2006 JHU SOM Phenotyping Symposium:

METABOLIC PROFILING

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Metabolism: Definitions

- Metabolism defines the chemical (small molecule) processes within an organism necessary for survival.
- Metabolic pathways regulate the disposition of energy.
  - Catabolic pathways break down molecules to yield energy.
  - Anabolic pathways synthesize the macromolecules necessary for life.
- Metabolism provides an integrative view of the status of an organism.
- Metabolism determines biological outcomes, i.e., physiology--behavior.
  - Reproductive competence
  - Social behavior
  - Aggression
- Metabolomics is the systems biology of small molecules.

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Metabolism: Why Monitor It?

- It is now recognized that metabolic pathways not only provide substrates for cellular processes, but that fluxes through these pathways are monitored to ultimately decide what biological events will or will not occur.
- For example, cell cycle regulation relies on metabolic signals to determine whether a cell will proliferate, differentiate, or die.
- Abnormalities of metabolism-- obesity, diabetes, and cancer-- are identified as the major threats to health by the NIH.
- Screening of the flux through metabolic pathways provides an integrated view of how a mutation or drug agent affects an organism.
- Metabolism builds upon genomics and proteomics to provide insight into function of genes.

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Aspects of Metabolic Screening

Metabolomics:

- Mass Spectrometry
  - FFA
  - Acyl carnitines
  - Organic acids
  - Amino acids
  - Nucleotides & nucleotide derivatives
- NMR-based mass isotopomer sampling to measure metabolic pathway fluxes
  - Measure metabolic fuels: ^{13}C-glucose, $^{2}H_2O$
  - Monitored in cells, tissues, and animals
- Hormone/cytokine profiling
- Metabolic modeling

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Aspects of Metabolic Screening

Mouse Physiology/Behavior

- Oxymax: VO_2, RER
- Activity

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Oxymax Monitoring:
Equations That Reveal Energy Utilization

•It is often important to understand the consequences of a mutation or drug effect on aspects of physiology/metabolism.
•To do this, we calculate oxygen consumption, or energy production.
•Oxygen consumption and carbon dioxide production are important to understand energy production:

$$VO_2 = V_iO_{2i} - V_oO_{2o}$$

$$VCO_2 = V_iCO_{2i} - V_oCO_{2o}$$

•Respiratory Exchange Ratio (RER) tells us the fuel choice of the organism-- carbohydrates (1.0) *vs* fats (0.7):

$$RER = VCO_2 / VO_2$$

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Oxymax Monitoring:

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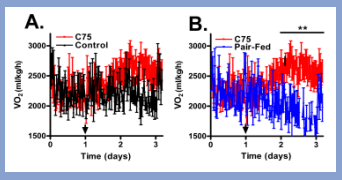
Oxymax Monitoring:

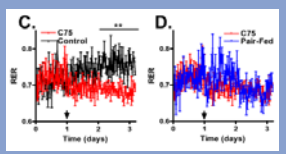
Example:
- C75 is a compound that inhibits food intake and produces rapid and profound weight loss. How does it work?
- Compare Control, C75-treated, and Pair-fed (they get what C75-treated animals got the day before) mice.
- Calculate VO_2-- energy production. Usually, animals decrease VO_2 when they eat less.
- C75-treated produce more energy than Control or Pair-fed.



3/22/06 Thupari *et al.* PNAS 99: 9498, 2002.

Oxymax Monitoring:

- Then look at RER--what is the source of fuel that the animals are using?
- C75-treated have a lower RER-- they are burning more fat as their fuel.
- These and other studies inform us that C75 facilitates fat mobilization and utilization as opposed to storage.



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Activity Monitoring:

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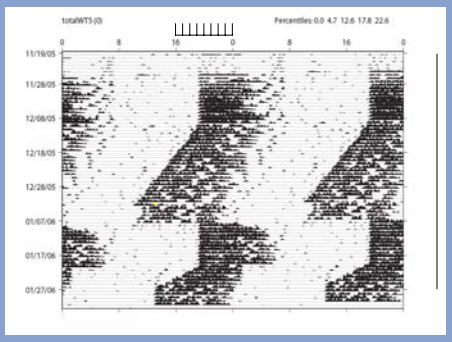
Activity Monitoring:



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Samer Hattar, unpublished data

Summary

- Metabolic monitoring provides insight into the overall state of an organism:
  - Activity
  - Stress/inflammatory responses
  - Anabolic *vs* catabolic status
  - The overall physiology
- This information is critical to understanding the consequences of mutation or pharmacological intervention to the overall health of the animal.

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