# Effect of Hydrogen Peroxide on Yeast Division

Diego Landeros


## Abstract

**The purpose of this project is to determine the effect of inducing higher levels of oxidative stress on the proliferation of fermenting *S. cerevisiae*. These cells have a similar metabolism to tumor cells, which can develop drug resistance to modern chemotherapy. Previous studies have simultaneously linked reactive oxygen species to tumor progression and revealed that the promotion of antioxidants in tumor models escalates precancerous tissue growth (Tran & Green, 2019; Sayin et al., 2014). This experiment tests if the administration of exogenous reactive oxygen species is a viable means to reduce the proliferation of cancer cells. Absorbance of culture samples at wavelength 600 nm was collected every thirty minutes for five hours and converted to cells/mL. The hypothesis is that if *S. cerevisiae* cultures are administered with varying concentrations of hydrogen peroxide ($H_2O_2$), then the culture with the highest concentration of $H_2O_2$ - 400 µM - will divide the least, because the ability of *S. cerevisiae* cells to divide will be impaired as oxidative stress results in the degradation of biomolecules crucial to cellular function - effectively decreasing total growth (Turrens, 2003). The mean results revealed a dose-dependent impairment of *S. cerevisiae* proliferation by $H_2O_2$: $28056 \pm 222 * 10^3$ cells/mL without $H_2O_2$, $20734 \pm 76 * 10^3$ cells/mL in 100 µM $H_2O_2$, $20550 \pm 58 * 10^3$ cells/mL in 200 µM $H_2O_2$, $20454 \pm 49 * 10^3$ cells/mL in 300 µM $H_2O_2$, and $20320 \pm 34 * 10^3$ in µM $H_2O_2$. The cultures with 400 µM $H_2O_2$ generated the lowest absorbance readings and thus the lowest concentrations of *S. cerevisiae*. The hypothesis was supported. Future research should be performed to improve the selective druggability of tumor cells by ROS.**


***Keywords:*** **Oxidative Stress, Oncology, *S. cerevisiae***

## Introduction

Oncologists face the issue of increased drug resistance in tumor cells upon the application of chemotherapeutics. Known as multi-drug resistance (MDR), this process often results from the extraction of chemotherapy drugs from cancer cells by transporter proteins. In over 90% of patients with spreading cancer, MDR prevents treatment success, hindering therapeutic function and vitiating patient prognosis (Catalano et al., 2022; Rueff & Rodrigues, 2016). If novel therapeutic approaches to cancer treatment are not under study, improvements to reduced patient mortality may not be achieved.

## Literature Review

Within eukaryotic cells, mitochondrial reactive oxygen species (ROS) are used as adaptive redox cell signaling molecules to maintain homeostasis; in oxidative phosphorylation, electron leaks also produce ROS (Milkovic et al., 2019; Turrens, 2003). ROS are molecular-oxygen-derived molecules and free radicals, species such as hydrogen peroxide ($H_2O_2$) with at least one unpaired electron (Jakubczyk et al., 2020). However, cancer can exploit the growth-signaling capacity of ROS to encourage tumor proliferation (Milkovic et al., 2019; Zhang et al., 2022). ROS overproduction induces an imbalance between endogenous ROS and cellular antioxidant power, named oxidative stress. Oxidative stress expedites "free radical-mediated chain reactions which indiscriminately target proteins, lipids, polysaccharides and DNA," leading to both apoptosis and necrosis as cell function is altered (Bardaweel et al., 2018; Turrens, 2003, p. 339).

Winterbourn (2013) maintains that as an ROS, $H_2O_2$ is a chemical compound and oxidizing agent that can induce cellular damage and cell death at high concentrations. Peroxiredoxins (Prxs) regulate the intracellular concentration of $H_2O_2$, decreasing its harmful potential and allowing for its local accumulation as a cellular signaling molecule (Winterbourn, 2013). As a signalling molecule, $H_2O_2$ is used to oxidize cysteine residues in order to activate transcriptional programs of transcription factors such as KEAP1-NRF2 and NF-κB (Konno et al., 2021).

Tumor cells induce major cellular, physical, and molecular changes to host tissues to maintain heightened levels of cell growth. MDR in cancer cells is maintained by drug efflux molecules, particularly upregulated ABC transporter proteins (Catalano et al, 2022). Furthermore, cancer cells must rewire their metabolism to thrive in the metabolically altered conditions of the tumor microenvironment (TME). The Warburg effect describes the proclivity of rapidly dividing cells, such as cancerous ones, to consume glucose and maintain aerobic glycolysis (Elia & Haigis, 2021). Since aerobic glycolysis does not involve oxidative phosphorylation, the Warburg effect decreases tumoral ROS levels.

The genetics of the unicellular Saccharomyces cerevisiae, baker's yeast, are 23%homologous to the genetics of humans, making it a model eukaryotic organism in the investigation of human gene function (Liu et al., 2017). As a eukaryotic organism, the *S. cerevisiae* cell cycle is mitotic and separated into four distinct phases in which cells prepare for the generation of daughter cells. Throughout this highly regulated progression, genetic information and cellular components of a parent cell are duplicated and partitioned, with daughter cells finally budding off the surface of parental cells (Jiménez et al., 2015). *S. cerevisiae* are inexpensive to maintain and exhibit rapid generation times, engendering their wide adoption in research (Duina et al., 2014). Furthermore, as asserted by Farrugia and Balzan (2012), production and defense systems of ROS are conserved in *S. cerevisiae*, resulting in its use for research on oxidative stress. Parallel metabolic fluxes in cancer and baker's yeast allow for the mechanisms of pathways in cancer to be further investigated (Natter & Kohlwein, 2013). For instance, *S. cerevisiae*

undergoes upregulated aerobic glycolysis in the presence of high levels of glucose via the Crabtree effect, similar to the Warburg effect in cancer cells (Pfeiffer & Morley, 2014).

A limited number of antioxidants offer yeast cells protection against ROS, such as glutathione (GSH) and superoxide dismutase (SOD), which respectively protect thiol groups in proteins from oxidation and detoxify progenitors of the most harmful ROS. Additionally, the yeast activator protein 1 (Yap1p) promotes antioxidant gene transcription; for instance, the GSH1 and GSH2 sequences are upregulated to promote the GSH system (Farrugia & Balzan). Furthermore, yeast cells can develop a tolerance to significant levels of oxidative stress through moderate concentrations of oxidants or exposure of external antioxidants (Eleutherio et al., 2018).

Previous studies have revealed that several oncogenic stimuli - carcinogens, radiation, aging, and more - have promoted the production of $H_2O_2$ within cells, leading researchers to hypothesize that the species may play a role in primary tumor-initiating occurrences (Lisanti et al., 2011). Tran and Green (2019) linked the NADPH oxidase enzymes NOX4, DUOX1, and DUOX2, major sources of $H_2O_2$ production, to metastatic, invasive, and chemoresistant tumors, further connecting $H_2O_2$ to tumor progression. Finally, Sayin et al. (2014) conducted a study on the effect of antioxidants on tumor progression in mouse models of lung cancer. By reducing ROS and DNA damage, antioxidants N-acetylcysteine and vitamin E in diets of mice with induced lung cancer escalated the growth of precancerous tissue. This resolves that fluctuations in oxidative stress are significant to rates of survival in models of cancer progression, suggesting pro-oxidants may serve as detrimental agents to cancer growth. Increased knowledge about intratumoral thresholds of ROS will aid the scientific community in pursuing pro-oxidants as a means of precision therapy.

## Methodology

### Safety

Standard laboratory protocol was followed, including the wearing of personal protective equipment. All flasks were labeled. The workstation was disinfected before and after experimentation. To dispose of flask contents, they first were poured with distilled water into a glass bottle. 15 mL of bleach was added to the bottle and it was left capped until the turbidity reduced. The bleach solution was poured down the sink followed by 30 seconds of steady water flow.

### Preparation of the Experiment

1. The Vernier Spectral Analysis application was downloaded on a Chromebook. A Go Direct SpectroVis Plus Spectrophotometer was connected via USB. "Absorbance vs. concentration (Beer's Law)." was selected.
2. 1 stir bar was slid into an Erlenmeyer flask and 100 mL distilled water was poured into the same flask using a funnel. 100 mL of 3% $H_2O_2$ diluted to desired molarity (100, 200, 300, or 400 µM) was poured in if the trial required. 5 g Sabouraud-Dextrose broth was suspended into the flask. This was the growth medium.
3. The flask was centered onto a magnetic stirrer. The stirring speed was increased to 200 rpm for 5 minutes.
4. The blank solution was prepared using a pipette to fill a cuvette ¾ full with growth medium.
5. The spectrophotometer was calibrated using the blank solution. The wavelength was set to 600 nm, the maximum absorbency of *S. cerevisiae*.
6. Steps 2-5 were repeated when collecting data for cultures with a distinct $H_2O_2$ concentration than that in the blank solution.

### Experiment

1. A Fleischmann's RapidRise Instant Yeast packet was emptied into the flask used to prepare the blank solution as it stirred.
2. 30 minutes passed until the absorbance of the flask culture sample was recorded in a prepared data table. Absorbency was collected every 30 minutes until the culture had grown for 5h.
3. 5 g Anhydrous Dextrose was added to the flask every hour to stimulate the Crabtree effect. Data was collected before adding the Anhydrous Dextrose.
4. After 5h growth, the magnetic stirrer was turned off and all flask contents but the stir bar were disposed of.

**Fig. 1.** Blank Solution


**Fig. 2.** Flask Culture

**Fig. 3.** Cuvete sample

## Results

**Table 1.1**

| Effect of Hydrogen Peroxide on S. cerevisiae Absorbance (600 nm) | | | | | |
|---|---|---|---|---|---|
| | Concentration of Hydrogen Peroxide (µM) | | | | |
| | 0 | 100 | 200 | 300 | 400 |
| **Trial 1** | 2.810 | 2.074 | 2.069 | 2.056 | 2.036 |
| **Trial 2** | 2.779 | 2.069 | 2.045 | 2.045 | 2.020 |
| **Trial 3** | 2.879 | 2.055 | 2.038 | 2.048 | 2.029 |
| **Trial 4** | 2.815 | 2.068 | 2.063 | 2.051 | 2.036 |
| **Trial 5** | 2.745 | 2.101 | 2.060 | 2.027 | 2.039 |
| **Mean** | **2.806** | **2.073** | **2.055** | **2.045** | **2.032** |
| **SD** | **0.050** | **0.017** | **0.013** | **0.011** | **0.008** |
| **SE** | **0.022** | **0.008** | **0.006** | **0.005** | **0.003** |

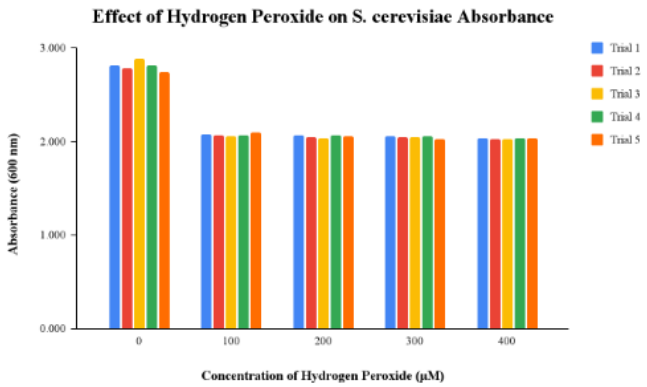
Table 1.1 shows the effect of hydrogen peroxide on *S. cerevisiae* culture absorbance at 600 nm after 5h growth, with the mean, standard error, and standard deviation.

**Table 2.1**

| Effect of Hydrogen Peroxide on S. cerevisiae Cell Concentration (10^3 cells/mL) | | | | | |
|---|---|---|---|---|---|
| | Concentration of Hydrogen Peroxide (μM) | | | | |
| | 0 | 100 | 200 | 300 | 400 |
| **Trial 1** | 28100 | 20740 | 20690 | 20560 | 20360 |
| **Trial 2** | 27790 | 20690 | 20450 | 20450 | 20200 |
| **Trial 3** | 28790 | 20550 | 20380 | 20480 | 20290 |
| **Trial 4** | 28150 | 20680 | 20630 | 20510 | 20360 |
| **Trial 5** | 27450 | 21010 | 20600 | 20270 | 20390 |
| **Mean** | **28056** | **20734** | **20550** | **20454** | **20320** |
| **SD** | **497** | **169** | **130** | **111** | **76** |
| **SE** | **222** | **76** | **58** | **49** | **34** |

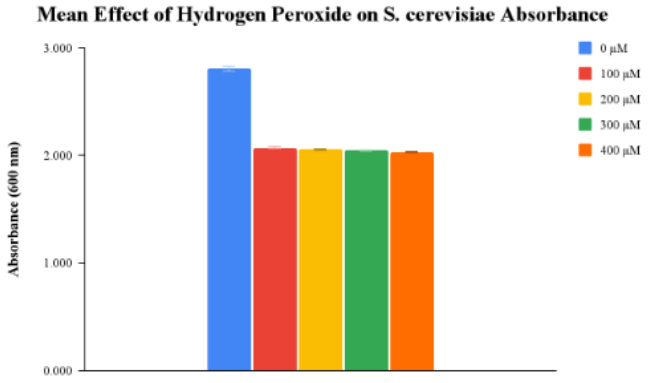
Table 2.1 shows the effect of hydrogen peroxide on *S. cerevisiae* concentration in 103 cells/mL after 5h, calculated using the corresponding scale factor for *S. cerevisiae*, 1 unit OD600 to 107 cells/mL. Mean, standard deviation, and standard error are also calculated.

**Graph 1.1**



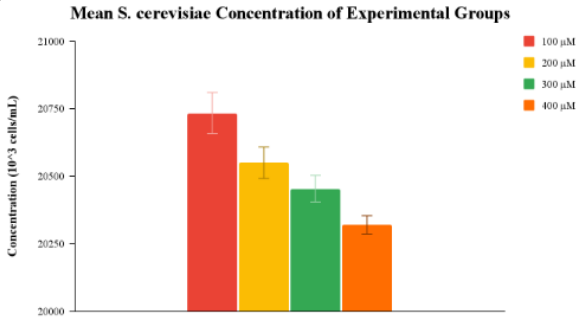

Graph 1.1 shows the effect of hydrogen peroxide concentration on *S. cerevisiae* culture absorbance at 600 nm over 5 trials after 5h growth.

**Graph 1.2**



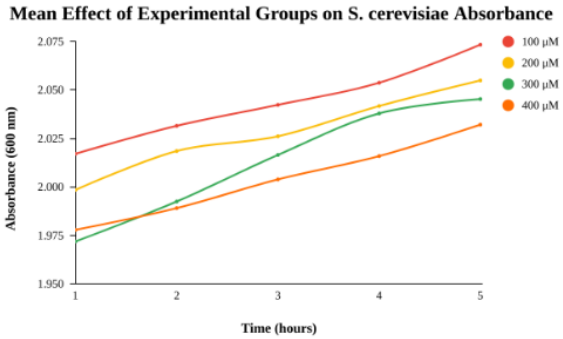

Graph 1.2 shows the mean effect of hydrogen peroxide concentration on *S. cerevisiae* absorbance at 600 nm after 5h growth.

**Graph 2.1**




Graph 2.1 portrays the mean *S. cerevisiae* cell concentrations in 103 cells/mL of the experimental groups after 5h growth, using the corresponding scale factor for *S. cerevisiae*, 1 unit OD600 to 107 cells/mL.

**Graph 3.1**




Graph 3.1 depicts the mean increases in absorbance (600 nm) over time as *S. cerevisiae* was cultured in each experimental condition.

The mean concentration of *S. cerevisiae* yielded in the liquid culture with 400 μM $H_2O_2$ was the lowest of all means: $20320 \pm 34 * 10^3$ cells/mL. The concentration of the control culture was the highest cell concentration at $28056 \pm 222 * 10^3$ cells/mL. The administration of 400 μM $H_2O_2$ was the most effective in its inhibition of novel cell growth within the flask cultures, but concentration margins within experimental groups were small compared to their margins with the control group. Graphs 2.1 and 3.1 depict a relationship between higher concentrations of $H_2O_2$ and more growth inhibition in culture. Standard error in mean data represented through Graphs 1.2, 1.3, and 2.1 overlaps between 200 and 300 μM $H_2O_2$.

## Discussion

The data revealed that higher doses of oxidant relate to lower levels of *S. cerevisiae* growth in culture. This is supported by the linear decrease in the average *S. cerevisiae* concentrations in cells/mL as the concentrations of $H_2O_2$ increased. A steep decrease in final *S. cerevisiae* concentration from the control to 100 μM $H_2O_2$ was observed. This indicates that even the least concentrated oxidant, 100 μM $H_2O_2$, was a profound stressor of the oxidative homeostasis within the *S. cerevisiae* culture. Therefore, in all experimental groups, *S. cerevisiae* cultures underwent increasing amounts of $H_2O_2$-mediated programmed cell death through either apoptosis or necrosis. Due to the role of $H_2O_2$ as a signalling molecule at moderate concentrations in the cellular environment for growth

signalling and protein activation, smaller concentrations of the oxidant would have possibly promoted the growth of cells within the culture.

## Conclusion

The hypothesis was that if *S. cerevisiae* cultures are grown in varying concentrations of hydrogen peroxide ($H_2O_2$), then the culture with the highest concentration of $H_2O_2$ - 400 µM - will divide the least, due to its larger onslaught of oxidative stress, decreasing the total amount of cells within the flask as degradation of biomolecules crucial to cellular function increases cell death and decreases total growth within the culture. This hypothesis was supported with the linear decrease in *S. cerevisiae* concentration in flasks with higher concentrations of $H_2O_2$. Higher doses of oxidant relate to higher levels of *S. cerevisiae* growth inhibition, with a steep decrease in cell concentration from the control to 100 µM $H_2O_2$. This dose-dependent impairment of culture proliferation by $H_2O_2$ is likely because the exogenous ROS promoted oxidative stress, decreasing the number of viable parent cells in culture even as the *S. cerevisiae* performed aerobic glycolysis. This is supported by the work of Sayin et al. (2014), which revealed that a decrease in oxidative stress damages by ROS increased the growth of precancerous tissue. Thus, it may be extended that levels of exogenous ROS would also have a dose-dependent reaction on tumor proliferation, where less aggressive tumors could be treated with a lower dose of pro-oxidant. Oxidative stress is a key pathway for cancer pathogenesis, but exogenous ROS can be used to exploit and weaken the ROS-dependent proliferation and function of cancer cells through nonspecific cellular damages to proteins vital for regulation of drug resistance. However, the steep margin between the control and experimental groups reveals the need to investigate a threshold of growth inhibition when cells performing aerobic glycolysis are administered with an $H_2O_2$ concentration less than 100 µM. Possible errors could have stemmed from the lack of micropipette accessibility for sample dilutions or from variations in culture environment. To address this, four flasks were cultured simultaneously with each experimental session. The ability of $H_2O_2$ to reduce proliferation on this scale through biomolecule degradation suggests its potential as a synergistic sensitizer for modern chemotherapeutics. Sayin et al. (2014) determined that antioxidants actually accelerate tumor progression, highlighting the mercurial nature of antioxidant species. This research serves as a stepping stone to characterizing the role pro-oxidative species can play in synergistic precision medicine. Future research should advance the development of prodrugs that intelligently release a proxidant agent within tumor cells for selective cytotoxicity.

## Reference List


Bardaweel, S. K., Gul, M., Alzweiri, M., Ishaqat, A., AlSalamat, H. A., & Bashatwah, R. M.. (2018, October). Reactive oxygen species: The dual role in physiological and pathological conditions of the human body. *The Eurasian Journal of Medicine,* 50(3), 193–201. https://doi.org/10.5152/eurasianjmed.2018.17397

Catalano, A., Iacopetta, D., Ceramella, J., Scumaci,, D., Giuzio, F., Saturnino, C., Aquaro, S., Rosano, C., & Sinicropi, M. S. (2022, January 18). Multidrug resistance (MDR): A widespread phenomenon in pharmacological therapies. *Molecules*, 27(3): 616. https://doi.org/10.3390/molecules27030616

Duina, A. A., Miller, M. E., & Keeney, J. B. (2014). Budding yeast for budding geneticists: A primer on the saccharomyces cerevisiae model system. *Genetics*, 197(1), 33.-48 https://doi.org/10.1534/genetics.114.163188

Eleutherio, E., Brasil, A. D., Franca, M. B., de Almeida, D. S. G., Rona, G. B., & Magalhaes, R. S. S. (2018, June). Oxidative stress and aging: Learning from yeast lessons *Fungal Biology,* 122(6), 514-525. https://doi.org/10.1016/j.funbio.2017.12.003

Elia, I., & Haigis, M. C. (2021, January 4). Metabolites and the tumour microenvironment: From cellular mechanisms to systemic metabolism. *Nature Metabolism*, 3, 21–32. https://doi.org/10.1038/s42255-020-00317-z

Farrugia, G. & Balzan, R. (2012, June 21). Oxidative stress and programmed cell death in yeast. *Frontiers in Oncology*, 2: 64. https://doi.org/10.3389/fonc.2012.00064

Jakubczyk, K., Dec, K., Kaldunska, J., Kawczuga, D., Kochman, J., & Janda, K. (2020, April 22). Reactive oxygen species - sources, functions, oxidative damage. Polski Merkuriusz Lekarski: Organ Polskiego Towarzystwa Lekarskiego, 48(284), 124–127. https://pubmed.ncbi.nlm.nih.gov/32352946/.

Jimenez, j., Bru, S., Ribeiro, M., & Clotet, J. (2015, March 2). Live fast, die soon: Cell cycle progression and lifespan in yeast cells. *Microbial Cell*, 2(3), 62-67. https://doi.org/10.15698/mic2015.03.191

Konno, T., Melo, E. P., Chambers, J. E., & Avezov, E. (2021, January 25). Intracellular sources of ROS/$H_2O_2$ in heath and neurodegeneration: Spotlight on endoplasmic reticulum. *Cells*, 10(2): 233. https://doi.org/10.3390/cells10020233

Lisanti, M. P., Martinez-Outschoorn, U. E., Lin, Z., Pavlides, S., Whitaker-Menezes, D., Pestell, R. G., Howell, A., & Sotgia, F. (2011, August 1). Hydrogen peroxide fuels aging, inflammation, cancer metabolism and metastasis: The seed and soil also needs 'fertilizer.' *Cell Cycle* (Georgetown, Tex.), 10(15), 2440-2449. https://doi.org/10.4161/cc.10.15.16870

Liu, W., Li, L., Ye, H., Chen, H., Shen, W., Zhong, Y., Tien, T., & He, H. (2017, July 6). From saccharomyces cerevisiae to human: The important gene co-expression modules. *Biomedical Reports,* 7(2), 153–158. https://doi.org/10.3892/br.2017.941

Milkovic, L., Gasparovic, A. C., Cindric, M., Mouthuy, P., & Zarkovic, N. (2019, July 30). Short overview of ROS as cell function regulators and their implications in therapy concepts. *Cells*, 8(8): 793. https://doi.org/10.3390/cells8080793

Natter, K. & Kohlwein, S. D. (2013, February). Yeast and cancer cells – common principles in lipid metabolism. *Biochimica et Biophysica Acta*, 1831(2), 314–326. https://doi.org/10.1016/j.bbalip.2012.09.003

Pfeiffer, T. & Morley, A. (2014, October 20). An evolutionary perspective on the crabtree effect. *Frontiers in Molecular Biosciences*, 1: 17. https://doi.org/10.3389/fmolb.2014.00017

Rueff, J., & Rodrigues, A. S. (2016). Cancer drug resistance: A brief overview from a genetic viewpoint. *Methods in Molecular Biology,* 1395, 1-18. https://doi.org/10.1007/978-1-4939-3347-1_1

Sayim, V. I., Ibrahim, M. X., Larsson, E., Nilsson, J. A., Lindahl, P., 7 Bergo, M. O. (2014, January 29). Antioxidants accelerate lung cancer progression in mice. *Science Translational Medicine*, 6(221):221ra15. https://doi.org/10.1126/scitranslmed.3007653

Tran, K. & Green, E. M. (2019, January 20). Assessing yeast cell survival following hydrogen peroxide exposure. *Bio-Protocol,* 9(2): e3149. https://doi.org/10.21769/BioProtoc.3149

Turrens, J. F. (2003, August 22). Mitochondrial formation of reactive oxygen species. *The Journal of Physiology*, 552(2), 335-344. https://doi.org/10.1113%2Fjphysiol.2003.049478

Winterbourn, C. C. (2013). The biological chemistry of hydrogen peroxide. *Methods in Enzymology,* 528, 3-25. https://doi.org/10.1016/b978-0-12-405881-1.00001-x

Zhang, B., Pan, C., Feng, C., Yan, C., Yu Y., Chen, Z., Guo, C., & Wang, X. (2022, December) Role of mitochondrial reactive oxygen species in homeostasis regulation. *Redox Report*, 27(1), 45–52. https://doi.org/10.1080/13510002.2022.2046423