

Research Article

Effect of photoperiodic alterations and binge eating on biochemical and metabolic parameters in zebrafish (*Danio rerio*)

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Abstract

Altered sleep-wake cycles and irregular eating patterns are among the most prominent lifestyle changes observed due to the increasing trend of shift work worldwide. Though circadian rhythm disruptions and unhealthy eating practices are increasingly recognized as contributors to metabolic disorders in humans, the underlying mechanisms remain unclear. The present study investigated the combined effects of binge eating and altered photoperiods on the metabolic and biochemical profiles of zebrafish (*Danio rerio*), a well-established vertebrate model. Adult zebrafish were subjected to excessive feeding and altered photoperiod (20 h light: 4 h dark) for two weeks, while the age-matched control group were maintained in standard laboratory conditions. Important biochemical indicators, such as blood glucose, nitric oxide, and the activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), were measured in addition to total protein. The present results showed that the blood glucose levels in the experimental group were significantly higher compared to the control group (76.2 ± 1.13 mg/dL vs. 52.9 ± 1.27 mg/dL). The antioxidant enzyme superoxide dismutase activity was significantly higher in the experimental group (56.02 ± 2.14 U/mg) compared to controls (3.18 ± 0.18 U/mg). The enzyme catalase also showed a slight but significant increase in its activity in the experimental group. However, Nitric oxide levels and total protein levels did not significantly change. These results showed that altering photoperiod in conjunction with altered feeding can lead to elevated glucose levels and increased oxidative stress, thereby affecting zebrafish metabolic homeostasis. This work provides a basic framework to understand the intricate relationship between circadian disruption and dietary excess in metabolic dysregulation, potentially impacting overall health.

Keywords: Binge eating, Circadian disruption, Homeostasis, Metabolism, Oxidative stress, Photoperiodic alterations, Zebrafish

INTRODUCTION

The circadian timing system represents one of the most fundamental biological processes governing life on Earth, orchestrating a complex network of molecular, physiological, and behavioral rhythms that synchronize organisms with their environment (Takahashi, 2017; Fagiani *et al.*, 2022). This endogenous timekeeping mechanism, driven by approximately 24-hour oscillations, regulates critical functions including sleep-wake cycles, hormone secretion, metabolism, and cellular repair processes. At the molecular level, circadian clocks are governed by transcriptional-translational feedback loops involving core clock genes such as *Clock*, *Bmal1*, *Period* (*Per1*, *Per 2*, *Per3*), and *Cryptochrome* (*Cry1* and *Cry2*), along with *Rev-erbs* and

RoRs which create rhythmic gene expression patterns that coordinate downstream physiological processes (Partch *et al.*, 2014).

Light-dark cycles serve as the primary environmental zeitgeber that entrains circadian rhythms, ensuring proper synchronization between internal biological clocks and external environmental conditions (Reid *et al.*, 2014). Recent research has demonstrated that the circadian clock is optimal when photoperiod is coupled with regular feeding during daylight hours, highlighting the intricate relationship between photic and metabolic cues in maintaining temporal homeostasis (Thraya *et al.*, 2025). The timing of feeding has emerged as a powerful non-photic zeitgeber capable of entraining peripheral circadian clocks independently of central pacemakers (Morbiato *et al.*, 2019). Feeding times con-

trol mutually inclusive physiological events by affecting metabolic homeostasis (a direct effect) and/or by their synchronization effects on the underlying circadian rhythms (an indirect effect) (López-Olmeda and Sánchez-Vázquez, 2011). This temporal regulation of nutrient intake is crucial for optimal metabolic function, as it coordinates digestive processes, hormone release, and energy metabolism with anticipated feeding periods (Hatori *et al.*, 2012).

The disruption of natural circadian rhythms has become increasingly prevalent in modern society, particularly through shift work and irregular lifestyle patterns. Shift work, which affects millions of workers globally, imposes chronic circadian misalignment by forcing activity, feeding, and light exposure during periods when the internal biological clock expects rest and darkness (Boivin *et al.*, 2023). Emerging studies have consistently demonstrated that relative to normal daytime workers, shift workers experience elevated oxidative stress, compromised antioxidant defenses, and increased metabolic dysfunction (Teixeira *et al.*, 2019; Demir *et al.*, 2021; Zou *et al.*, 2023). Recent systematic reviews have indicated that shift work disrupts circadian cycles and misaligns endogenous rhythms, leading to impaired metabolic functions and increased propensity for metabolic disorders (Schettini *et al.*, 2023).

Similarly, social jetlag—the misalignment between sleep-wake times on workdays versus free days—affects up to 70% of the population worldwide and represents a more subtle but widespread form of circadian disruption (Dial *et al.*, 2025). Recent research has revealed that social jetlag potentially disrupts circadian rhythms and is associated with increased risk for cardiometabolic diseases, altered gut microbial composition, and impaired exercise-induced mitochondrial adaptations (Dial *et al.*, 2025; Beaumont *et al.*, 2023). A large-scale cohort study demonstrated that social jetlag affects diet quality, postprandial metabolism, and gut microbiome diversity, establishing clear links between circadian misalignment and metabolic health parameters (Beaumont *et al.*, 2023).

The metabolic consequences of circadian disruption are particularly concerning, as they affect multiple physiological systems simultaneously. Recent evidence shows that shift workers have significantly lower levels of antioxidant defenses and higher levels of oxidative stress damage compared to day workers, with social jetlag serving as a critical mediator of these effects (Teixeira *et al.*, 2019; Kyung *et al.*, 2025). These findings underscore the critical importance of maintaining proper circadian alignment for metabolic health and overall well-being (Bass and Takahashi, 2010; Liu *et al.*, 2022).

Total protein levels serve as indicators of overall metabolic status and protein synthesis capacity, which follow distinct circadian patterns influenced by feeding timing and photoperiod (Khapre *et al.*, 2011). Blood glucose regulation is fundamentally tied to circadian rhythms,

with glucose tolerance exhibiting marked diurnal variation under normal conditions (Morris *et al.*, 2015). Recent studies have confirmed that disruption of these rhythms leads to impaired glucose homeostasis and increased diabetes risk, particularly in shift workers (Schettini *et al.*, 2023). Nitric oxide (NO) levels reflect vascular function and inflammatory status, with NO synthesis showing circadian oscillations that can be disrupted by irregular light-dark cycles and feeding patterns (Zhao *et al.*, 2016). The antioxidant enzymes superoxide dismutase (SOD) and catalase represent critical cellular defense mechanisms against oxidative stress, with their activity patterns normally synchronized to anticipate periods of increased metabolic activity and potential oxidative damage (Demir *et al.*, 2021; Zou *et al.*, 2023).

Despite extensive research on circadian rhythms and metabolism, significant gaps remain in our understanding of how combined photoperiodic and feeding disruptions affect biochemical and metabolic parameters. Most previous studies have examined either light-dark cycle alterations or feeding pattern changes in isolation, failing to capture the complex interactions that occur when both zeitgebers are simultaneously disrupted—a situation that closely mimics real-world shift work conditions (Potter *et al.*, 2016; Boivin *et al.*, 2023). Furthermore, while the individual effects of circadian misalignment on various metabolic parameters have been documented, comprehensive studies examining multiple biochemical markers simultaneously in response to combined photoperiodic and nutritional challenges remain limited (Zimmet *et al.*, 2019; Schettini *et al.*, 2023).

The findings from the present study will enhance the present understanding of the mechanistic relationships between circadian disruption, metabolic dysfunction, and oxidative stress, potentially informing strategies for mitigating the adverse health effects associated with shift work and social jetlag (Kyung *et al.*, 2024).

The present study examined the combined impact of binge eating-like behaviour and altered photoperiods on the metabolic and biochemical profiles of zebrafish (*Danio rerio*), intending to understand the synergistic effect of circadian misalignment in light and food cues. The biochemical parameters examined in this study represent key markers of metabolic function and cellular health that are particularly sensitive to disruptions in the circadian rhythm.

MATERIALS AND METHODS

Zebrafish maintenance

Adult zebrafish (*Danio rerio*) were procured from an authorized supplier and maintained in standard laboratory conditions for two weeks. The water temperature was maintained at $28 \pm 1^\circ\text{C}$, and 14-hour (h):10 h Light (L)-Dark (D) cycles were maintained in the laboratory. The fish were fed once a day with standard micropel-

lets (Champion premium fish feed), which contain fish meat, wheat flour, soybean meal, fish oil, vitamins, and phosphorus. The tanks were aerated for 24 hours before fish were introduced, and aeration was maintained throughout the acclimation and experimental periods.

Animal ethical approval

All experiments were conducted in accordance with the guidelines for Experimentation on Fishes (CPCSEA, 2021) and in compliance with the Institutional Animal Ethics Committee (IAEC) guidelines.

Photoperiod alterations and excessive feeding

The fish in the control tank were exposed to standard photoperiodic conditions (14 h:10 h L-D cycles) and the fish in the experiment tank were exposed to altered photoperiodic conditions (20 h: 4 h L-D cycles). The 20 h: 4 h L-D cycle represents a significant extension of the light phase, known to robustly disturb circadian regulation and mimic environmental light pollution or shift work, while 14 h: 10 h L-D cycles closely resemble natural day-night conditions for zebrafish (López-Olmeda, 2006). The fish in the control tank were fed with normal fish food once a day (at 9 AM) and the fish in the experiment tank were fed with an excess amount of food three times a day (at 9 AM, 4 PM, and 10 PM). The control and experimental groups were exposed to the respective L-D and feeding regimen for two weeks.

Biochemical analysis

All the biochemical assays were done in triplicate from the control and experimental groups ($n = 5$). The parameters measured included total protein concentration, glucose levels, nitric oxide (NO) levels, superoxide dismutase (SOD) activity, and catalase (CAT) activity. These tests were selected to evaluate the metabolic profile and oxidative stress response in zebrafish exposed to altered photoperiods and feeding regimes.

Protein estimation

The fish homogenate was prepared in phosphate-buffered saline (PBS) (0.1 M, pH 7.4). 0.5 g of muscle tissue was weighed, and to this, 0.5 mL of 0.1 M PBS buffer was added and homogenized using a micro pestle. The homogenate was centrifuged at 4°C at 10,000 rpm for 10 minutes. The supernatant was used for further tests. Protein estimation was carried out by modified Lowry's method (Peterson, 1977). The protein working standard of 1 mg/mL bovine serum albumin was prepared. This was pipetted to a series of 6 test tubes labelled as 0.0, 0.2, 0.4, 0.6, and 0.1 mL. The fish homogenate of 0.3 mL from each fish was pipetted into different test tubes. All the test tube volumes were made up to 1 mL with distilled water. A tube with 1 mL of distilled water was taken as a blank. To all the test tubes, 5 mL of Lowry's reagent was added, including the one labeled as blank and unknown. The contents in the test tubes were vortexed and incubated for 10

minutes. To the solution, 0.5 mL of Folin Ciocalteu reagent was added, mixed well, and allowed to stand for 30 minutes in the dark until a blue color developed. The absorbance at 660 nm was taken using a UV-Visible spectrophotometer (SHIMADZU, UV 1800). The absorbance values were noted, and a standard curve was plotted by taking the amount of protein along the X-axis and absorbance along the Y-axis. The unknown concentrations were found using the standard graph equation in MS Excel.

Blood glucose estimation

The amount of blood glucose was determined using a glucometer (Dr. Morepen BG-03 Gluco One Glucometer) as per the manufacturer's instructions. Blood was drawn from the tail of the fish ($n = 5$ in each group). Before collecting blood, the fish were anaesthetized using ice-cold water and the anaesthetized fish were transferred to a petri plate. Using a sharp steel blade, the tail end of the fish was cut and the fish was transferred to an Eppendorf tube and centrifuged at 200 rpm for 5 minutes. The blood collected was immediately used for glucose determination (Pedroso, 2012).

Nitric oxide estimation

The nitric oxide levels were determined by Griess assay (Yucel *et al.*, 2012). The fish homogenate was prepared using the salt alkaline method (Hanne K. Mæhre, 2016). The estimation of nitric oxide was done in triplicates in 96-well flat-bottomed microplates. To prepare the homogenate, 0.1g of fish tissue was homogenized with 10 mL of 0.1 M sodium hydroxide in 3.5% sodium chloride in a 10 mL homogenizer. The homogenates were incubated at 60°C for 90 minutes. The homogenates were centrifuged at 10,000 rpm for 30 minutes at 4°C. The supernatant was used for the NO analysis. A sodium nitrite (NaNO_2) standard of 1 mg/mL was prepared using distilled water and concentrations of 0, 1, 2, 4, 8, and 16 μM . In the microplate well, 100 μL of the sample was added. To all the wells, 20 μL of Griess reagent (SRL) was added. All the wells were made up to 300 μL . This was incubated for 30 minutes at 37°C. The absorbance was read at 540 nm using a microplate reader. The mean of triplicates was taken and nitrite concentration was analyzed and expressed in $\mu\text{mol}/\mu\text{L}$.

Assay of superoxide dismutase activity

The SOD activity was determined using the nitro blue tetrazolium salt reduction method (Beauchamp *et al.*, 1971). The fish homogenate was prepared by the method of salt alkaline extraction ($n = 5$ in each group), and all the tests were done in triplicate. To 0.5 g of the fish muscle tissue, 0.5 mL of PBS was added and homogenized using micro pestle. The homogenate was centrifuged at 4 °C at 10,000 rpm for 10 minutes, and the supernatant was used for further tests. For the assay, 0.5 mL of PBS (pH 7.4), 0.1 mL of EDTA, 0.1 mL

of methionine, 0.1 mL of NBT, and 0.1 mL of 10 mM riboflavin were added. Distilled water was used to make up the volume to 3 mL. To this, 0.1 mL of homogenate was added and incubated under light (15 W) for 20 minutes. A control was taken without adding the homogenate. The absorbance was measured at 560 nm (Gardner and McQUILLIN, 1980).

Catalase activity

Catalase activity was determined by the method of hydrogen peroxide reduction. The homogenate was prepared by the salt alkaline method. To 0.5 g of the fish muscle tissue 0.5 mL of PBS was added and homogenized using micro pestle. The homogenate was centrifuged at 4°C at 10,000 rpm for 10 minutes and the supernatant was used for further tests. To a 96-well plate, 280 µL of PBS, 10 µL of H₂O₂, and 10 µL of homogenate were added. The absorbance was read every 30 seconds for 3 minutes in a microplate reader (Hadwan *et al.*, 2024).

Statistical analysis

The statistical analysis was performed using MS Excel and GraphPad Prism software (version 10.4). The data were analysed using Student's t-test to determine significant differences, with a significance level of 0.05 and a 95% confidence interval. All t-tests were unpaired. All average data were presented as mean ± SD. The statistical acceptance level was $p < 0.05$ in all the presented data.

RESULTS

In the present study, significant differences were observed between the control and experimental groups in the concentrations of blood glucose levels, SOD activity, and catalase activity, while nitric oxide levels and

total protein content did not differ significantly, suggesting that the impact of altered feeding and photoperiodic regimen on selected biochemical parameters was significant. The data of all the biochemical assays are given in Table 1, and the statistical comparisons between the experimental group and the control group are given in Supplementary Tables S1-S5.

The amount of total protein was found to be 0.58 ± 0.02 mg/mL in the control group, and it was also found to be 0.25 ± 0.02 mg/mL in the experimental group (Table 1). It was observed that despite excessive feeding, the experimental group subjected to an extended light phase showed a slight decline in the total protein content compared to the control group, though the difference was not statistically significant.

Further, the amount of nitric oxide was found to be 10.25 ± 0.77 µM in the control group and the concentration was found to be 10.37 ± 0.57 µM in the experiment group (Table 1). The nitric oxide levels were comparable between the two groups ($p > 0.05$).

The blood glucose levels in the control group were found to be 52.9 ± 1.27 mg/dL, and in the experimental group, they were 76.2 ± 1.13 mg/dL (Table 1). This significant increase was consistent with the binge eating regimen that the experimental fish were subjected to ($p < 0.05$).

Assays for antioxidant enzymes like SOD and catalase showed a significant difference, indicating an altered stress response in the experimental group (Table 1). Both antioxidant enzymes exhibited a significant increase in activity within the experimental group compared to the control group. In the control group, SOD activity was observed to be 3.18 ± 0.18 U/mg and it was 56.02 ± 2.14 U/mg in the treated group (Table 1). The result suggested an 18-fold increase in the enzyme activity upon altering the photoperiod and feeding regimen ($p < 0.05$). The catalase activity was found to be

Table 1. Results of the assays carried out to understand the combined impact of the altered photoperiods and altered feeding regimen on selected biochemical parameters in zebrafish

Parameter	Control (Mean ± SD)	Experiment (Mean ± SD)	Statistical Significance (p-value, Student's t test)	Remarks
Protein Concentration (mg/mL)	0.58 ± 0.02	0.25 ± 0.02	$p > 0.05$	No significant difference
Nitric Oxide (µM)	10.25 ± 0.77	10.37 ± 0.57	$p > 0.05$	No significant difference
Glucose Level (mg/dL)	52.9 ± 1.27	76.2 ± 1.13	$p < 0.05$	Significant increase indicating hyperglycemia
SOD Activity (U/mg)	3.18 ± 0.18	56.02 ± 2.14	$p < 0.05$	Marked increase indicating oxidative stress
Catalase Activity (U/mg)	39.84 ± 1.38	54.57 ± 0.92	$p < 0.05$	Elevated activity reflecting antioxidant response

39.87 U/mg in the control group, whereas it was 54.57 U/mg in the experimental group, suggesting a significant upregulation in enzyme activity (Table 1).

DISCUSSION

The present study demonstrates that combined alterations in photoperiod and feeding patterns have a significant impact on metabolic and biochemical parameters in zebrafish, providing valuable insights into the mechanisms underlying metabolic dysregulation induced by circadian disruption. Photoperiod-induced stress activates numerous oxidative stress-related genes and has been shown to alter behavioral patterns, including locomotor activity, feeding behavior, and anxiety-like responses (Villamizar *et al.*, 2014). Moreover, reduced daylight duration in vertebrates is linked to seasonal affective disorder and related mental health issues (Sorokin, 2022). Long photoperiods have also been reported to disrupt endocrine functions in zebrafish, affecting pituitary hormones and transcription factors (Lucon-Xiccato, 2022; Jin, 2009).

The present findings reveal elevated glucose levels and increased antioxidant enzyme activity in zebrafish exposed to prolonged light exposure combined with excessive feeding, supporting the hypothesis that disruption of the circadian rhythm, coupled with dietary excess, promotes metabolic dysfunction. The observed hyperglycemia in the experimental group aligns with previous research, which demonstrates that circadian disruption impairs glucose homeostasis. A similar glucose elevation in zebrafish subjected to constant light conditions, attributed to disrupted insulin signalling pathways, has been reported earlier (Mason *et al.*, 2020). Furthermore, studies in mammalian models have consistently demonstrated that shift work and irregular eating patterns are associated with glucose intolerance and an increased risk of diabetes. The mechanisms underlying this glucose dysregulation likely involve disruption of the molecular clock machinery that normally coordinates metabolic processes with environmental cues (Scheer *et al.*, 2009; Antunes *et al.*, 2010).

The dramatic increase in SOD activity observed in the present experimental group suggests a compensatory response to elevated oxidative stress. This finding is consistent with previous studies conducted in Wistar rats, which demonstrate an increase in reactive oxygen species production associated with circadian disruption, necessitating enhanced antioxidant defence mechanisms (Reiter *et al.*, 2011). The concurrent increase in catalase activity further supports this interpretation, as both enzymes work synergistically to neutralize oxidative damage. Similar patterns have been reported in shift workers, where chronic circadian misalignment leads to elevated markers of oxidative stress and inflammation (Knutsson and Kempe, 2014).

The combination of altered photoperiod and excessive

feeding appears to create a synergistic effect on metabolic dysfunction, as neither factor alone typically produces such pronounced changes. It has been demonstrated that feeding schedules can entrain peripheral circadian clocks independently of the central pacemaker, suggesting that irregular feeding patterns may exacerbate the metabolic consequences of photoperiodic disruption (Yamazaki *et al.* 2000). The interaction between feeding rhythms and light-dark cycles may explain the severity of glucose elevation observed in our study. Interestingly, nitric oxide and total protein levels remained unchanged, indicating that the metabolic perturbations observed were specific rather than a general physiological stress response. This selectivity suggests that the experimental conditions primarily affected glucose metabolism and antioxidant systems while leaving other biochemical pathways relatively intact. Similar findings have been reported in human shift work studies, where specific metabolic parameters are affected while others remain normal (Karlsson *et al.*, 2001).

The relevance of the zebrafish model to human circadian biology is well-established, with conserved clock genes and metabolic pathways, making it an excellent model for studying the effects of circadian disruption (Whitmore *et al.*, 2000). The present findings contribute to the growing body of evidence linking circadian rhythm disruption with metabolic dysfunction, providing mechanistic insights that may inform interventions for shift workers and individuals with irregular eating patterns. These results have important implications for understanding the health consequences of modern lifestyle factors, particularly the increasing prevalence of shift work and irregular eating habits. The elevated glucose levels and oxidative stress markers observed suggest that combined disruptions to the circadian rhythm and dietary habits may predispose individuals to metabolic syndrome and related complications. Future research should investigate potential therapeutic interventions that target the restoration of the circadian rhythm and antioxidant supplementation to mitigate these adverse effects.

Conclusion

This study demonstrates that combined photoperiodic disruption and excessive feeding significantly alter metabolic homeostasis in zebrafish, as evidenced by elevated blood glucose levels and increased activity of antioxidant enzymes. The dramatic increase in SOD activity (18-fold) and modest elevation of catalase indicate a compensatory response to heightened oxidative stress, while hyperglycemia suggests impaired glucose regulation, similar to patterns observed in human shift workers. The synergistic effects of altered light-dark cycles (20h:4h) and binge eating patterns provide mechanistic insights into how modern lifestyle factors contribute to metabolic dysfunction. These findings establish the zebrafish as a valuable model for studying

circadian disruption-induced metabolic disorders, highlighting the importance of maintaining regular sleep-wake cycles and feeding schedules for optimal metabolic health. Future research should investigate therapeutic interventions aimed at restoring the circadian rhythm to mitigate these adverse metabolic consequences in both preclinical and clinical settings. Furthermore, the zebrafish model system employed here provides a valuable platform for future investigations into the temporal aspects of metabolic regulation and the development of chronotherapy approaches for metabolic disorders.

Supplementary Information

The author(s) are responsible for the content or functionality of any supplementary information. Any queries regarding the same should be directed to the corresponding author. The supplementary information is available for download from the article's webpage and will not be included in the print copy.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

- Antunes, L. C., Levandovski, R., Dantas, G., Caumo, W., & Hidalgo, M. P. (2010). Obesity and shift work: chronobiological aspects. *Nutrition Research Reviews*, 23(1), 155-168. DOI: <https://doi.org/10.1017/S0954422410000016>
- Bass, J., & Takahashi, J. S. (2010). Circadian integration of metabolism and energetics. *Science*, 330(6009), 1349-1354. DOI: <https://doi.org/10.1126/science.1195027>
- Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44(1), 276-287. DOI: [https://doi.org/10.1016/0003-2697\(71\)90370-8](https://doi.org/10.1016/0003-2697(71)90370-8)
- Beaumont, M., Mordret, A., Gaggini, M., Weill, G., Coudé, M., Kessler, L., & Vinoy, S. (2023). Exploring the relationship between social jetlag with gut microbial composition, diet and cardiometabolic health, in the Zoe Predict 1 cohort. *European Journal of Nutrition*, 62(6), 2419-2437. DOI: <https://doi.org/10.1007/s00394-023-03204-x>
- Boivin, D.B., Boudreau, P. and Kosmadopoulos, A. (2022). Disturbance of the circadian system in shift work and its health impact. *Journal of biological rhythms*, 37(1), 3-28. DOI: <https://doi.org/10.1177/07487304211064218>
- CPCSEA (2021). Guidelines of the Committee for the purpose of control and supervision of experiments on animals *CPCSEA for experimentation on fishes*, Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying, Government of India. Link: https://ccsea.gov.in/Content/54_1_Acts,rulesandguidelines.aspx
- Demir, I., Toker, A., Aksoy, H., Tasyurek, E. & Zengin, S. (2021). The Impact of Shift Type on Oxidative Stress, Inflammation, and Platelet Activation. *Journal of Occupational and Environmental Medicine*, 63(3), e127-e131. DOI: <https://doi.org/10.1097/JOM.0000000000002124>
- Dial, M.B., Malek, E.M., Cooper, A.R., Neblina, G.A., Vasileva, N.I. & McGinnis, G.R. (2025). Social jetlag alters markers of exercise-induced mitochondrial adaptations in the heart. *npj Biological Timing and Sleep*, 2(1), 4. DOI: <https://doi.org/10.1038/s44323-024-00019-9>
- Fagiani, F., Di Marino, D., Romagnoli, A., Travelli, C., Voltan, D., Di Cesare Mannelli, L., Racchi, M., Govoni, S. & Lanni, C. (2022). Molecular regulations of circadian rhythm and implications for physiology and diseases. *Signal transduction and targeted therapy*, 7(1), 41. DOI: <https://doi.org/10.1038/s41392-022-00899-y>
- Gardner, P., & McQUILLIN, J. (1980). Immunofluorescence techniques, control of specificity and non-specific fluorescence. *Elsevier eBooks*, 56-91. DOI: <https://doi.org/10.1016/b978-0-407-38441-5.50010-x>
- Hatori, M., Vollmers, C., Zarrinpar, A., DiTacchio, L., Bushong, E. A., Gill, S., & Panda, S. (2012). Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metabolism*, 15(6), 848-860. DOI: <https://doi.org/10.1016/j.cmet.2012.04.019>
- Jin, Y., Shu, L., Sun, L., Liu, W., & Fu, Z. (2009). Temperature and photoperiod affect the endocrine disruption effects of ethinylestradiol, nonylphenol and their binary mixture in zebrafish (*Danio rerio*). *Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology*, 151(2), 258-263. DOI: <https://doi.org/10.1016/j.cbpc.2009.11.004>
- Karlsson, B., Knutsson, A. & Lindahl, B. (2001). Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27 485 people. *Occupational and environmental medicine*, 58(11), 747-752. DOI: <https://doi.org/10.1136/oem.58.11.747>
- Khapre, R. V., Kondratova, A. A., Susova, O., & Kondratov, R. V. (2011). Circadian clock protein BMAL1 regulates cellular senescence in vivo. *Cell Cycle*, 10(23), 4162-4169. DOI: <https://doi.org/10.4161/cc.10.23.18381>
- Kyung, M., Park, S., Park, C.G. & Hong, O. (2024). Association between sleep duration, social jetlag, and the metabolic syndrome by shift works. *International Journal of Environmental Research and Public Health*, 21(6), 668. DOI: <https://doi.org/10.3390/ijerph21060668>
- Knutsson, A., & Kempe, A. (2014). Shift work and diabetes—a systematic review. *Chronobiology International*, 31(10), 1146-1151. DOI: <https://doi.org/10.3109/07420528.2014.957308>
- López-Olmeda, J. F., Madrid, J. A., & Sánchez-Vázquez, F. J. (2006). Light and Temperature Cycles as Zeitgebers of Zebrafish (*Danio rerio*) Circadian Activity Rhythms. *Chronobiology International*, 23(3), 537-550. DOI: <https://doi.org/10.1080/07420520600651065>
- López-Olmeda, J. F., & Sánchez-Vázquez, F. J. (2011). Thermal biology of zebrafish (*Danio rerio*). *Journal of Thermal Biology*, 36(2), 91-104. DOI: <https://doi.org/10.1016/j.jtherbio.2010.12.005>
- Lucon-Xiccato, T., Montalbano, G., Frigato, E., Loosli, F., Foulkes, N. S., & Bertolucci, C. (2022). Medaka as a model

- el for seasonal plasticity: Photoperiod-mediated changes in behaviour, cognition, and hormones. *Hormones and Behavior*, 145, 105244. DOI: <https://doi.org/10.1016/j.yhbeh.2022.105244>
20. Mason, I. C., Qian, J., Adler, G. K., & Scheer, F. A. (2020). Impact of circadian disruption on glucose metabolism: implications for type 2 diabetes. *Diabetologia*, 63(3), 462-472. DOI: <https://doi.org/10.1007/s00125-019-05059-6>
21. Mæhre, H., Jensen, I., & Eilertsen, K. (2016). Enzymatic Pre-Treatment Increases the Protein Bioaccessibility and Extractability in Dulse (*Palmaria palmata*). *Marine Drugs*, 14(11), 196. DOI: <https://doi.org/10.3390/md14110196>
22. Morbiato, E., Frigato, E., Dinarello, A., Maradonna, F., Facchinello, N., Argenton, F., Carnevali, O., Valle, L. D., & Bertolucci, C. (2019). Feeding entrainment of the zebrafish circadian clock is regulated by the glucocorticoid receptor. *Cells*, 8(11): 1342. DOI: <https://doi.org/10.3390/cells8111342>
23. Hadwan, M. H., Hussein, M. J., Mohammed, R. M., Hadwan, A. M., Saad Al-Kawaz, H., Al-Obaidy, S. S., & Al Talebi, Z. A. (2024). An improved method for measuring catalase activity in biological samples. *Biology Methods and Protocols*, 9(1) bpae015. DOI: <https://doi.org/10.1093/biomethods/bpae015>
24. Morris, C. J., Yang, J. N., Garcia, J. I., Myers, S., Bozzi, I., Wang, W., & Scheer, F. A. (2015). Endogenous circadian system and circadian misalignment impact glucose tolerance via separate mechanisms in humans. *Proceedings of the National Academy of Sciences*, 112(17), E2225-E2234. DOI: <https://doi.org/10.1073/pnas.1418955112>
25. Teixeira, K.R., Dos Santos, C.P., de Medeiros, L.A., Mendes, J.A., Cunha, T.M., De Angelis, K., Penha-Silva, N., de Oliveira, E.P. & Crispim, C.A. (2019). Night workers have lower levels of antioxidant defenses and higher levels of oxidative stress damage when compared to day workers. *Scientific reports*, 9(1), 4455. DOI: <https://doi.org/10.1038/s41598-019-40989-6>
26. Partch, C. L., Green, C. B., & Takahashi, J. S. (2014). Molecular architecture of the mammalian circadian clock. *Trends in Cell Biology*, 24(2), 90-99. DOI: <https://doi.org/10.1016/j.tcb.2013.07.002>
27. Pedroso, G. L., Hammes, T. O., Escobar, T. D., Fracasso, L. B., Forgiarini, L. F., & Da Silveira, T. R. (2012). Blood collection for biochemical analysis in adult zebrafish. *Journal of Visualized Experiments*, (63), e3865. DOI: <https://doi.org/10.3791/3865>
28. Peterson, G.L. (1977). A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Analytical biochemistry*, 83(2), 346-356. DOI: [https://doi.org/10.1016/0003-2697\(77\)90043-4](https://doi.org/10.1016/0003-2697(77)90043-4)
29. Potter, G. D., Skene, D. J., Arendt, J., Cade, J. E., Grant, P. J., & Hardie, L. J. (2016). Circadian rhythm and sleep disruption: causes, metabolic consequences, and countermeasures. *Endocrine Reviews*, 37(6), 584-608. DOI: <https://doi.org/10.1210/er.2016-1083>
30. Reid, K. J., Santostasi, G., Baron, K. G., Wilson, J., Kang, J., & Zee, P. C. (2014). Timing and intensity of light correlate with body weight in adults. *PLoS One*, 9(4), e92251. DOI: <https://doi.org/10.1371/journal.pone.0092251>
31. Reiter, R. J., Rosales-Corral, S., Coto-Montes, A., Antonio Boga, J., Tan, D. X., Davis, J. M., Konturek, P. C., Konturek, S. J. & Brzozowski, T. (2011). The photoperiod, circadian regulation and chronodisruption: the requisite interplay between the suprachiasmatic nuclei and the pineal and gut melatonin. *Journal of Physiology and Pharmacology*, 62(3), 269-274. Link: <https://pubmed.ncbi.nlm.nih.gov/21893686/>
32. Scheer, F. A., Hilton, M. F., Mantzoros, C. S., & Shea, S. A. (2009). Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proceedings of the National Academy of Sciences*, 106(11), 4453-4458. DOI: <https://doi.org/10.1073/pnas.0808180106>
33. Schettini, M.A.S., do Nascimento Passos, R.F. & Koike, B.D.V. (2023). Shift work and metabolic syndrome updates: a systematic review. *Sleep Science*, 16(02), 237-247. DOI: <https://doi.org/10.1055/s-0043-1770798>
34. Sorokin, I. E., Evsyukova, V. S., & Kulikov, A. V. (2022). Effect of Short Photoperiod on the Behavior and Brain Serotonin System in Zebrafish *Danio rerio*. *Bulletin of Experimental Biology and Medicine*, 173(3), 293-297. DOI: <https://doi.org/10.1007/s10517-022-05536-w>
35. Takahashi, J. S. (2017). Transcriptional architecture of the mammalian circadian clock. *Nature Reviews Genetics*, 18 (3), 164-179. DOI: <https://doi.org/10.1038/nrg.2016.150>
36. Thraya, M., Patel, A., Stewart, K., Abou-Akl, H., Roberts, D., Heath, D., Pitcher, T.E., Carmona-Alcocer, V. & Karpowicz, P. (2025). Integration of photoperiod and time-restricted feeding on the circadian gene rhythms in juvenile salmon. *Scientific Reports*, 15(1), 16156. DOI: <https://doi.org/10.1038/s41598-025-01069-0>
37. Villamizar, N., Vera, L. M., Foulkes, N. S., & Sánchez-Vázquez, F. J. (2014). Effect of lighting conditions on zebrafish growth and development. *Zebrafish*, 11(2), 173-181. DOI: <https://doi.org/10.1089/zeb.2013.0926>
38. Whitmore, D., Foulkes, N. S., & Sassone-Corsi, P. (2000). Light acts directly on organs and cells in culture to set the vertebrate circadian clock. *Nature*, 404(6773), 87-91. DOI: <https://doi.org/10.1038/35003589>
39. Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., & Tei, H. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. *Science*, 288(5466), 682-685. DOI: <https://doi.org/10.1126/science.288.5466.682>
40. Yucel, A., Gulen, S., Dincer, S., Yucel, A., & Yetkin, G. (2012). Comparison of two different applications of the Griess method for nitric oxide measurement. *Journal of Experimental and Integrative Medicine*, 2(2), 167. DOI: <https://doi.org/10.5455/jeim.200312.or.024>
41. Zhao, M., Wan, J., Zeng, K., Tong, M., Lee, A. C., Ding, J., & Chen, Q. (2016). The reduction in circulating melatonin level may contribute to the pathogenesis of ovarian cancer: a retrospective study. *Journal of Cancer*, 9(5), 831-839. DOI: <https://doi.org/10.7150/jca.14573>
42. Zimmet, P., Alberti, K. G. M., Stern, N., Bilu, C., El-Osta, A., Einat, H., & Kronfeld-Schor, N. (2019). The circadian syndrome: is the metabolic syndrome and much more! *Journal of Internal Medicine*, 286(2), 181-191. DOI: <https://doi.org/10.1111/joim.12924>
43. Zou, Y., Ma, X., Chen, Q., Xu, E., Yu, J., Tang, Y., Wang, D., Yu, S. & Qiu, L. (2023). Nightshift work can induce oxidative DNA damage: a pilot study. *BMC Public Health*, 23(1), 891. DOI: <https://doi.org/10.1186/s12889-023-15742-4>