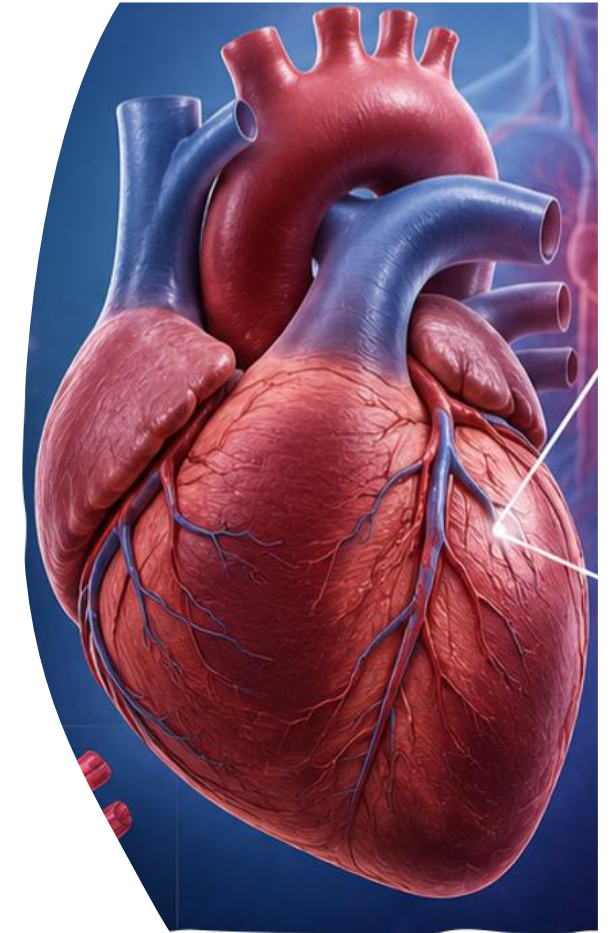


COULD SUCCINATION LEAD TO CARDIOTOXICITY?



AFAF FIZAZI

Mons University - Belgium
Human Biology and Toxicology Laboratory
Promotor: **Jean-Marie COLET**
Co-promotor: **Anne-Emilie DECLEVES**

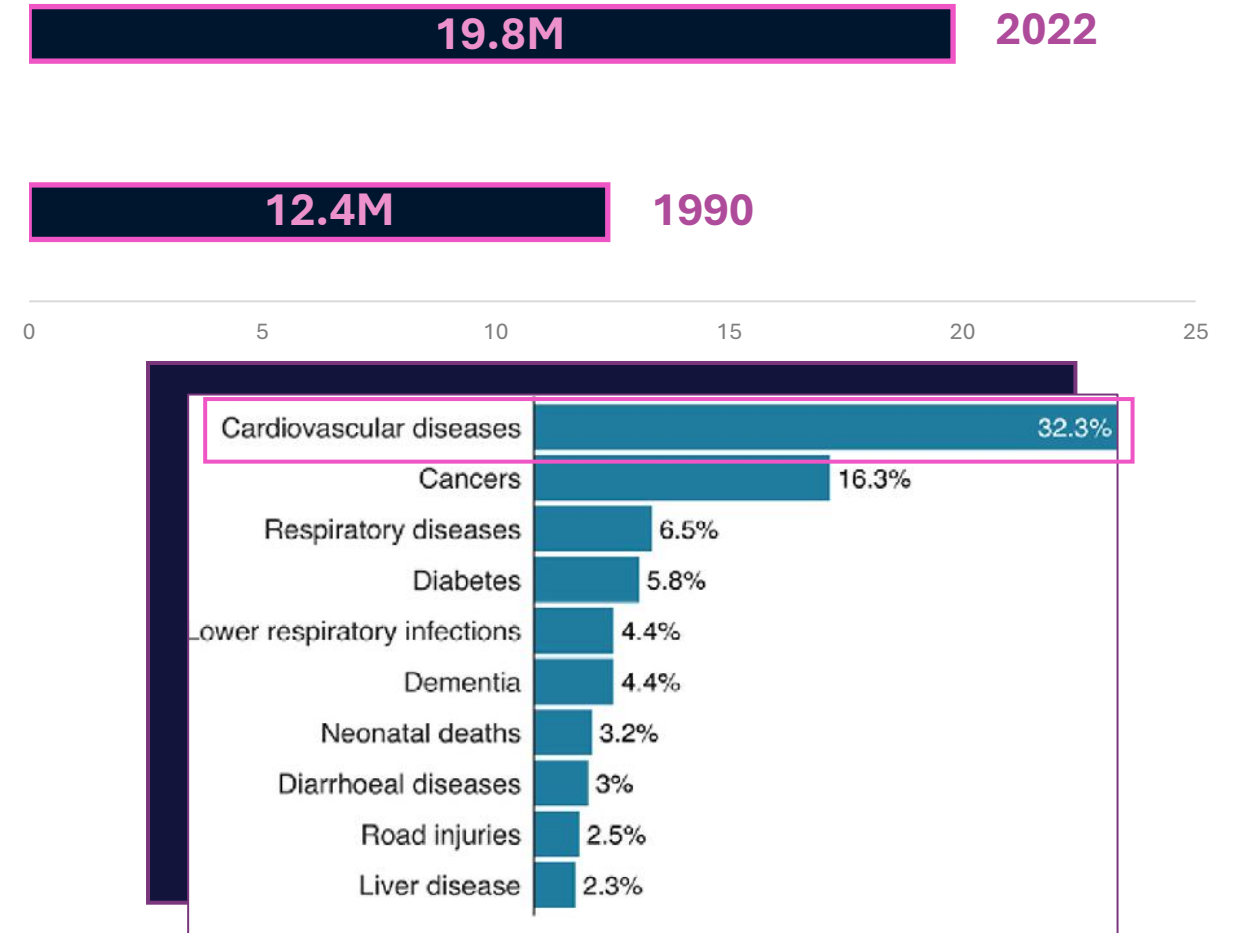


Cardiovascular diseases (CVD)

- Leading cause of mortality worldwide
- 19.8 million deaths annually
- 32% of all deaths worldwide

- Global Burden of Cardiovascular Diseases and Risks 2023 Collaborators, 2025
- WHO, 2022

Global burden of CVDs - 2022



Risk factors

NON-MODIFIABLE	BEHAVIORAL	IATROGENIC	ENVIRONMENTAL	METABOLIC
				
 Age	 Smoking	 Anthracyclines	 Air pollution	 Hypertension
 Sex	 Diet	 Trastuzumab	 Heavy metals	 Diabetes
 Genetics	 Physical inactivity	 TKIs	 Toxins	 Dyslipidemia
	 Alcohol	 Other cardiotoxic drugs		 Obesity

WHO, 2025

Figure generated with OpenAI ChatGPT

ESC Cardio-Oncology Guidelines, 2022

Metabolic diseases are major cardiovascular risk factors

The increasing prevalence of obesity and diabetes is reshaping cardiovascular disease



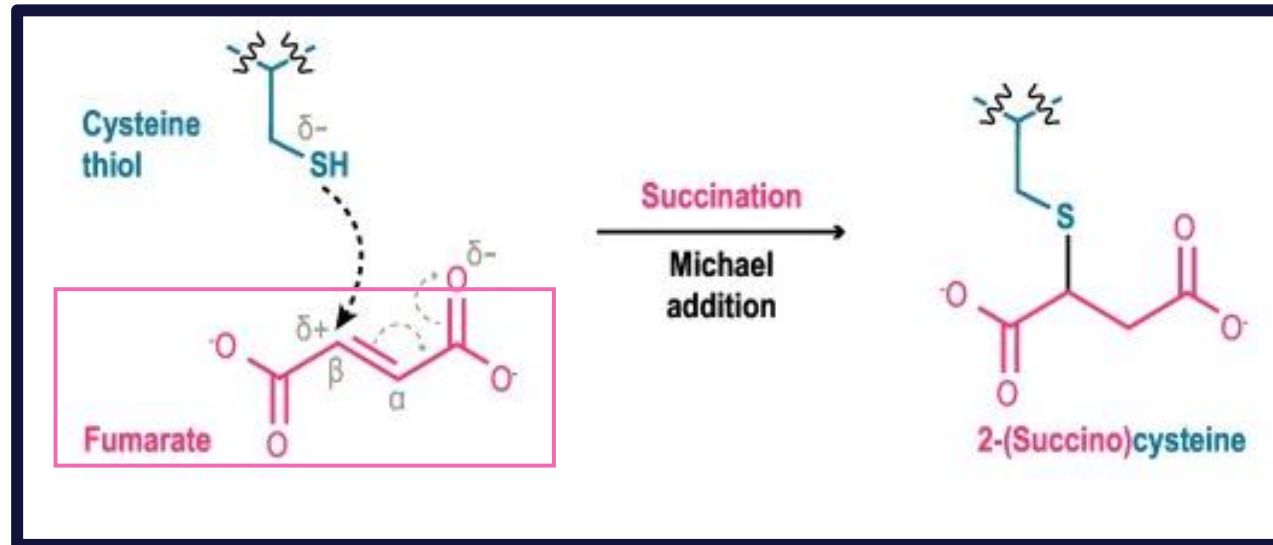
- Oxidative stress
- Mitochondrial dysfunction
- Altered glucose and fatty acid metabolism
- Heart failure development

Figure generated with OpenAI ChatGPT

- Modified from Guberovic, *et al.*, 2024
- Kühner *et al.*, 2025
- Gong *et al.*, 2025

Beyond classical mechanisms, emerging evidence suggests that **metabolic intermediates** themselves may become pathogenic

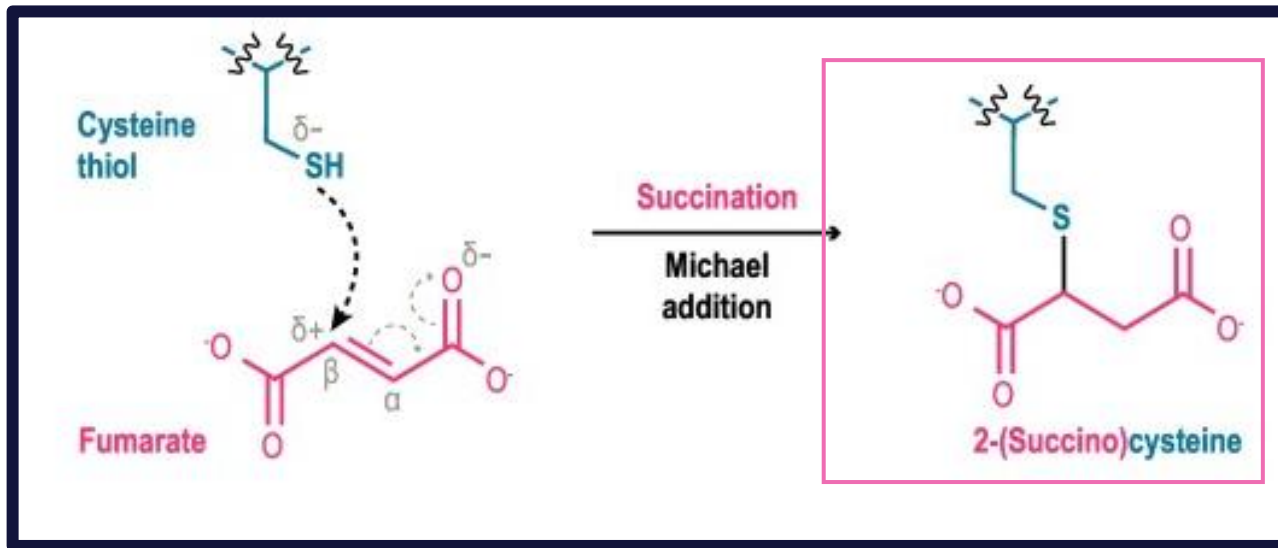
Fumarate: A metabolic intermediate



Guberovic, Iva *et al.*, (2024)

Fumarate: a TCA cycle intermediate that becomes **harmful when accumulated**

Succination



Guberovic, Iva *et al.*, (2024)

- **Spontaneous**
- Non-enzymatic
- Irreversible
- Marker of metabolic stress
- Affects protein function

Central question

**Could elevated fumarate levels
and protein succination induce
cardiotoxicity ?**

Hypothesis



Guberovic, Iva *et al.*, (2024)

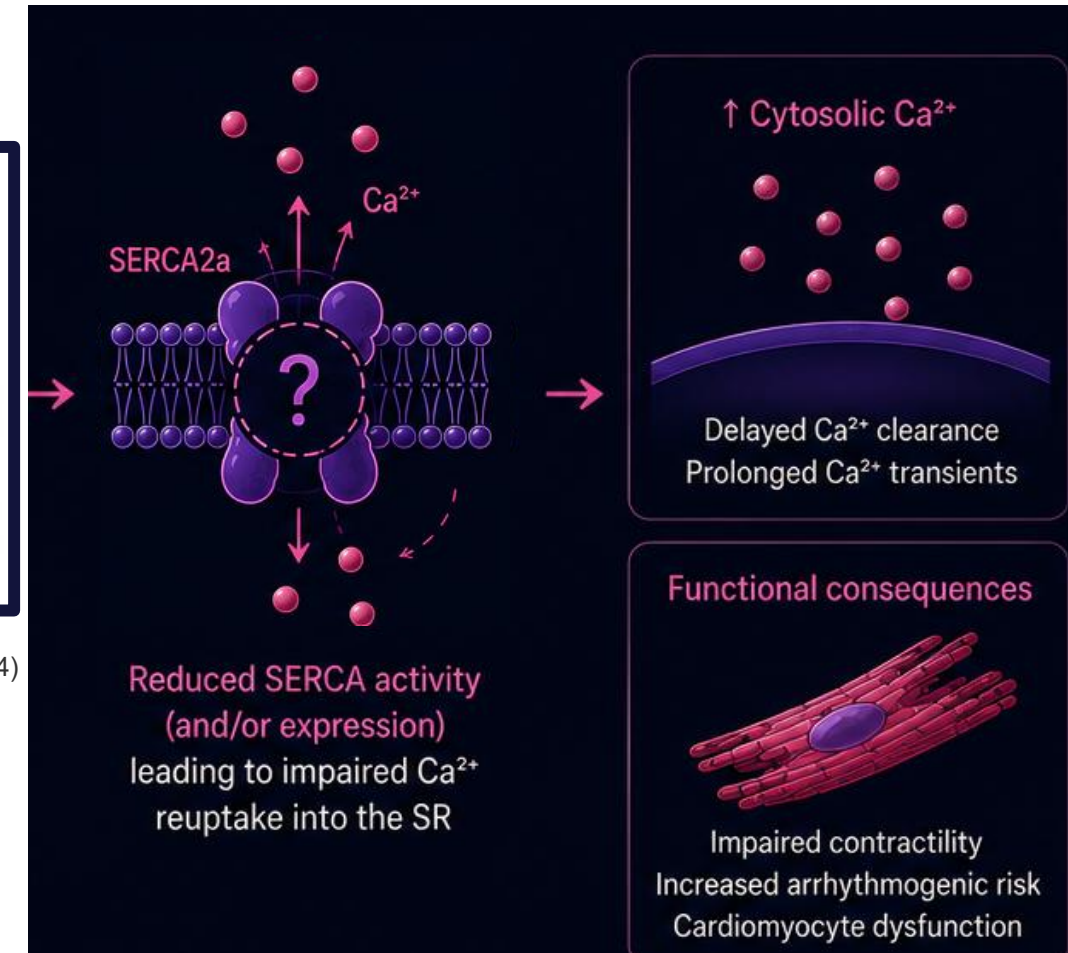
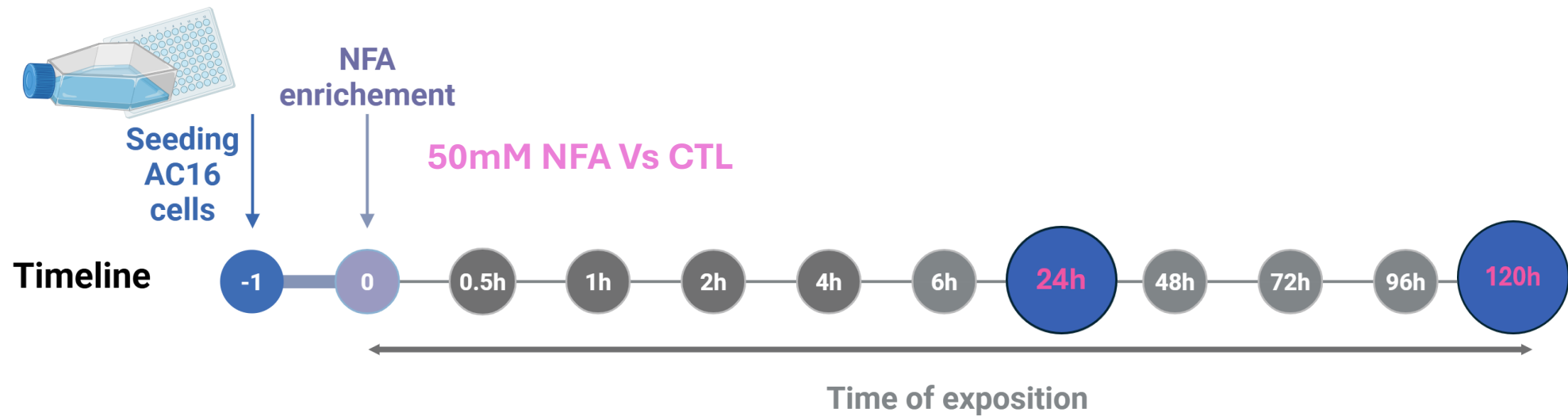


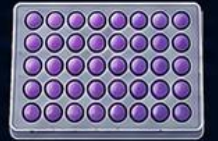
Figure generated with OpenAI ChatGPT

EXPERIMENTAL DESIGN



NFA = Disodium Fumarate

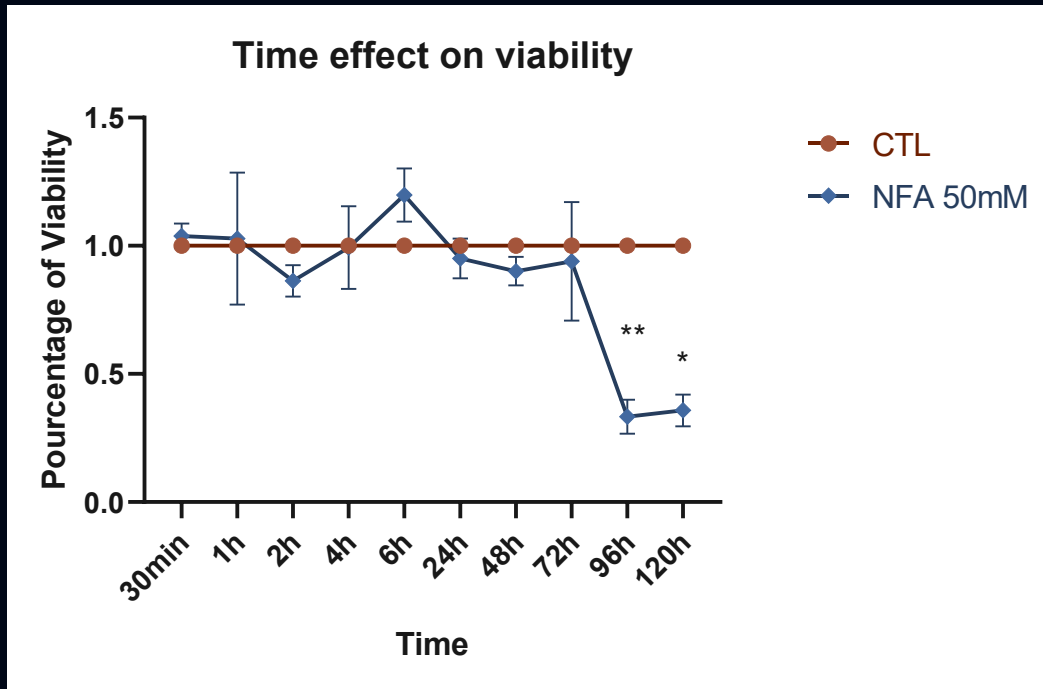
Results - Viability



Cell viability

Initial cellular toxicity

Krystal Violet



Disodium fumarate (NFA) reduced viability in AC16 cells after 96 & 120h



This suggested that uninterrupted exposure to fumarate induces cellular stress

Data are presented as means $\pm$ SEM; $n=6$. Statistical analyses were performed using Two-Way ANOVA followed by Dunnet posttest. * $p = 0,0121$ vs CTL group, ** $p = 0,0084$ vs CTL group

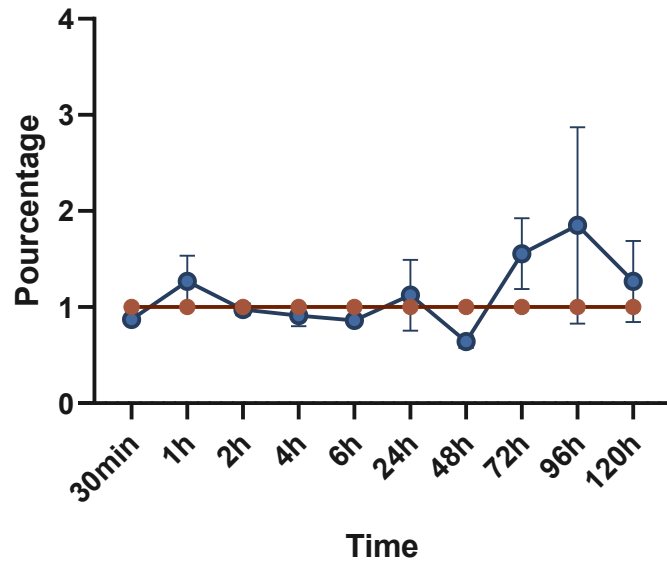
Results – Oxidative stress

Initial cellular toxicity

Oxidative stress – DCFH-DA probe

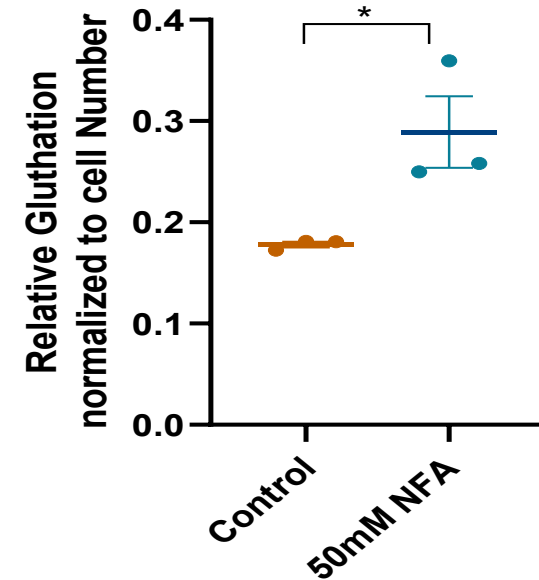


1 Time effect on oxidative stress



Glutathione ratio

2 24h



NFA may activate oxidative stress response through NRF2 pathway!

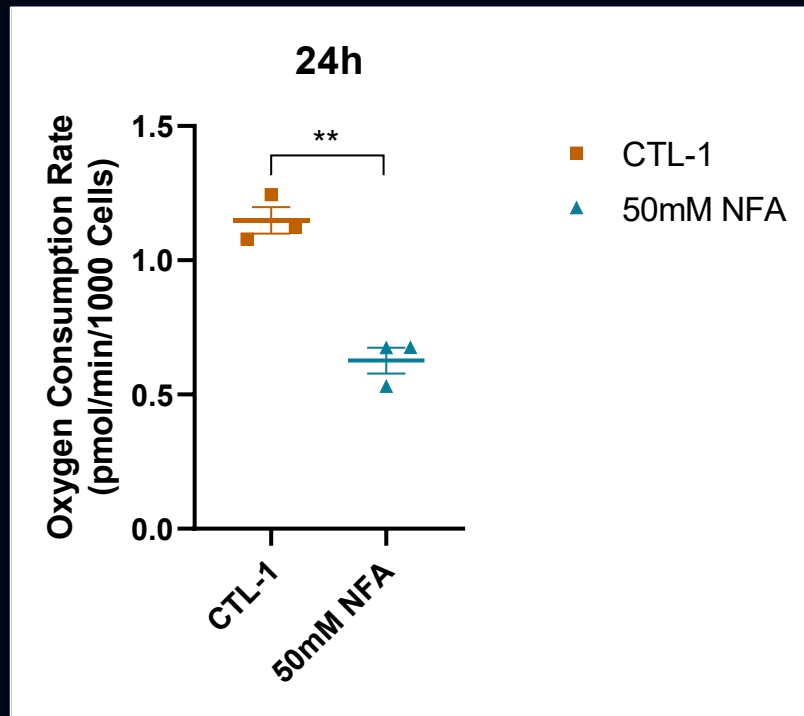
1. Data are presented as means $\pm$ SEM; $n=3$. Statistical analyses were performed by

2. Data are presented as means $\pm$ SEM; $n=3$. Statistical analyses were performed by Unpaired t test. * $p = 0,035$ vs CTL group

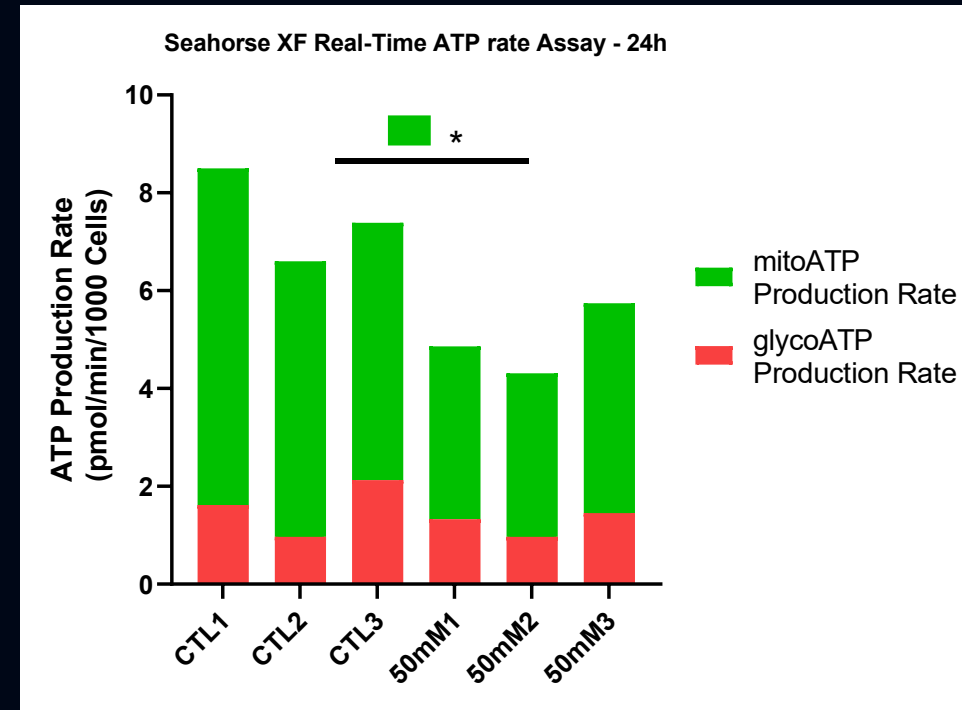
Results – Energetics dysfunction



Oxygen consumption rate (OCR)



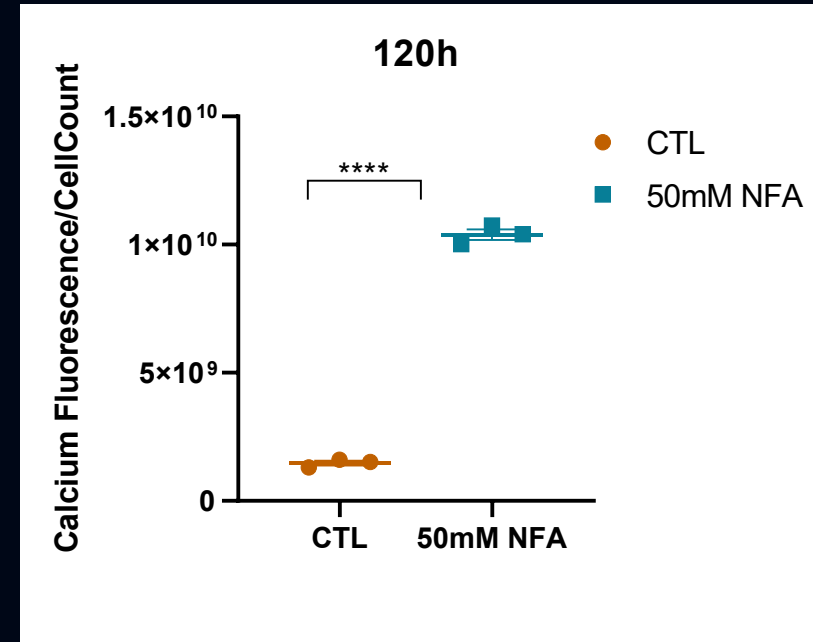
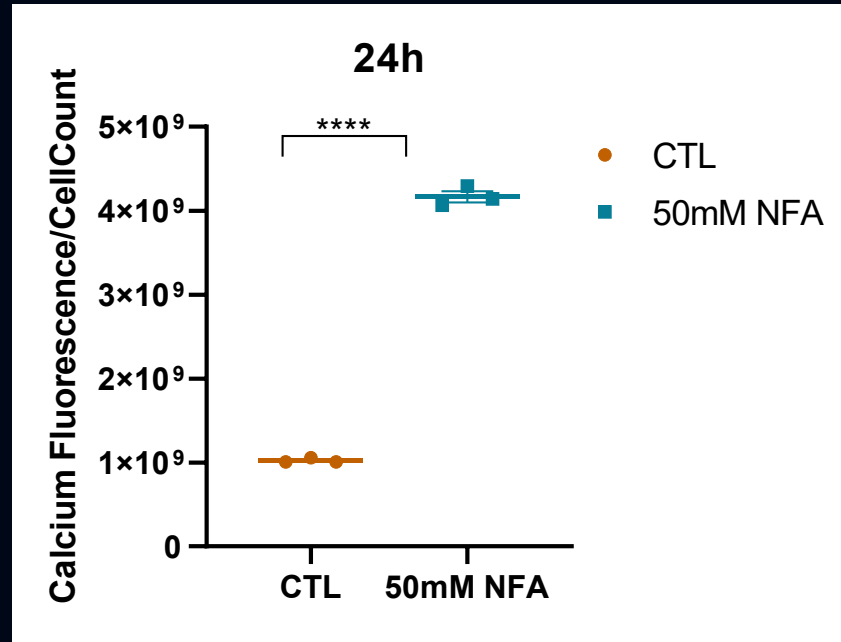
ATP production



- Mitochondria are consuming less oxygen, and mitochondrial ATP production is decreased
- This could reflect an adaptive response, mild metabolic inhibition, or reduced cellular activity

Results – Calcium handling

Does fumarate disrupt calcium handling?



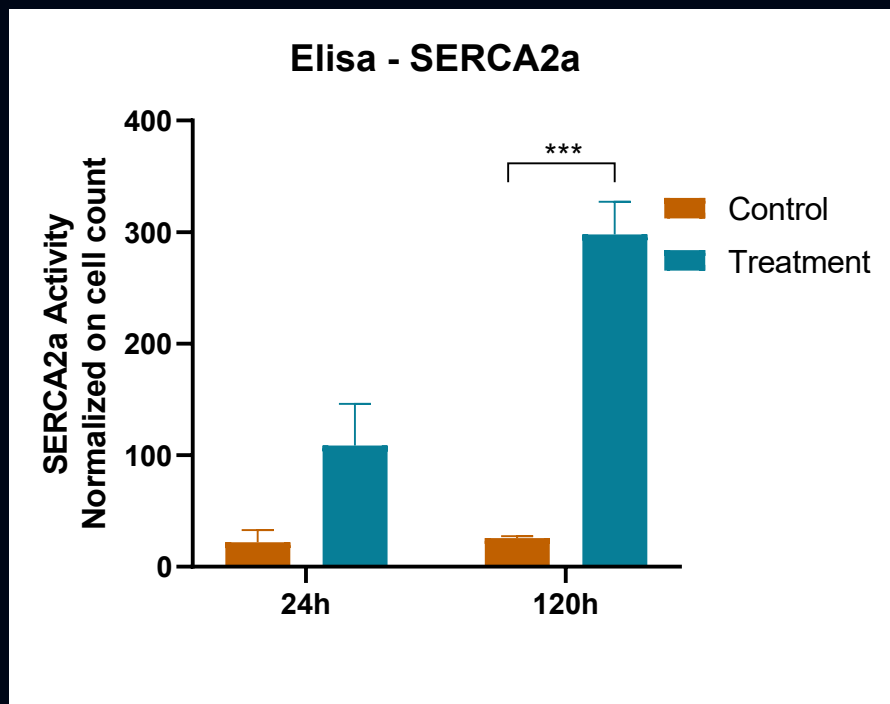
Abnormal intracellular calcium

Data are presented as means $\pm$ SEM; $n=3$. Statistical analyses were performed by Unpaired t test. **** $p < 0,0001$ vs CTL group

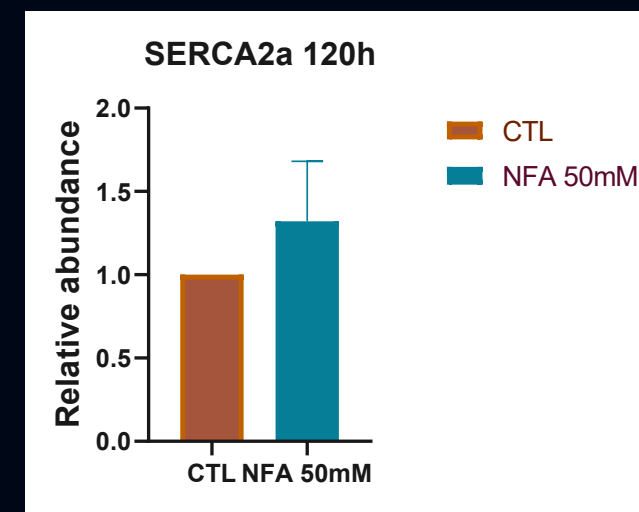
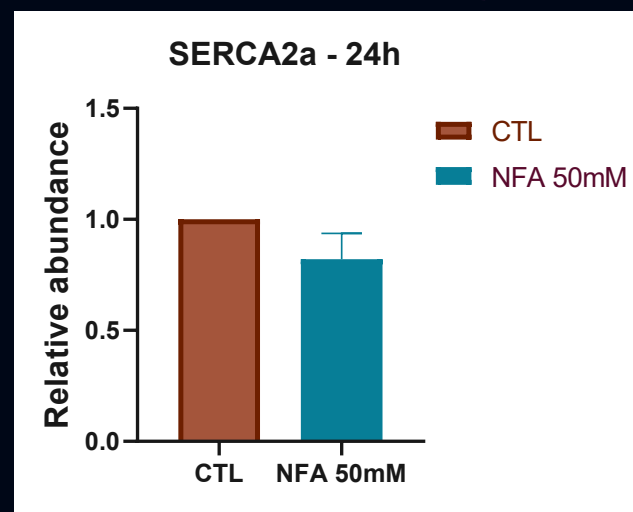
Results – SERCA2a



Elisa results



Western Blot results



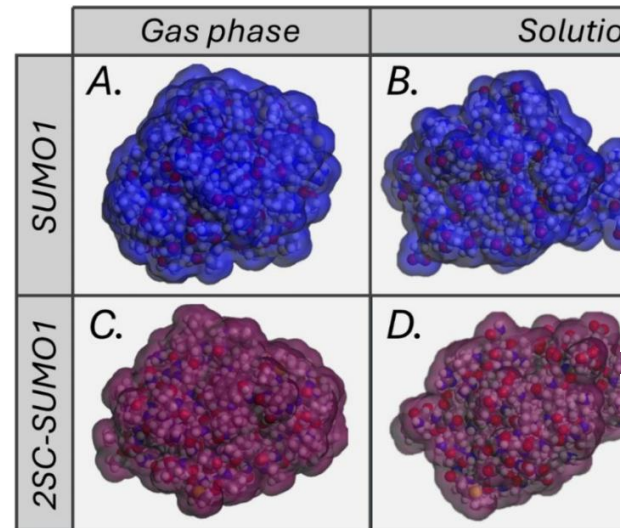
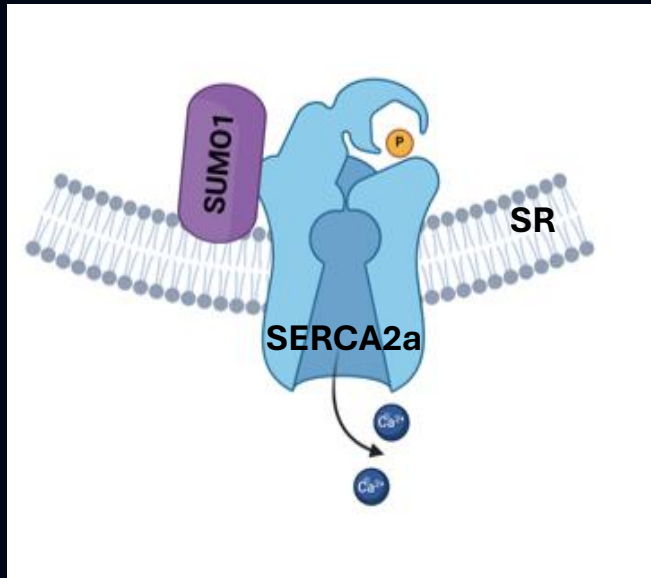
SERCA2a
Tubulin



- SERCA2a's dysregulation is perhaps associated with changes in calcium metabolism
- The change in activity of SERCA2a does not implicate a change in its abundance

Elisa. Data are presented as means $\pm$ SEM; $n=6$. Statistical analyses were performed using Two-Way ANOVA followed by Tukey's multiple comparisons test. *** $p = 0.0002$ vs CTL group. **Western Blot.** Data are presented as means $\pm$ SEM; $n=3$. Statistical analyses were performed using an Unpaired t test.

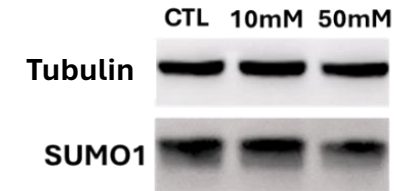
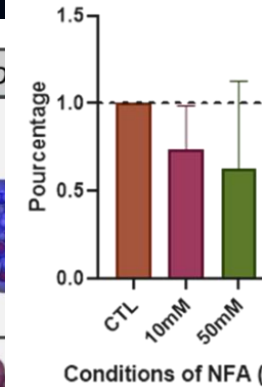
Results – SUMO1



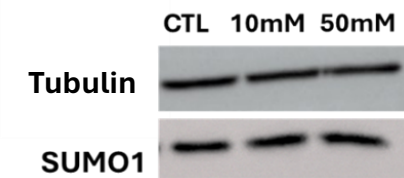
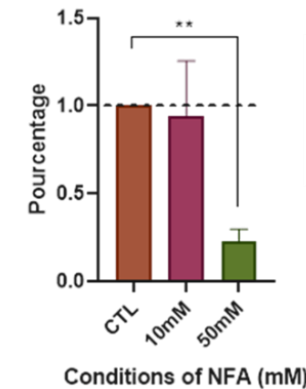
Groignet *et al.*, 2025

Western Blot results

Relative abundance of SUMO1 24h



Relative abundance of SUMO1 120h



Gas-phase (A and C) and solution phase (B and D) Molecular Dynamics simulations. The blue structures represent SUMO1 ions (6+ in the gas phase and 4- in solution), while the purple structures represent 2SC-SUMO1 ions (6+ in the gas phase and 4- in solution)

Results – Section 6

Metabolic remodeling : ^1H -NMR

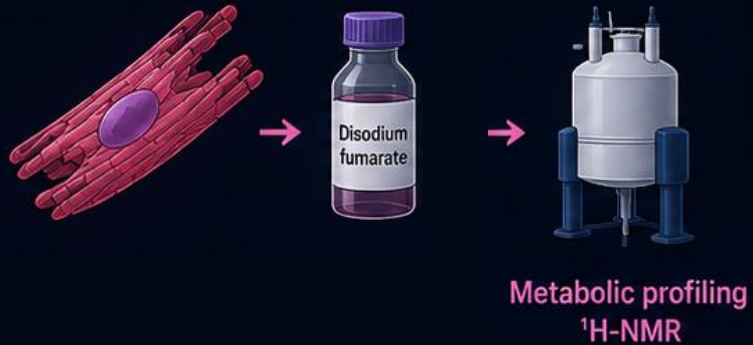
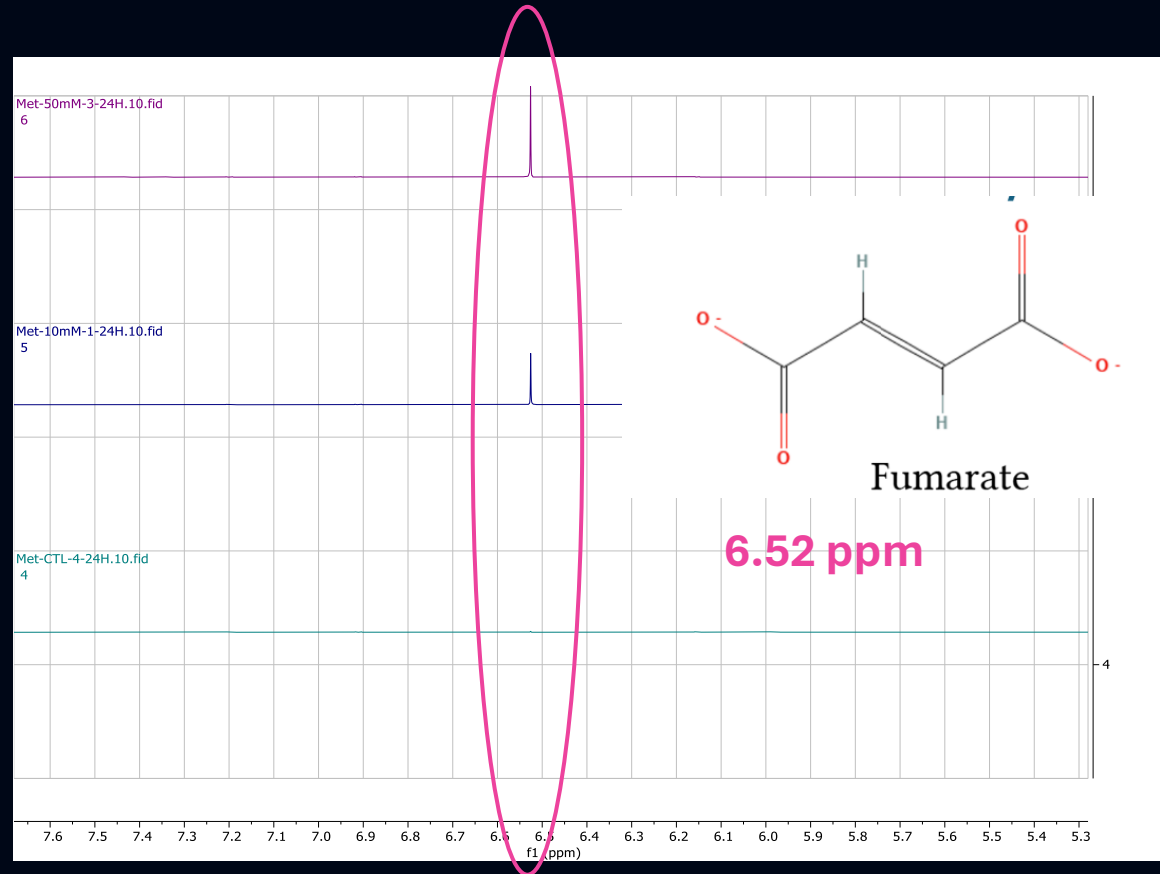
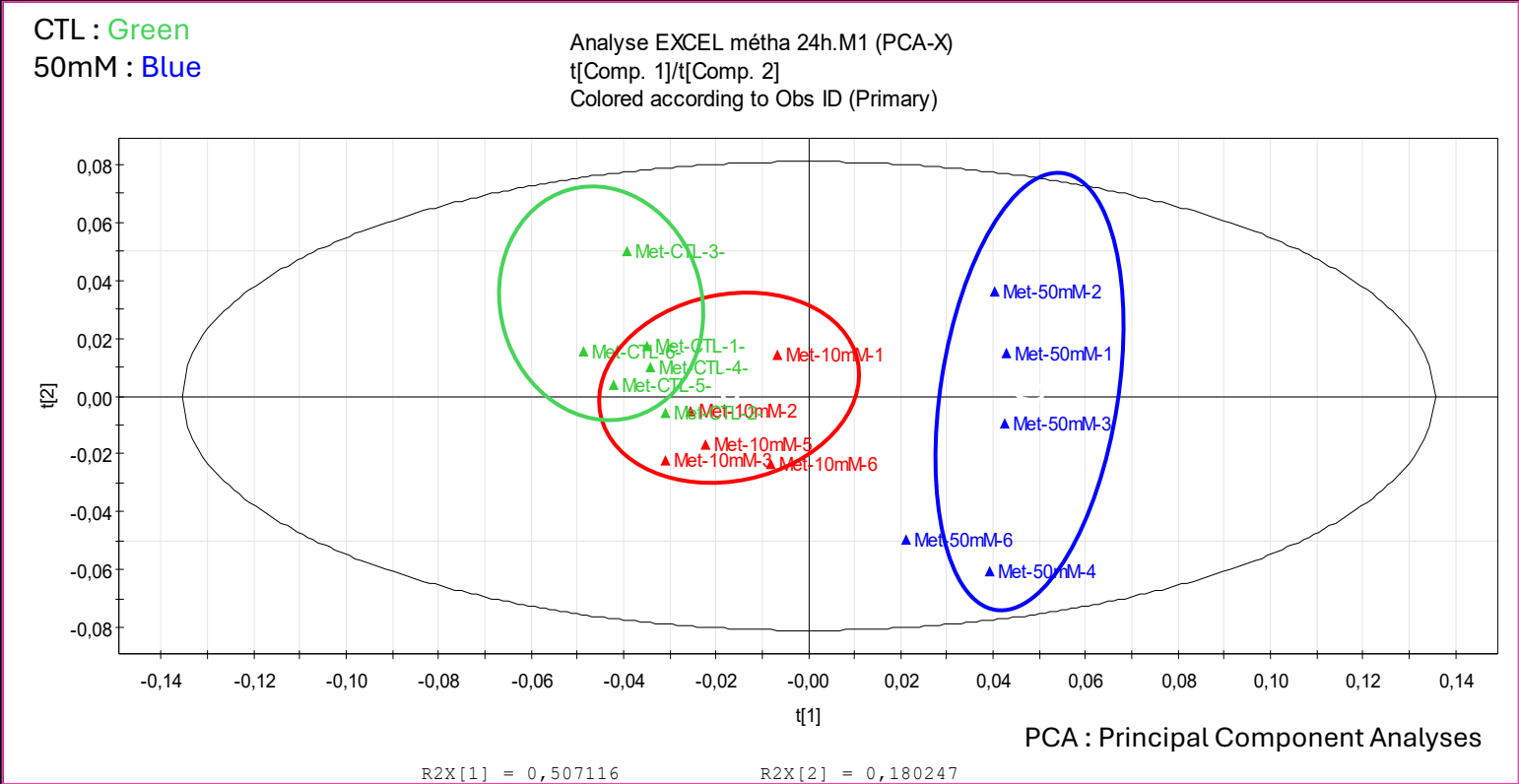


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Semi-quantitative analysis



Metabolic remodeling : ¹H-NMR



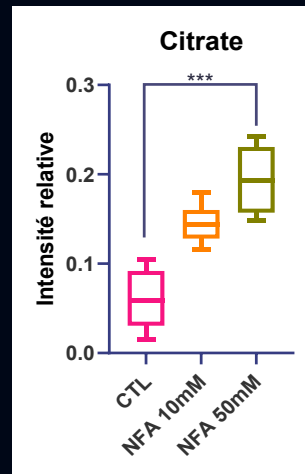
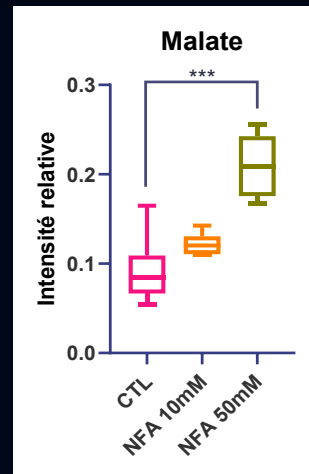
INTRACELLULAR EXTRACT

Results – Section 6

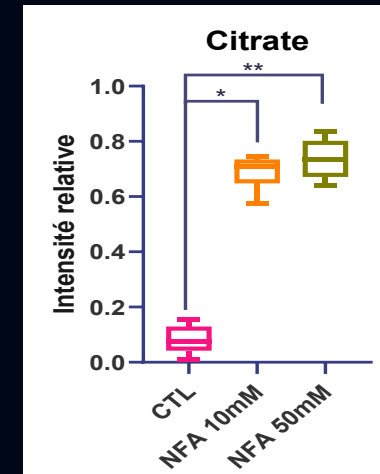
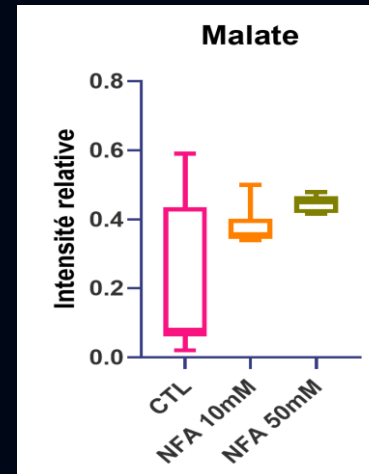
^1H -NMR reveals metabolic remodeling

1 – TCA cycle modification

24h - extracellular media



120h - extracellular media



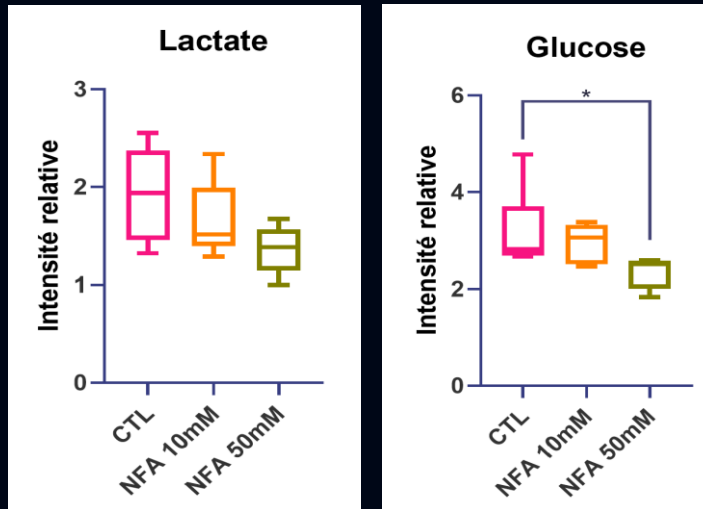
- The cardiomyocyte tries to create a balance due to the high levels of fumarate

The data are presented as an average $\pm$ SEM. At 24h and 120h, $n = 6$ for all conditions. Statistical analyses were performed using the non-parametric Kruskal-Wallis test followed, if significant, by a post hoc Dunn's test. * $p = 0.05$ vs CTL group. ** $p = 0.001$ vs CTL group. *** $p = 0.0001$ vs CTL group

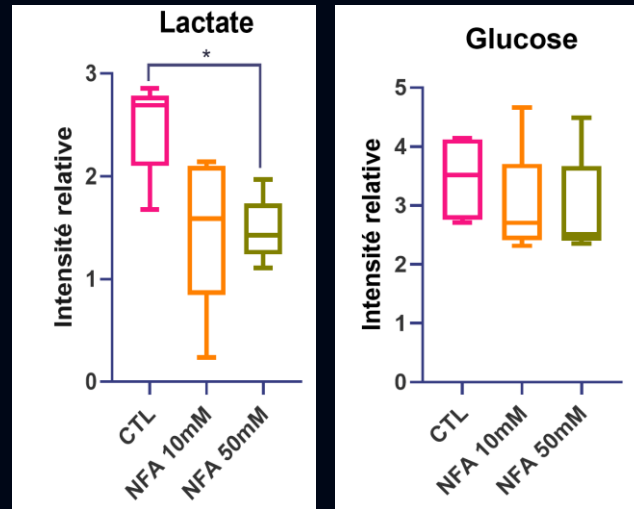
Results – Section 6

Metabolic reprogramming

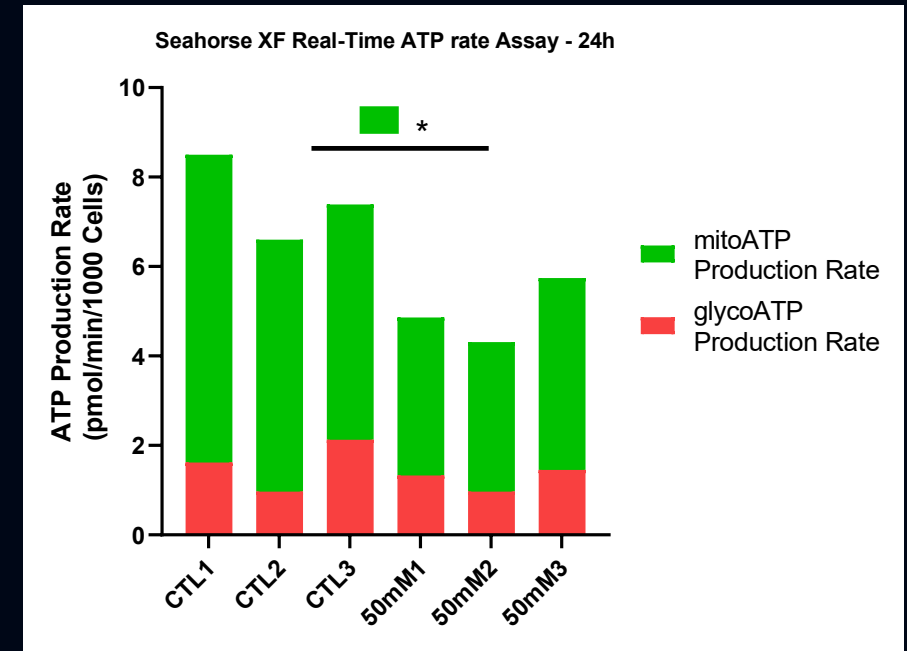
24H - Intracellular Environment



120H - Intracellular Environment

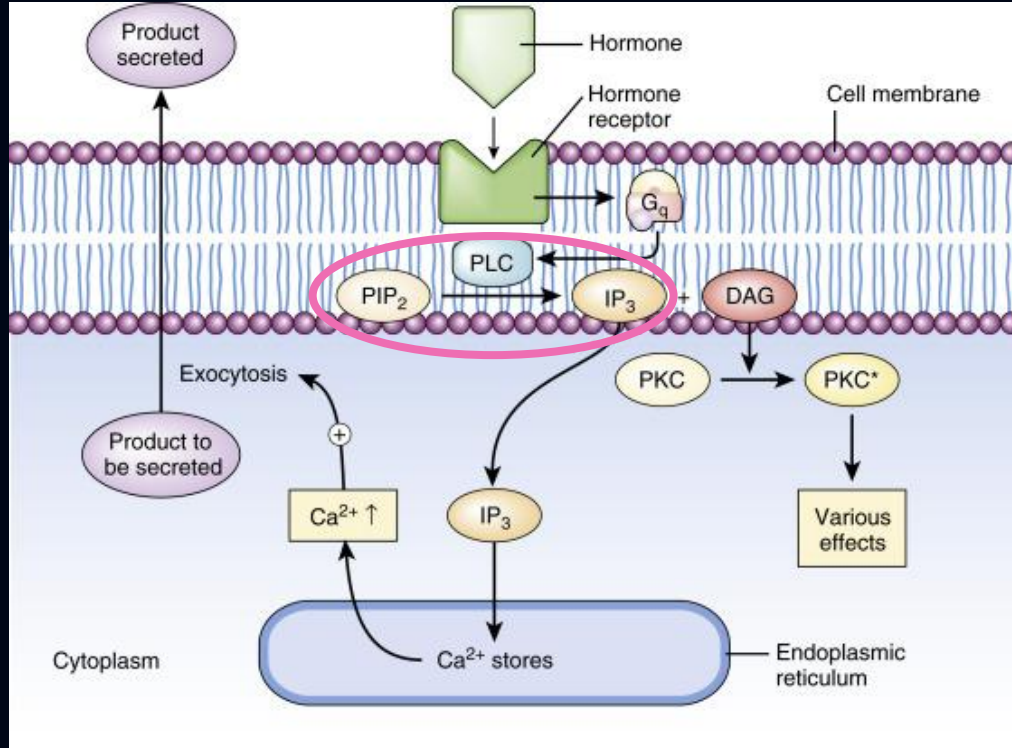


ATP production



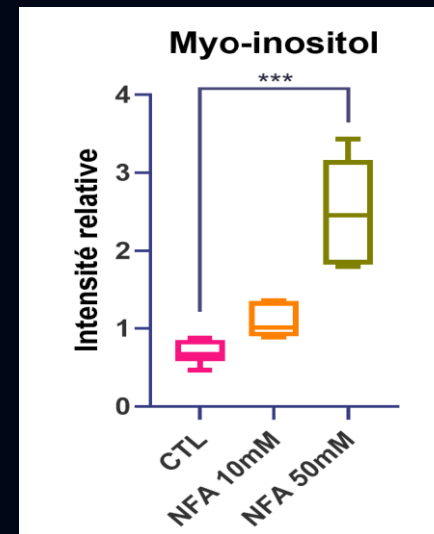
Data are presented as an average $\pm$ SEM. At 24h, $n = 6$ CTRL and $n = 5$ for NFA 10mM and 50mM and at 120h, $n = 5$ for all conditions. Statistical analyses were performed using non-parametric Kruskal-Wallis test followed, if significant, by a post hoc Dunn's test. * $p \leq 0.05$ vs CTL group.

Results – Section 6

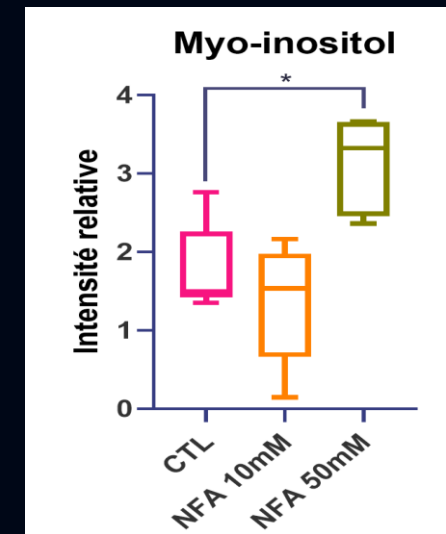


Norris D. *et al.*, (2013)

24h Intracellular media



120h Intracellular media



Data are presented as whiskers min to max; n=7 CTL; n=6 10mM & 50mM. Statistical analyses were performed by Kruskal-wallis test followed by multiple comparison Dunn's test. *** $p \leq 0.001$ vs CTL group. * $p \leq 0.05$ vs CTL group.

Summary

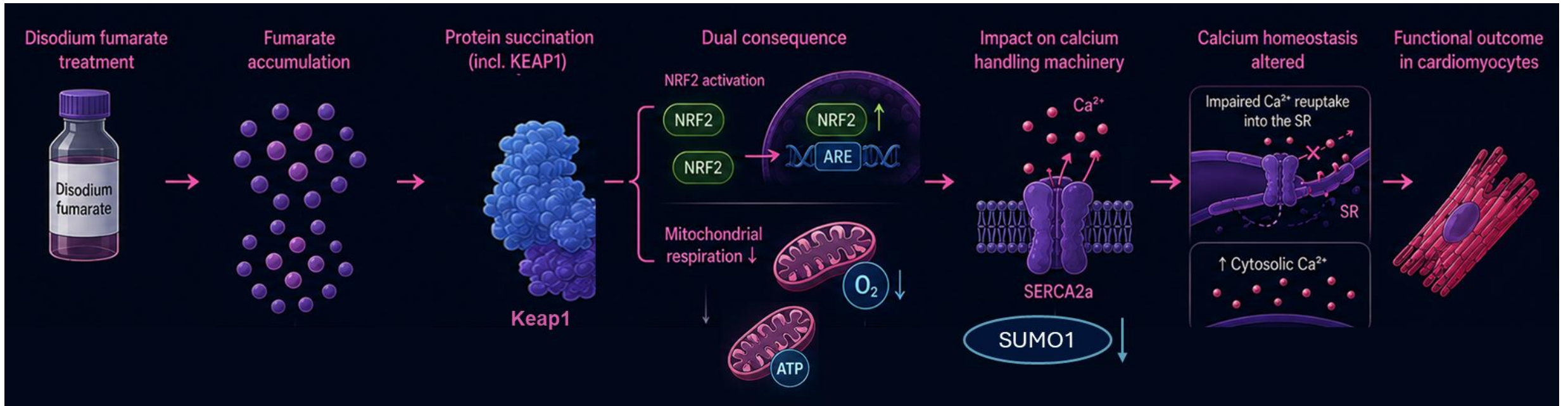


Figure generated with OpenAI ChatGPT

Take home message

Our findings support the hypothesis that fumarate accumulation induces coordinated cellular alterations. Whether protein succination represents the mechanistic link between these observations remains the central question that we are currently investigating

Acknowledgements

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- Anne-Emilie Declèves



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- Groignet Louis



Contributors

- Cloé Schoeling
- Groignet Louis
- Raphaël Conotte
- De Winter Julien
- Anne-Emilie Declèves
- **Jean-Marie Colet**



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