Metabolic Flux Analysis in Mitochondria
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Abstract
The emergence of high-throughput data has enabled the study of mitochondria as systems. We have reconstructed and characterized the human mitochondrial metabolic network based on proteomic and biochemical data. Linear programming and Monte Carlo sampling methods were applied to identify candidate steady states consistent with the imposed physiological and chemical observations. Analysis of equivalent optimal flux distributions, calculated with respect to each of the three metabolic functions, identified a group of flux distributions that were highly correlated, and thus are likely to be physiologically relevant. Samples of steady-state flux distributions showed that the experimentally observed reduced activity of pyruvate dehydrogenase in diabetic and ischemic patients could be a result of stoichiometric constraints, and may not necessarily require enzymatic inhibition. Application of isotopomer data from isolated mouse hearts identified the fate of perfused [U-13C6]glucose and [U-13C3]pyruvate and flux redistribution at key substrate branch points.

Methods
The constraint-based approach for analyzing reconstructed network involves the application of a series of constraints arising from reaction stoichiometry, thermodynamics, enzymatic capacities, and regulatory and isotope balance constraints when they are available.

Results
ATP production slightly reduced
Excess fatty acid increases phospholipid synthesis
Minimal pyruvate dehydrogenase activity
Effects of reduced glucose and ketone body uptake are positive but small.

Conclusions
1. An in silico framework integrating multiple datasets is useful for studying mitochondrial metabolism under normal and stress conditions, and provides a basis for assessing effects of potential disease treatments.
2. Metabolic flux profiles with isotopomer data can uncover details about substrate utilization, substrate redistribution at network branch points, and quantitative information about enzyme activity.

References