# Nutrient-sensitized screening for drugs that shift energy metabolism from mitochondrial respiration to glycolysis

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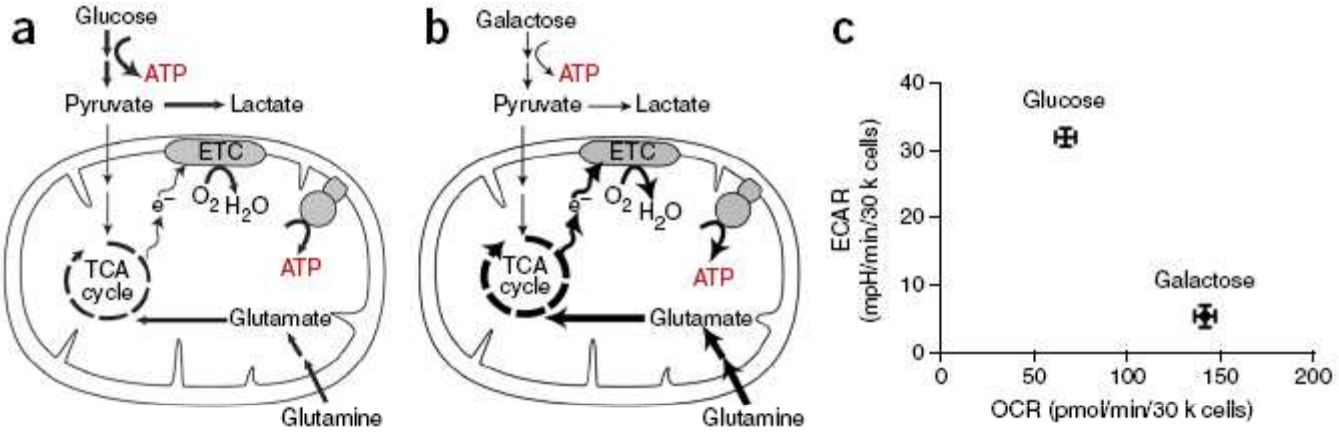
# Background

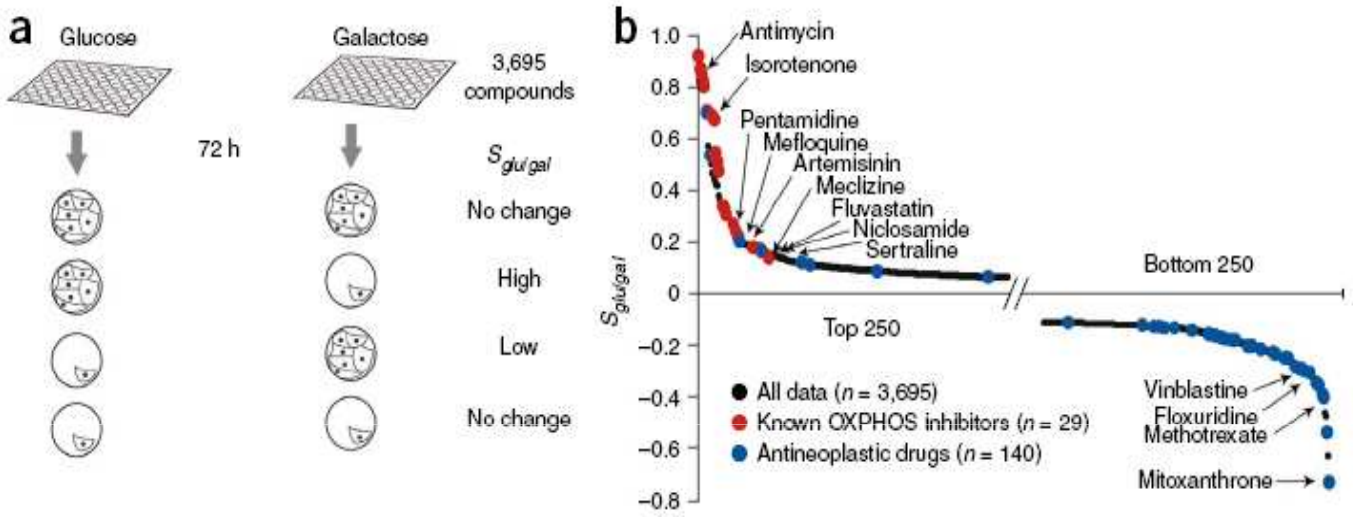
- Most cells have the inherent capacity to shift their reliance on glycolysis relative to oxidative metabolism.
- Targeting such shifts to treat/prevent diseases (e.g., cancer, ischemic injury).
- Currently a limited number of mechanistically distinct classes of drugs alter the relative activities of these two pathways.

# Methods




Metabolic plasticity of human fibroblasts. (a) Cells grown in glucose-rich medium derive ATP from glycolysis as well as from glutamine-driven respiration. (b) Replacing glucose with galactose forces cells to generate ATP almost exclusively from glutamine-driven oxidative metabolism. (c) Measurement of ECAR (extracellular acidification rate), a proxy for the rate of glycolysis, and OCR (oxygen consumption rate), a proxy for mitochondrial respiration, of fibroblasts grown in media containing.

# Methods and Results



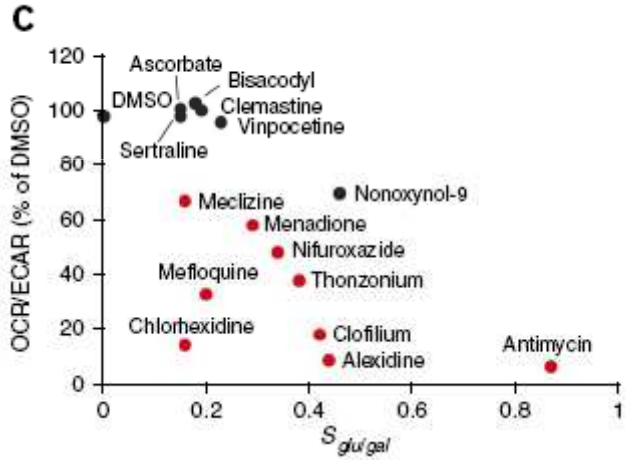

A nutrient-sensitized screen to discover agents that shift energy metabolism. (a) Fibroblasts grown on glucose- or galactose-containing media are exposed to a chemical library of 3,695 compounds (span **nearly half of all FDA-approved drugs**, as well as other bioactives and natural products). The logarithm of the normalized cell number in glucose versus galactose provides a summary statistic ($S_{glu/gal}$) for each compound. (b) Results from a nutrient-sensitized screen. $S_{glu/gal}$ is plotted for top and bottom 250 compounds.

# Results

- For most drugs, $S_{glu/gal}$ is close to zero, indicating similar effects on growth and viability in glucose- and galactose-containing media.
- The upper tail of the $S_{glu/gal}$ distribution - respiratory chain and OXPHOS inhibitors and compounds that directly interrupt mitochondrial respiration or uncouple it from ATP synthesis.
- The lower tail - antineoplastic agents, chemotherapeutic agents that are likely to retard the growth and viability of cells rapidly proliferating in glucose (Warburg effect?).

# Results - Subtle Metabolic Shifts

- Subtle metabolic shifts = safe drugs.
- Focused on commercially available drugs exhibiting low to intermediate, positive $S_{glu/gal}$ scores (0.15–0.45).




- Secondary assays of OCR, ECAR and cell viability and confirmed that eight of these agents induce statistically significant ($P < 0.05$) metabolic shifts.

# Large Scale ⟶ Small Scale

- Meclizine (an over-the-counter drug most commonly used to inhibit nausea and vomiting) is the chosen one!
- It induced reduction in OCR and concomitant increase in ECAR in all cell types tested, including immortalized mouse striatal cells, human embryonic kidney cells (HEK293) and HeLa cells.

# Mechanism of Action

Brookes: ”Hi, Meclizine is a histamine receptor (H1) antagonist, maybe it has something to do with that?”

Mootha: ”Nope, other 64 H1 receptor antagonists from the chemical library did not exhibit elevated $S_{glu/gal}$ scores, and if that isn’t enough, two classic antihistamines did not inhibit cellular OCR. ”

Brookes: ” Hi, Meclizine is a weak muscarinic acetylcholine receptor antagonist, maybe it has something to do with that?”

Mootha: ”Nope, 33 annotated antimuscarinic antagonists from the chemical library did not exhibit elevated $S_{glu/gal}$ scores, and if that doesn’t convince you, two well-characterized antimuscarinic agents did not inhibit cellular OCR.”

# Mechanism of Action

- Why is the suppression of oxygen consumption slow?
  - It takes time for meclizine to accumulate in mitochondria
  - Act indirectly.
- To distinguish between these alternatives study the effect of meclizine on isolated mitochondria (they can actually do that!).
- No effect on isolated mitochondria.

# Cell Line ⟶ Organism

- Meclizine pretreatment provided cytoprotection in *in vitro* and *ex vivo* models of cardiac ischemia-reperfusion injury.
- Prophylaxis with meclizine significantly reduced infarct volume in an *in vivo* model of cerebral ischemia.
- The utility of pretreatment paradigms described in this study arises in clinical settings in which ischemic insults can be anticipated.

# Conclusions

- Several clinically used drugs never linked to energy metabolism were identified, including the antiemetic meclizine, which attenuates mitochondrial respiration through a mechanism distinct from that of canonical inhibitors.
- Nutrient-sensitized screening may provide a useful framework for understanding gene function and drug action within the context of energy metabolism.