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HORMONIT STUDY on changes in cortisol and anxiety levels associated with rotating shift work

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LIST OF ABBREVIATIONS

AUC	Area under the curve
DAG	Directed acyclic graph
GMD	Geometric mean difference
GMR	Geometric mean ratio
SD	Standard deviation
STAI	State-trait Anxiety Inventory

ABSTRACT

Introduction:

Prior research suggests that cortisol levels and anxiety levels are regulated by daily circadian rhythms. Changes in cortisol and anxiety levels may exist in night shift workers as a result of circadian rhythm disruption.

Methods:

Analyses included 49 male shift workers between the ages of 18-65 who were employed in a car factory in Barcelona. Workers were employed in backward rotating shifts of 3 weeks duration: night shift, evening shift and day shift. Workers were assessed twice, once during the day and once during the night shift. Anxiety levels were measured using the State-Trait Anxiety Inventory (STAI). Cortisol levels were measured from 24-hour urine samples, and total cortisol production and peak cortisol production time was derived for the 42 participants that had complete cortisol data at both shifts. Univariate and multivariate linear mixed models were used to examine within-individual associations between shift work and anxiety levels, shift work and cortisol levels, and cortisol and anxiety levels comparing values during the night shift to the referent day shift and adjusting for hours of daylight.

Results:

The average age of the population was 38 years (± 9). STAI scores were slightly elevated during the night shift compared to the referent day shift score (coefficient 1.14, 95%CI -1.17, 1.40). Furthermore, during the night shift, cortisol production was blunted (GMR 0.83, 95%CI 0.71, 0.96) and peak production time and results suggested a several hour shift in peak production time (GMD 0.416 95%CI -0.18, 8.51). Finally, our results suggest that greater cortisol production is associated with an increase in reported anxiety levels (coefficient 1.84, 95%CI -0.56, 3.11), and that a larger shift in time of cortisol peak production is associated with a slightly higher reported anxiety level (coefficient 0.94, 95%CI -0.07, 1.95).

Conclusion:

These results suggest that shift work causes circadian disruption, which is associated with changes in cortisol and anxiety levels.

RÉSUMÉ

Introduction:

Des recherches antérieures suggèrent que les niveaux de cortisol et d'anxiété sont régulés par les rythmes circadiens quotidiens. Des changements dans les niveaux de cortisol et d'anxiété peuvent exister chez les travailleurs de nuit en raison de la perturbation du rythme circadien.

Méthodes:

Les analyses ont porté sur 49 travailleurs postés masculins âgés de 18 à 65 ans, employés dans une usine automobile de Barcelone. Les travailleurs étaient employés dans des équipes rotatives inversées d'une durée de 3 semaines: équipe de nuit, équipe du soir et équipe de jour. Les travailleurs ont été évalués deux fois, une fois pendant la journée et une fois pendant l'équipe de nuit. Les niveaux d'anxiété ont été mesurés à l'aide du Inventaire d'état d'anxiété (STAI). Les niveaux de cortisol ont été mesurés à partir d'échantillons d'urine de 24 heures, et la production totale de cortisol ainsi que l'heure du pic de production de cortisol ont été calculées pour les 42 participants qui disposaient de données complètes sur le cortisol aux deux quarts de travail. Des modèles linéaires mixtes univariés et multivariés ont été utilisés pour examiner les associations intra-individuelles entre le travail posté et les niveaux d'anxiété, le travail posté et les niveaux de cortisol, et les niveaux de cortisol et d'anxiété en comparant les valeurs pendant l'équipe de nuit à celles de l'équipe de jour de référence et en ajustant pour les heures de lumière du jour.

Résultats:

L'âge moyen de la population était de 38 ans (± 9). Les scores STAI étaient légèrement élevés pendant l'équipe de nuit par rapport au score de l'équipe de jour de référence (coefficient 1,14, 95%CI -1,17, 1,40). De plus, pendant l'équipe de nuit, la production de cortisol était émoussée (GMR 0,83, 95%CI 0,71, 0,96) et l'heure de production maximale et les résultats suggèrent un décalage de plusieurs heures de l'heure de production maximale (GMD 0,416 95%CI -0,18, 8,51). Enfin, nos résultats suggèrent qu'une production plus importante de cortisol est associée à une augmentation des niveaux d'anxiété déclarés (coefficient 1,84, IC95% -0,56, 3,11), et qu'un décalage plus important de l'heure du pic de production du cortisol est associé à un niveau d'anxiété déclaré légèrement plus élevé (coefficient 0,94, IC95% -0,07, 1,95).

Conclusion:

Ces résultats suggèrent que le travail posté provoque une perturbation circadienne, qui est associée à des changements dans les niveaux de cortisol et d'anxiété.

INTRODUCTION:

Work and its associated conditions are two of the key social determinants of health (Torres, Mata-Greve and Harkins, 2018). During the past several decades, working hours have evolved, resulting in a greater number of workers engaging in non-traditional working hours, including shift work. The International Labour Organization defines working in shifts as a "method of organization of working time in which workers succeed one another at the workplace so that the establishment can operate longer than the hours of work of individual workers", which includes different hours during the day and the night (Cannizzaro *et al.*, 2020). Shift work is one of the most common types of occupational exposures around the world. An estimated 20% of the workforce in the European Union Member States, Australia, and the United States, among others, involve shift work (Statistics, 2010; Mandl *et al.*, 2015; Mancio *et al.*, 2018). Shift work systems differ in various settings, but across the board, this type of work exposure has various risks involved. This work schedule is hazardous for workers due to the desynchronization of physiological function of the human body, especially on circadian rhythms. Studies indicate an association between rotating shifts and negative health consequences, such as the increased risk of breast and prostate cancer in workers doing rotating shifts compared to permanent night shift workers (Humans, 2010a). For this reason, it is important to highlight the significance of adaptation and the role it plays as a major health determinant for shift workers. In this sense, adaptation refers to the slow process involving changes in physiology or behaviors that reduce the stress caused by changes in the environment. For night shift workers, being able to adapt to their shift work is associated with fewer work accidents during the shift, less sleepiness, and better sleep quality during the day (Boudreau, Dumont and Boivin, 2013).

Circadian rhythms are the physical, mental, and behavioral changes that follow a 24-hour light-dark cycle. These natural processes affect most living things, including animals, plants, and microbes that have adapted to this light-dark cycle dictated by the Earth's natural rotation. Because shift work disrupts the synchronicity between work/daylight and sleep/darkness, it deeply disturbs the circadian rhythm; in fact, some studies suggest these disruptions may result in carcinogenicity (Humans, 2010b). Additionally, effects of circadian disruption due to shift work can impact various hormones, such as cortisol secreted by the adrenal gland. Upon awakening, cortisol levels peak then decline gradually throughout the day until nighttime. Cortisol serves as an excellent biomarker that can be tracked over a 24-hour period to accurately predict the impact of cumulative stress on the human body or other mental health issues (Boudreau, Dumont and Boivin, 2013). Staying awake during the biological night impacts the normal rhythm of cortisol production. Some studies have shown that the type of shift work affects cortisol profiles differently.

For instance, one study found that the cortisol profiles of permanent night workers appeared to be blunted during night work and days off. However, circadian cortisol profiles were not disturbed in former night workers who recently switched to the fast-rotating shift schedule. In contrast, implementation of night work in former day workers seemed to lead to initially blunted cortisol profiles that normalize after a short adjustment period (Kudielka *et al.*, 2007).

It is well established that shift work causes disruption in the circadian rhythm and cortisol levels, but this secondarily has an impact on mental health, most notably stress, mood, depression, and even anxiety (Kalmbach *et al.*, 2015; Bani-Issa *et al.*, 2020; Khan *et al.*, 2021). Anxiety levels may change according to the speed of rotation; those that are in faster rotations, experience greater levels of anxiety. Notably, few studies have examined the relationship between mental health and shift work. The majority of the existing studies were set in the healthcare sector. A study including female healthcare professionals, found that around 57% of women reported moderate levels of perceived stress and 60% of women reported poor sleep quality. Approximately 36% of women had impaired morning cortisol levels and 14% had impaired bedtime cortisol levels. As a result, a large number of participants, more than 50%, had an impaired response to stress and demonstrated abnormal cortisol levels; this was most pronounced in those working night shifts and longer shifts (more than 8 hours) (Bani-Issa *et al.*, 2020). One study observed paramedics who were monitored for a period of eight consecutive days across pre-shift, day shift, night shift, and two days off. In this population, night shift work was also associated with higher levels of stress, fatigue, and sleepiness (Khan *et al.*, 2021). Night shift workers are at risk of developing night shift disorder, which consists of symptoms of insomnia or excessive sleepiness that occur as transient phenomena in relation to work schedules ('The International Classification of Sleep Disorders Revised 1997', 2004). A longitudinal study reported that nearly 90% of shift work disorder sufferers were accurately identified as high risk one year prior to disease onset. Finally, analyses revealed significant indirect effects wherein high sleep reactivity increased risk for shift work disorder, which led to greater severity of anxiety and depression symptoms in this population (Kalmbach *et al.*, 2015). Even though there is ample research on this topic pertaining to healthcare workers, few studies have examined these correlations among shift workers in non-healthcare settings, such as the manufacturing industry.

Although prior studies have investigated the effects of circadian rhythm on a specific target population, the effects of circadian disruption on mental health and physiological responses are still not well understood. Moreover, previous studies have included mainly females as their target population, hence we wanted to focus on male shift workers for this research. The present study

aims to fill existing gaps and investigate the association between cortisol and anxiety and circadian rhythm disruption among rotating night shift workers.

OBJECTIVES:

General Objective:

Examine the effect of circadian disruption due to night shift work on changes in cortisol and anxiety levels.

Specific Objectives:

1. Analyze total cortisol production and cortisol peak time among male shift workers during their night shift compared to their day shift.
2. Analyze anxiety levels among male shift workers during their night shift compared to their day shift.
3. Determine if there is an association between changes in cortisol and anxiety levels within the male shift workers during their night shift compared to their day shift.

MATERIALS AND METHODS:

Shift work is recognized as a serious risk factor for workers' health, but there is little research on the impact that anxiety and fluctuating cortisol levels can have among shift workers. Therefore, our study will examine the associations of shift work, anxiety levels, and cortisol levels among male workers (*Figure 1*). Data from the HORMONIT study, which focused on examining the changes in hormone levels associated with circadian disruption in night-shift workers will be used.

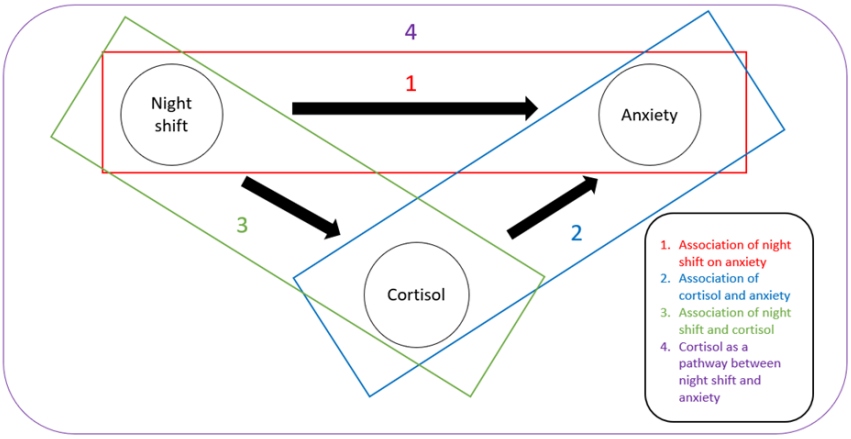

Figure 1.

Study Population:

Out of an initial total of 57 male shift workers who volunteered for this study, 8 were excluded for not reporting State-Trait Anxiety Inventory (STAI) scores for both shifts, which resulted in a total population of 49 workers. Of these 49 workers, 7 were excluded due to missing data on cortisol levels on one or more shifts, resulting in a sample size of 42 male workers between the ages of 18 and 65 who were included in the present study (*Figure 2*).

Figure 2. Flow chart of anxiety and cortisol levels reported by study participants during shifts. *State-Trait Anxiety Inventory.

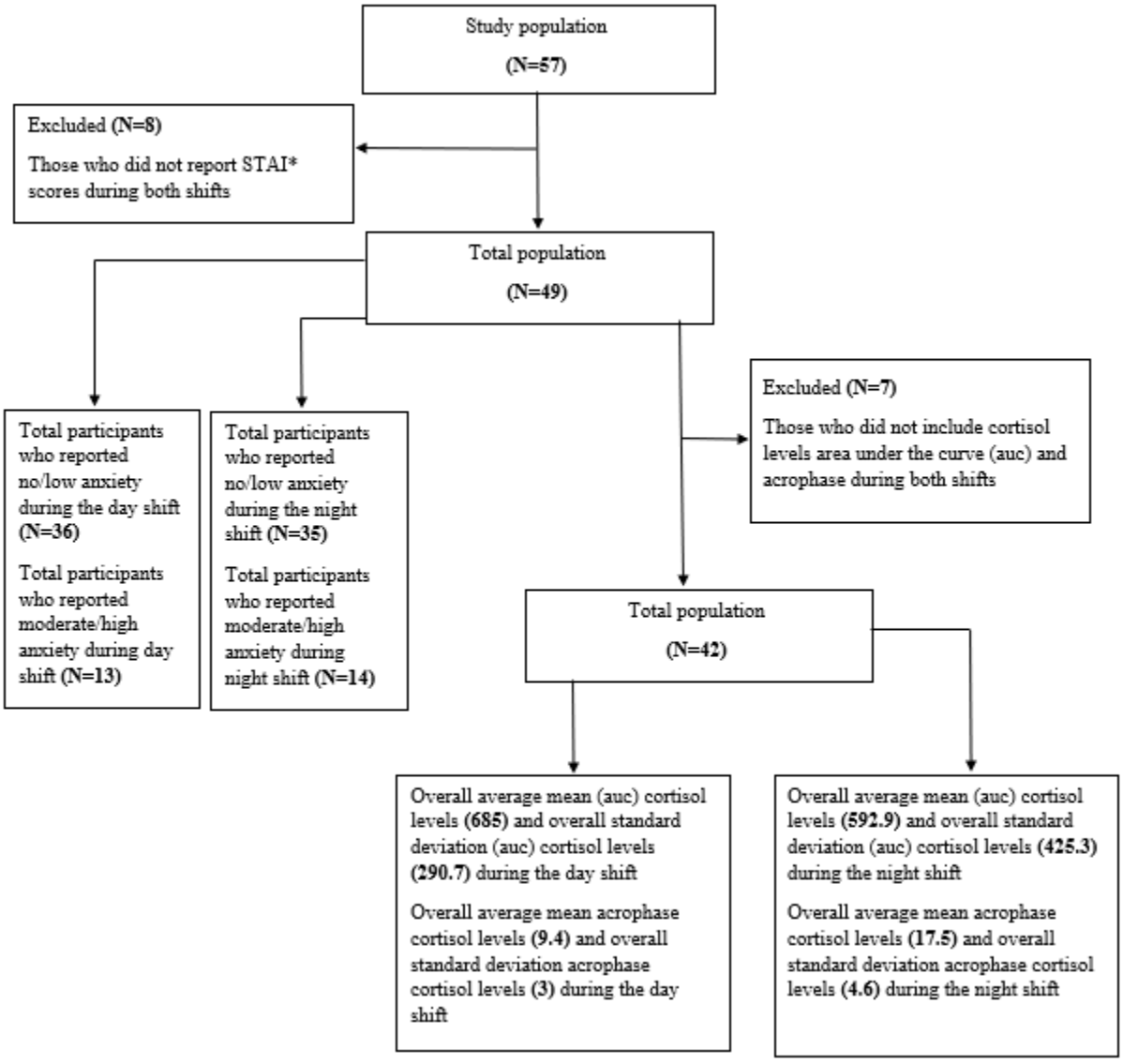

Figure 2.

All the workers were employed in a car factory in the province of Barcelona where workers are employed in rotating shifts of 3 weeks duration in which they rotated clockwise: evening shift (14:00-22:00 hrs.), night shift (22:00-06:00 hrs.), and morning shift (06:00-14:00 hrs.). Participants were sampled twice; once during the day shift (during the 2nd or 3rd week of the 3-week rotation) and again during the night shift (during the 2nd or 3rd week of the 3-week rotation).

Participation in the study was offered through the company's health and safety departments. Subjects with a significant past medical history of cancer were not eligible to participate in the study. The study was approved by the Parc de Salut Mar Clinical Research Ethics Committee (#2015/6351). Participants were given information about the purpose of the study and were given an informed consent form to sign.

Statistical analyses:

Directed acyclic graphs (DAGs) were created using the software DAGitty and then analyzed. By using the DAGs and reviewed literature, key confounders and important covariates were determined (*Figure 3*). We described participant characteristics for the entire population and we separately described STAI scores and cortisol levels during the night shift and the day shift.

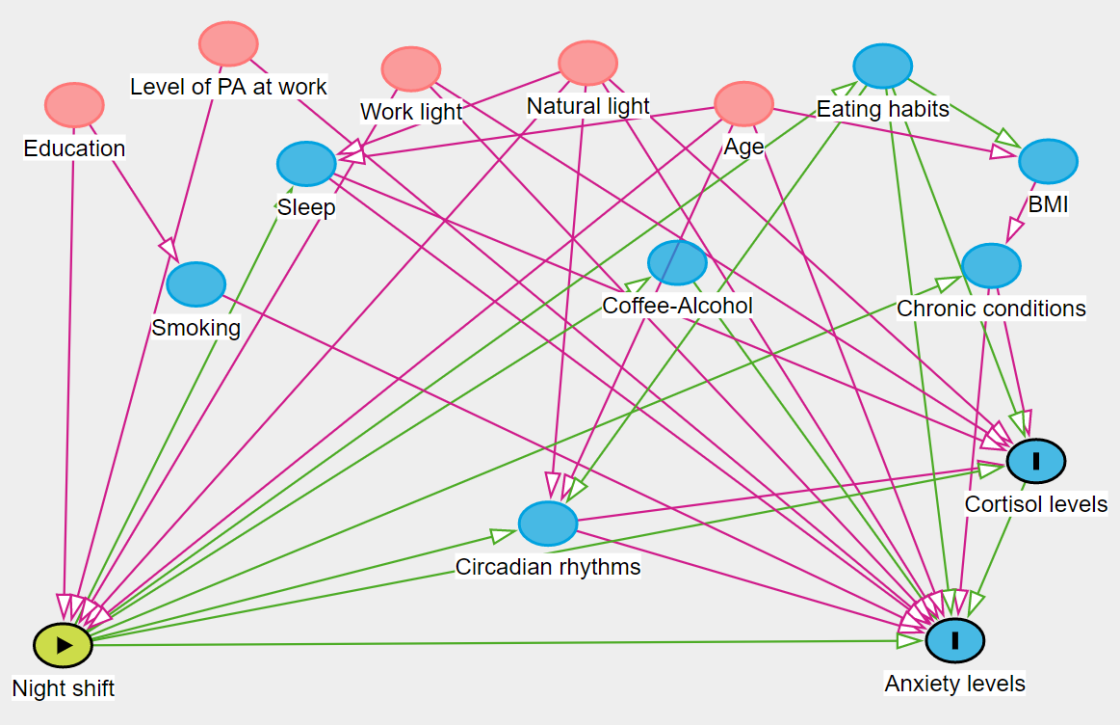

Figure 3.

This DAG was generated using DAGitty and shows an assumption that working night shift causes changes in circadian rhythms which causes an increase in anxiety levels and cortisol levels. Additionally, it shows education, type of work (in terms of physical activity), work light,

daylight hours, and age as confounders either for increased anxiety and cortisol levels or both due to working night shift. It also shows that night shift can cause changes in sleeping patterns, eating habits, coffee and alcohol consumption, which then impact either anxiety and cortisol levels or both. Furthermore, it shows the assumption that cortisol levels impact anxiety levels.

STAI Questionnaire

The State-Trait Anxiety Inventory (STAI) is a common tool utilized to measure the trait and state of anxiety (*The State-Trait Anxiety Inventory (STAI)*, 2011). It is used in clinical settings to detect anxiety and differ it from depression. The STAI is a validated 20 item self-report assessment device which includes separate measures of state and trait anxiety, but a short six-item form has been created that produces scores similar to the original 20-item STAI. For our study, we used the six-item STAI to measure state-trait anxiety and not pathological anxiety, since we wanted an assessment of the subject's anxiety levels at the time of assessment. Various reliability and validity tests have been conducted on the STAI and have provided sufficient evidence that the STAI is an appropriate and adequate measure for studying anxiety in research (*The State-Trait Anxiety Inventory (STAI)*, 2011).

Participants were asked to respond to six phrases that are commonly used to describe oneself: "*I feel comfortable (I'm at ease)*", "*I feel anxious*", "*I feel comfortable*", "*I feel nervous*", "*I am worried*", and "*Right now I feel good"* at the start of their shift. All items are then rated on a 4-point scale ("Not at all", "Somewhat", "Moderately", "Very much", which corresponded to numeric scores of 1-4). Responses to each item were summed together— totaling a score of 6-24, and then scores were scaled by 20/6 so that final scores ranged from 20-80 (Marteau and Bekker, 1992). Notably, they responded to this form in Spanish using a validated Spanish translation (Perpiñá-Galvañ *et al.*, 2011). STAI scores are commonly classified as "no or low anxiety" (20-37), "moderate anxiety" (38-44), and "high anxiety" (45-80) (Kayikcioglu *et al.*, 2017). We visually examined STAI scores during each shift using boxplots and individual trajectory plots (*Figure 4*).

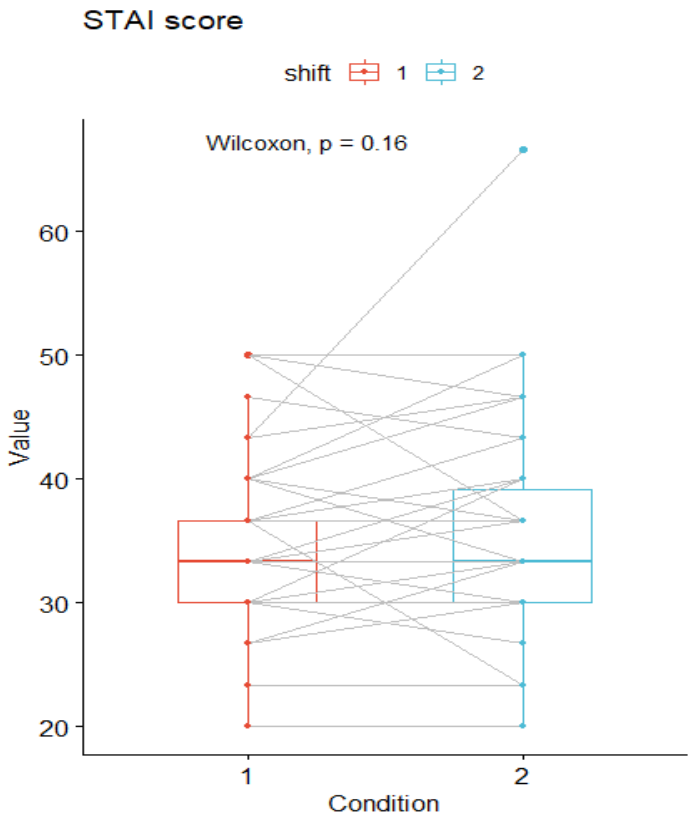

Figure 4.

We also analyzed the deviation of the STAI score, so we observed if participants had a significant change of their scores from night shift to day shift using a 7-point increase or decrease of score by one standard deviation (± SD 1).

Cortisol levels were derived from urinary samples. Participants collected all natural urine voids over 24 hours and then sent their samples to the lab. A cosinor analysis, which uses the least squares method to fit repeatedly measured analyte levels to a sinusoidal wave was used. This method is used in analysis of biologic time series that show predictable rhythms. The cosinor analysis allowed us to extract the total cortisol production [area under the curve (AUC)], the mean 24-hour production and the time of peak production of cortisol (acrophase).

We applied linear mixed models to analyze the relationship between within-person cortisol levels during the night shift compared to the referent day shift. We examined: 1) the association between shift and STAI score, 2) the association between shift and cortisol levels (AUC and acrophase), as well as 3) the association between cortisol and STAI scores. In models examining relationships between shift and cortisol levels, we present results for the total cortisol production as the geometric mean ratio (GMR) produced during the night shift compared to the referent day

shift. Furthermore, we present results for the shift in peak production time as the geometric mean differences (GMD) in production time during the night shift compared to the referent day shift. Finally, in models examining the associations between cortisol levels and STAI score, we use non-log transformed cortisol values and report changes in STAI scores corresponding to a 1SD change in cortisol production and a 1 SD change in cortisol peak production time. Analyses were run using STATA software.

RESULTS:

The average (± SD) age of the population was 38 (± 9) years, and 53% of the participants had a BMI ≥25 kg/m^2. Approximately 40% of the population smoked during the last 24 hours, and 24% reported having a chronic disease. More than half of the population (82%) reported moderately or very active levels of physical activity at work (*Table 1*).

Table 1. Descriptive characteristics

Characteristics	**Overall N= 49**	**Mean (SD) or %**
Demographic		
Age		38 (9)
BMI		
<25	23	47%
25-30	18	37%
>= 30	8	16%
Education		
Primary	14	29%
Professional training	35	71%
Occupation		
Work light		
Normal	21	43%
Bright	26	53%
Level of PA at work		
Sedentary	2	4%
Low intensity activity	7	14%
Moderately active	23	47%
Very active	17	35%
Tobacco		
Smoking last 24h-day		
No	29	63%
Yes	17	37%
Smoking last 24h-night		
No	29	60%

Yes	19	40%
Coffee-alcohol use		
Coffee (cups)- day		
0	14	29%
1	8	16%
2 to 3	17	35%
4 to 5	8	16%
Wine or cava (24hrs)-day		
No	45	96%
Wine or cava (24hrs)-night		
No	49	100%
Chronic disease No/Yes[1]		
No	37	76%
Yes	12	24%

[1] Chronic diseases that were reported included anemia, anxiety, cholesterol, hypertension, thyroid, fasciitis, asthma, mite allergy and cat epithelium allergy, depressive cholesterol and arterial hypertension, hepatitis c, uric acid, attention deficit disorder (ADD).

Of the 49 participants with complete STAI data, 35 reported “no/low anxiety” levels during the night shift compared to 36 during their day shift and 14 reported “moderate/high anxiety” levels during the night shift compared to 13 during their day shift (*Figure 2*). Out of the 49 participants, 15 reported the same score on both shifts. However, 18 participants were more anxious during the night shift, in which 3 decreased their scores by more than 7 points (corresponding to a change larger than 1 ±) compared to 16 who were more anxious during the day shift, in which 4 increased their scores by more than 7 points (corresponding to a change larger than 1 ±). Of those 42 participants with complete STAI and cortisol data, the average mean cortisol AUC was 592.9 (± SD 425.3) during the night shift compared to 685 (± SD 290.7) during the day shift. Moreover, the average mean cortisol secretion acrophase was 17:30 (+/- 4.6hr) (± SD 4.6) during the night shift compared to 9:24 (+/- 3hr) (± SD 3) during the day shift (*Figure* 2).

Some preliminary analyses were done using a 2x2 approach to observe shift versus anxiety level and cortisol level versus anxiety level during the day shift and during the night shift (*Tables 2, 3, 4*). We classified the area under the curve as “low cortisol level” (283-526) and “high cortisol level (527-2897). Then, acrophase was classified as “less acrophase shift” (0-11) and “more acrophase shift” (12-24). We log transformed the cortisol values to generate variables with normal distribution. The results from these comparisons suggested that most of the participants

reported “no/low anxiety” during shift work and when looking at STAI scores with cortisol levels, there were more participants who also reported “no/low anxiety”.

Table 2. Shift versus anxiety level

	Anxiety level		**Total**
Shift	No/low anxiety	Moderate/high anxiety	
Day	36 73 51	13 27 48	49 100 50
Night	35 71 49	14 29 52	49 100 50
Total	71 72 100	27 28 100	98 100 100

Table 3. Cortisol level versus anxiety level during the day shift

	Anxiety level		**Total**
Cortisol level			
Cortisol area under the curve	No/low anxiety	Moderate/high anxiety	
Low cortisol (AUC<527)	14 82 44	3 18 30	17 100 40
High cortisol (AUC>=527)	18 72 56	7 28 70	25 100 60
Total	32 76 100	10 24 100	42 100 100
Cortisol acrophase			
	No/low anxiety	Moderate/high anxiety	
Less acrophase shift (ACRO<12)	24 71 75	10 29 100	34 100 81
More acrophase shift (ACRO>=12)	8 100 25	0 0 0	8 100 19
Total	32 76 100	10 24 100	42 100 100

Table 4. Cortisol level versus anxiety level during the night shift

	Anxiety level		**Total**
Cortisol level			
Cortisol area under the curve	No/low anxiety	Moderate/high anxiety	
Low cortisol (AUC<527)	19 79 61	5 21 45	24 100 57
High cortisol (AUC>=527)	12 67 39	6 33 55	18 100 43
Total	31 74 100	11 26 100	42 100 100
Cortisol acrophase			
	No/low anxiety	Moderate/high anxiety	
Less acrophase shift (ACRO<12)	3 100 10	0 0 0	3 100 7
More acrophase shift (ACRO>=12)	28 72 90	11 28 100	39 100 93
Total	31 74 100	11 26 100	42 100 100

Analyses were adjusted for daylight hours because the date of sample collection during the day and night shift for a given person, in some cases, differed by several weeks and the amount of daylight is known to be associated with cortisol production. Our results suggest that STAI scores were slightly elevated during the night shift compared to the referent day shift score (coefficient 1.14, 95%CI -1.17, 1.40) (*Table 5*). The total production of cortisol was lowered during the night shift compared to the referent day shift average (GMR 0.83, 95%CI 0.71, 0.96) and the acrophase shifted about four hours (GMD 4.16, 95%CI -0.18, 8.51) during the night shift (*Table 6*). Furthermore, in models examining the relationship between a 1 SD change in cortisol production or a 1 SD change in peak production time and a higher level of total cortisol production was associated with an increase in STAI score (GMR 1.84, 95%CI -0.56, 3.11) (*Table 7*), and a

larger acrophase shift during the night shift that is related to a GMD of 0.94, which is about an hour (GMD 0.94, 95%CI -0.07, 1.95) compared to the day shift (*Table 7*).

Table 5. Regression table for STAI and shift

	Unadjusted	95% CI	Adjusted[1]	95% CI
STAI[2]	4.40	(-5.12, 1.39)	1.14	(-1.17, 1.40)

[1]Estimate adjusted for hours of daylight.

[2]STAI score ranges from 20-80 and scale from 1-4.

Table 6. Regression table for cortisol area under the curve & acrophase and shift

	Unadjusted	95% CI	Adjusted[1]	95% CI
Cortisol AUC geometric mean ratio (GMR)	0.82	(0.71, 0.95)	0.83	(0.71, 0.96)
Cortisol acrophase geometric mean difference (GMD)	3.75	(2.72, 4.78)	4.16	(-0.18, 8.51)

[1]Estimate adjusted for hours of daylight.

Table 7. Regression table for cortisol area under the curve & acrophase and STAI

	Unadjusted	95% CI	Adjusted[1]	95% CI
Cortisol AUC geometric mean ratio (GMR)	1.80	(0.53, 3.07)	1.84	(-0.56, 3.11)
Cortisol acrophase geometric	0.92	(-0.05, 1.89)	0.94	(-0.07, 1.95)

mean difference (GMD)				

[1]Estimate adjusted for hours of daylight.

DISCUSSION:

We observed that participants had slightly elevated anxiety levels and slightly lower cortisol levels during the night shift compared to their referent day shift. Overall, as cortisol production was higher, STAI scores were also higher. Additionally, we saw that the more the acrophase was delayed, the STAI score was slightly elevated. Our results suggested a small change when looking at cortisol production and STAI scores as well as when looking at acrophase and STAI score, but these associations did not reach statistical significance.

Previous studies have shown that rotating shift work may result in higher anxiety scores when rotating too quickly or rotating during the night shift (Chang *et al.*, 2014). This in accordance with our finding that STAI scores were slightly elevated during the night shift. State-trait anxiety data was collected because changes in anxiety levels may be guided by cortisol fluctuations.

We focused on cortisol levels because of its association to stress and its fluctuation among night shift workers. For instance, one study concluded that shift work changes the diurnal cortisol pattern and that it is predictive of increased cortisol secretion in professionals in the health sector (Li *et al.*, 2018). Our research findings suggest that peak production does shift during the night compared to during the day; however, there was not an overproduction of the hormone. In our study, cortisol acrophase was delayed, which meant that it took a bit longer for certain participants to recover their circadian rhythm; this finding corroborates a previous study. The recovery of urinary cortisol secretion after one night or 24 hour shift was successful for individuals working in 9 day cycles, but the recovery period was delayed for those working in 6 or 21 day cycles (Lim *et al.*, 2020).

Strengths of our study include its study design, which was used to explore if there were any associations between shift work, cortisol levels, and anxiety levels. We used a repeated measures approach, which allowed the comparison of within-person levels of anxiety and cortisol between the two shifts. This means that we are a little less worried about power although the sample is still small. We also avoided a lot of normal epidemiologic concerns about confounding. Another strength lies in the use of the STAI questionnaire, which was a reliable and validated tool to measure levels of anxiety. The participants were compared to themselves during their night

shift versus their day shift, which allowed for minimal confounding. Also, since past studies have mainly involved women, we focused on filling gaps in existing data by including additional data on men. Nevertheless, our study had some limitations. The overall sample size was small, resulting in minimal power for secondary analyses and wide confidence intervals of our effect estimates. Since our study included only men, it limits the generalizability of findings to female populations.

Recommendations for future analyses include studying other variables that may be important when considering anxiety and cortisol among night shift workers. Such variables of interest may include sleep or other lifestyle behaviors such as alcohol consumption, eating habits, or smoking habits, which may impact workers' ability to cope with stress or limit the ability to adapt to night shift work. Furthermore, analyses examining potential effect modification of the relationships studied here by chronotype would be interesting. Potential future interventions could incorporate administering the STAI questionnaire as a pre- and post-examination, in which participants would have to fill out the questionnaire before the start of the shift and at the end of their shift to observe if any changes occurred during their work shift. Moreover, future analyses may include studying other cortisol metabolites, which may be valuable to the study. Another recommendation when looking at the relationship between anxiety levels and cortisol production would be to use a larger sample of male and female shift workers from different occupations. This would also look at the differences seen among shift work and gender. Another consideration is to use animal models to highlight associations between circadian rhythm changes and cortisol secretion.

In conclusion, night shift was associated to changes in anxiety levels and cortisol levels. More evidence-based research needs to be done in the area of shift work, occupational health, and mental health to ultimately develop policies that will make a positive impact in the occupational setting.

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FIGURES:

Figure 1.

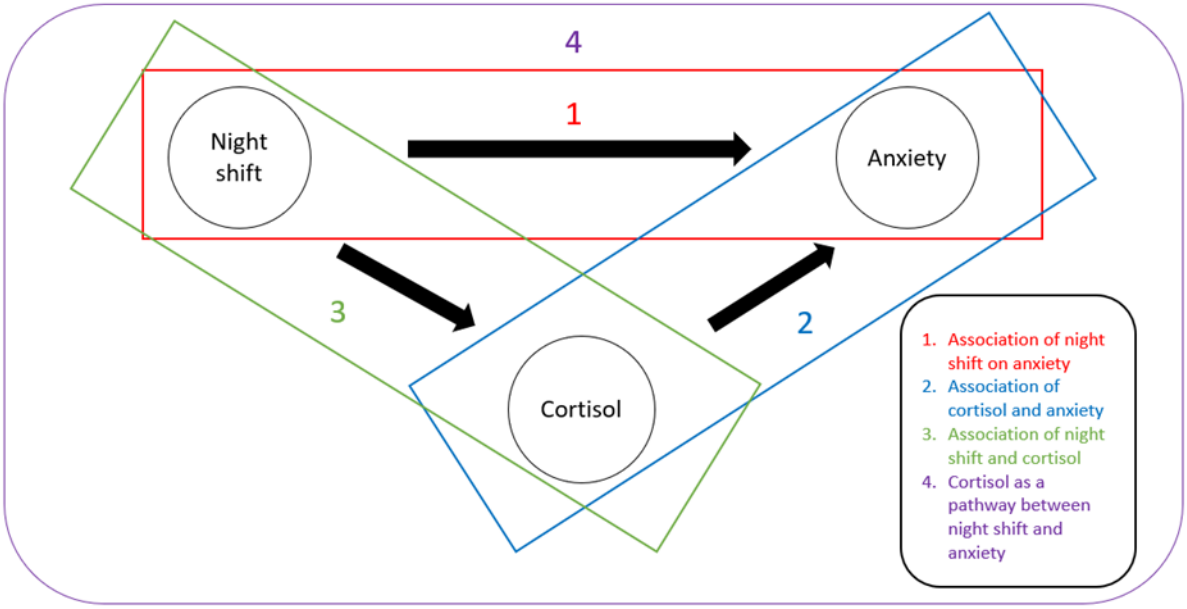


Figure 2.

Figure 2. Flow chart of anxiety and cortisol levels reported by study participants during shifts. *State-Trait Anxiety Inventory.

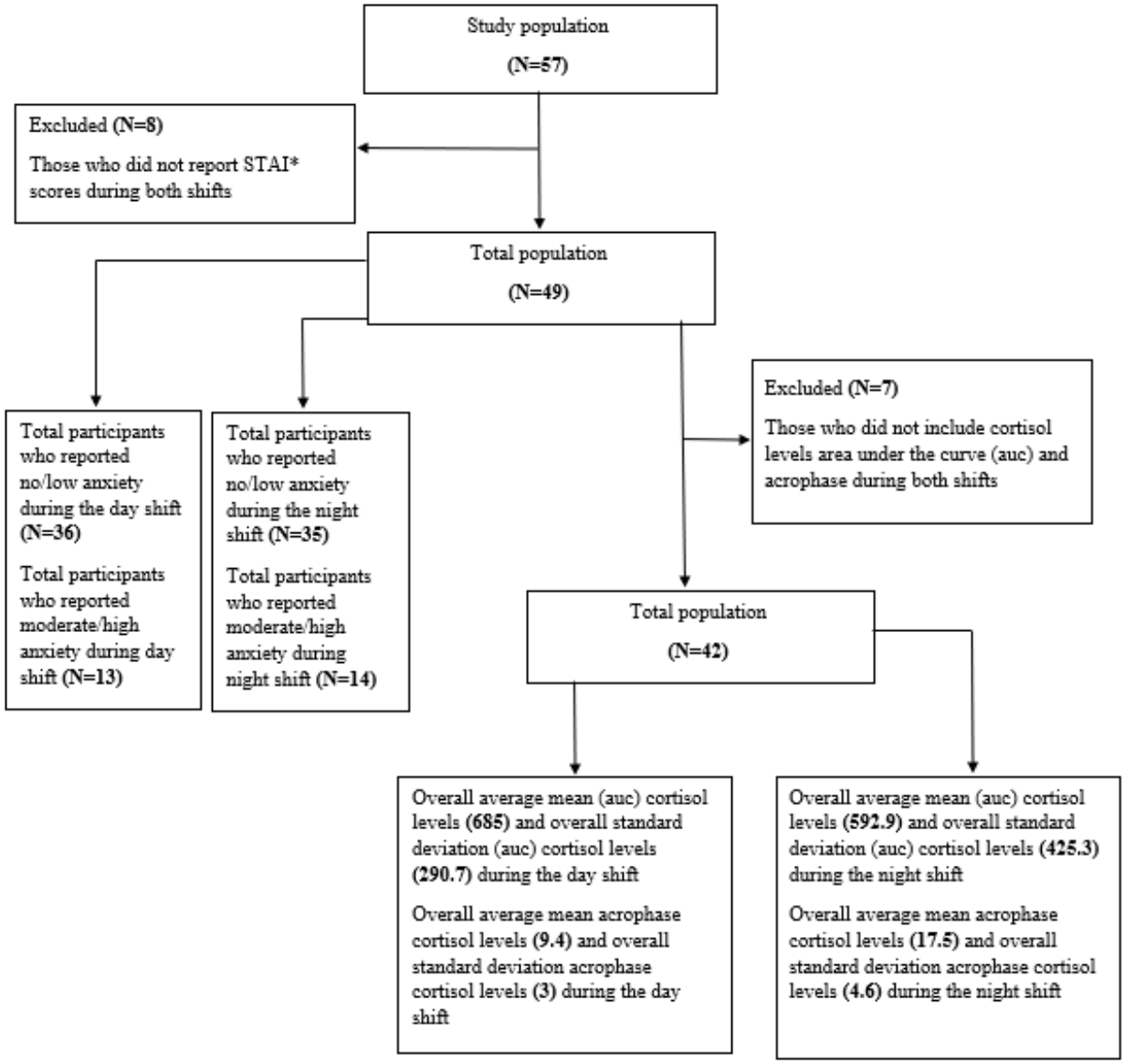
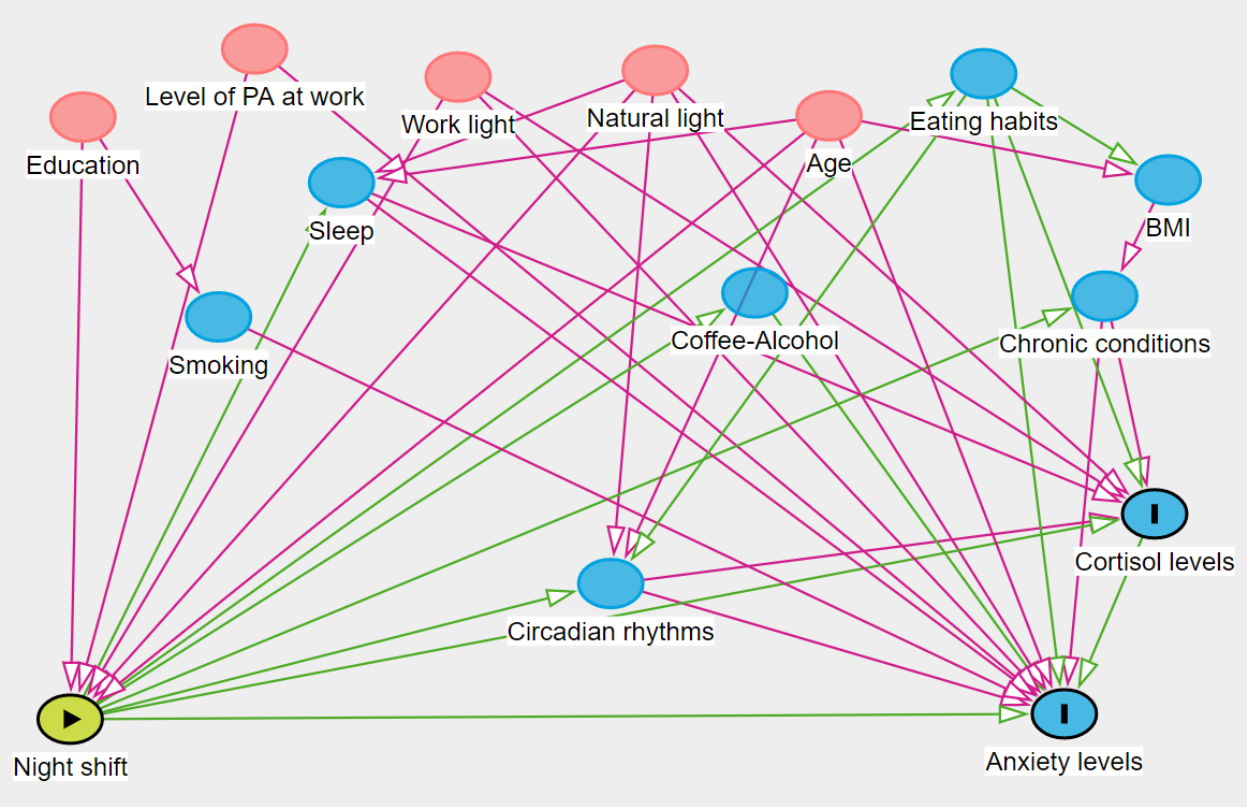
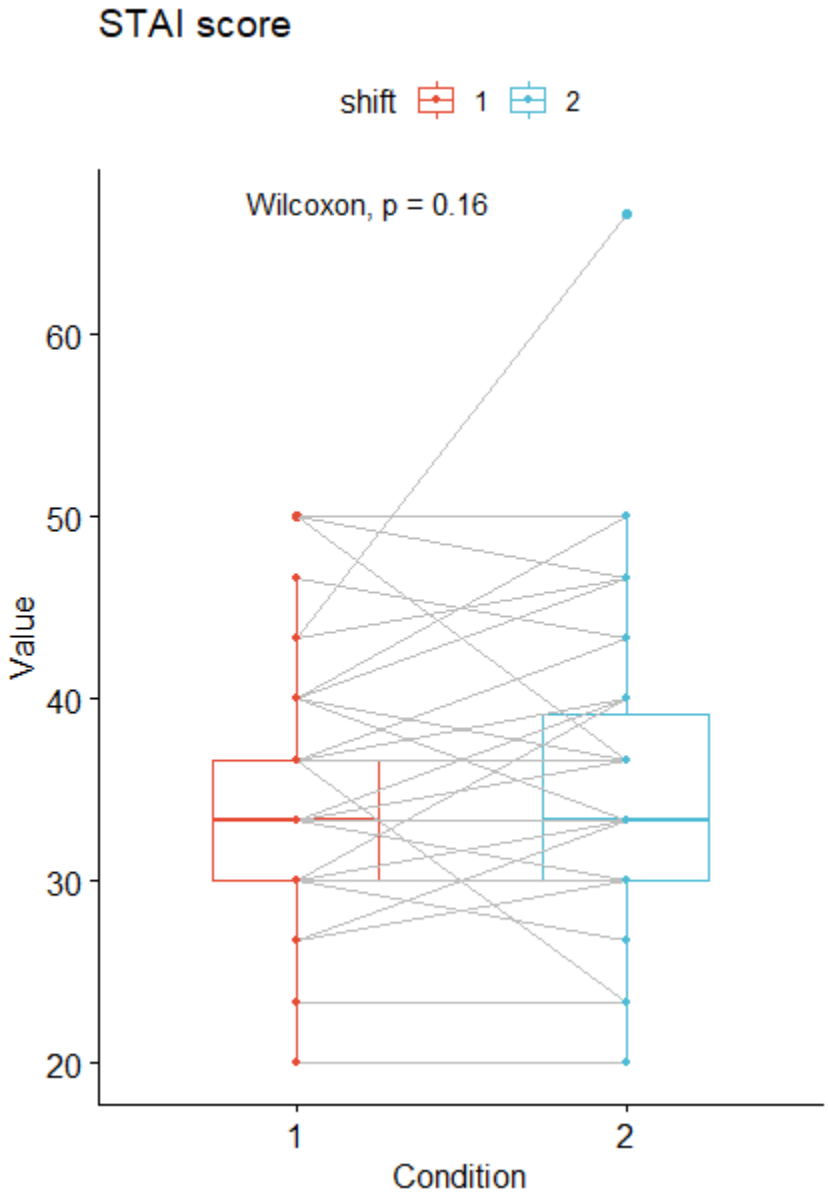


Figure 3.



This DAG was generated using DAGitty and shows an assumption that working night shift causes changes in circadian rhythms which causes an increase in anxiety levels and cortisol levels. Additionally, it shows education, type of work (in terms of physical activity), work light, daylight hours, and age as confounders either for increased anxiety and cortisol levels or both due to working night shift. It also shows that night shift can cause changes in sleeping patterns, eating habits, coffee and alcohol consumption, which then impact either anxiety and cortisol levels or both. Furthermore, it shows the assumption that cortisol levels impact anxiety levels.

Figure 4.


STAI score
shift 1 2
Wilcoxon, p = 0.16
60
50
40
30
20
Value
1
2
Condition

TABLES:

Table 1. Descriptive characteristics

Characteristics	Overall N= 49	Mean (SD) or %
Demographic		
Age		38 (9)
BMI		
<25	23	47%
25-30	18	37%
>= 30	8	16%
Education		
Primary	14	29%
Professional training	35	71%
Occupation		
Work light		
Normal	21	43%
Bright	26	53%
Level of PA at work		
Sedentary	2	4%
Low intensity activity	7	14%
Moderately active	23	47%
Very active	17	35%
Tobacco		
Smoking last 24h-day		
No	29	63%
Yes	17	37%
Smoking last 24h-night		
No	29	60%
Yes	19	40%
Coffee-alcohol use		
Coffee (cups)- day		
0	14	29%
1	8	16%
2 to 3	17	35%
4 to 5	8	16%
Wine or cava (24hrs)-day		
No	45	96%
Wine or cava (24hrs)-night		
No	49	100%
Chronic disease No/Yes[1]		
No	37	76%

Yes	12	24%

[1] Chronic diseases that were reported included anemia, anxiety, cholesterol, hypertension, thyroid, fasciitis, asthma, mite allergy and cat epithelium allergy, depressive cholesterol and arterial hypertension, hepatitis c, uric acid, attention deficit disorder (ADD).

Table 2. Shift versus anxiety level

	Anxiety level		**Total**
Shift	No/low anxiety	Moderate/high anxiety	
Day	36 73 51	13 27 48	49 100 50
Night	35 71 49	14 29 52	49 100 50
Total	71 72 100	27 28 100	98 100 100

Table 3. Cortisol level versus anxiety level during the day shift

	Anxiety level		**Total**
Cortisol level			
Cortisol area under the curve	No/low anxiety	Moderate/high anxiety	
Low cortisol (AUC<527)	14 82 44	3 18 30	17 100 40
High cortisol (AUC>=527)	18 72 56	7 28 70	25 100 60
Total	32 76 100	10 24 100	42 100 100
Cortisol acrophase			
	No/low anxiety	Moderate/high anxiety	
Less acrophase shift (ACRO<12)	24 71 75	10 29 100	34 100 81
More acrophase shift (ACRO>=12)	8 100 25	0 0 0	8 100 19
Total	32 76 100	10 24 100	42 100 100

Table 4. Cortisol level versus anxiety level during the night shift

	Anxiety level		**Total**
Cortisol level			
Cortisol area under the curve	No/low anxiety	Moderate/high anxiety	
Low cortisol (AUC<527)	19 79 61	5 21 45	24 100 57
High cortisol (AUC>=527)	12 67 39	6 33 55	18 100 43
Total	31 74 100	11 26 100	42 100 100
Cortisol acrophase			
	No/low anxiety	Moderate/high anxiety	
Less acrophase shift (ACRO<12)	3 100 10	0 0 0	3 100 7
More acrophase shift (ACRO>=12)	28 72 90	11 28 100	39 100 93
Total	31 74 100	11 26 100	42 100 100

Table 5. Regression table for STAI and shift

	Unadjusted	95% CI	Adjusted[1]	95% CI
STAI[2]	4.40	(-5.12, 1.39)	1.14	(-1.17, 1.40)

[1]Estimate adjusted for hours of daylight.

[2]STAI score ranges from 20-80 and scale from 1-4.

Table 6. Regression table for cortisol area under the curve & acrophase and shift

	Unadjusted	95% CI	Adjusted[1]	95% CI
Cortisol AUC geometric mean ratio (GMR)	0.82	(0.71, 0.95)	0.83	(0.71, 0.96)
Cortisol acrophase geometric mean difference (GMD)	3.75	(2.72, 4.78)	4.16	(-0.18, 8.51)

[1]Estimate adjusted for hours of daylight.

Table 7. Regression table for cortisol area under the curve & acrophase and STAI

	Unadjusted	95% CI	Adjusted[1]	95% CI
Cortisol AUC geometric mean ratio (GMR)	1.80	(0.53, 3.07)	1.84	(-0.56, 3.11)
Cortisol acrophase geometric mean difference (GMD)	0.92	(-0.05, 1.89)	0.94	(-0.07, 1.95)

[1]Estimate adjusted for hours of daylight.