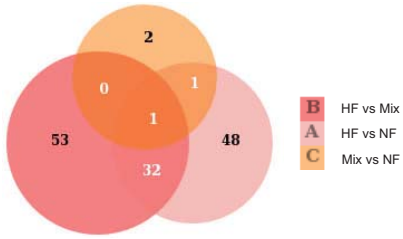


TABLE 1. Proteins over-expressed in the HF diet group that map to the Peroxisome Proliferator-Activated Receptor (PPAR) signaling pathway.

FIGURE 9. Over-represented pathways from over-expressed proteins in the HF diet group. Proportion of Red/Green bars represent observed and expected odds. Bar length represents *-Log(FDR p-value)*. PPAR signaling pathway is highlighted with an arrow.

FIGURE 10. Over-represented pathways from highly expressed proteins in the LF diet group. Glycolysis/Gluconeogenesis signaling pathway is highlighted with an arrow.

FIGURE 11. Two ceramide species among select significant lipids (2014).

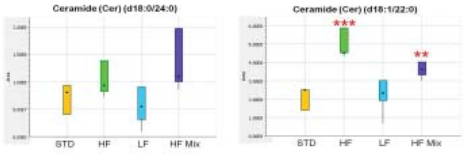


FIGURE 12. Significant compounds including carnitine (2014 metabolomics).

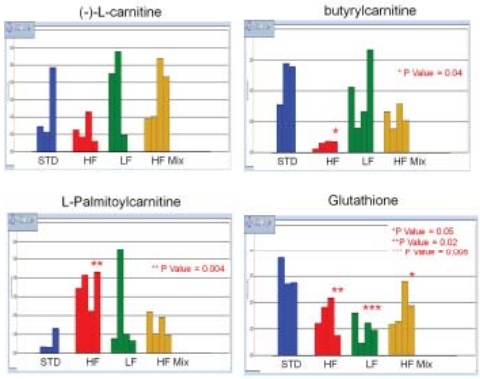


FIGURE 13. Schematic of the carnitine acylcarnitine translocase (CAT) shuttle.

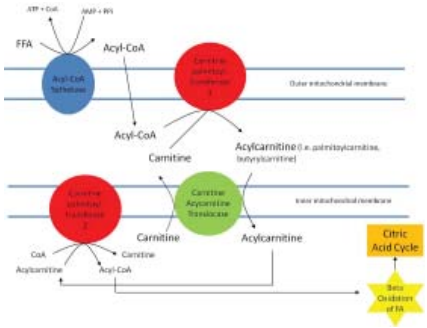
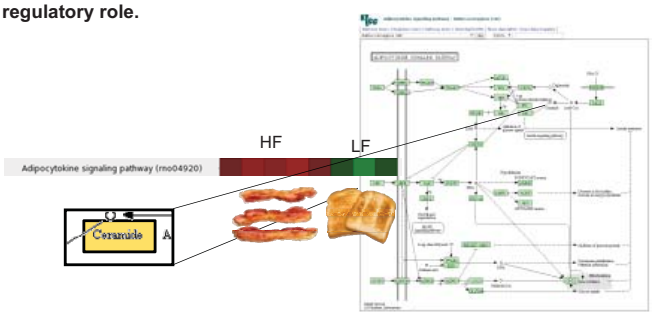


FIGURE 14. ProteinCenter software heatmap of proteins in 2015 dataset that map to the adipocytokine signaling pathway were ceramide plays a regulatory role.



Conclusion

Good differentiation was observed between high fat (HF) and low fat (LF) high carbohydrate diets. Differential and significant pathways by protein analysis agree well with previous small molecule observations including ceramide signaling, glutathione abundance, and carnitine processing and shuttling enzymes.

Proteins identified as over-expressed in the HF liver samples show highly significant enrichment of PPAR Signaling Pathway (rno03320 : 1.0E-11; 1.9E-10), the fatty acid degradation pathway (rno00071: 1.9E-13; 7.2E-12), the fatty acid metabolism pathway (rno01212: 3.7E-11; 4.6E-10), and the valine, leucine and isoleucine degradation pathway (2.0E-7; 1.8E-6).

The peroxisome pathway observations corroborate our previous metabolomics analyses which showed elevated glutathione levels in the same samples (ref 1), indicative of peroxisomal activity and corresponding elevation of reactive oxygen species (creating endogenous oxidative stress).

Among 11 identified proteins from the fatty acid metabolic pathway (of 45 proteins) is the liver isoform of carnitine O-palmitoyltransferase which catalyzes the transfer of the acyl group of long-chain fatty acid-CoA conjugates onto carnitine. This agrees with our previous report showing significant alteration of carnitines and related metabolite levels in HF samples, relative to standard chow.

References

1. Schlatzer, D. M.; Puchowicz, M. A.; Pallante, G.; Peake, D. A.; Stratton, T.; Chance, M. R.; Wang, J. Metabolomic and Lipidomic Analysis of Diet-Induced Inhibition of Hepatic De Novo Lipogenesis with Carbohydrate Restriction ASMS Poster Sessions, 2014.
2. http://perseus-framework.org/.

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