The urinary 6-sulfatoxymelatonin level after three different work schedules with 2, 4 and 7 consecutive night shifts among Danish police officers

Marie Aarrebo Jensen a*, Julie Boye Kjærgaard a, Jindong Ding Petersen c,d, Åse Marie Hansen a,b, Jesper Kristiansen a, Anne Helene Garde a,b

a National Research Centre for the Working Environment, Denmark

b Department of Public Health, University of Copenhagen, Denmark

c Research Unit for General Practice, Department of Public Health, University of Southern Denmark, Denmark

d Research Unit for General Practice, Department of Public Health, University of Copenhagen, Denmark

* Correspondence should be addressed to Marie Aarrebo Jensen, National Research Centre for the Working Environment, Denmark [E-mail: maa@nfa.dk]
Abstract

Night shift work suppresses excretion of melatonin, but little is known about the needed time for recovery. We aimed to compare levels of 6-sulfatoxy melatonin after three different night shift schedules, including recovery days. In a quasi-experimental, within-subject crossover study, 73 male police officers in Denmark collected morning urine after the last recovery day in three different work schedules with two, four, and seven consecutive night shifts followed by a corresponding number of days for recovery. We found no significant differences for 6-sulphatoxymelatonin concentrations in morning urine between the three different work schedules indicating similar recovery of melatonin suppression in the studied work schedules.

Key words: consecutive night shifts, 6-sulphatoxymelatonin, recovery, urine, shiftwork
Introduction

The ability to recover after night shift work is of importance for employee health and well-being (Geurts & Sonnentag, 2006). During recovery, psycho-physiological systems that have been affected during work unwind to and stabilize at a baseline level (Geurts & Sonnentag, 2006). However, when recovery is impeded, the effects of night work might carry on and incomplete recovery of physiological systems is a possible mechanism leading from shift work to chronic health impairment (Geurts & Sonnentag, 2006). Night shift work leads to circadian disruption of rhythms of melatonin (Jensen et al., 2016) and suppression of excretion of melatonin (Hansen et al., 2006). Melatonin is involved in regulating body temperature, blood pressure, and hormone levels. During a typical sleep-wake cycle of a non-night worker, melatonin production reaches the highest level during the night and lowest level during the day (Haus & Smolensky 2006). Nighttime work influences the excretion of melatonin and extended periods with night work results in melatonin suppression compared with day work (Yamauchi et al., 2001; Hansen et al., 2006). Melatonin suppression can be assessed by urinary excretion of 6-sulfatoxymelatonin, which is the major metabolite of melatonin and reflects the approximate blood concentration of melatonin (Arendt et al., 1985). Recovery of melatonin suppression can be used as a proxy for the recovery of the circadian disruption seen after night shifts (Arendt 2010).

It is important to find out the most optimal way to schedule night shift work and the number of consecutive night shifts and recovery days are important components. Yet, information about recovery, particularly in relation to suppression of excretion of melatonin when working specific night work schedules is limited, and it is not known how many days are needed to recover the suppression of melatonin. The aim of this study is to investigate the effects on the concentrations of 6-sulphatoxymelatonin in urine of three different night shift schedules with two, four, and seven consecutive night shifts and a corresponding number of recovery days.
Methods

This paper presents results from the project “In the Middle of the Night”. The study was approved by The National Committee on Health Research Ethics in Denmark (protocol number H-4-2012-155). The experimental protocol conforms to international ethical standards for biological rhythm research studies (Portaluppi et al., 2010).

Recruitment procedure

We recruited participants from the five police districts on Zealand, Denmark. The inclusion criteria were that the participants had to be non-smoking male police officers with night shifts as a part of their regular schedule. We invited potential participants to participate in the study by e-mail. A total of 121 police officers showed interest in participating in the study. Among these, 99 police officers received individual, detailed information about the project. In total, 73 police officers completed the self-reported baseline questionnaire and provided urine samples.

The study was designed as a quasi-experimental, within-subject crossover study exposing the participants to three different work schedules: 2 night shifts followed by 2 recovery days (‘2+2’), 4 night shifts followed by 4 recovery days (‘4+4’), and 7 night shifts followed by 7 recovery days (‘7+7’). Recovery days were defined as day shifts or days off.

The data collection period was April-June (spring) 2013 and September-November (fall) 2013. The three work schedules lasted 26 d in total and were conducted within three months, and each individual only participated in either the spring (n=69) or the fall (n=5). The participants were required not to work any night shifts during the 7 d proceeding the first day in a shift system. For more detailed information about the recruitment procedure and data collection, see previously published papers from the study (Jensen et al., 2016; Nabe-Nielsen et al., 2016; Garde et al., 2020).
Participants

The 73 participants were men between age 25 and 62 y with a mean age of 38 y. Some 22% had <3 y of night shift experience, 38% had 3-10 y of night work experience, and 40% had >10 y of night work experience. Most rated their health to be excellent or very good, and they were generally physically active.

Questionnaire

The participants completed a background questionnaire before starting their first work schedule in the study. From the questionnaire, we obtained information about tenure within the police force, night work experience, physical activity, self-rated overall health, general job satisfaction, and chronotype.

Collection of urine samples

Each participant collected urine directly in 10 ml tubes the morning after the last recovery day in each work schedule for determination of 6-sulphatoxymelatonin. The participants kept the urine samples in the freezer at home until all three work schedules were completed. Samples were returned to laboratory by mail within 7 d after the last sampling. The urine samples were stored at -20° in the laboratory until analysis. A total of 204 urine samples were collected; 65 urine samples from participants of the 2+2 work/recovery schedule, 67 urine samples from participants of 4+4 work/recovery schedule, and 72 urine samples from 7+7 work/recovery schedule. Sixty-three out of 73 participants provided urine samples under all three work/recovery schedule conditions.

Measurement of 6-sulfatoxymelatonin in urine
A competitive enzyme-linked immunosorbent assay (ELISA) (IBL, Hamburg, Germany) was used to determine 6-sulphatoxymelatonin in the urine. The analysis was performed according to the manufacturer’s specifications.

Epoch™ Microplate Spectrophotometer (BioTek Instruments, Winooski, USA) and the program Gen5, version 2.04 (for Windows) were used to calculate concentrations. The limit of detection (LOD) was 3.05 nmol/L. Urinary creatinine was used to standardize the results. Urinary creatinine was measured with an enzymatic method utilizing a multi-step approach (ABX Pentra Enzymatic Creatinine CP, Horiba medical, USA).

**Statistical analysis**

All statistical analyses were done in the statistical software SAS 9.3 (SAS Institute, Cary, NC). We did multilevel regression analyses using the PROC MIXED procedure. The three work schedules were included as categorical variables in three levels (‘2+2’; ‘4+4’; ‘7+7’). We used a random intercept for each individual with a variance component covariance structure. All concentrations of 6-sulphatoxymelatonin were expressed as logarithmic value and treated as a continuous variable.

**Results**

**Urinary melatonin**

We found no significant difference in 6-sulphatoxymelatonin concentration between the three different work schedules (2+2, 4+4, and 7+7) (p = 0.971) (Figure 1). The average morning concentration of 6-sulphatoxymelatonin was 6.5 nmol/mol creatinine across the three different work/recovery schedules.
Discussion

In this quasi-experimental, within-subject crossover study, we investigated the effect of two, four and seven consecutive night shifts with a corresponding number of recovery days on the concentration of 6-sulphatoxymelatonin. We found no statistically significant difference in the morning urine concentration of 6-sulphatoxymelatonin between the three different work schedules.

In accordance with the results from our study, a study of Danish nurses with night shifts as part of their both fixed night shift and mixed (day, evening, and night) schedules found no difference in concentrations of 6-sulphatoxymelatonin between days with night shifts and days off for mixed shift workers (Hansen et al., 2006). The morning concentrations of 6-sulphatoxymelatonin reported for both shift and day workers in that study are in addition very close to the average concentrations found in the present study (6.5 nmol/mol creatinine), which is consistent with the hypothesis that the 6-sulphatoxymelatonin was fully recovered on the last recovery day in our present study. In the nurses’ study, the authors found an indication of lower 6-sulphatoxymelatonin on off days for workers with fixed night shifts, indicating insufficient recovery. However, this could be due to a higher number of night shifts (not specified in the paper) compared to recovery days than in the present study. It could, therefore, be speculated that a lack of difference between recovery days and workdays indicates full recovery of the suppression of melatonin.

Mirick et al. found that male night shift workers have substantially lower 6-sulfatoxymelatonin during night work and daytime sleep, and levels remained low when night shift workers slept at night, indicating a chronic suppression of melatonin (Mirick et al., 2013). Davis et al. also found similar results to Mirick et al. in a study of shift working women (Davis et al., 2012). However, in both of these studies, the measurement of 6-sulfatoxymelatonin was done after only one day off, and we speculate that this might not be a sufficient duration of time to recover the suppression of melatonin.
Since the purpose of this project was to compare recovery of melatonin secretion after suppression in relation to three different night shift schedules, we did not include a baseline day. However, in the same population we found that the circadian rhythm of melatonin in saliva on all recovery days was identical and all of the profiles were as expected for a normal routine of daytime wakefulness and nighttime sleep (Jensen et al., 2016). Combined with the results presented here, this indicates recovery of the suppression of melatonin.

Recovery after night shifts are pivotal for shift worker health and the design of shift schedules should consider recovery. This study implies that corresponding number of recovery days after night shifts are sufficient for recovery of melatonin. More detailed sampling would make it possible to assess if recovery occurs earlier and how quickly after night shift work.

In conclusion, we find no difference in concentrations of 6-sulfatoxy-melatonin after two, four, and seven consecutive night shifts with a corresponding number of recovery days thereby indicating that the recovery time is sufficient per each specific work/recovery schedule.

References


Figure 1. The excreted average concentration of 6-sulfatoxymelatonin in urine (nmol/mol creatinine) the morning after the last recovery day of each work schedule in a quasi-experimental, within-subject crossover study. Error bars indicate 95% Confidence Interval.

2+2 refers to the concentration after the last day of the schedule 2+2; 2 night shifts followed by 2 recovery days (N= 65)

4+4 refers to the concentration after the last day of the schedule 4+4; 4 night shifts followed by 4 recovery days, (N=67)

7+7 refers to the concentration after the last day of the schedule 7+7; 7 night shifts followed by 7 recovery days. The error bars indicate standard deviations (N=72)