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**When does stress end? Evidence of a prolonged stress reaction in
shiftworking truck drivers**

Melissa Araújo Ulhôa¹, Elaine Cristina Marqueze¹, Thomas Kantermann², Debra Skene², and Claudia Moreno¹

¹University of São Paulo - School of Public Health
Dr. Arnaldo Avenue, 715. Cerqueira César, São Paulo 01246-904
Brazil

²University of Surrey - Centre for Chronobiology
Guildford, Surrey GU2 7XH,
United Kingdom

Corresponding author:
Moreno Claudia University of São Paulo – School of Public Health
Dr. Arnaldo Avenue, 715. Cerqueira César
São Paulo, São Paulo 01246-904 Brazil
T: +55 (11) 3061-7905
F: +55 (11) 3061-7732

E-Mail: crmoresno@usp.br

ABSTRACT

This study aimed to analyze individual cortisol levels in relation to work conditions, sleep, and health parameters among truck drivers working day shifts ($n = 21$) compared to those working irregular shifts ($n = 21$). A total of 42 male truck drivers (39.8 ± 6.2 yrs) completed questionnaires about sociodemographics, job content, work environment, health, and lifestyle. Rest-activity profiles were measured using actigraphy, and cardiovascular blood parameters were collected. Salivary cortisol samples were obtained (i) at waking time, (ii) 30 min after waking, and (iii) at bedtime, during both one workday and one off day from work. Irregular shift workers showed significantly higher waist-hip ratio, VLDL-cholesterol, tiredness after work, years working as a driver, truck vibration, and less job demand compared to day workers ($p < .05$). High cortisol levels in irregular shiftworkers were correlated with certain stressors, such as short sleep duration and low job satisfaction, and to metabolic parameters, such as total cholesterol, HDL, LDL, VLDL, and triglycerides. Day workers had higher cortisol levels collected 30 min after waking ($p = .03$) and a higher cortisol awakening response (CAR, $p = .02$) during workdays compared to off days. Irregular shiftworkers had higher cortisol levels on their off days compared to day shiftworkers ($p = .03$). In conclusion, for the day workers, a higher cortisol response was observed on workdays compared to off days. Although no direct comparisons could be made between groups for work days, on off days the irregular shiftworkers had higher cortisol levels compared to day workers, suggesting a prolonged stress response in the irregular group. In addition, cortisol levels were correlated to stressors and metabolic parameters. Future studies are warranted to investigate further stress responses in the context of irregular work hours. (Author's correspondence: crmoreno@usp.br)

Key words: Cortisol, Stress, Irregular shift, Truck drivers

Introduction

Understanding the mechanisms leading to work-related stress is of major importance to improve the work environment and to maintain workers' health, especially in those workers employed in shiftwork schedules (Bara el al., 2009; Harma et al., 2006; Wirtz & Nachreiner, 2009). Previous studies have clearly shown that shiftwork is associated with chronic health problems, for example, mental health disorders (Bara el al., 2009; Driesen et al., 2009), cardiovascular disease (Puttonen et al., 2010), and the metabolic syndrome (Chen et al., 2010; Karlsson et al., 2003; Padilha et al., 2009). These health problems are often discussed in the context of elevated psychosocial stress arising from a mismatch between job demand and job control (Alves et al., 2004; Bara el al., 2009; Rystedt et al., 2008; Thomas et al., 2009), which can result in pronounced levels of the stress hormone cortisol (Rystedt et al., 2008). In this respect, night work, in particular, has been found to increase levels of cortisol both independently of job strain and work hours (Thomas et al., 2009) as well as in association with an adverse psychosocial work environment (Rystedt et al., 2008) and elevated subjective stress (Dahlgren et al., 2009). Furthermore, the effect of work stress on mental and physical health is potentiated by insufficient or poor sleep, which is very common in shiftwork populations (Harma et al., 2006; Meerlo et al., 2008; Ohayon et al., 2010).

Truck drivers constitute an occupational group that is regularly exposed to night work, insufficient sleep, job strain, and elevated stress (de Croon et al., 2002; Horne & Reyner, 1999; Moreno et al., 2004), resulting, *inter alia*, from high levels of noise and vibration during driving, in combination with pressure to deliver goods on time and extended hours driving in a seated position (Horne & Reyner, 1999; Tamrim et al., 2007). Driving at night, in particular, and for extended hours has been shown to result in sleep deprivation, sleepiness, and increased risk of traffic accidents (Pinho et al., 2006), putting this population at a considerable health risk. Moreover, young subjects

have been shown to be sleepier while driving at night compared to elderly subjects, increasing the risk of accidents (Lowden et al, 2009). In addition, the work conditions of truck drivers have been proposed to lead to an unhealthy lifestyle with high alcohol intake, cigarette smoking, and poor physical activity (Moreno et al., 2004). A recent study on professional drivers found a significant positive association between the number of consecutive working days and urinary cortisol levels (Sluiter et al., 1998), which was highest on workdays compared to off days. It was unclear from this study, however, what the influence of the work environment on the elevated cortisol levels was. Aiming to address this gap in knowledge, we have investigated the effect of the work environment on cortisol levels in regular day working and in irregular shiftworking truck drivers. The objective of our study was to analyze cortisol levels in relation to work conditions, sleep, and health parameters among truck drivers working day shifts compared to those working irregular shifts and to determine the validity of cortisol as a physiological stress marker in this population.

Methods

This study was approved by the Ethics Committee of the School of Public Health of the University of São Paulo and adhered to the guidelines for human research (Portaluppi et al., 2010). Written informed consent was obtained from all participants.

Participants

This cross-sectional survey comprised 101 male full-time truck drivers from a transportation company in São Paulo (Brazil). The study took place between April and July 2009. Initially, all of the workers were interviewed. Those who met one or more of the exclusion criteria were excluded (e.g., gastric disorders, cardiovascular, and others diseases [n = 34], surgery in the last year [n = 5], taking medication [n = 23], or having another second job [n = 0]). Workers not presenting these exclusion criteria were invited to participate in the next phase of the study, which comprised completing questionnaires and having physiological parameters measured. After applying the exclusion criteria, 44 workers were invited to participate in the study; 21 were day shiftworkers and 23 irregular shiftworkers. Two irregular shiftworkers were later excluded because of noncompliance with the study protocol, leaving an equal number of 21 workers in each group for the final analysis. Comparison between the excluded and selected participants revealed no statistically significant differences between the two groups in relation to age and BMI (*t*-test, $p > .05$). Moreover, there was no relationship between the excluded participants and the shiftwork group (day or irregular) (chi-squared, $p > .05$).

The day workers worked from 08:00 to 18:00 h Mondays to Fridays, with 1 h for lunch. These workers only drove during the day, with no night shifts. In contrast, the work hours of the irregular shift group were arranged and specified according to the work demands. Each employee working irregular shifts received his work schedule 1

wk in advance of working from Mondays to Fridays, although the workers may have also undertaken extra shifts on Saturday. Irregular shiftworkers usually arrived at work at 20:30 h, waited for the truck to be loaded, and started driving around 22:30 h. They finished their duty in the morning at ~08:00 h, but this time was unpredictable because it depended on the distance between cities as well as traffic congestion. In the same working week, the irregular shiftworkers could also work morning or afternoon shifts. The irregular working shift included night work, which entailed an additional 20% payment.

Questionnaires

All participants completed a self-administered questionnaire on sociodemographics (e.g., age, marital status, education), lifestyle (e.g., smoking, alcohol intake), and work conditions (e.g., traffic accidents, type of shift, hours driving/day, number of yrs employed as full-time driver at the current company). In addition, participants completed the Portuguese short version (Alves et al., 2004) of the job content questionnaire (Karasek & Theorell, 1990). The latter questionnaire is composed of 17 questions, subdivided into three scales: six questions about job control (e.g., autonomy at work, scores from 6 to 24), five questions about job demand (e.g., time pressure to do the job, scores from 5 to 20), and six questions about social support (e.g., hostile supervisor and coworker relations, scores from 6 to 24). Data from these questionnaires were analyzed both on a continuous and a dichotomic scale. In order to define high job demand, low job control, and low social support, median scores were used set at thresholds of 16, 14, and 18, respectively (Alves et al., 2004; Karasek & Theorell, 1990; Ulhoa et al., 2010). Combinations of high or low job demands and job control were divided into four categories based upon median levels. The "passive"-job group consisted of those below the median for both demands and control, and the "active"-job group were those above the median for both variables. A "high-strain" group was

defined as participants above the median for demands in this population and below the median for control. A “low-strain” group was the combination of low job demand and high job control (Alves et al., 2004; Karasek & Theorell, 1990).

Participant’s mental health was evaluated using the Self-Report Questionnaire (SRQ-20) developed by Harding et al. (1980), which was translated into Portuguese by Mari and Williams (1986). This 20-item questionnaire asks about fatigue, physical symptoms (e.g., headache, tremor in the hands), and depressed thoughts. Questionnaire data were analyzed on a continuous scale only.

All workers further completed a questionnaire asking about job satisfaction, extracted from the Occupational Stress Indicator (OSI) validated and translated into Portuguese by Swan and colleagues (1993). The questionnaire allows participants to rate their feelings about 22 different aspects of their job, such as the worker’s motivation, type of job, and job security. Scores range from 22 to 132, with higher scores indicating higher job satisfaction.

To assess sleep quality, degree of tiredness, and levels of truck noise and vibration, all participants completed a visual analog scale (VAS, ranging from 0 to 10) for one complete week at two time points each day, namely once before and once after work.

Actigraphy

Actigraph devices (Ambulatory Monitoring, NY, USA) were placed on the non-dominant arm of participants for 1 wk (same week as VAS completion). The actigraph is the size and appearance of a wrist-watch and was programmed to record individual activity and rest profiles continuously in 1-min epochs. Action W software (Ambulatory Monitoring, NY, USA) was used for data analysis. Individual activity data were used to calculate the actigraphic sleep duration, sleep latency, body movements

during sleep, and sleep efficiency. All participants also completed an activity diary to record, for example, how long they spent driving, how much time they spent waiting for the truck to be loaded with goods, and how long they slept.

Physiological measures

In the morning (between 07:00 and 12:00 h) of one work day, all participants had their resting blood pressure (BP) and heart rate (HR) measured three times in 2-min intervals. This was performed directly after completion of the questionnaires. The average value of these three measures of BP and HR was used for data analysis. Fasting blood samples were collected from the antecubital vein by a trained technician and immediately stored at -20°C. The blood samples were subsequently analyzed for plasma glucose, cholesterol, and triglycerides (enzymatic colorimetric method CHOP-PAP). Body weight and height of the participants were measured after blood sampling to calculate the body mass index (BMI) and the hip and waist circumferences were measured to derive the waist-hip ratio (WHR).

Salivary cortisol

The day shift (n = 21) and irregular shiftworkers (n = 21) collected their saliva samples at three time points per day, during both one working day and one off day from work. This sampling procedure was carried out in the same week that the participants wore the actigraph devices, did the physiological measures, and completed the VAS. Each participant received six commercial sampling kits (Salivette®, Sarstedt, Nümbrecht, Germany) to collect saliva samples: (i) at waking, (ii) 30 min after waking, and (iii) at bedtime. The time of the sample collection was recorded on each tube. Participants were instructed not to brush their teeth, nor eat nor smoke prior to the sampling, and to avoid contaminating the sample with blood or food. They were instructed to wait 3 h after their main meal (lunch or dinner) and 30 min after teeth brushing before sampling. After

saliva collection, the samples were kept in the workers' refrigerator before returning them to the research team. Samples were analyzed at the Genese laboratory (São Paulo, Brazil), and salivary cortisol concentrations were determined by ELISA with a competitive antibody-capture technique, with a detection limit of 0.011 $\mu\text{g/dL}$ (Kit DSL-10-671000 Active® Salivary Cortisol, Diagnostic Systems Laboratory, Webster, Texas, USA). A total of 198 saliva samples from the 21 day and 21 irregular shiftworkers were analyzed. Salivary cortisol values above the recommended levels for laboratory analyses of 2.0 $\mu\text{g/dl}$ were excluded. Samples that were below the detection limit of the assay (0.011 $\mu\text{g/dl}$) were set equal to the detection limit (12.5% of samples measured at bedtime).

Statistical analysis

Student's *t*-test was performed to compare means of the variables (health, biochemistry, work, and psychosocial factors) between the day and irregular shiftworkers. Two-way ANOVA was performed to compare cortisol levels between day and irregular shiftworkers on off days, with type of shift (day and irregular) and time of the sample collection as factors (waking time, 30 min after waking time, and bedtime).

The cortisol awakening response (CAR) was calculated by subtracting the cortisol level at waking from the cortisol level 30 min after waking (Dahlgren et al., 2009). The CAR can be indicative of a stress response as a predictor of the subsequent demands of the day (Fries et al., 2009). Paired Student's *t*-test was performed to compare cortisol levels and the CAR between work and off days for each shift group.

Spearman correlation was performed between cortisol levels and additionally collected variables (as described in methods and Table 1) only for the days off work when the sampling times between the two shift groups were comparable. All statistical analyses were performed using SPSS 17.0 (SPSS Inc. Chicago, USA).

Results

Of the 21-day workers (mean \pm SD: 39.0 \pm 4.9 yrs of age), 65.4% consumed alcohol, 11.5% smoked cigarettes, and 88.5% lived together with a partner. For the 21-irregular shiftworkers (mean \pm SD: 40.6 \pm 7.2 yrs of age), the corresponding figures were 54.8%, 16.1%, and 90.3%. There were no significant differences between the groups in these parameters.

Irregular shiftworkers, as opposed to the day workers, had a significantly higher waist-hip ratio (WHR) and VLDL-cholesterol levels (Table 1). There was a non-significant trend for higher diastolic and systolic BP in the irregular shiftworkers. Irregular shift workers reported significantly more tiredness after work, less job demand, and more years working as a truck driver. They also reported that they experienced more truck vibration compared to day workers (Table 1). The day workers, however, reported that they spent more time driving and waiting for the truck to be loaded.

Table 1 about here

Average (\pm SD) actigraphic sleep duration for the day and irregular shiftworkers was 393.5 (\pm 70.9) min and 410.7 (\pm 89.3) min, respectively, and the mean (\pm SD) sleep efficiency was 89 (\pm 6.2%) in the day and 89 (\pm 8.7%) in irregular shiftworkers. These differences were not statistically significant between the groups. Sleep quality was also not significantly different between the groups. The irregular shiftwork group spent on average (\pm SD) 384.4 (\pm 88) min/day driving, while the day shiftworkers spent on average (\pm SD) 497.1 (\pm 111) mins/day driving.

Figure 1 shows the mean salivary cortisol levels for the two shift groups, obtained from a total of 198 salivary samples. Day workers had a similar cortisol

pattern, with higher cortisol in the morning compared to bedtime, on both work days and off days. The pattern for irregular shiftworkers, in turn, differed, probably due to their different sampling times on work and off days.

Figure 1 about here

Table 2 about here

Table 2 shows the mean clock time (\pm SD) the workers collected their samples on the work and off day. There was a significant effect of sampling time (at waking, at 30 min after waking, and at bedtime) on the cortisol levels on work and off days, in both the day (repeated-measures ANOVA; $F_{(2, 30)} = 42.0, p = .001$) and irregular shiftworkers (repeated-measures ANOVA; $F_{(2,22)} = 16.5, p < .001$). There was no significant effect of sampling day on cortisol levels in the day workers, but there was a significant interaction between sampling time and day of collection (work day or off day; repeated-measures ANOVA; $F_{(2,30)} = 5.0, p = .013$). For day workers, cortisol at 30 min after waking was significantly higher on work compared to off days (paired Student's *t*-test $p = 0.03$). No statistical significance for this comparison was observed in the irregular shiftworkers.

Two-way ANOVA showed a significant difference ($F = 4.7, p = .03$) between the two shift groups on off days with significantly higher cortisol at 30 min after waking in the irregular shiftworkers (mean \pm SEM: $0.96 \pm 0.12 \mu\text{g/dl}$) compared to day workers ($0.67 \pm 0.10 \mu\text{g/dl}$). The sampling time at 30 min after waking on off days was significantly later ($p < .001$) in the irregular shift (09:13 h) compared to day workers (08:05 h).

The statistically significant correlations between work, health, and sleep parameters and cortisol levels on the off days in both shift groups are presented in Table 3. Cortisol levels in the day shiftworkers were only correlated with subjective variables

(job control score, sleep quality) and length of years working. In irregular shiftworkers, there were also significant correlations with physiological parameters (cardiovascular and metabolic), in addition to subjective variables (tired after working and job satisfaction). Higher levels of cortisol were correlated with high BP and low job satisfaction. Metabolic parameters were correlated with cortisol levels collected in the morning and at bedtime. High cortisol levels were positively correlated with high levels of cholesterol, LDL, triglycerides, VLDL and negatively correlated with low HDL and short sleep duration ($p < .05$).

Table 3 about here

The cortisol awakening response (CAR) in the day and irregular shiftworkers is presented in Figure 2. The CAR was higher on work days compared to off days in both groups. This difference between work days and off days was only significant for day workers (paired Student's t -test $p < .02$).

Figure 2 about here

We did not perform any statistical analysis to compare cortisol levels for both groups when separated into the four categories of the job content questionnaire because of the low sample number in some categories. Describing the job categories of the day workers, 28.6% ($n = 6$) had high job strain, 14.3% ($n = 3$) had low job strain, 14.3% ($n = 3$) were categorized for a passive job, and 42.8% ($n = 9$) fell into the category for an active job. For irregular workers, 14.3% ($n = 3$) were categorized for high job strain, 28.5% ($n = 6$) for low job strain, 52.4% ($n = 11$) for a passive job, and 4.8% ($n = 1$) for an active job.

Discussion

In agreement with previous publications (Harma et al., 2006; Kantermann et al., 2010), the present study found that irregular shiftworkers had a poorer health status compared to day workers. Furthermore, both cortisol levels at 30 min after waking and cortisol levels at bedtime were correlated with total cholesterol, HDL, LDL, VLDL, and triglyceride levels, but only for the irregular shiftworkers. This finding is in line with a previous study showing shiftwork to be associated with higher triglycerides, lower HDL, and central obesity compared to day workers (Karlsson et al., 2003). Possible reasons for this finding in the irregular workers might be elevated psychosocial stress, disrupted circadian rhythms, sleep deprivation, a lower quality and/or irregular timing of the diet, physical inactivity, and insufficient time for rest and revitalization (Lowden et al., 2010; Scheer et al., 2009). It is also important to highlight that the irregular shiftwork group was more negatively affected by environmental conditions, since they reported being more disturbed by truck vibration than the day workers.

In addition, the present study shows that the factors “low job satisfaction”, “number of years working as a truck driver” and “short sleep duration” were significantly positively correlated with cortisol levels in the irregular shiftworkers (Table 2). This we interpret as a stress reaction to these factors in this group. Indeed, low job satisfaction and many years working as a driver has recently been shown to be associated with poor mental health in a sample of 460 truck drivers (Ulhoa et al., 2010).

Sleep deprivation and circadian misalignment are well known consequences of working shifts and might feasibly act as stressors affecting cortisol release and an individuals' perception of stress. Environmental stressors, such as elevated noise, have been shown to significantly affect sleep. Sleep deprivation and stress are thus conditions that are interlinked and often feedback on each other (Meerlo et al., 2008). Circadian misalignment does not only affect sleep but also cardiovascular and metabolic function

(Scheer et al., 2009). To date, however, the mechanisms underlying circadian misalignment and adverse health in shiftworkers are still poorly understood (Kantermann et al., 2010). One strategy that has been suggested to improve sleep and to promote circadian adjustment in (irregular) shiftworkers is appropriately timed light exposure and melatonin administration as well as timed meals and physical exercise (Crowley et al., 2003; Skene & Arendt, 2006).

Concerning the individual stress response, the present study found that day workers had higher morning cortisol and a higher cortisol awakening response (CAR) on work compared to off days. This result could be explained by a stress response in anticipation of the subsequent work on that respective day (Fries et al., 2009). The sampling time of saliva collection on work days was earlier in the morning than on the off days. We, therefore, assume that on off days the time of the sample collection was nearer the endogenous cortisol peak time (Scheer et al., 2009; Viola et al., 2007). Despite this, cortisol levels on work days were higher than on off days, emphasizing a positive stress response on these work days. The present study was designed to measure cortisol before and after sleep (awakening time, plus 30 min after waking, and at bedtime) to track the biological rhythm instead of fixing sampling to clock time. This method has been used in previous studies (Thomas et al., 2009) and was considered especially important to compare day workers to irregular workers on their off days, as irregular workers have a completely different schedule on work days.

Due to the highly irregular sleep-wake times in the irregular shiftworkers on work compared to off days (e.g., because of the night work schedule and subsequent daytime sleep), it was not possible to compare work days and off days directly in this group. Although the CAR on work days was higher compared to off days, this result did not reach statistical significance for the irregular shiftworkers. Because of these differences in work hours between day and irregular shiftworkers on work days, it was only

reliable, therefore, to compare cortisol profiles on off days between the two shift groups. On days off, the irregular shiftworkers showed on average 30% higher cortisol (at 30 min after waking) compared to the day workers. There was a significant effect of work shift (day compared to irregular shiftworkers) on cortisol, but this finding has to be interpreted with caution, since there was also a significant difference in the sampling times between the two groups. On off days, irregular shiftworkers woke up later than day workers, and due to correspondingly later sampling times the cortisol concentrations and other physiological parameters could have been affected. Previous studies have shown that night shifts (Thomas et al., 2009) and counterclockwise rotating shift schedules (Vangelova et al., 2008) can increase cortisol secretion. The present results suggest that irregular shiftwork may also affect cortisol to a similar extent as reported previously for night shiftwork.

In both work groups, some truck drivers showed a positive CAR while others showed a negative CAR. This finding is in agreement with Dahlgren et al. (2009), who investigated 14 office workers across 4 wks and found that in the morning the workers with a negative CAR had stayed in bed longer after their first awakening, which was interpreted by the authors as a sign of snoozing; thus, the actual CAR was not captured. Unfortunately, in the present study information as to how long the participants remained in bed after their first waking was not collected, and the drivers were advised to collect saliva immediately after getting out of bed. Future studies should take respective sleep times as well as time in bed into consideration. Moreover, it is also possible to have a reduction in cortisol levels 30 min after waking, as cortisol levels are also affected by time of day, an individual's circadian phase, and the sleep time (Fries et al., 2009). Although Dahlgren et al. (2009) suggest that the CAR is affected by the time remaining in bed, a recent review (Fries et al., 2009) concluded that it is the physiological anticipation of the day that is mainly relevant for the magnitude of the CAR. Our results

support this idea. It is also important to take age into consideration, since age can affect the magnitude of the CAR. Our results, however, show that there were no differences in age between the work groups (day and irregular shift), and neither was age correlated with the cortisol levels. We, thus, assume that age was not a factor to affect the present results.

Individual factors or certain personality can influence the stress response and cortisol levels; for example, a very competitive, extremely committed person is more susceptible to stress and sleep problems (Soehner et al., 2007; WHO, 2008). In the present study, however, personality bias was not evaluated. Another point to note is the extra 20% payment to night workers, which could be a reason why some workers choose to do shift work and would hence confound the data. Moreover, the distribution of the job categories was different between the work groups, with day workers showing a higher level of job demand compared to the irregular workers, which may also affect stress markers (de Croon et al., 2002; Karasek & Theorell, 1990).

The strengths of the present study include an extensive data collection about the truck driver's work, sleep, and health, and the comparison between two different work regimes in male workers from the same transportation company. A potential limitation of the present study is its cross-sectional nature. Due to the correlational approach used, any cause and effect between cortisol levels and health parameters remains unanswered and requires follow-up investigation. Although there are many studies only comparing cortisol and other physiologic parameters between two days or two work situations (Axelsson et al., 2006; Lowden et al., 2009), we recommend that future studies collect saliva samples on more days. Multiple sampling days would also help to obtain stable CAR estimates by minimizing possible sampling inaccuracies (Chida & Steptoe, 2009; Okun et al., 2010). It should be noted, however, that in the present study there were logistical difficulties in collecting saliva. For truck drivers who have to deliver goods on

time to many different destinations often miles apart, it was very difficult to collect saliva samples on the road and to store these samples adequately for several days.

In summary, our results show that day workers had higher cortisol on their work compared to their off days, showing a higher stress response at work. Irregular shiftworkers had higher cortisol levels on their off days compared to the day shiftworkers, possibly indicating a prolonged stress response in the irregular workers. This may be due in part to sleep loss caused by the characteristics of the job, which was also reflected in the job satisfaction scores. Although subjective psychosocial stress was not directly correlated to cortisol levels in this population, a correlation between cortisol and other individual stress factors, such as low job satisfaction and short sleep duration as well as metabolic parameters was observed. Future studies are warranted to investigate additional stress responses in the context of irregular work hours.

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