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Epigenetics: Examining Female Adolescent Clock Genes to Glucocorticoids Amidst the COVID-19 Pandemic

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Abstract: The COVID-19 pandemic has had a significant impact on adolescents, necessitating changes to online learning and increased screen time. These alterations, coupled with an increase in stress, can disrupt sleep and circadian rhythms. Isolation has increased the worldwide need for digital devices. Teens' excessive screen usage increased by a factor of two because of academic and extracurricular obligations. The purpose of the study was to determine whether the COVID-19 pandemic had any significant effects on circadian rhythm alterations in glucocorticoids. Six healthy female participants weighing $25\ kg/m^2$ (mean = 21.7) and aged 18 ± 1 were divided into 2 groups and underwent a 3-day experiment that encompassed a pandemic and a pre-pandemic lifestyle. A 24-hour urine collection with the analysis techniques of UV-Vis spectroscopy. The patterns from the groups' respective calibration curves of urine were this distinct. Peak changes from the pandemic-controlled outbreak were significant. A follow-up chromatographic comparison of pure cortisol with one participant from each group under high-performance liquid chromatography (HPLC) was also performed. The observation suggests it is plausible that target glucocorticoids such as cortisol, melatonin, and estradiol fluctuate during COVID-19. More HPLC testing can accurately find glucocorticoid biomarkers of 6-sulfatoxymelatonin (aMT6s) for cortisol, estrone-3-glucuronide (E3G), and the NR3c1 gene, as well as other hormones linked to the event's circadian rhythms. This can further open discussions about DNA methylation. Physiological studies of neuroscience, endocrinology, and genetics performed on these groups indicated that a pandemic lifestyle might have a significant effect on glucocorticoids in adolescent women.

Keywords: circadian rhythm, glucocorticoids, sleep, stress, pandemic

1. INTRODUCTION

The COVID-19 pandemic has significantly impacted adolescents, necessitating adjustments to online learning and increased screen time. These changes, combined with heightened stress, can disrupt sleep patterns and circadian rhythms (Dusang, 2019). Home isolation has made the use of digital gadgets rise dramatically throughout the world. All ages are being driven to depend on digital platforms for work, education, and purchases (Pandya & Lodha, 2021). This led to the rate of excessive screen time doubling for adolescents due to both school-related and recreational activities. According to Adrianna Rodriguez (2021), a study conducted by JAMA Pediatrics indicated that teens' non-academic screen time significantly increased from 3.8 hours a day pre-pandemic up to 7.7 hours a day after the outbreak. This makes adolescent screen time more than eight hours per day if online classes and digital studying are included in the calculation. Children who often use electronic devices at night or before sleeping are less likely to get enough good sleep and are more likely to be exhausted the next day (Pacheco, 2022).

Circadian rhythms, or clock genes, are critical in the regulation of physiological and behavioral functions in organisms such as humans (Duffield, 2016). These were *CLOCK, Per1, Cry, and BMAL1*. These rhythms have a

24-hour cycle and regulate processes like sleep-wake cycles, and metabolism. They also control hormones from the adrenal cortex, such as Glucocorticoids and stress. The Glucocorticoid diurnal cycle harmonizes the human endocrine glands, which include metabolism, mood, growth, and reproduction ("NCI Dictionary of Cancer Terms," n.d.).

Cortisol and melatonin are glucocorticoid hormones that control the diurnal cycle (Chan & Debono, 2010). The predominant metabolite discharged in urine is 6-sulfatoxymelatonin (aMT6s), making it possible to quantify its role as a circadian clock. The level of melatonin in the blood is reflected in urinary aMT6s between urination episodes (Mirick & Davis, 2008). The aMT6s concentration is stable throughout time and may be accurately tested using standard methods. Because melatonin concentration is essentially unaffected by extrinsic variables like stress, physical exercise, and high carbohydrate consumption, it is more reliable to estimate circadian phase position than other circadian indicators like cortisol. Its metabolites in the urine, blood, and saliva provide accurate indirect measurements. In conclusion, melatonin is a biomarker that may help us understand how circadian disturbance affects our brain's neurophysiology, behavior, and metabolism. Furthermore, cortisol may serve as a biological biomarker of anxiety, psychological distress, and chronic exhaustion.

The stress response integrates repressive and stimulating glucocorticoid effects for the purpose of self-preservation. These steroid hormones exert a wide variety of actions throughout the body, some of which have significant effects on fertility. Stress and high levels of glucocorticoids can have a negative impact on reproduction, in addition to their many other negative effects on body systems. In order for the gonadal follicles to operate properly, glucocorticoids must be present in very specific amounts. If this delicate balance is upset, the estradiol hormone or fertility will suffer (Whirledge & Cidlowski, 2010). Furthermore, a significant association between late night shift work and polycystic ovarian syndrome (PCOS) and genome-wide chronodisruption exists in ovarian granulosa cells (Wang et al., 2021). Both women with PCOS and permanent night shift workers experience melatonin rhythm disruption. Women with PCOS have higher melatonin levels and metabolites in their urine and serum. Due to the bidirectional nature of the relationship between reproductive hormones and sleep, sleep disruptions may change the pattern of menstrual hormone release (Goldstein & Smith, 2016).

Overall, the COVID-19 pandemic serves as a significant event that highlights the relationship between epigenetic changes, circadian rhythms, and the overall well-being of individuals during this challenging time. Irregular sleep due to excessive screentime can affect clock genes as exposure to shorter wavelengths suppresses more melatonin and therefore tolerates higher cortisol (Ishihara et al., 2021). Examples of these short wavelengths include blue and purple light which LED lights obtain and are commonly found in electronic devices. Disruptions in circadian rhythms due to pandemic-related factors have been associated with mood disorders, insomnia, and mental health issues (LeGates et al., 2014). Additionally, it can impact hormonal regulation, potentially leading to menstrual irregularities and health complications in women (Gupta, 2022).

These findings highlight the complex relationship between environmental factors, gene expression, and their influence on behavior and physiological processes during the pandemic. The objective of this study is to consider cortisol, melatonin, and estradiol as biomarkers of stress and circadian dysregulation, find pathways of circadian rhythms to adolescent glucocorticoids, and investigate the impact of the COVID-19 pandemic on women's physiological, physical, and behavioral well-being.

2. METHODOLOGY

2.1 Design of the Experiment

The framework of this case study will provide an overview of whether increased screen time and changes in sleeping habits and lifestyle contribute to a physiological change in the female body, focusing on and relating the physiological effects to the aforementioned glucocorticoids (cortisol, melatonin, and estradiol). A comparative analysis will be conducted on participants living their pandemic lifestyle versus their pre-pandemic lifestyle in a 3-day experiment. Distinguishing parameters would be sunlight exposure, screen time, time of sleep and wake cycles, exercise, and diet, all derived from pandemic studies. It would take six healthy female adolescent participants, ages 15 to 19, who have a body mass index (BMI) of 25 kg/m^2. They should have a regular menstruation cycle and not have consumed any form of steroids such as contraceptive pills,

over the last 4 months.

A 24-hour urine collection will be the basis of the experiment, considering that this is non-invasive and the analysis techniques for this sample are within reach in terms of availability and budget. Furthermore, urine can maintain the stability of metabolites within a year in a freeze-thaw process as long as it is stored at -80 °C. Biohazard and sanitation protocols for urine collection and storage were observed.

Prior to accomplishment, the researchers ensured that the participants understood the implications of participation and reached a fully informed, considered, and freely given decision about their participation in the study. Informed consent and assent forms were acquired by the De La Salle University Research Ethics Office about voluntary participation, data privacy, anonymity, and withdrawal.

2.2 Experimental Parameters

Table 1. *Protocols for Group 1 pandemic-controlled and Group 2 non-pandemic-controlled*

	Group 1 (pandemic)	Group 2 (non-pandemic)
Time for sleeping Time for waking	3:00 AM 9:00 AM	10:30 PM 5:30 AM
Foods recommended	Sugar, Processed, Caffeine, alcohol, & high calories	Balanced Diet No caffeine and alcohol
Calorie Intake	319 kcal + from the standard intake derived from BMI	Fit for BMI
Light intensity of gadgets	≥250 lux (50% in brightness scale)	≥250 lux (50% in brightness scale)
Screen Time	≥7 hours	≤6 hours
Outdoor Sunlight Exposure	< 15 mins	> 15 mins
Sleep hours	6 hours	7 hours
Exercise	0-30 mins/day	30-60 mins/day

Table 2. *Urine Collection Time*

	Group 1 (pandemic) Time	Group 2 (non-pandemic) Time
Day B: 1st collection (2nd urine of the day)	11:00 AM	7:00 AM
Day B: 2nd collection	6:00 PM	2:00 PM
Day B: 3rd collection (Urine before bedtime)	2:00 AM	9:00 PM
Day C: (1st urine of the day)	9:00 AM	5:30 AM

2.3 Sample Preparation for Analysis

The frozen urine samples are brought to the De La Salle University Chemistry Laboratory for lyophilization (*Telstar LyoQuest)* or *freeze-drying f*or 48 to 72 hours to convert them to dried urine and protect the sample's chemical and biological structure. Each dried urine sample was weighed and dissolved with methanol and acetonitrile, then sonicated using the ultrasonic cleaner machine for 5 minutes (*SONER 206H)* to ensure maximum efficiency. Samples were eluted with 10 mL of MeOH (55%), H2O (42%), and ACN (3%) for the test preparation that will be utilized using ultraviolet-visible spectroscopy. Consequently, a series of samples were injected into vials with a syringe filter of 13 mm with a 0.45 pore size for HPLC analysis.

2.4 Characterization Technique

The researchers utilized solvent analysis, ultraviolet-visible spectroscopy, or UV-VIS (Hitachi U-2900 Spectrophotometer). The temperature was not controlled. UV spectra were recorded over a range of 220-600nm with a scan speed of 400 nm/min, a 340nm lamp change, a Path length of 10nm, and a range of 0-2.0 absorption units. Chromatograms were acquired at 220-400nm. The cuvette eluted solution ratio was 1 mL from the diluted urine with 2 mL of water.

Consequently, Chromatographic separation with High-performance liquid chromatography, or HPLC (DKSH Philippines model Agilent 1200 Series), was performed. The retention time temperature was 25 °C, with the mobile phase of acetonitrile/methanol/water (3%/42%/55%). The flow rate was 1.5 mL/min, and the time of equilibration between injections was 20 minutes. Both techniques were selected as they have the capability to rapidly separate the sample components with high resolution and can adequately provide a preview of biomarkers' existence in the urine samples. The entire process took place at the De La Salle University Chemistry Lab.

3. RESULTS AND DISCUSSION

In this investigation, a comparison of calibration curves of urine from non-pandemic (Fig. 1) and pandemic-controlled participants (Fig 1.2) was constructed with optimum experimental conditions of 600 nm to 220 nm of wavelength to propose a variety of compounds, such as hormones.

Figure 1

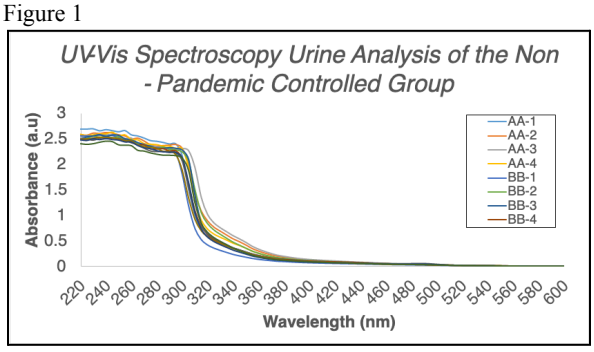
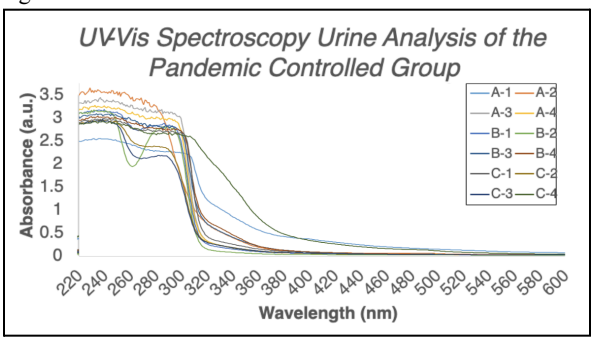
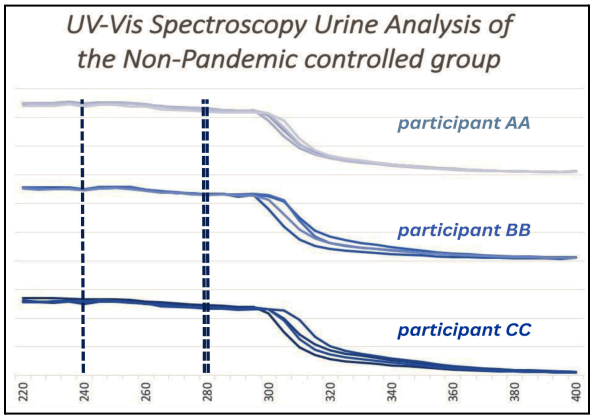
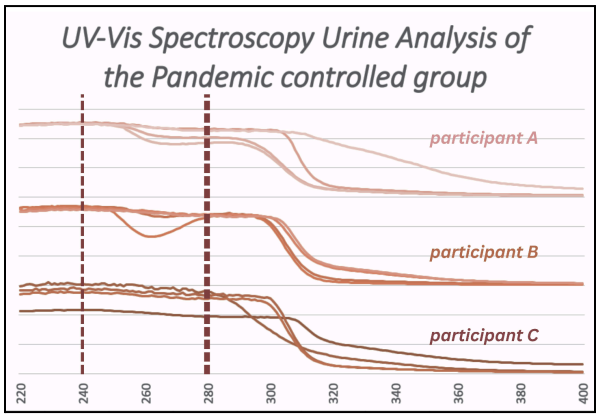


Figure 1.2



*Note: Figures 1.1 and 1.2 demonstrate stacked data from UV-Vis Spectroscopy (Hitachi U-2900) of 24-hour urine collection coming from the non-pandemic controlled (top panel) and pandemic controlled (bottom panel) (n =12). Fig. 1.1 shows a maximum absorbance unit of 2.7 a.u. While Fig. 1.2 is 3.1 a.u.*Figure 2





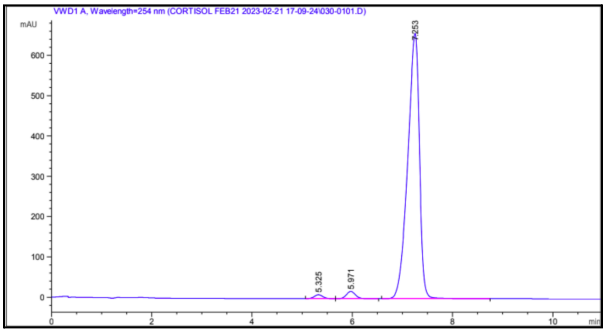
Note: These figures demonstrate stacked data of individual graphs of each urine sample in the course of the 24-hour collection from the pandemic-controlled group (top) and the non-pandemic controlled group (bottom). Each participant had four urine samples.

Based on pre-existing studies, the selected identification absorption wavelengths were 240 nm for cortisol (max of CRT) by Plenis et al. (2010), 278 nm for melatonin (max of MEL) by Simionescu and Ion (2015), and 280 nm for estradiol (max of E2) by Yilmaz and Kadioglu (2013). While methanol and acetonitrile thoroughly absorb UV at shorter wavelengths (205-210 nm), these wavelengths are ideal for further optimization, providing good resolution and peak shape for the analytes.

In *Figure 2,* a general comparison of calibration curves from two groups will be the basis for analysis. From the non-pandemic group (top), peak patterns showed minimal differences, appearing almost uniformly. Compared to the pandemic group (bottom), the graph of each participant showed distinction. Changes in peak areas were significant and fell between wavelengths 240nm to 340nm. However, due to the mixture and potential interactions between components, as well as possible changes in the chemical environment, the exact position of the intended absorbance peak may be altered.

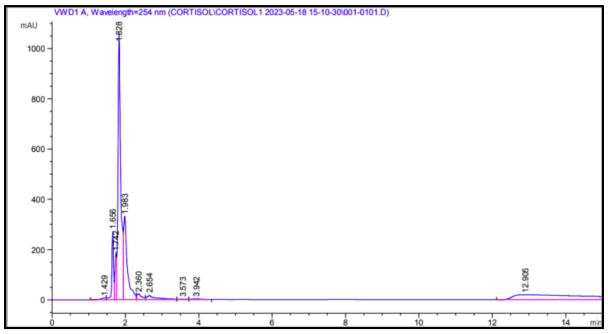
With the non-controlled bearing consistent data and the controlled doing the opposite, this may suggest that the objective of this research is promising and a candidate for advanced study. It is plausible that target glucocorticoids such as cortisol, melatonin, and estradiol may exist within each sample as their absorption wavelengths fit the significant peak changes of wavelength provided in the results. A series of high-performance liquid chromatography tests will precisely determine if the glucocorticoid biomarkers of 6-sulfatoxymelatonin (aMT6s) for cortisol, estrone-3-glucuronide (E3G) for estradiol, and the glucocorticoid receptor gene (NR3c1) for melatonin are viable.

Figure 3. *Standard solution of Cortisol 100 ppm*



*Note: This figure presents the chromatographic analysis of pure cortisol at 100 ppm with a maximum peak height of 657.39 mAU in 7.253 s. The x-axis measures retention time, and the y-axis measures a specific signal generated by the detector. Peak Area is 1.06 mAU*s.*

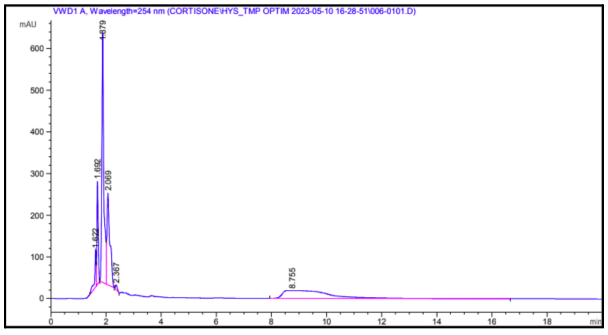
Figure 3.1
HPLC chromatogram - Participant A sample 1



*Note: This figure presents the chromatographic analysis of a single urine sample from the pandemic-controlled group. It acquires a maximum peak height of 1,057 mAU in 1.828 s. Peak Area is 5,645 mAU*s.*

Figure 3.2

HPLC chromatogram - Participant AA sample 1



*Note: This figure presents the chromatographic analysis of a single urine sample from the non-pandemic-controlled group. It acquires a maximum peak height of 597.76 mAU in 1.879 s. Peak Area is 3,008.9 mAU*s.*

The availability of pure cortisol made initial testing for cortisol reference and sample comparison possible. With peak heights from *Figures 3.1 and 3.2* exceeding and falling behind *Figure 3,* HPLC can help this study provide a statistical point of view of analyst concentrations and accurately affirm if our biomarkers are truly present.

4. CONCLUSIONS

The study verified that the pandemic lifestyle may have a significant impact on glucocorticoids in adolescent women. The evident peak patterns, together with our identified absorbance spectrum (Figure 2.1), suggest that biomarkers for cortisol, melatonin, and estradiol can be asserted. Following these findings, it insists that performing HPLC would confirm their certainty; thus, UV-vis findings cannot be conclusive. The additional technique may give satisfactory and consistent results within the scope of the process by providing regression and correlation; it can even provide relevant biomarkers beyond the spectrum. Measured glucocorticoid biomarkers, when HPLC is completed, can be used for investigating circadian rhythms. With the pandemic lasting for more than 2 years, there are chances that epigenetics may occur. The DNA electrophoresis method can extend a deeper analysis with the activation or deactivation of the circadian rhythm's reference genes. DNA methylation can be further illustrated.

5. ACKNOWLEDGMENTS

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