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Supplemental information

Tolerance to NADH/NAD$^+$ imbalance anticipates aging and anti-aging interventions

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Supplementary Methods

Methods S1, related to STAR Methods

Flux balance analysis is a widely used approach for studying biochemical networks. The first step of FBA consists on representing metabolic reactions in the form of a tabulation, a matrix S, of the stoichiometrix coefficients of each reaction. This is a matrix in which each row represents a particular metabolite, like NADH, and each column represent a specific metabolic reaction. In addition to this matrix, the flux through all of the reactions in the metabolic network is represented by a vector v. In this way, the change of each metabolite is given by the product of S and v ($dx/dt = Sv$).

While this actual value is not accessible by definition of the method that only considers steady-state, i.e., $dx/dt = 0$, and that exclusively provides fluxes in steady state that satisfy the mass balance constrains provided by S; the framework also reduces drastically the quantity of information necessary to build the model.

To still manage to simulate an imbalance in the ratio between two metabolites in a significant manner in a framework where the actual value cannot be known, we modified the production or consumption of each metabolite in a way that it was immediately and unequivocally rerouted to the other.

A similarly important and counter intuitive hallmark of the study, the analysis of homeostatic nutrients, works around natural flux balance analysis limitations by calculating the amount of a metabolite that is used by enzyme pools per unit of time, rather than the total amount concentration that is made available during that time, therefore obtaining a quantitative measure of the necessity of these metabolites even if concentrations are inaccessible.

TOLERANCE PROFILES ACROSS ORGANISMS ARE EXPLAINED BY SIMILAR METABOLIC RESPONSES

A comparison with other organisms can help evaluate the generality of the previous patterns (Methods, **Fig. S2**). The *Caenorhabditis elegans* metabolic response was very similar to that of yeast when the NADH/NAD^+ imbalance reaction was placed in the mitochondria. The preceding pseudohypoxic behavior followed tightly that found in the yeast model, and so did most of the oxidative response. The only difference was that during very extreme oxidative imbalances the worm model drew heavily on a partial

reverse configuration of the Krebs cycle (sometimes referred to as the glyoxylate cycle) (**Figs. S2A-B**).

The response of the worm model to cytosolic imbalances diverged slightly more from that of *S. cerevisiae*. For once, glutamine metabolism did not change much from its normal level under any point of the tolerance profile. Some features however did emerge in a similar fashion, like the increased glycolysis to Krebs cycle ratio in response to NAD^+ reduction, and the elevated gluconeogenesis, pentose phosphate and oxidative phosphorylation flux following NADH oxidation (**Figs. S2A-B**).

In the case of the more intricate human metabolic network, we obtained a much wider tolerance profile, which made for more elaborate metabolic strategies and a more diverse response (**Figs. S2C-D**). However, some tendencies emerged that recover many of the patterns already described. Yet, the human recon differs from the other models in that these changes are far tamer, with fluxes rising and declining within a 10% to 2-fold of the normal reaction rates (compared to the much broader changes in *S. cerevisiae* and *C. elegans*).

Between more or less advanced models of the same organism, more specifically yeast (yeast 5, yeast 6, iND750, iMM904, iAZ900, yeast 7 and yeast 8), results varied to an extent. The speed at which pseudohypoxic metabolism deploys with reductive imbalance differed, and the specific tolerances of the model were slightly different. The late oxidative regime was only present in yeast 4-6 and iAZ900, but not in iND750 and the supposedly more predictive yeast7 to 8, suggesting it may be an artifact. It may be counterintuitive for a richer network to result in a loss of function, but more demanding biomass pseudo reactions such as that present in yeast 8 ('r_4041') could make the late oxidative growth unfeasible.

ALTERNATIVE SOLUTIONS AND NUTRIENT DEPENDENCE

FBA provides alternative solutions for certain environmental conditions, and to every calculation there is always a certain flux variability that can be estimated through flux variability analysis. Throughout our study, we noted that the tolerance profile is very robust, representing a very restricted range of feasible solutions for each redox imbalance condition. The growth rate of yeast under imbalance seemed to be not only a phenotype convergent across different flux distributions presented by different

organism models, but also convergent to different flux distributions due to variability in feasible enzyme reaction rates. This is so since, while alternative solutions for the tolerance profile were notoriously absent, alternative solutions for particular reaction fluxes lying underneath were very much a thing. It is however worth mentioning that tolerance profiles calculated in earlier yeast models, like iND750, did include alternative solutions for the tolerance profile. Such alternative solutions were conservative in shape for the most part and sometimes were only feasible for some imbalance conditions, but not others. There were also some null solutions in those cases. More predictive models like iaz900, or yeastGEM 7 and 8, did not show this.

As for the alternative solutions to specific fluxes and sets of fluxes, they occur when a mode of metabolism can supply for another. These did not largely impact out study of the metabolic profile, since that was based on flux averaged through standardized metabolic pathways, and fringe alternative solutions often involve alternative metabolic configurations. Flux variability within the same model didn't change the conclusions of the flux analysis. The point where it did become a concern was when dealing with the nutrient study. Somewhat different alternative solutions here were pervasive and modified the dependence of the tolerant state on the main homeostatic nutrients. They introduced considerable changes in the dependence lines, mostly in terms of the slopes, and regime changes. That did not change the selection of nutrients overall, but it did change their priority and the scores of dependences to a reasonable (up to 60% above or below) degree. The solution we found for this was using a regressed mean of the flux variability distribution as an estimate of the actual dependence profile of a nutrient, so the ultimate scalar summary (the slope) accounted for alternative solutions. Alternative solutions are thus another reason why we used regressed linear approximations of the actual dependence profiles instead of the profiles themselves.

Supplementary Figures

Figure S1: Tolerance profiles of different coenzymes in yeast, related to Figure 1. A) NADH/NAD^+, cytosolic perturbation. B) NADH/NAD^+, mitochondrial perturbation. C) NADPH/$NADP^+$ cytosolic perturbation. D) NADPH/$NADP^+$, mitochondrial perturbation. E) Thioredoxin, cytosolic perturbation. F) Thioredoxin, mitochondrial perturbation.

Figure S2. Metabolic signature of redox imbalance in different organisms, related to Figure 2. A) Pathway flux under NADH/NAD+ cytosolic imbalance in the *C. elegans* model iCEL1273. B) Pathway flux under NADH/NAD+ mitochondrial imbalance in the *C. elegans* model iCEL1273. C) Pathway flux under NADH/NAD+ cytosolic imbalance in the human metabolic reconstruction 2.02. D) Pathway flux under NADH/NAD+ mitochondrial imbalance in the human metabolic reconstruction 2.02.

Fig S1

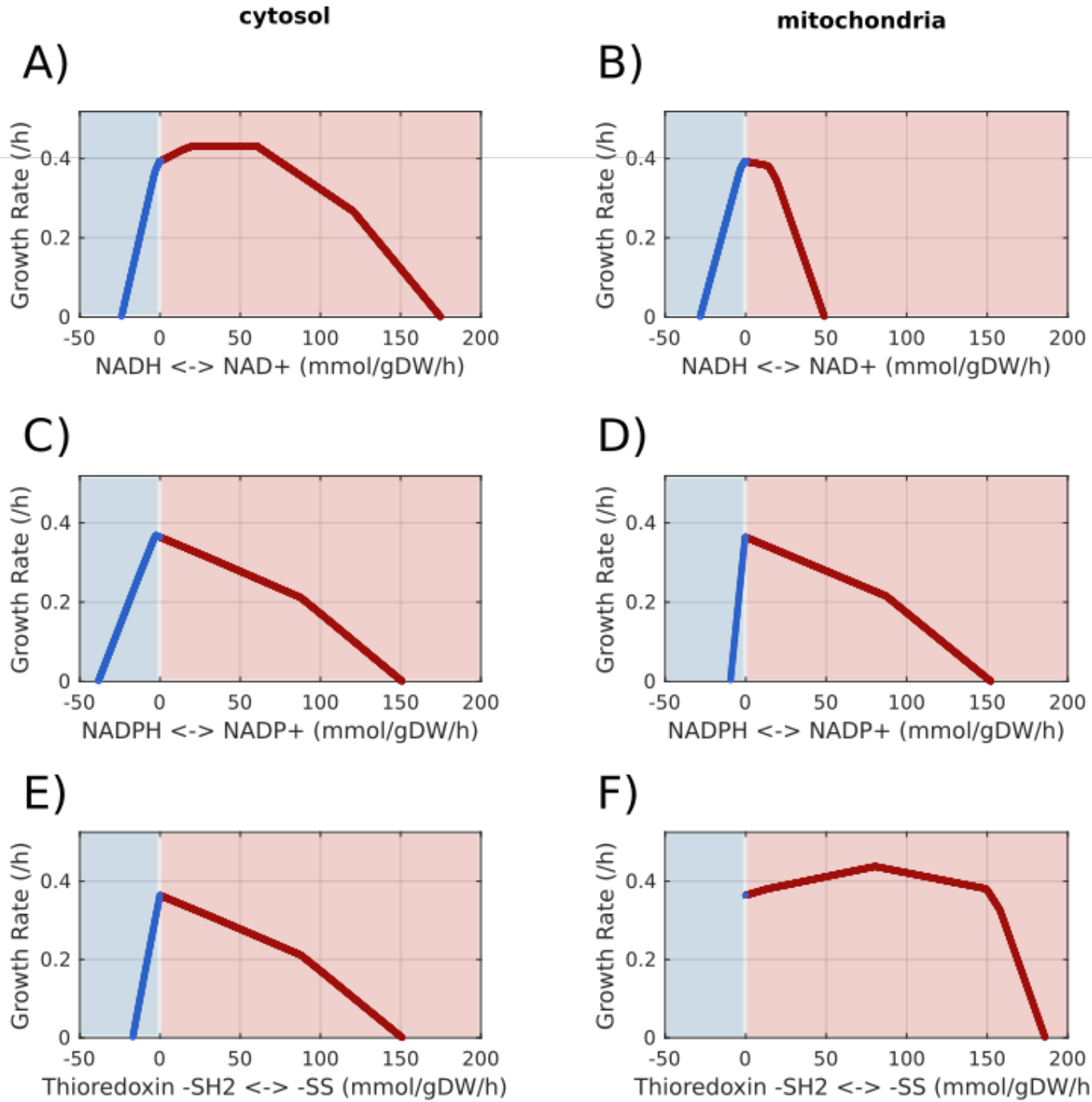
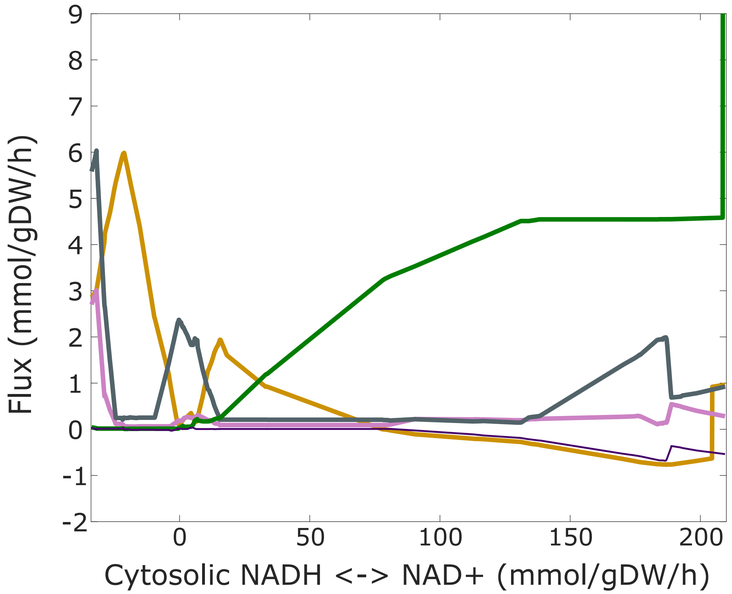
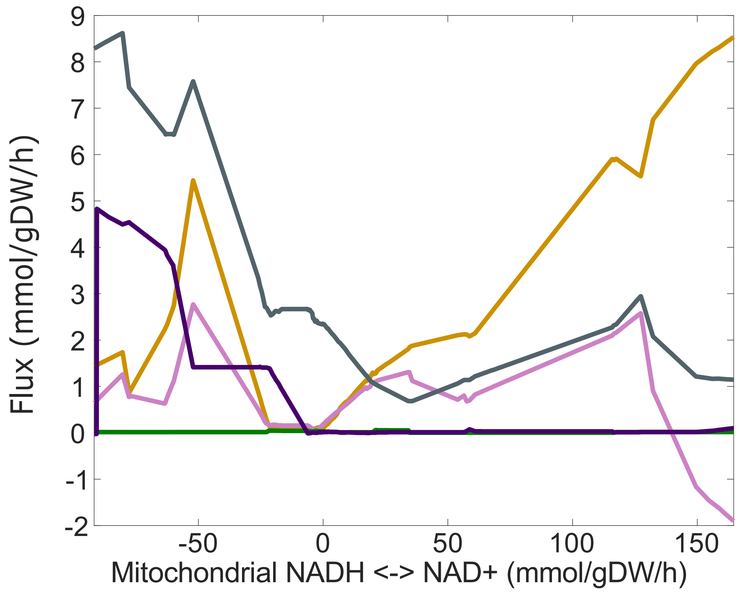

cytosol
mitochondria
A)
B)
C)
D)
E)
F)
Growth Rate (/h)
0
0.2
0.4
-50
0
50
100
150
200
NADH <-> NAD+ (mmol/gDW/h)
NADPH <-> NADP+ (mmol/gDW/h)
Thioredoxin -SH2 <-> -SS (mmol/gDW/h)

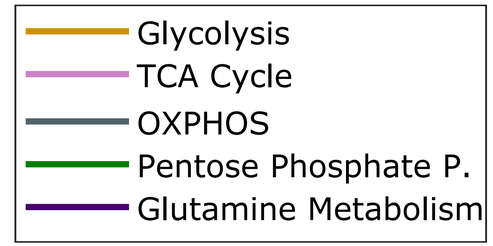
Fig S2

CYTOSOL **MITOCHONDRIA**

C. elegans
model iCEL1273







H. sapiens
recon 2.2