Heliyon 11 (2025) e42898

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Yeast cells experience chronological life span extension under prolonged glucose starvation

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ARTICLE INFO

Keywords:
Saccharomyces cerevisiae
Glucose starvation
Longevity
Mitochondria and autophagy

ABSTRACT

Budding yeast, *Saccharomyces cerevisiae,* is an ideal model organism for genetic research due to its similarity in life cycle and cellular structure to higher eukaryotes as well as its ease of cultivation and manipulation in the laboratory. Yeast cells benefit from being cultured in calorie-restricted media, which can be achieved by reducing glucose concentration from 2 % to 0.5 %. Cell metabolism depends on glucose and therefore, affects the physiology of the cell. This study aimed to investigate the effects of long-term glucose starvation on the lifespan of yeast cells by culturing in both standard and glucose-starved conditions. In this investigation yeast cells (BY4743 strain) were cultured in glucose-restricted YPD media (0.5 percent dextrose) to assess lifespan, growth-proliferation, autophagy, apoptosis, mtDNA abundance. The findings revealed that prolonged glucose restriction significantly extended chronological lifespan in yeast ($p < 0.05$). In order to decipher how starved yeast live chronologically longer, we tested mitochondrial association and found that calorie deprivation lowered the rate of mtDNA spontaneous mutation and increased mtDNA abundance which is a suggestive sign of mitobiogenesis. Furthermore, cells cultured on glucose-restricted media led to more autophagosome formation but less cell death. These results suggested that glucose restriction can enhance lifespan by improving overall cellular conditions. These findings may serve as a foundation for future research in aging, cancer and diabetes.

1. Introduction

Saccharomyces cerevisiae, yeast is used in molecular biology, had its genome sequenced first among eukaryotic organisms [1]. It shares 32 % of amino acids with humans, and human homologs can replace 50 % of critical yeast genes [2]. Yeast also serves as a valuable model in medical studies with homologs for 30 % of genes related to human illnesses [3,4]. They also share many signaling pathways and cellular process such as cell cycle [5], metabolism [6], apoptosis [7], protein folding, quality inspection, and degeneration [8],vesicular transport.

Yeast is one of the many organisms that exhibit the benefits of calorie restriction under low glucose concentration settings [9]. Yeasts, nematodes, fish, hamsters, fruit flies, and various mouse and rat strains all exhibit consistently longer lifespans when nutrition

Abbreviations: CLS, chronological lifespan; OD, optical density; CR, calorie restriction; mTOR, molecular target of rapamycin; IGF-1, insulin growth factor 1; mtDNA, mitochondrial DNA; rpm, rotation per minute.

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https://doi.org/10.1016/j.heliyon.2025.e42898
Received 10 November 2024; Received in revised form 19 February 2025; Accepted 20 February 2025
Available online 20 February 2025

availability falls between 30 % and 75 % of the typical calorie supplementation, depending on the species in question [10]. Moderate CR can be applied to yeast by lowering the content of glucose in rich media from 2 % to 0.5 % [11] that is 75 % reduction in glucose availability. The metabolism of cells depends on glucose, which also affects the physiology of cells. The pace of cell growth, metabolism, and the production of cellular byproducts such reactive oxygen species can all be significantly impacted by changes in the availability of glucose [9].

The duration of a nondividing yeast cell's life is known as its chronological lifespan or CLS [12]. Evolutionarily conserved nutrient-sensing mechanisms such as mTOR, (IGF-1)-signaling, adenosine monophosphate-activated protein kinase (AMPK), and sirtuins can regulate lifespan, at least in part. The insulin/IGF-1 and mTOR pathways are triggered by excess nutrient while the AMPK and sirtuin pathways are triggered by nutrient limitation. An organism's longevity can be altered by changing its nutrient-sensing pathways by either stimulating the AMPK and sirtuins pathways or inhibiting the mTOR and insulin/IGF-1 pathway [13].

Yeast cells grow in a highly predictable way when cultured in a stable environment. These stages are lag, log, deceleration and stationary [14]. *Saccharomyces cerevisiae* enters a stationary phase due to starvation, halting cell cycle and undergoing physiological, biochemical, and morphological changes. This allows for prolonged viability without additional nutrition but they also maintain the potential to grow again quickly when the right nutrients are available [15].

Autophagy is a crucial cellular process where autophagosomes transport intracellular materials to lysosomes for destruction, replacing baseline constitutive autophagy in response to stimuli like food constraint, DNA damage, and oxidative stress [16]. In nutrient-rich environments, Gcn5 acetylates Atg8 at K13 and keeps Atg8 in the nucleus and inhibit autophagy. But during hunger TOR inactivation lowers the amount of acetylation of Atg8 by encouraging Gcn5 degradation via the 26S proteasome. The autophagic process is subsequently initiated by translocating deacetylated Atg8 into the cytoplasm. Furthermore, Hdf1 a lysine deacetylase increases autophagy by deacetylating Atg8 [17]. Autophagy is a process that is necessary for cellular viability but it is also one of the mechanisms that leads to programmed cell death(PCD) [18].Apoptosis is a specific type of PCD. Yeast cells experience apoptosis after being exposed to acetic acid, sugar- or salt-stress, or hydrogen peroxide [19]. Apoptosis in yeast is characterised by the release of cytochrome *c* from mitochondria [20,21], phosphatidylserine externalization to the outer leaflet of the plasma membrane, DNA cleavage and apoptosis-typical chromatin condensation (margination) [22]. Besides, ROS also accumulate in yeast cells after induction of apoptotic death by various stimuli [23].

Mitochondria are a significant focus in the field of aging research due to their crucial role in metabolic functions, energy production, cellular redox balance, and various other processes such as maintaining cellular balance and overall organism health [24]. A yeast cell has fifty to one hundred copies of mtDNA or more depending on the strain. Mitochondria often undergo dynamic morphological changes, fusions and divisions based on the culture circumstances and the stage of the yeast cells' life cycle [25]. Yeast mt-nucleoids appeared as strings-of-beads in tubular mitochondria in log-phase cells but as many fluorescent dots in stationary-phase cells using the DAPI-staining approach [26]. Besides,somatic mtDNA point mutations rise with standard ageing because mtDNA is prone to mutation [27]. As mtDNA is vulnerable to damage from reactive oxygen molecules generated as a byproduct during OXPHOS, the rate of mutation in mtDNA is 10 times higher than that of nuclear DNA [28]. Furthermore, little to no recombination [29,30] which leads to greater mutation, and the DNA repair processes seen in the nucleus [31] are absent from the mtDNA. The frequency of mutants resistant to antibiotics that target different mitochondrial activities can be used to determine the levels of mitochondrial point mutagenesis [32].

As glucose availability can regulate cell growth, metabolism, production of cellular byproducts, apoptosis, autophagy, mtDNA content we assumed that glucose starvation ultimately might affect the longevity through these processes. Therefore, we reduced the glucose percentage in the current experiment compared to standard media and looked at a number of cellular factors that might have an impact on lifespan in order to evaluate the effects of long-term glucose starvation on yeast cell's longevity.

2. Materials and methods

2.1. Yeast strains and media

This study included the BY4743 (mat a/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0) [33] yeast strain that was obtained from EUROSCARF. Solid YPD culture medium was made with 2 % agar, 2 % dextrose, 2 % peptone, and 1 % yeast extract, while the liquid YPD culture medium contained 1 % yeast extract, 2 % dextrose, and 2 % peptone [34]. In the glucose-restricted YPD liquid and solid media, the percentages of other constituents were same as in the corresponding YPD media, with the exception of 0.5 % of dextrose [35].

2.2. Chronological lifespan assessment

The yeast chronological lifespan assay is an experiment that examine the variation in longevity between BY4743 strain grown in standard and glucose-restricted media during the stationary phase. This assay showed whether glucose restriction has an effect on aging. To obtain consistent results, the assay was run again over a period of thirty days. In a 250-mL conical flask, standard and calorie-restricted liquid YPD mediums were inoculated with yeast cells with a flask volume: media volume ratio of 5:1. This assay showed whether glucose restriction has an effect on aging. Every three days, the spectrophotometer was used at 600 nm to measure the optical density (OD) of the cells in the liquid cultures. First serial dilutions were carried out using formula (0.4*1000/OD) and the later with liquid culture (including cells): distilled water ratio of 5 μL:995 μL. 30 μL of the diluted culture were spread out on both standard and calorie-restricted YPD plates. Following a 48-h incubation period at 30 °C, the number of colony forming units (CFU) were measured.

To compare the longevity of yeast cells in regular and calorie (CR) media, the Online Application for Survival Analysis or OASIS programme was performed for the survival analysis. The Kaplan-Meier Estimator was used to create survival percentage and the Log-rank test was used to determine the significance difference.

2.3. Growth and proliferation status

Both liquid standard and glucose-restricted media were prepared. A single colony of yeast cells was cultured in 100 ml liquid YPD media in 500 ml conical flask (1:5 ratio). Incubation was performed in a shaking incubator at 30 °C and 150 rpm. The experiment was conducted for 30 days. Using a spectrophotometer, 1 mL of the sample culture from each tube was taken on day one, and the optical density (OD600) was determined. The optical density was also assessed every single day from the start date to day 15 and then every three consecutive days until the 30th day.

2.4. Autophagy assessment

The BY4743 strain was grown in regular and glucose deprived media for 2 days and 25 days. After that, the cells were observed under a light microscope at 100x magnification to find out the autophagic vacuole-containing cells. Following around equal cell counts, autophagic vacuole-containing cells were distinguished by a yellow arrow, whereas apoptotic cells were indicated by a black arrow sign.

2.5. Apoptosis (survival) test

2.5.1. Light microscopy

This experiment was performed to observe whether apoptosis has occurred or not. Cells from BY4743 strain were cultured in both standard and starved YPD media for 2 and 25 days. After that, cell cohesion on slides was done by methanol and then were stained with Giemsa stain for 2 min. Later, the slides were washed with PBS, followed by distilled water. Cells were observed under a light microscope at 40X after placing cover slips on slides.

2.5.2. CFU (colony forming unit)

The purpose of the study is to determine the extent of cell death. On 2nd,7th,14th,21st,30th days of culture in both types of media, the optical density was adjusted to 0.4. After second dilution, 30 μL of culture were spread out on both standard and calorie restricted YPD plates. The numbers of colony forming units (CFU) were measured after 2 days, and the percentages of dead cells were calculated.

2.6. mtDNA abundance and distribution analysis by fluorescence microscopy with DAPI

mtDNA was visualized on 2nd and 25th days after culturing in both media types, and the results were compared. In 1.5 mL microcentrifuge tubes, 300 μL of each strain's culture were combined with 600 μL of 100 % ethanol. The cells were then allowed to sit at room temperature for 30–60 min. After centrifuging the tubes for 1 min at 2500 rpm, the supernatant was disposed of. After that, the pellets were suspended in 1 mL of 1x PBS buffer solution and centrifuged for 1 min at 2500 rpm. The pellets were suspended once again in 200 μl of a 1x PBS/1:2000 dilution DAPl mixture (to make the PBS-DAPI solution, 2.5 mg of DAPI stain was added to 1 mL of distilled water, and then 0.5 μL was added to 1x PBS buffer) after the supernatant was poured off and was left for half an hour at room temperature in the dark. Once the supernatant has been removed by centrifugation, a single drop of the mixture is placed on a different microscope slide and covered with a cover slip. The yeast cells were viewed under a "Nikon Eclipse 50i fluorescence microscope.

CTCF (Corrected Total Cell Fluorescence) value quantification was done with "ImageJ" software for three times with the same number of cells from three different views of the same slide, and a graph was generated from the mean CTCF value to compare the fluorescence level from the cells. The formula CTCF = Integrated Density - (Area of selected cell × Mean fluorescence of background readings) was used to calculate the CTCF value.

2.7. mtDNA spontaneous mutation rate analysis by erythromycin assay

The rate of mitochondrial DNA mutation was measured to assume the probable cause of the rise in mitochondrial abundance. Although the entire investigation was conducted in glucose media, glycerol was used in this specific experiment. Since yeast cells may develop by mitochondrial respiration in the presence of respiratory substrates like glycerol, they can be used to examine mtDNA mutation. The solid YPG culture media contained 1 % yeast extract, 3 % glycerol, 2 % peptone, and 2 % agar, while liquid YPG was devoid of agar [34]. All other elements in calorie-restricted YPG medium were kept constant, with the exception of glycerol, which was added at a rate of 2 % [36].

The BY4743 strain was cultured in both regular and calorie restricted (CR) YPG liquid media. "Erythromycin containing YPG" plates were made by combining 0.2 g of the antibiotic with 100 % ethanol and then adding it to 200 ml of YPG media (both regular and CR). The optical density was set to 0.4, and 30 μl of liquid culture was put onto plates and cultivated at 30 °C for 21 days to see if any colony forming units (CFU) had grown. If CFUs form, it indicates the resistance to antibiotics due to mutation. The experiment was repeated to get a consistent result. The formula μ = -In (Po)/n* In2 was used to compute the mutation rate. In this case, "n" denotes the total number of cultures, while "Po" denotes the percentage of cultures free of erythromycin-resistant mutations. Lastly, the difference

between mutation rate was statistically assessed with single factor ANOVA.

3. Results and discussion

3.1. Glucose restriction enhanced yeast cell's longevity

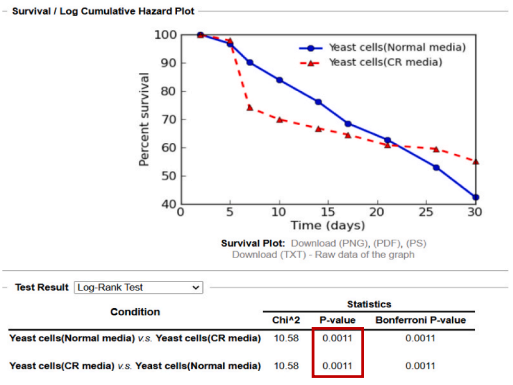
The yeast chronological lifespan assay is an experiment that examines the variation in longevity during the stationary phase. The BY4743 strain was grown in standard and glucose-restricted media to show whether glucose restriction has an effect on aging. After the culture of 30 days, yeast cells in CR media showed an increased lifespan ($P < 0.05$) than the cells in control YPD media (Fig. 1A). When the cells were in regular media, there was 882 CFU on day 2, and at the end of 30 days, the CFU count was 468 with 42.40 % survivability, whereas cells in glucose restricted media had 55.239 % survival rate that started with 813 colonies and ended at 484 colonies on the 30th day.

Reducing the glucose concentration of growth media from 2 % to 0.5 % (or less) allows yeast cells to be subjected to calorie restriction (CR), which lengthens their life span by 30 %–40 % [35]. By regulating the cell cycle, maintaining quiescence, entering a non-quiescent state, and surviving in it, caloric restriction prolongs the chronological longevity of yeast [37]. Mitochondria play a great role in aging studies because of their role in metabolic processes, energy production, and cellular redox state, as well as in cellular homeostasis and physiology [33]. Several organisms have been the subject of recent studies on calorie restriction, and these studies have shown that there is an increase in mitochondrial activity linked to the beneficial effects of this dietary restriction plan [38]. Again, eukaryotic cells can live longer when adaptive autophagy is induced through calorie restriction or intermittent fasting [39]. It's possible that variations in autophagic activity correlate with longevity and that increasing autophagic function could be a useful strategy for delaying aging and extending life in a variety of species, including mammals [40]. Restricting calories also helps to extend life span during aging by delaying the death of apoptotic cells [41]. On the basis of these previous studies, we performed some assays that were suspected to play a role in improving lifespan.

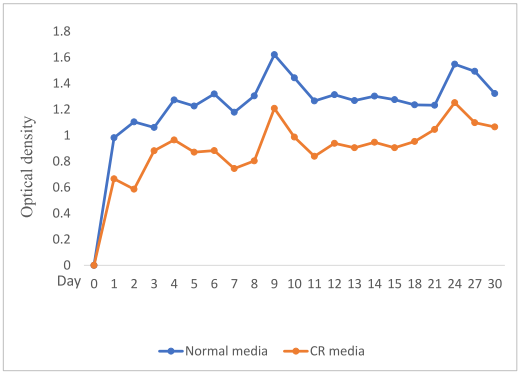
3.2. Glucose restriction resulted in slower proliferation rate

This experiment was performed to determine the growth and proliferation state in order to perceive the impact of glucose restriction on chronological longevity. The findings from measuring the optical density at 600 nm showed that, opposed to conventional media, the proliferation in CR media was found to be lower from the beginning. OD values ranged between 0.586 and 1.621 (Fig. 1B). Overall, there was a modest daily variation in OD.

Newcomb et al. suggested that proliferation is extremely fast in glucose-rich medium during log-phase fermentative growth, but it drastically slows down when the glucose is depleted. After then, the cells go through a plateau phase during which G1 is extended and proliferation stops. This is followed by a sluggish post-log phase of oxidative growth [42]. When there is a shortage of glucose, cells undergo a temporary growth arrest to transition from a fermentative to a respiratory metabolism. Subsequently, they grow again gradually by consuming the ethanol that has built up in the medium. When ethanol levels are also lowered, cells cease to divide and enter the stationary phase, a state of quiescence that deepens over time while the cells are in it [43].



(A)



(B)

Fig. 1. Chronological lifespan analysis: Survival/Log Cumulative Hazard Plot suggested that glucose starved cells survived over the next 30 days with higher survivability compared to control. The survival percentage curve of cells in glucose restriction was found to surpass that of cells in normal media, particularly after 20 days. Again, Log-rank test assessed the significance difference ($p < 0.05$) in lifespan between cells from two cultures (A); Growth curve analysis: BY4743 strain from regular media (N) showed an almost similar proliferation pattern compared to glucose-restricted (CR) media with little OD fluctuation per day. However, cells had lower growth and proliferation in glucose-restricted media over the course of the experiment (B).

3.3. Prolonged glucose starvation triggered remarkable autophagosome formation

A catabolic membrane-trafficking process called autophagy is triggered when yeast cells experience significant fluctuations in their nutritional availability [44]. To understand the long-term effects of glucose starvation on autophagy, cells with autophagic vacuoles were identified under a light microscope at 100X after being cultured in standard and CR media for two and twenty-five days.

Y. Wang et al. suggested that TFEB is the master transcription factor for autophagy- and lysosome-related gene expression. GN5 is a particular TFEB acetyltransferase. Acetylation by GCN5 causes a decrease in TFEB transcriptional activity. Thus, autophagy is adversely regulated by GCN5 [45]. Another study by J. Wang et al. found that Gcn5 inhibits autophagy by acetylating Atg8, maintaining Atg8 in the nucleus. This process occurs in nutrient-rich conditions. But when calories are restricted, TOR inactivation encourages Gcn5 degradation, which lowers Atg8's acetylation level. After being translocated into the cytoplasm, deacetylated Atg8 starts the autophagic process [17]. Compatible with the above study, it was found that, upon calorie restriction, the number of autophagosome containing cells was higher (Fig. 2C and D) than that of cells in regular media (Fig. 2A and B) on both days 2 and 25. Again, glucose restriction for a long time was found to be more helpful to form extensive autophagic vacuoles, as their number was greater on 25 days's glucose restricted cells (Fig. 2D) than day 2. Besides, on the 25th day, cells grown in glucose restricted condition had few apoptotic cells compared to the 25 day's cells from regular media (Fig. 2B and D). But a previous study suggested that uncertainty surrounds the causal relationship between autophagy and different types of regulated or unregulated cell death. Research has demonstrated that autophagy and apoptosis share cross-talk between signal transduction elements and can be antagonistic or coincident, depending on the experimental environment [46]. Therefore, further study is needed in this aspect.

3.4. Yeast cells grown in glucose restricted media facilitated delayed apoptosis

As cells age chronologically, they accumulate oxygen radicals, display caspase activation, and show signs of apoptosis [19]. To detect the incidence of apoptosis, an experiment was carried out with Giemsa staining. It was found that, in both types of culture media, the nuclei of the yeast cells showed pink coloration on day 2 (Fig. 3A and C), but the nuclei from the 25-day culture showed a deep purple color (Fig. 3B and D). According to McCarthy & Evan, Giemsa staining allows for the differentiation of living from apoptotic cell populations based on their DNA state. Apoptotic nuclei are usually visible as purple nucleoli, whereas living nuclei stain pink [47]. Result from Giemsa staining reveals that if cells are cultured for a long time in the same media without refilling, some cells die and the rest of the cells have apoptotic nuclei, indicating the occurrence of apoptosis.

It has been shown that apoptosis happens when colonies grow on solid media [48]. So, for measuring the mortality rate, CFU was counted on 2nd,7th,14th,21st,30th day from both media conditions. On the 2nd day, the CFU number was considered to be 0 percent mortality for both instances. At 7th and 30th days, BY4743 strain in CR media showed 25.83 and 44.72 percent cell death respectively, whereas in the case of control media mortality was only 9.86 % on day 7 that dramatically increased to 57.60 % on day 30th. Therefore, cells grown in regular media showed higher cell death at the end of 30th day opposed to cells in glucose restricted media. This decreased apoptosis rate in calorie restriction supports the result from CLS assay. A former study indicated that calorie restriction increases life span by delaying apoptotic cell death. Early cell death from starvation increases the likelihood that the remaining cells will survive and proliferate, increasing the likelihood that the clone will survive. Furthermore, in these circumstances, the higher formation of ROS raises the likelihood of somatic mutations and consequently the emergence of genetic variants that are able to adapt to constantly shifting environments [41].

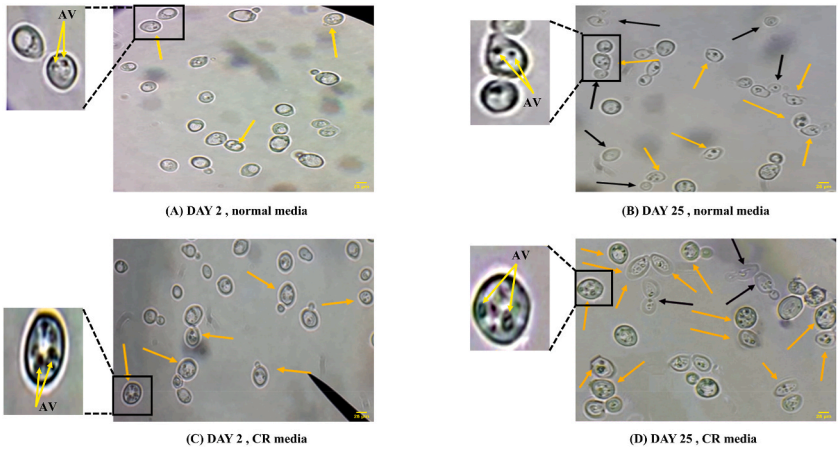


Fig. 2. Visualization of cells with autophagic vacuoles: The light microscopic analysis was performed after the cells were cultured in both standard media (N) and CR media. In 2 days' culture (regular and glucose-restricted), there were a smaller number of autophagosome-containing cells (A and C). After being cultured for 25 days, it was found that cells showed an increased number of autophagic vacuole formations (B and D). However, a higher number of these vacuoles were found during glucose restriction for 25 days (D). Yellow arrows indicate autophagic vacuole-containing cells; black arrows show apoptotic cells. (AV: Autophagic Vacuole).

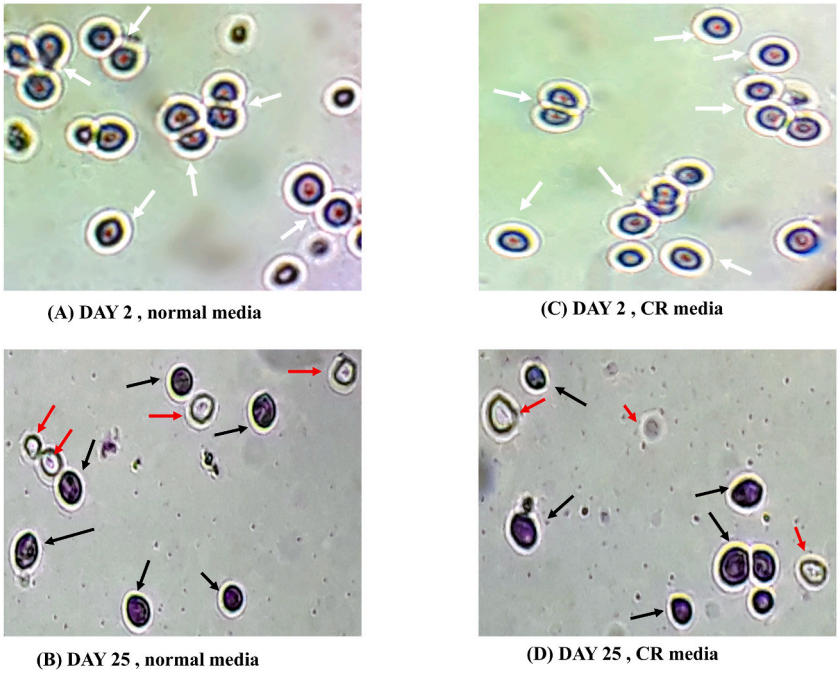


Fig. 3. Nuclear staining with Giemsa to study apoptosis: Results from Giemsa staining varied depending upon culture duration. The viable nuclei displayed pink coloration on day two (A and C), but the apoptotic nuclei from the 25-day culture displayed a deep purple coloration (B and D) in both types of culture media. After 25-day culture in both media, some already dead cells were also seen. (White arrow indicates Viable Nuclei, Black arrow indicates Apoptotic Nuclei and Red arrow indicates Dead cell).

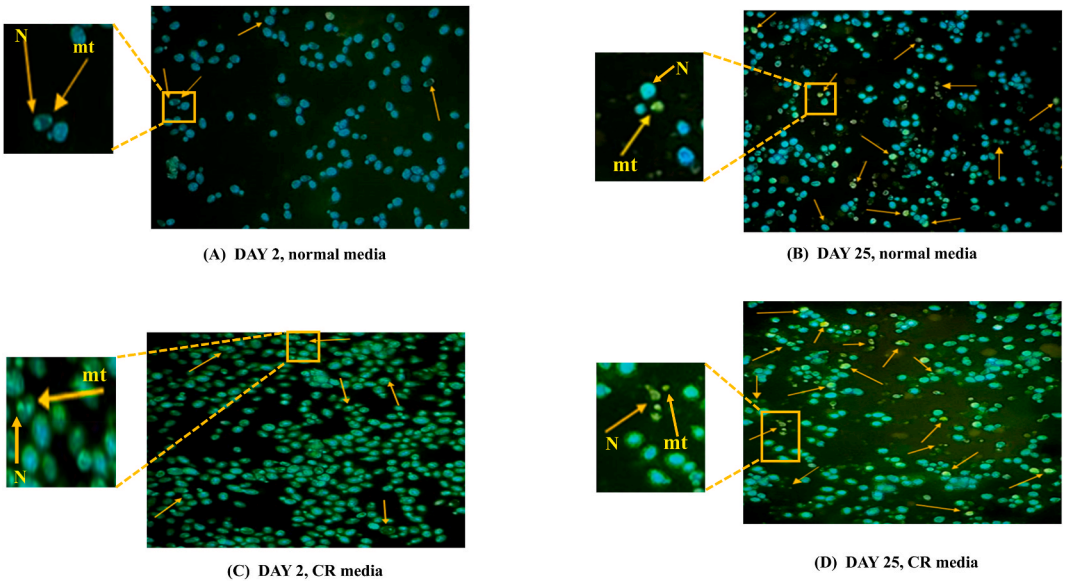


Fig. 4. mtDNA abundance: observation of yeast cells under a fluorescence microscope at 40x and analysis of mtDNA abundance and distribution (mt: mitochondrial DNA and N: nucleus). In both media, there were more mitochondria in cells grown for twenty-five days (B and D) compared to cells that were cultured for only 2 days (A and C). However, the highest number of mitochondria was found in 25 days' glucose-restricted cells (D). (E) Bar chart for fluorescence level analysis. The CTCF value also indicated that cells grown in glucose-restricted medium for 25 days exhibited a higher level of fluorescence. This improvement in fluorescence level was statistically significant (*$p < 0.05$); mtDNA spontaneous mutation rate: An erythromycin assay was carried out to determine the mutation rate. In regular medium, the rate of mutation was 3.25615 × 10-7 but in glucose-restricted medium this rate decreased to 1.1327 × 10-7. Mutation rate was significantly (*$p < 0.05$) lower in glucose-restricted yeast cells (E).

3.5. Glucose starvation raised mtDNA abundance

The 4′, 6-diamidino-2-phenylindole (DAPI) staining method is employed in fluorescent DNA staining to determine the state of the mtDNA [49–51]. DAPI stains mtDNA regardless of the mitochondria's metabolic condition; it can help differentiate between yeast cytoplasmic petite mutants with and without mtDNA. DAPl has minimal cytoplasmic background staining and is a potent nuclear and mtDNA marker due to these properties [52].

DAPI-stained cells were examined at 40x magnification using a fluorescent microscope to determine the abundance and distribution of mtDNA. The large, round, labeled substance in the cells was thought to represent the nucleus (N), while the smaller, scattered ones were mitochondrial DNA (mt) [53]. According to a microscopic analysis, cells that had been cultivated for 25 days had more mitochondria (Fig. 4B and D) compared to the cells for 2 days (Fig. 4A and C). Further analysis was carried out by ImageJ software. The mean CTCF value-derived graph also shows that, for both media, the fluorescence levels on day two were lower than those in 25 day's yeast cells. However, cells maintained for 25 days in glucose-restricted media showed higher fluorescence levels than cells under standard conditions (Fig. 4E). The mitochondrial distribution and abundance in the two-day cells from CR medium increased slightly, but this difference is not statistically significant. The mitochondrial content of cells cultivated in CR conditions for 25 days, however, showed a statistically significant ($P = <0.05$) improvement over cells from standard medium when tested with single factor ANOVA.

A previous study also suggested that, during the fast growth of glucose fermentation, *S. cerevisiae*'s mtDNA copy number is relatively low (9 copies per haploid genome) and it significantly increases during the diauxic shift and respiratory phases. The number of copies of mtDNA increases prior to the complete depletion of glucose [54]. According to Nisoli et al., mice given 30 % calorie restriction (CR) for three months showed significant increases in mitochondria in their brain, heart, liver, and adipose tissue [55]. Another study mentioned, restricting calories increases the energy efficiency of the entire body by promoting the biogenesis of mitochondria that use less oxygen and generate fewer reactive oxygen species (ROS) [56]. It might be predicted, therefore, that when the cells were subjected to prolonged glucose restriction, mitobiogenesis enhanced mitochondrial quantity and distribution and thus played role in longevity.

To understand the possible reason for higher mitochondrial abundance we hypothesized that, there might be a relationship between mtDNA mutation rate and abundance under calorie restriction. So, we performed erythromycin assay to determine mtDNA mutation rate. It was found that yeast cells in standard YPG media exhibited erythromycin resistance with a mutation rate of 3.25615 $\times$ 10-7, which decreased to 1.1327 $\times$ 10-7 when cultured in calorie-restricted media (Fig. 4F). The mutation rate in calorie restricted yeast cells was about three times lower than control ($p < 0.05$) indicating the association of calorie restriction with mitochondrial genome mutation. A study with rats showed long-term CR dramatically lowers the rate of Mitochondrial ROS production [57]. Another study founded that dietary practices like "Calorie Restriction" may be crucial for maintaining the integrity of mtDNA and extending life [58]. Nevertheless, Gureev et al. opined that, dietary restriction, or caloric restriction (DR), appears to be one potential strategy for reducing age-dependent mtDNA damage [59]. So, it can be assumed that, lower mitochondrial DNA mutation rate in calorie restricted condition could be a potential reason for higher mitochondrial abundance and distribution.

4. Conclusion

This study aimed to ascertain the consequences of calorie restriction specifically prolonged glucose starvation in yeast (*Saccharomyces cerevisiae*) cells. After successfully completing the targeted experiments, it can be concluded that, glucose restricted condition can positively regulate yeast cells life span. We also hypothesized so far, the overall effects of glucose reduction on growth-proliferation, apoptosis, autophagy, mtDNA content contributed to extend longevity. However, the expression of various genes related to these cellular processes under calorie restriction was not studied here and needs further investigation. If further studied and validated, this study could potentially contribute to the development of novel therapeutic strategies for human disease.

CRediT authorship contribution statement

Setu Mallick: Writing – original draft, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Atia Shanjida Shormi:** Writing – review & editing, Visualization, Validation, Supervision, Investigation. **Hawa Jahan:** Writing – review & editing, Visualization, Supervision, Resources. **Mohammad Shamimul Alam:** Writing – review & editing, Visualization, Supervision, Resources, Investigation. **Rowshan Ara Begum:** Writing – review & editing, Visualization, Validation, Supervision, Investigation. **Rakha Hari Sarker:** Writing – review & editing, Visualization, Resources, Formal analysis. **Khandaker Ashfaqul Muid:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

SM received funding from the Ministry of Science and Technology, Bangladesh through the National Science and Technology

Fellowship. The authors express their gratitude to Dr. Shefali Begum, Professor & Chairman, Department of Zoology, University of Dhaka for her help. For assistance with research facilities, the authors also thank Dr. Md. Ismail Hosen, Associate Professor, Department of Biochemistry, University of Dhaka.

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