Published in final edited form as:

Trends Endocrinol Metab. 2018 April ; 29(4): 201–202. doi:10.1016/j.tem.2018.01.005.

Squeezing Flux Out of Fat

Alba Gonzalez-Franquesa¹ and Mary-Elizabeth Patti²

¹Section for Integrative Physiology, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark

²Research Division, Joslin Diabetes Center, and Harvard Medical School, Boston, MA

Graphical abstract

Adipocytes can be categorized into three broad categories: white adipocytes that store fat, brown adipocytes that burn fat, and beige adipocytes that are located in white adipose depots but can be reprogrammed to acquire metabolic characteristics of brown adipocytes, a process known as “browning”. These differences may have clinical applications, since activation of brown fat with cold exposure improves insulin sensitivity. Thus, better understanding of the underlying metabolic differences could offer novel therapeutic targets to improve whole-body energy expenditure and metabolism.

Unfortunately, comprehensively assaying metabolic flux is experimentally challenging. Furthermore, estimating flux from high-throughput data requires quantitative measurement of abundance of all metabolites and active enzymes along with their kinetic parameters. Since this is not practical, a common strategy is to use mRNA measurements, such as RNA-seq. However, there are many regulatory steps from mRNA to protein to active enzyme, and expression and enzyme activity are not strongly correlated. An alternative experimental approach to study metabolic flux is measurement of extracellular fluxes, such as oxygen consumption rate (OCR) and extracellular acidification rate (ECAR)/proton production rate (PPR), in the presence of different substrates and enzyme inhibitors to infer respiration and glycolytic flux.

In a recent issue of Cell Reports, Ramirez et al. [1] introduce a method to model cellular metabolic flux from such extracellular flux data, and apply it to identify differences between brown and white adipocytes. Although such assays only offer a few measurements, these authors linked the measured fluxes in central metabolism to the rest of the human metabolic network using a mathematical approach called flux balance analysis (FBA)[2]. FBA assumes that the cell is temporarily at steady-state, where each metabolite's input and output fluxes balance or equalize. Thus, central metabolic fluxes are connected via both upstream fluxes related to the cell's nutrient uptake and downstream fluxes linked to secretion, growth, and storage (e.g. lipid storage for adipocytes). FBA has been used in bacteria [3], fungi [4], and human diseases such as cancer [5], insulin resistance [6], and diabetes [7]. Using this new method, together with a revised version of the genome-wide metabolic network, Ramirez et al. predicted flux changes in brown compared to white adipocytes across the metabolic map. They experimentally validated several of them, such as increased glucose, glutamine, and palmitate uptake, 4-aminobutyrate (GABA) transaminase flux, and urea secretion. By contrast, ammonia secretion was reduced despite increased overall metabolic activity; this was linked to increased formation of urea via the enzyme arginase-2 (ARG2) in brown fat, an enzyme suggested to increase the activity of this tissue [8].

Ramirez et al. then compared their findings to microarray datasets from human brown and white adipocytes and paired preadipocytes. Gene expression and predicted flux were concordant in several pathways, including the TCA cycle, cholesterol metabolism, and branched-chain amino acid (BCAA) metabolism. These findings are of interest for several reasons. Firstly, brown adipose tissue is distinct from white adipose tissue in multiple oxidative metabolic pathways, not just in the heat-generating uncoupling protein UCP1. Higher overall substrate oxidative flux may suggest that broad targeting of oxidative metabolism, perhaps during the differentiation process, may be required to fully mimic the increased cellular energy expenditure characteristic of brown adipose tissue. Secondly, differential metabolism within BCAA pathways has been postulated to play a role in insulin sensitivity, since higher plasma BCAA levels mark both prediabetes and type 2 diabetes [9]. We have previously related decreases in BCAA metabolic pathway flux to insulin resistance phenotypes in muscle [6, 10], and reduced uptake of BCAA in white adipose tissue has been linked to increased plasma BCAA levels [11]. The new study predicts that BCAA uptake is higher in brown than white adipose, potentially implicating brown adipose tissue as another regulator of systemic BCAA metabolism which might be harnessed therapeutically. Taken together, these results suggest that BCAA catabolism in brown adipose tissue may be one of

the mechanisms by which this tissue is associated with improved insulin sensitivity and metabolism. This hypothesis will need to be tested in future experiments.

Much of a cell's activity revolves around regulating its metabolism to yield cell-specific endpoints (e.g. lipid synthesis and storage in white adipose). While high-throughput transcriptomics or metabolomics data can illuminate many processes, these remain insufficient for flux estimation. Ramirez et al. offer a new method to squeeze more flux estimation out of extracellular flux analyzers. What remains to be deciphered is how the magnitude of observed differences in extracellular flux can be used with whole-genome metabolic modeling to predict differential fluxes in cells and tissues with more subtle differences in metabolic functionality. Regardless of the future potential of this method, it provides more information from currently used assays about the flux of fat, and should be also utilized to query metabolic flux regulation, and new targets mediating the impact of insulin resistance, in other tissues.

REFERENCES

1. Ramirez A et al. (2017) Integrating Extracellular Flux Measurements and Genome-Scale Modeling Reveals Differences between Brown and White Adipocytes. *Cell reports* 21 (11), 3040–3048. [PubMed: 29241534]
2. Orth J et al. (2010) What is flux balance analysis? *Nature Biotechnology* 28 (3), 245–248.
3. Covert M et al. (2004) Integrating high-throughput and computational data elucidates bacterial networks. *Nature* 429 (6987), 92–96. [PubMed: 15129285]
4. Dreyfuss J et al. (2013) Reconstruction and Validation of a Genome-Scale Metabolic Model for the Filamentous Fungus *Neurospora crassa* Using FARM. *PLoS computational biology* 9 (7), e1003126. [PubMed: 23935467]
5. Frezza C et al. (2011) Haem oxygenase is synthetically lethal with the tumour suppressor fumarate hydratase. *Nature* 477 (7363), 225–228. [PubMed: 21849978]
6. Nogiec C et al. (2015) Metabolic modeling of muscle metabolism identifies key reactions linked to insulin resistance phenotypes. *Molecular Metabolism*.
7. Thiele I et al. (2005) Candidate metabolic network states in human mitochondria. Impact of diabetes, ischemia, and diet. *Journal of Biological Chemistry* 280 (12), 11683–11695. [PubMed: 15572364]
8. McKnight JR et al. (2010) Beneficial effects of L-arginine on reducing obesity: potential mechanisms and important implications for human health. *Amino Acids* 39 (2), 349–57. [PubMed: 20437186]
9. Newgard CB et al. (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 9 (4), 311–26. [PubMed: 19356713]
10. Lerin C et al. (2016) Defects in muscle branched-chain amino acid oxidation contribute to impaired lipid metabolism. *Molecular metabolism* 5 (10), 926–936. [PubMed: 27689005]
11. Lynch CJ and Adams SH (2014) Branched-chain amino acids in metabolic signalling and insulin resistance. *Nat Rev Endocrinol* 10 (12), 723–36. [PubMed: 25287287]