Studying the effects of hypoxia on mitochondrial metabolism in human heart using a genome-wide metabolic network model

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Hypoxia is a common feature of many human diseases including heart failure, chronic lung disease and anaemia. In order to study the effect of hypoxia on cardiac mitochondrial metabolism we used a genome-wide metabolic network model constructed from proteomic and bibliomic data.

Methods: The model has been described elsewhere (Thiele *et al.*, 2005), but essentially comprises every metabolic reaction thought to be associated with or located in a human myocardial mitochondrion. Rather than attempting to estimate the large range of kinetic parameters required for full dynamic simulations, we used a linear optimization approach known as flux balance analysis (FBA). To study the effect of changes in gene transcription in response to hypoxia we used bibliomic data to simulate a core response to hypoxia (Table 1). Finally, we ran four flux balance optimisations: basic model/normoxia (BN); basic model/hypoxia (BH); adapted model/normoxia (AN); and adapted model/hypoxia (AH). All flux values are in μM/min/mg of mitochondrial protein (U).

Results: Due to the nature of the objective function (to maximize ATP production), glycolytic flux was maximized, while haeme and phospholipid biosynthesis were abolished in all simulations. Glycolytic flux was higher in AN *vs* the BN due to the increased maximal flux through the glucose transporter (Table). However, the increased glycolytic flux was insufficient to compensate for the reduced flux through fumarase, resulting in a 3% reduction in maximal ATP production in normoxia (AN: 66.3 U vs. BN: 68.4 U). In hypoxia the reduction in available O_2 lessened the impact of the loss of fumarase flux, meaning that the adapted model now produced ATP at rates that were 5% higher than the basic model (AH: 40.6 U *vs* BH: 38.8 U). Interestingly, oxygen uptake rates were identical in the two models, both in hypoxia and normoxia (BN/AN: −31 U, BH/AH: −7 U). Thus the loss of fumarase in the adapted model did not result in an insufficiency of the supply of reducing equivalents to the respiratory chain. This suggests that the reduced maximal ATP production in AN was due to the role of fumarase in other pathways. There was a net production of urea in the basic model in normoxia only. However, complete inhibition of urea cycle flux had no effect on maximal ATP production, suggesting that this was one of a class of alternate optimal solutions. Glutamate uptake was present in normoxia in the basic model but was effectively zero in hypoxia (BN: −1.1 U *vs* BH: 0 U) and under all conditions in the adapted model. Acetoacetate uptake, while high in BN, was almost completely absent in hypoxia (*e.g.* BN: −201 U *vs* BH: −0.1 U), AN or AH. Hydroxy-butanoate release was substantial in normoxia (BN: -182 U, AN: −137 U), but was heavily suppressed in hypoxia (BH: −6 U, AH: −6 U). C16 and C18 fatty acids were oxidised in AN only (C16: 0.9 U, C18: 1 U). C20 fatty acids were oxidized by both models in hypoxia. Only C22 fatty acids were utilized in all four simulations.

Conclusions: Our results show a) that mapping gene expression data onto an existing network model improved the functional capacity of the model under appropriate conditions and b) that the role of fumarase in the human metabolic adaptation to hypoxia merits closer examination.

The core human mitochondrial metabolic response to hypoxia.

Gene	Reaction	Up/Down	References
ALDOC, Aldoart1	Fructose-bisphosphate aldolase	Up	Benita *et al.*
PGAM1	Phosphoglycerate mutase	Up	Benita *et al.*
LDHA, Ldha	L-lactate dehydrogenase	Up	Benita *et al.*; Chi *et al.*
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Up	Benita *et al.*
PGK1, Pgk1	Phosphoglycerate kinase	Up	Benita *et al.*; Chi *et al.*
PFKP, Pfkl	Phosphofructokinase	Up	Chi *et al.*
SLC2A1	Glucose transport (uniport)	Up	Chi *et al.*
SLC2A3	Glucose transport (uniport)	Up	Chi *et al.*
FH	Fumarase, mitochondrial	Down	Benita *et al.*

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