

A yeast model for investigating mitochondrial reprogramming

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1. Introduction

- Inflammation, as part of the programmed immune response, is a key focus of disease research for all leading causes of morbidity and mortality.
- Cell autonomous immunity enables individual cells to respond, similarly to the immune system, to the threats posed by pathogens (PAMPs), damage (DAMPs), and poisons.
- When looking at multicellular organisms, inflammatory responses by immune cells encompass both an initial proinflammatory phase, favouring glycolysis (which eliminates possible threats), followed by an anti-inflammatory phase, favouring oxidative phosphorylation (facilitating healing and repair).
- We argue that in general, eukaryotic cells respond to challenges via mitochondrial adjustments in relation to supply of and demand for energy, in accordance with a programmed regulation of respiratory coupling efficiency.
- There is currently no way to determine if the mitochondria of an organism are programmed by environmental factors, such as diet, and if so, how they might be reprogrammed to restore healthy function. To this end, a model is needed to test new technologies.

Study Aim:

- To demonstrate whether *S. cerevisiae* could be reprogrammed to metabolically respond differently to a specific environment, compared to 'normally' programmed yeast.

Hypotheses:

We hypothesised that the progeny of Fasted yeast would be reprogrammed, compared to a High Glucose control to:

- be more tightly coupled (higher efficiency),
- show greater metabolic flexibility, 'tilting' more toward oxidative phosphorylation over fermentation even in the same environment (higher PDC activity),
- respond differently to a PAMP (LPS) challenge (Higher OCR when lowering RCR).

2. Methods

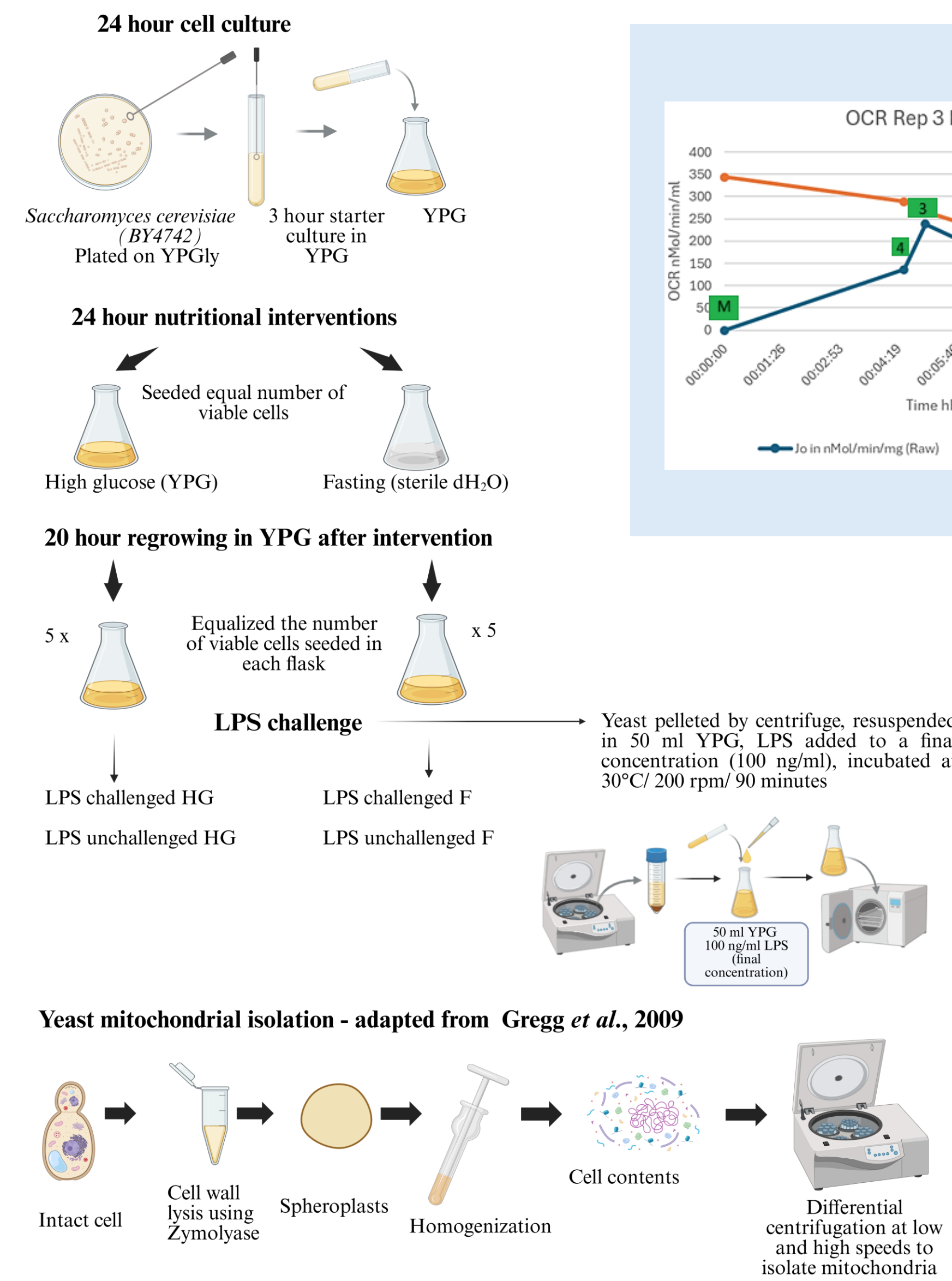


Figure 1. Shows the yeast culture method. Created in <https://BioRender.com>

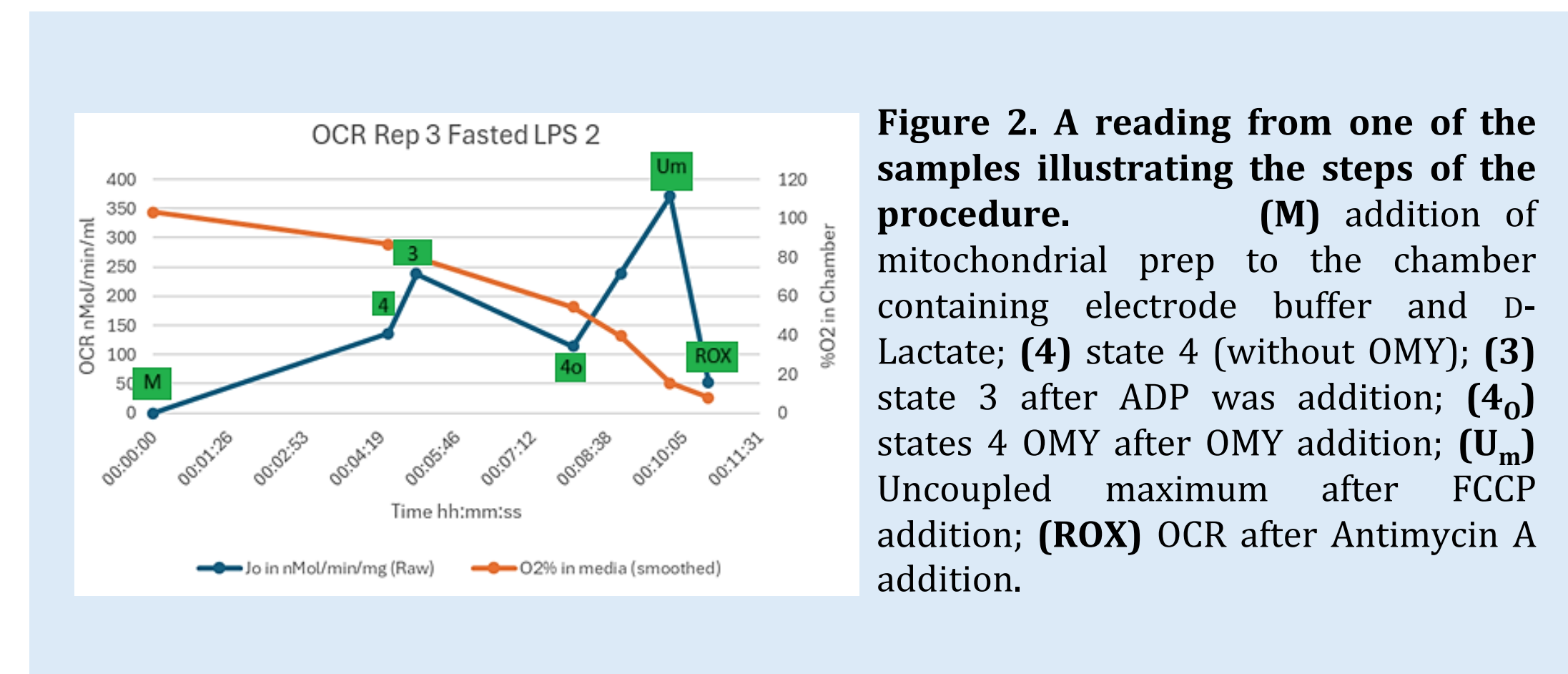


Figure 2. A reading from one of the samples illustrating the steps of the procedure. (M) addition of mitochondrial prep to the chamber containing electrode buffer and D-Lactate; (4) state 4 (without OMY); (3) state 3 after ADP was added; (4_o) states 4 OMY after OMY addition; (U_m) Uncoupled maximum after FCCP addition; (ROX) OCR after Antimycin A addition.

Pyruvate dehydrogenase complex (PDC) activity assay based on NADH production:

Mitochondria (40 µg) were suspended in 25 µl homogenization buffer (BSA-free). Membranes were lysed with 25 µl of 0.25% Triton X-100, mixed, and incubated at room temperature for 10 min. Samples were then incubated at 30°C for 10 min in assay buffer. The reaction was initiated by adding 100 µl Acetyl CoA (final 0.13 mM). NADH levels were measured at 340 nm every 60 s for 10 min. Negative controls lacked addition of NAD⁺.

3. Results

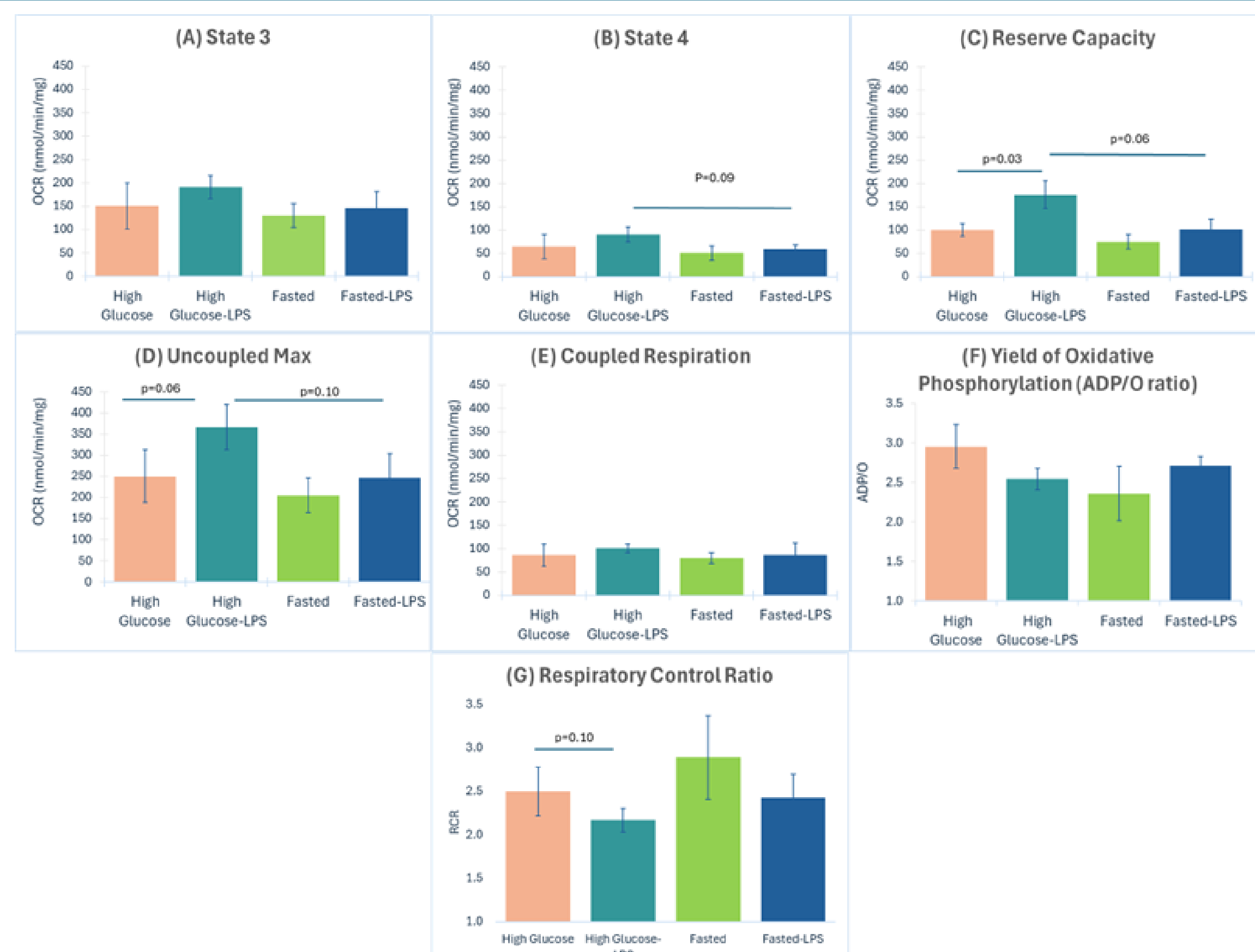


Figure 3. Variations in oxygen consumption rate (OCR) characteristics of the mitochondria isolated from the progeny of the yeast *S. cerevisiae* that experienced pre-conditioning. (A) State 3, OCR immediately post ADP addition; (B) State 4, OCR after the addition of Oligomycin A (known to block ATP synthase); (C) Reserve Capacity, Uncoupled max - State 3; (D) Uncoupled maximum respiration, after the addition of the uncoupler FCCP at a concentration pre-determined to achieve maximum uncoupling without inhibiting respiration; (E) Coupled respiration, State 3 - State 4; (F) Yield of Oxidative Phosphorylation (ADP/O ratio), nmol of oxygen consumed over the State 3 duration after the addition of 125nmol ADP; (G) RCR, State 3 / State 4. (1-tailed Student T tests were performed to test for significance. For all graphs, paired when comparing data from the same conditioning and unpaired, assuming equal variance, when comparing across the two conditions). Each sample was assayed in duplicate and the results are the mean of three independent experiments ±SEM.

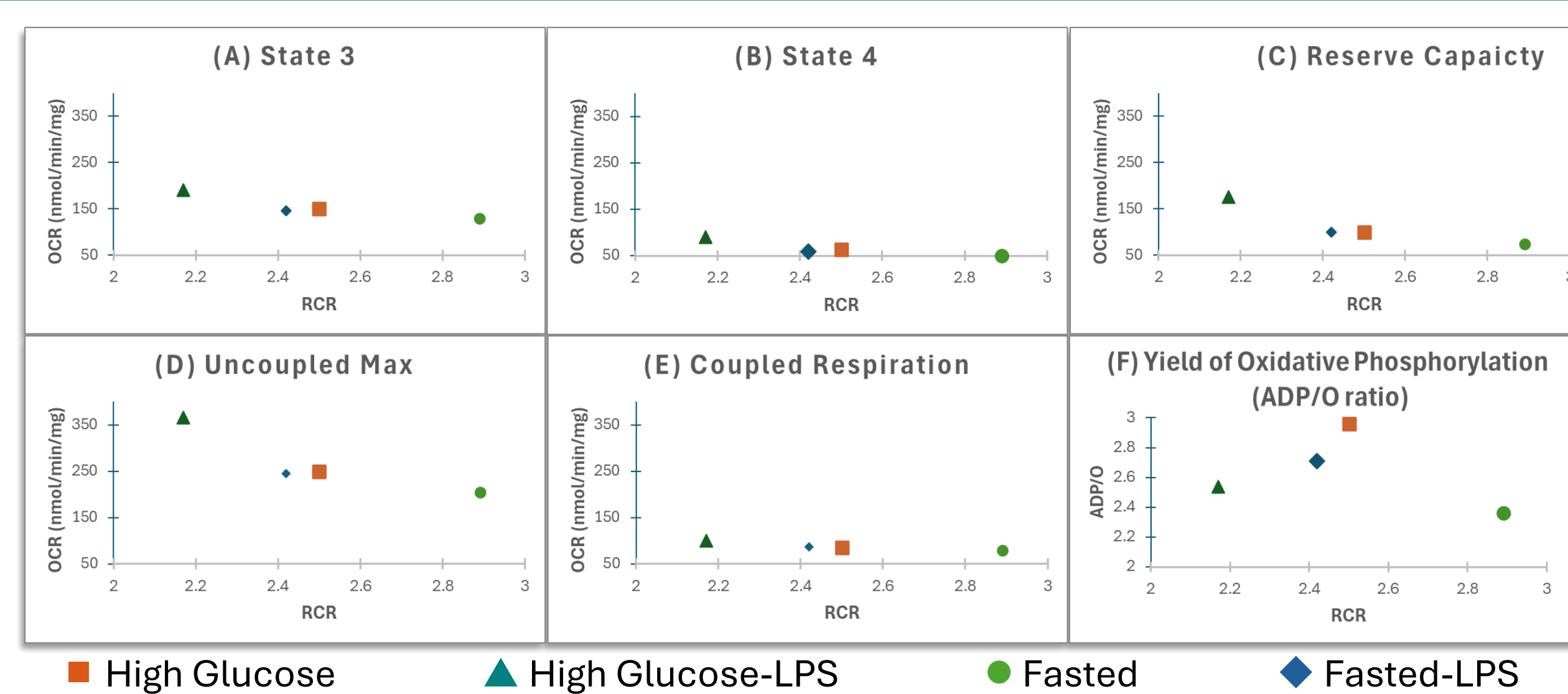


Figure 4. Data shown in figure 1 relative to the Respiratory Control Ratio (RCR) (State 3/State 4): (A) State 3, OCR immediately post ADP addition; (B) State 4, OCR after the addition of Oligomycin A (known to block ATP synthase); (C) Reserve Capacity, Uncoupled Max - State 3; (D) Uncoupled Maximum respiration after the addition of the uncoupler FCCP; (E) Coupled respiration, State 3 - State 4; (F) Yield of Oxidative Phosphorylation (ADP/O ratio), nmol of oxygen consumed over the state 3 duration after the addition of 125nmol ADP.

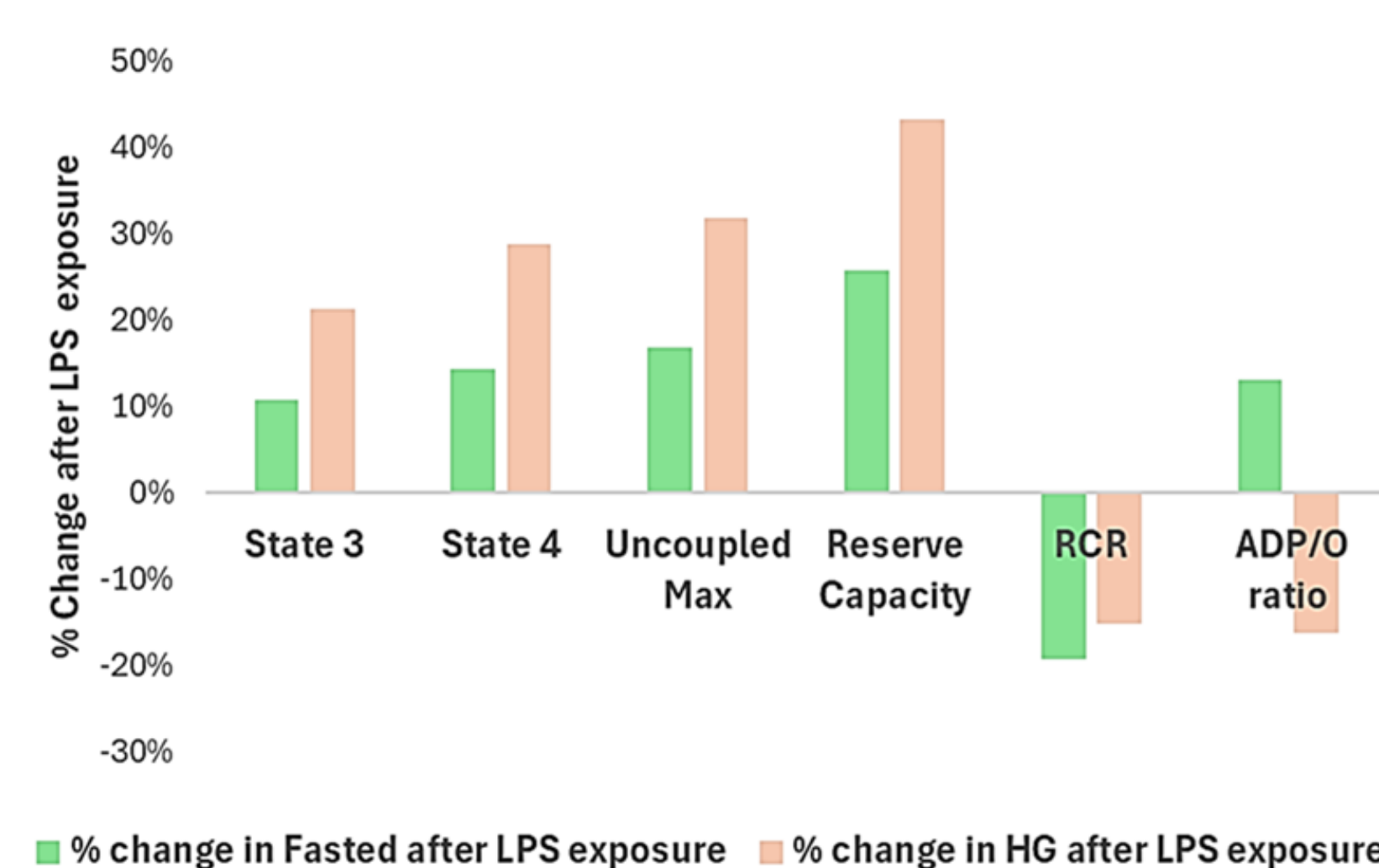


Figure 5. Percent changes in mitochondrial respiration responses. Comparison between pre-conditional programming (Fasted and HG), in response to being incubated for 90 minutes in HG broth containing LPS (100ng/ml) immediately prior to isolation.

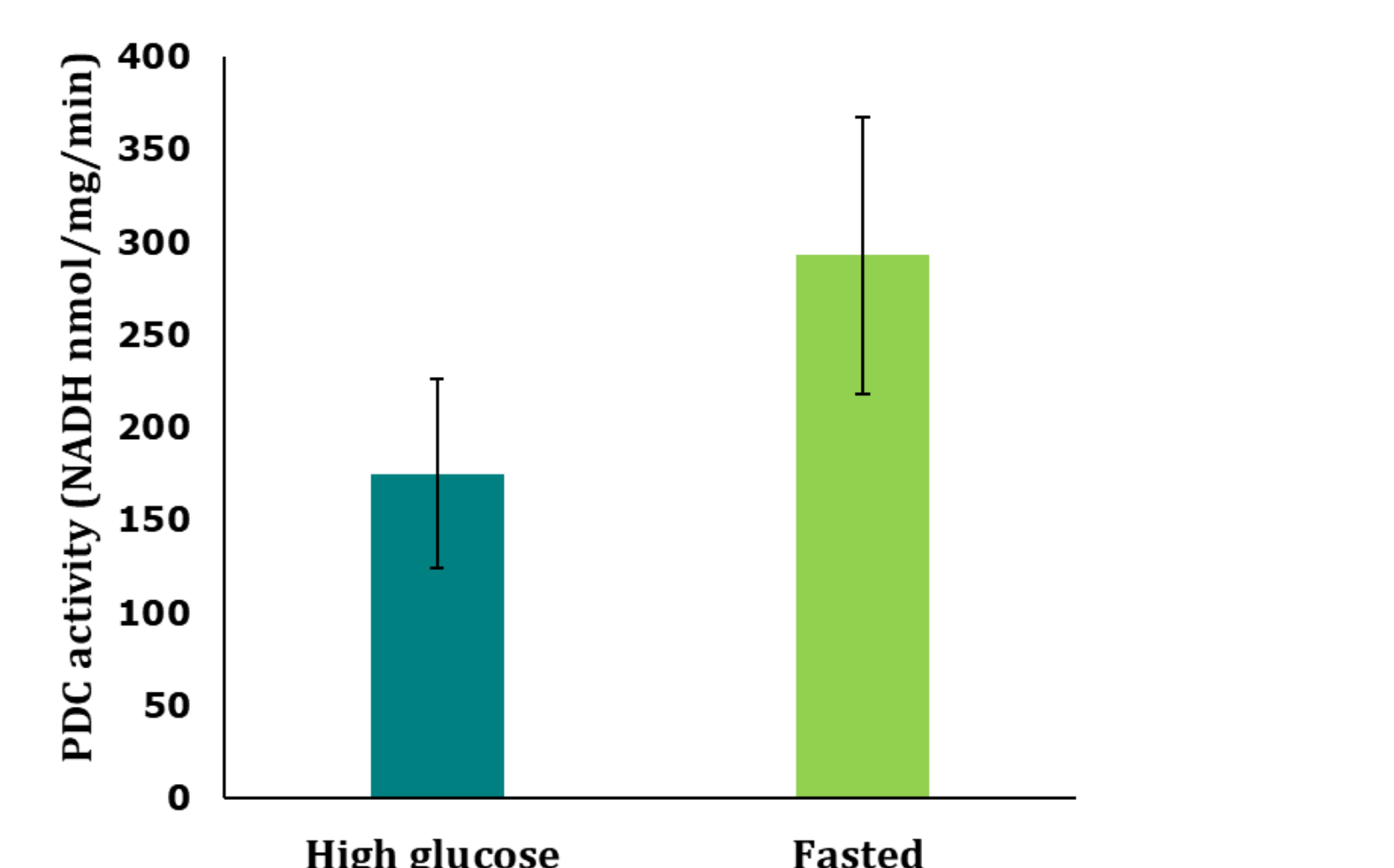


Figure 6. Pyruvate Dehydrogenase Complex (PDC) activity based on NADH production. Mitochondria isolated from cells of High Glucose and Fasted progeny regrown in high glucose after nutritional conditioning. Data represent the averages from three independent experiments ±SEM

4. Discussion

- It was clearly evidenced that there were changes in the way the mitochondria utilized fuel in relation to the rate at which they consumed oxygen. When exposed to LPS, the differences between the two groups reached statistical significance for State 4, reserve capacity, and uncoupled max OCR (Fig 3).
- The PDC activity data (Fig 6), suggests that the progeny of the fasted cells had been reprogrammed to make more use of oxidative phosphorylation even under the same condition. This reprogramming possibly shows that the HG progeny were 'tilted' toward fermentation before exposure to the LPS.
- The data appears to indicate that when the F progeny were exposed to LPS, their State 4 respiration was possibly achieving roughly the same OCR at a lower membrane potential (Fig 4.B). The possibility that the same OCR can be achieved at different membrane potentials could reveal a new understanding of how the regulation of coupling efficiency is programmed.
- Coupled Respiration (Fig 4.E) had virtually the same OCR for all conditions, i.e., reprogramming did not affect OCR via a change in the difference between state 3 and state 4 (of a particular cell population) when dealing with a high glucose environment. Nor did it affect this difference when challenged with a PAMP. Programming, however, possibly caused them to adjust such that they maintained this difference while at different membrane potentials, resulting in different RCRs. We believe this is fundamental to understanding what is happening.
- In both F and HG progenies LPS caused a decrease in the RCR, but it was a greater decrease in the F progeny (19% compared to 15% [Fig.5]) which when untreated were at a higher RCR than the HG Progeny. This is also quite striking when considered with the ADP/O ratio, again supporting the fact that the protocol has in fact reprogrammed the yeast.

5. Conclusion

- In summary, we first demonstrated that *S. cerevisiae* could be reprogrammed to metabolically respond differently to a specific environment, compared to 'normally' programmed yeast.
- To our knowledge, this is the first time it has been shown that programming, at the level of coupling efficiency, could be altered over generations by dietary changes; with reprogramming the regulation of coupling efficiency being the novel contribution.
- Furthermore, the reprogrammed yeast were also shown to respond differently to a PAMP (LPS), with regard to bioenergetic changes associated in mammalian cells with the switch to a proinflammatory and proliferative metabolic state.
- It will now be exciting to move to the next phase and use the model to relate these changes quantitatively to membrane potential.

