Working in the “Middle of the Night”

The physiological effects of consecutive night shifts

PhD thesis

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Title
Working “In the Middle of the Night” – the physiological effects of consecutive night shifts

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Preface
My Ph.D. project was a part of the “In the Middle of the Night” project. The aim of the project was to compare the effects of working two, four, or seven consecutive night shifts on sleep and risk factors related to cardiovascular disease and metabolic disorders. The project was funded by The Danish Working Environment Research Fund under Grant 10-2011-09 and a Ph.D. grant from the University of Copenhagen.

The “In the Middle of the Night” project was collaboration between The National Research Centre for the Working Environment and the University of Copenhagen.

I held a key position in the “In the Middle of the Night” project throughout the entire process: planning, ethical approval, recruitment of participants, data collection and analysis of collected material. This work resulted in this thesis and data which can be used in future research on the effects of consecutive night shifts.

The thesis is based on the following papers:

**Paper I:** The effect of the number of consecutive night shifts on diurnal rhythms in cortisol, melatonin and heart rate variability (HRV) – a systematic review of field studies

*Submitted to International Archives of Occupational and Environmental Health, considered for publication conditioned on minor revisions*

**Paper II:** Heart rate variability during sleep after 2, 4 and 7 consecutive night shifts and recovery days - a cross-over intervention study

*Submitted to Chronobiology International, under review*

**Paper III:** Changes in the diurnal rhythms of cortisol, melatonin and testosterone after 2, 4 and 7 consecutive night shifts in male police officers

*In preparation, plan is to submit to Chronobiology International in September 2015*

**Paper IV:** An interlaboratory comparison between similar methods for determination of melatonin, cortisol and testosterone in saliva.

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Introduction
Night work is common in many occupations both now and in the future and it has an impact on health both in the short and possibly also in the long run (2-5). The causality between night shift work and increased risk of certain diseases is, however, heavily debated in the public as well as in the scientific community, and is yet unsettled. This is also true for the role of specific shift work schedules e.g. the number of consecutive nights in a schedule. When work at night is inevitable the question is how to organize it in order to reduce potential health impairment? Negative effects related to number of mistakes, reaction time and concentration increases with an increasing number of consecutive night shifts (6;7). Sleep length between night shifts tend to increase with the number of consecutive nights (8). Thus, there are good reasons to encourage the employees to work many consecutive nights in order to allow adaptation and thereby fewer turns in diurnal rhythms. However, negative metabolic effects appear after just one day without sleep (9) and other studies show that sleep loss is accumulated over consecutive night shifts (10). Thus, there are also good reasons to work only few consecutive nights rather than longer periods with night work. In spite of a rather comprehensive scientific literature on this subject, studies with focus on the potential mechanisms linking numbers of consecutive night shifts to disease are needed (11).

The existing knowledge on the physiological effects of consecutive night shifts has primarily been found through laboratory studies (12;13), whereas only few studies have been performed among employees at work (14;15). Performing field studies with self-sampling a number of implications have to be handled, such as sampling procedure at home or at the work place, storage of samples until analysis, compliance of the sampling procedure, and comparability with measurement in other studies.

The perspective of this thesis is to investigate what is the best: Many consecutive nights and thereby fewer turns of the diurnal rhythm or few consecutive nights and thereby more turns of the diurnal rhythm? We know that shift work causes acute physiological responses, but we do not know to what extent these responses depend on the number of consecutive night shifts when exposed to night shifts in field studies. An important question is therefore, if there is an optimal way to organize night work, when this is inevitable?
Objectives and research questions

This thesis addresses some of the challenges involved when conducting field studies and analyzing biological markers from field studies. In a field study context how does the human body react to consecutive night shifts? I wish to explore the effect of the number of consecutive night shifts using acute physiological responses to evaluate possible long term effects of night shifts. In specific I want to study the effects and possible health consequences of different shift systems physiological biomarkers are keys to understanding how the body responds to night shifts because they give objective measurement of the reactions to night shifts. To study the specific exposure for workers in their real lives field studies are an important part of shift work research. When conducting field studies data control and validity is important to ensure valid results, this thesis will also include a section on how to ensure optimal data quality.

This thesis includes results from a review of the existing literature on adaptation of diurnal rhythms in field studies, results from the “In the Middle of the Night” projects; a cross-over intervention study examining the effects of consecutive night shifts and a inter laboratory comparison of analytical methods for determination of melatonin, cortisol and testosterone in saliva.

The specific research questions investigated in the thesis are:

1) **How many consecutive night shifts are required for adaptation of diurnal rhythms in cortisol, melatonin and heart rate variability (HRV) to night work?**

This research question is investigated in a review of field studies that use the biological markers melatonin, cortisol and heart rate variability. The details of this study are presented in Paper I: The effect of the number of consecutive night shifts on diurnal rhythms in cortisol, melatonin and heart rate variability (HRV) – a systematic review of field studies.

2) **What are the effects of the number of consecutive night shifts on HRV during sleep?**

This research question will be investigated a part of the “In the Middle of the Night” project and the details of this study are presented in paper II: Heart rate variability during sleep after 2, 4 and 7 consecutive night shifts and recovery days - a cross-over intervention study.
3) **What are the effects of the number of consecutive night shifts on the rhythm of melatonin, cortisol and testosterone?**

This research question will be investigated as part of the “In the Middle of the Night” project and the details of this study are presented in paper III: Changes in the diurnal rhythms of cortisol, melatonin and testosterone after 2, 4 and 7 consecutive night shifts in male police officers.

4) **Can we compare analysis of hormones in saliva measured in different laboratories?**

As a part of ensuring data quality the method used for measuring hormones in the “In the Middle of the Night” projects took part in an interlaboratory comparison of methods for analyzing hormones in saliva. The details of this study are presented in paper IV: An interlaboratory comparison between similar methods for determination of melatonin, cortisol and testosterone in saliva.
**Background**

**Night shift work and health problems**

Between 15-20% of the working population in Europe and USA are estimated to have jobs which imply work at night (4;16). In Denmark 2-3% of the working population report that half or more of the working hours are between 23:00 and 04:00. In some occupations the segment of night work is much higher e.g. waiters/waitresses (31%), taxi drivers (40%), employees at hospitals (31%) and police and prison staff (27%) (www.arbejdsmiljoforskning.dk results from ‘Arbejdsmiljø og helbred i Danmark’, 2014.). Demands on increased effectiveness and flexibility in order to increase competitiveness in an increasingly globalized world may, together with the development towards a 24-7 society, imply that night work in the future will affect even more employees.

Shift work is associated with both short and long term health consequences. The short-term effects of shiftwork are relatively well known. Most of the short term effects are related to sleep problems, fatigue, disturbed work-life balance, reduced work performance, and risk of occupational accidents (17-21).

In the long term night shift work have been associated with increased risk of obesity (22), breast cancer (23), cardiovascular disease (CVD) (24), gastrointestinal disorders (25), and possibly elevated mortality risk. However, it is still debatable whether or not shiftwork itself is an independent risk factor for impaired health (26;27).

**How to organize night shift work**

There exists very little recommendation on how to organize shift work to prevent adverse health outcomes. This is partly due to lack of evidence for the mechanisms leading from shift work to disease and this makes it difficult to predict the effect of a specific work schedule. When looking at the question of few vs. many consecutive night shifts there are different reasons for preferring one or the other. Negative effects related to number of mistakes, reaction time and concentration are most pronounced in relation to the 1st to 3rd night shift, after which some adaptation to the night shift occurs (6;7). Sleep length between night shifts tend to increase with the number of consecutive nights (8). Thus there are good reasons to encourage the employees to work many consecutive nights in order to allow adaptation and thereby fewer turns in circadian rhythms because it may reduce the risk of accidents and increase overall sleep length. However, the opposite suggestion that the number of consecutive nights should be as few as possible also has solid justification. Negative metabolic effects appear after just one day without sleep (9).
studies show that sleep loss is accumulated (10), and that the circadian rhythms are displaced more. It may be argued that if a non-day shift worker completely adapted to the new 24 h schedule, maintaining this also on days off, circadian disruption and health impairment would presumably be minimal (28). In most cases non-day work is not carried out isolated from the rest of the society, which interferes with the possibilities to adjust diurnal rhythms. Night workers do not quite adjust their rhythm to being awake at night and sleep during the day (29). Thus, there are also good reasons to work only few consecutive nights rather than permanent night shift work.

**Chronobiology**

Circadian rhythms are pivotal for all life. Most physiological processes are rhythmic and many oscillate over 24 hours. Fluctuations in body temperature, blood pressure, hormone secretion, digestion, metabolism, and cell turnover all have distinct circadian rhythms. The biological master clock and central pacemaker of the circadian system is suprachiasmatic nucleus (SCN) located in the hypothalamus (30). The SCN synchronizes the internal rhythms to the external environment by receiving signals from external zeitgebers such as light and darkness, timing of meals, social rhythms, and activities such as physical activity and work (31). Circadian rhythms are rhythms that persist during constant environmental and behavioral conditions, however, when investigating rhythms in field studies it is not possible to distinguish the relative contribution of endogenous circadian timing system versus changes in posture, sleep/wake cycle, behavioral activity, light/dark cycles, and feeding/fasting cycles in the rhythms (32). Therefore the rhythms investigated in the field studies in this thesis are referred to as diurnal rhythms.

Humans are day-oriented and night shift work requires a change of our physiological rhythms and sleep/wake cycle. This is not an instant change, but happens gradually with increasing number of consecutive night shifts (4), however, how many consecutive night shifts are required for adaptation if adaptation occurs or if a complete adaptation is ever obtained is still debated. In this thesis full adaptation to night shifts is regarded as being achieved when the physiological rhythms are fully synchronized relative to the new work schedule and sleep times. Thus, being fully adapted to the new sleep/wake cycle will mean a disruption in relation to the ambient dark/light cycle. The main focus of this thesis is to investigate diurnal rhythms and the physiological effects of night shifts in field studies. In order to describe and characterize a rhythm and possible changes to this rhythm we need measures of amplitude and phase, see figure 1 (33). This will require measurements taken at several time points in order to describe all characteristics of a given rhythm and to evaluate possible adaptation. This is not easily obtained in an ambulatory setting (34).
Possible mechanisms linking shift work and health problems
Several mechanisms leading from night shift work to disease have been suggested. When humans work at night a disturbance occurs between behavioral and biological rhythms, stemming from being awake at night and sleeping during the day. This leads among other things to circadian disruption, sleep loss, melatonin suppression, and lack of coordination between diurnal rhythms (23;24;35;36).

Circadian disruption: One proposed mechanism for the link between night shift work and disease is circadian disruption. Circadian disruption is when the physiological rhythms are not in line with the external environment and if often defined differently. Stevens et al proposed the following definition: “A perturbation of the endogenous circadian rhythmicity, particularly by electric light exposure of the eyes during the night. Circadian rhythmicity includes both phase and amplitude characteristics of biologic markers that exhibit an endogenous, approximately 24-hour rhythm, such as circulating melatonin, circadian gene expression, and sleep. The term circadian disruption includes disturbances such as phase shifts of the entire circadian system, the displacement of sleep relative to the circadian clock, and/or the acute suppression of nocturnal melatonin production whether or not a phase shift also occurs.” (37). According to this definition of circadian disruption a night shift worker that fully adapts to night shifts in terms of his sleep/wake cycle will experience a very high level of circadian disruption according to the definition by Stevens.
Sleep deprivation and insufficient recovery: Another important possible mechanism linking night shift work and disease is sleep deprivation and insufficient recovery. Recovery after night shifts is an important part of shift worker health (38). Recovery enables stabilization at a baseline level of the physiological systems that have been activated during work (39). Sleep is reduced and poorer among night workers compared to day workers (40;41). Apart from an increased risk of mistakes and accidents (42) sleep deprivation leads to reduced secretion of growth hormone, increased cortisol secretion, and reduced insulin sensitivity (4). This is linked to increased risk of obesity, metabolic syndrome and type 2 diabetes, as well as hypertension and suppression of the immune system (4).

Melatonin suppression: The suppression of melatonin via exposure to light at night is also a possible mechanism linking night shift work and disease. It can be viewed a part of circadian disruption, but is often mentioned separately. Melatonin may act on initiation, promotion, and progression of tumors (23). Melatonin has also been linked to cancer risk, because it affects levels of estrogens, which are known to affect risk of breast cancer (26).

Lack of coordination between internal circadian rhythms: Besides the coordination of the external and internal environment the circadian system synchronizes and coordinates the relative phasing of a multitude of diverse internal physiological processes (43). Different biological rhythms adapt to night work with different speed and this can cause a lack of coordination between internal circadian rhythms (4). In animal experiments this has been shown to cause cardiovascular disease and renal failure (44). Laboratory studies of humans have shown that lack of synchronization of circadian rhythms, measured by sleep and cortisol release may cause pre-diabetic changes (45). The internal balance of testosterone and cortisol has an effect on the risk of developing cardiovascular disease and type 2 diabetes (46). Hence, the lack of coordination between the circadian rhythms of these hormones may increase the risk of these health outcomes by disturbing this balance.

The biomarkers used in the thesis: melatonin, cortisol and testosterone, and HRV
The four biomarkers evaluated in this thesis are the three hormones melatonin, cortisol and testosterone and a marker of the autonomic nervous system, heart rate variability (HRV). All four chosen biomarkers have distinct diurnal rhythms.

Melatonin: Melatonin is produced in the pineal gland and is the primary pacemaker of the circadian system. The function of melatonin is to synchronize the internal environment to the light-dark cycle of the external environment (47). The production of melatonin is directly affected
by light and can be completely suppressed by light of sufficient intensity (47). Melatonin is regarded as a strong indicator of the circadian rhythm and has been used to study circadian changes in both laboratory and field studies (47). Melatonin can be measured in blood, urine and saliva (48-50).

**Cortisol:** Cortisol is a steroid hormone and is produced in the adrenal gland (51). Cortisol is the principal marker of the activation of the hypothalamus-pituitary-adrenal (HPA) axis and released in response to stress. The primary functions of cortisol are to raise the level of blood sugar and to suppress the immune system (51). Cortisol has a pronounced diurnal rhythm and is viewed as a good and robust marker of the overall circadian rhythm (33). Cortisol has a characteristic rise 30 min. after awakening and then the concentration falls during the day. This characteristic rise in the level of cortisol is called the cortisol awakening response (CAR) (4). Awakening time, physical activity and stress can all influence the concentration of cortisol (52). Cortisol is often used in field studies since it can be measured in urine, blood and saliva (50;53).

**Testosterone:** Testosterone is an anabolic steroid produced in the testes in men and to a lesser extent, in the ovaries in women. Testosterone is also the principal male sex hormone. The primary functions of testosterone are in men the development of male reproductive tissues. Testosterone has strong anabolic affects such as include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation (54). Testosterone has a distinct diurnal profile since testosterone is released during deep sleep (54;55). However, unlike melatonin and cortisol, testosterone is not under direct circadian control and linked to the light-dark cycle, but is instead primarily dependent on sleep (56). Testosterone can be measured in blood and saliva (50), however, testosterone has not been used as frequently in field studies as melatonin and cortisol.

**HRV:** The autonomic nervous system (ANS) is an important part of the stress regulatory system in the body. The ANS exerts its effects on peripheral target organs via the central nervous system. HRV has gained popularity as a simple, non-invasive marker of autonomic regulation in both laboratory and field studies. The (ANS) also controls HRV. HRV is based on the fact that the heart rate constantly fluctuates. HRV is the physiological phenomenon of variation in the time interval between heartbeats and is measured by the variation in the beat-to-beat intervals also called RR intervals (57). The ANS combines influences from sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). Thus, autonomic regulation is reflected in variations of RR intervals, which characterize a healthy and adaptable regulation of the ANS HRV (58;59). When analyzing HRV the most commonly used measures are: High frequency (HF) variation which
reflects parasympathetic activity. Low frequency (LF) variation reflects sympathetic activity, but with a significant contribution from parasympathetic activity. LF/HF is often used to reflect the sympathetic/parasympathetic balance. RR-mean (that is, the average interbeat interval) is also often reported. RR-mean and heart rate have a direct inverse relationship and an increase in RR-mean equals a decrease in heart rate. (58-61). Most measures of HRV have diurnal rhythms that are mainly controlled by the sleep-wake cycle (62;63) and work versus leisure time (64;65).

**Field studies and shift work**

Field and laboratory studies can be used to evaluate the effects of specific shift systems and both laboratory and field studies have advantages and disadvantages (34). The laboratory studies have the advantage of a high level of control of exposure and can use measurement techniques that are not possible to use in a field study setting. When analyzing the effects of shift work laboratory studies can control the level of light, feeding times and control sleep times to get very exact exposures and isolate the effect of different exposures. This level of control is helpful in elucidating the mechanisms leading from shift work to disease. Laboratory studies also give the opportunity to control or monitor the participants to ensure high levels of compliance. Field studies lack the level of control of the environment. Participants are exposed to noise, light (both sun light and artificial light) in an uncontrolled manner and are still required to participate in their everyday life (preparing food, taking care of children and pets etc.). However, this uncontrollable exposure is part of what is means to work nights and is what the shift workers will be exposed to during their working life. Laboratory studies can use simulated work, however this will never be the same exposure as actually doing your real job. So laboratory and field studies can supplement each other to give a better understanding of the complex mechanisms that are involved in the progression from shift work to disease (34).

**Quality and validity of data in field studies**

Undertaking a field study of night shift work requires good reliable biomarkers and analytical methods, as well as a good compliance from the study participants. I will go through a few of the challenges and possibilities below.
**Biomarkers**

Field studies of diurnal rhythms require biomarkers that are accessible in the field. This means that blood sampling may not be feasible. Blood sampling requires trained staff and this will result in very high cost for the project. Blood sampling may also impede some people from participating. Ideal biomarkers for field studies are possible for the participants to collect themselves after instructions. Saliva sampling has the advantages of being noninvasive, painless and easy to perform (66). This especially important in field studies that require that samples can easily be taken during the day in field studies where and participants in a study can be instructed in the sampling procedure at home or at that work place (67). It is also important that the chosen biomarkers are stable during handling and potential shipping.

Other possible measurements that can be done in field studies include small devises that can measure activity and sleep. Actigraphy is often used in field studies as it is an easy noninvasive method of measuring rest and activity. It is a small actimetry sensor worn on the wrist of the participant to measure motor activity. The principle is that there is reduced movement during sleep and increased movement during wake periods (33).

HRV is also has been used in field studies since the measurement can be done with small portable monitors like Actiheart (CamNtech Ltd U.K.) Actiheart is a small lightweight system can be applied for monitoring HRV (68) and has been validated against standard clinical measurements (Holter recordings) (69). Actiheart for measuring HRV has been validated as for the use in field studies (70).

**Analytical methods**

The chosen biomarkers need to be analyzed by methods that have the necessary quality. In field studies participants are primarily healthy adults so both the variations from normality and concentrations of the biomarkers of interest are relatively small (71). Hence it is crucial to use a precise and sensitive method and insure that the methods used are in analytical control (72). Quality control is essential in of course both laboratory and field studies and ensures validity of the analyzed samples. This includes both internal and external quality control (73). Internal quality control can be archived be control charts and external quality control requires participation in proficiency-testing schemes like laboratory comparisons (74).
Compliance and control

The participants in a field study are often left to do sampling and filling out questionnaires by themselves, but whether they actually do as we expect them to do is often an open question. When analyzing hormones that have pronounced rhythmicity during the day it is of course important that samples are taken according to the study protocol. However, participant compliance is not given. A study that investigated compliance with saliva sampling protocols using electronic monitoring found that 76% followed the protocol and 26% failed to take at least one out of six samples (75).

Methods

Paper I: The effect of the number of consecutive night shifts on diurnal rhythms in cortisol, melatonin and heart rate variability (HRV) – a systematic review of field studies

Search strategy
We preformed the literature search in august 2014. We searched the databases ‘Web of Science’ and ‘PubMed’. To develop the search strategy we consulted a search specialist from The National Research center for the Working Environment. The search resulted in total of 900 articles. All titles and abstracts were read by the first author, and 65 papers were scrutinized further. Sixteen of the 65 papers fulfilled the inclusion criteria given below.

Inclusion criteria for the review were:

- Original research written in English and published in a peer-reviewed journal
- Data from humans in field studies
- Studies on one or more night shifts, i.e. primary working hours between 23.00 and 07.00
- Results on cortisol, and/or melatonin/6-sulfatoxy-melatonin, and/or HRV
- At least three measurements on the same day to ensure that there was an indication of diurnal rhythm
- A comparison of night work with day work or days off

Exclusion criteria were:

- Combined field and laboratory studies
- Intervention studies e.g. of the effect of light therapy and melatonin treatment
To complement the search strategy reference lists of selected papers were scanned for additional relevant studies. This resulted in two relevant papers which were included in the review. This gave a total for 18 papers for the review.

**Evaluation of the degree of adaptation to night work**

To classify the degree of adaptation to night work for cortisol, melatonin and HRV in the chosen studies papers were categorized into three categories: “no degree of adaptation”, “some signs of adaptation” and “a high degree of adaptation”. The categories were based on how much the diurnal rhythm of melatonin, cortisol and HRV on night shifts deviated from the diurnal rhythm observed during the day shift in relation to working hours. For studies with permanent night workers as part of the study population days off were used for comparison. The first category “No adaptation” was characterized by no change in the timing of the phase or amplitude of the diurnal rhythm compared to a day shift. The second category “some signs of adaptation” were characterized by changes in the timing of the phase or amplitude of the diurnal rhythm, but not a full phase shift. The last category “A high degree of adaptation” was characterized by a rhythm that followed the same pattern as on a day shift only shifted to match a night shift schedule.

**Papers II and III:**

**Heart rate variability during sleep after 2, 4 and 7 consecutive night shifts and recovery days - a cross-over intervention study**

**Changes in the diurnal rhythms of cortisol, melatonin and testosterone after 2, 4 and 7 consecutive night shifts in male police officers**

Papers II and III presents results from the project “In the Middle of the Night”. The overall aim of the project was to compare the effect of two, four and seven consecutive night shifts on a range of outcomes related to the risk of shift work related diseases. Paper II focuses on HRV during sleep and Paper III focuses changes in the rhythms of melatonin, cortisol and testosterone.

**Recruitment procedure**

The project was carried out in collaboration with the Danish police who volunteered to participate. The labor union also approved the participation in the study. Throughout all phases of the study there was intensive collaboration and strong support from both management and employee representatives within the Danish police.

Inclusion criteria were that the participants had to be non-smoking male police officers and that night shifts had to be a part of their regular schedule. We recruited participants from the five
police districts at Zeeland, Denmark. Before recruitment began information meetings were held for managers, the personal responsible for personnel-on-duty planning, and employee representatives and all districts were offered an initial information meeting for potential participants and this offer was accepted by two police districts.

An e-mail was sent to potential participants in the five invited police districts with an invitation to participate in the study. 121 police officers returned an email showing interest in participating in the study. Among these, 99 police officers proceeded to the next step and received individual, detailed information about the project either face-to-face or on the phone. 84 police officers had the three interventions planned as part of their work schedule. Of the 84 police officers who had the interventions planned, 73 filled in the baseline questionnaire and participated in at least one of the interventions. The main reasons for dropping out at all stages of recruitment were primarily due to problems with planning of the three interventions, due to holidays, change of jobs, and family considerations. Due to safety considerations, the Danish Police did not want officers on patrol to wear the HRV monitors, so only police officers from the call centers were included in the HRV measurements. Of the 73 participants who participated in the study, 18 worked at call centers and were invited to participate in the HRV measurements. One declined and 17 participants were included in the HRV study.

The interventions: Three different shift systems
The participants were exposed to three different interventions conditions: two night shifts followed by two recovery days (‘2+2’), four night shifts followed by four recovery days (‘4+4’), and seven night shifts followed by seven recovery days (‘7+7’) and the study was designed as a crossover intervention with each participant completing each intervention once. See figure 2. Recovery days were defined as day shifts or days off. Night shifts were from typically from 23:00 to 7:00 (we allowed the start of the shift to be from 22:00 to 00:00) and day shifts were typically from 7:00 to 15:00 (we allowed day shifts to end as late as 18:00).

The participants had to complete the three interventions in one of the two three month’s periods: April-June 2013 or September-November 2013. The three interventions lasted 26 days in total. The order of the three interventions was neither fixed nor random but could occur in the order that suited the person in charge of the personnel-on-duty planning. To ensure as much variation as possible the person in charge of the personnel-on-duty planning was instructed to mix the interventions so they occurred in different orders and to let the intervention conditions begin at different weekdays. The schedule was planned so that it also suited the individual employee thereby mimicking the usual way of scheduling the shifts. Before starting each intervention the participant had to have at least seven days without night shifts.
**Figure 2**: The three intervention conditions. Grey = night shift; white = recovery days. The three intervention conditions were not planned in a fixed order and before starting each intervention the participants had to have at least seven days without night shifts. X = intensive measurement days: saliva samples every 4th hours and 24 hours HRV recordings.

**Measures**

The participants filled out a background questionnaire before starting their first intervention and on each day of the interventions (a total of 26 days) the participants filled out a sleep diary. On each day of the intervention the participants also wore actigraphs to measure activity and sleep. On the intensive measurement days participants collected saliva samples and filled in a logbook approximately every 4th hour. The 17 participants who participated in the HRV study had 24 hours HRV recordings on the intensive measurement days. On the last day after each intervention the participants also had to collect a morning urine sample. After finishing all three interventions the participants filled out a follow-up questionnaire were they had to specify which intervention they preferred. Figure 3 shows an example of data collection during the 2+2 intervention.

**Figure 3**: Data collection during the 2+2 intervention.

The data collection was quite complex, since the participants had to fill in different questionnaires on different days and collect saliva and urine on specific times and days. To make sure that it was as easy as possible to complete this rather complex data collection it was carefully explained in the initial individual conversations with the participants. Each participant also received a personalized timetable that specified what he had to do on each day and examples of how an intensive measurement day could look like. The day before the intensive measurement days the
participants also got a text message as a reminder. All material and questionnaires given to each participant can be seen in the picture below and in Appendix I (in Appendix I only the sleep dairy for the 2+2 intervention is shown, however, the questions are the same for the 4+4 and 7+7 sleep interventions).

Questionnaires, time tables, examples of intensive measurement days (recovery day and night shift), saliva sampling kits, actigraphs, HRV monitors and urine sampling kits given to participants in the “In the Middle of the Night” project. See Appendix I for more details.

**Ethical considerations**
The person in charge of the personnel-on-duty planning had to be informed, so participation was not anonymous. All information provided by the participants was, however, treated confidentially, and the management was not informed about non-respondents and drop-outs. The person responsible for personnel-on-duty planning in each district and the management was, however, usually informed by the participants themselves. Although management strongly encouraged employees to participate in the study it was stressed that participation was voluntary and that it was possible to withdraw at any time during the study. The “In the Middle of the Night” project was approved by The National Committee on Health Research Ethics in Denmark (protocol number H-4-2012-155).
HRV

HRV measures were done on the intensive measurement days marked by X in Figure 2 and as indicated in figure 3. HRV measurements were done by Actiheart (CamNtech Ltd U.K.) a small lightweight system that has previously been applied for monitoring HRV (68). Actiheart has been validated against standard clinical measurements (Holter recordings) (69). A member of the project group carefully instructed each participant on how to put on and where to place the device. The participants were also given written instructions and a cell phone number they could contact in case they had any questions. The participants put on the device themselves on the following measurement days. HRV were analyzed during primary sleep. Primary sleep was defined as the first sleep after work for the night shifts and the sleep during the night for the recovery days. Sleep and wake times were noted by the participants in the sleep diaries and these times were used to determine time of sleep. The five 5-minute intervals with the lowest heart rate during each sleep period were extracted for spectral analysis of the heart interbeat interval time series. Periods with lowest heart rate reflects relatively dominant parasympathetic activity in the ANS and it is assumed to correspond to peak activity in restorative biological systems and hence the time of maximal physiological restitution during sleep (76). In the Actiheart recorder, the analog signal was band-pass filtered (10-35 Hz), sampled with a frequency of 128 Hz, and processed by a real time algorithm. Data analysis was done using a robust period detection (RPD) algorithm (77). For each 5-minute interval an average of the following variables were extracted: RR-mean (ms²), total power (ms²) HF (ms²), LF (ms²) and LF/HF. LF was 0.04-0.15 Hz, HF was 0.15-0.4 Hz and total power was 0.003 – 0.15 Hz.

Missing HRV data

Except for one participant who did not complete his ‘2+2’ due to problems with planning of his schedule all 17 participants in the HRV study completed all three interventions. The missed intervention resulted in loss of data from one 2+2 night shift and one 2+2 recovery day. 22 recordings were not initiated properly and no data was recorded due to a technical problem. This resulted in the loss of data from two 2+2 night shifts, two 4+4 night shifts, and four 7+7 night shifts, three 2+2 recovery days, five 4+4 recovery days and six 7+7 recovery days. No readable data during sleep resulted in the loss of 12 recordings from one 2+2 night shift, one 4+4 night shift, four 2+2 recovery days, two 4+4 recovery days and four 7+7 recovery days. This means that there was a total of a total of 26 recordings from the recovery days and 40 recordings from the night shifts.

Saliva sampling

The participants collected saliva samples on the last day with night shift and on the last recovery day in each intervention (the intensive measurement day marked X in Figure 2). The participants were instructed to collect the first sample right after awakening and the last sample right before
bedtime and the rest of the day participants were instructed to collect samples at 07:00, 11:00, 15:00, 19:00, 23:00 and 03:00, but only if they were awake. So if a participant woke at 13.30 and went to bed at 08.45 he collected saliva samples at 13:30 (awakening), 15:00, 19:00, 23:00, 03:00, 07:00 and 08:45 (bedtime). The participants were given both verbal and written instructions on how to collect the saliva samples and they were given a cell phone number to contact in case they had any questions. The day before saliva sampling the participants received a text message as a reminder. The saliva samples were collected by drooling directly into tubes. The participants were instructed to keep the samples in the freezer or refrigerator until they had completed each intervention and then mail the samples and the filled in questionnaires to the National Research Center for the Working Environment. Melatonin, cortisol and testosterone in saliva have been shown to be stable at room temperature for at least seven days (50) and all samples were received within seven days and therefore shipping should not affect the concentrations of the hormones.

**Analyses of hormones**

Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to analyze the concentration of hormones in saliva. Jensen et al. have described the analysis in detail (50). First hormones were extracted from the saliva using liquid-liquid extraction and then 25 µL was injected into an Agilent 1200 HPLC (Agilent technologies, Santa Clara, CA, USA) equipped with a C18 2.1 mm × 50 mm 2.6 µm Kinetex column (Phenomenex, Torrance, CA). The chromatic conditions were a linear gradient: 3 min from 10% to 100% MeOH and maintained at 100% MeOH for 2.5 min, followed by 1 min of equilibration at 10% MeOH. The analysis was done using an Agilent 6460 QQQ mass spectrometer (Agilent technologies, Santa Clara, CA) equipped with a jet stream ESI ion source and was operated in the positive ion mode. Quantification was achieved using multiple reaction monitoring mode. A single precursor ion-product ion transition was used for each hormone and its internal standard. The transitions were: m/z 233.2 → m/z 174.1 for melatonin; m/z 237.2 → m/z 178.1 for d-4-melatonin (internal standard); m/z 363.2 → m/z 121.1 for cortisol; m/z 367.2 → m/z 121.2 for d-4-cortisol (internal standard); m/z 289.2 → m/z 97 for testosterone; m/z 291.2 → m/z 97 for d-3-testosterone (internal standard). The detection limits were 3.61 pmol/L, 0.62 nmol/L and 6.64 pmol/L for melatonin, cortisol and testosterone respectively. To document that the analysis was in statistical and analytical control, control samples at two levels were analyzed with every 14 samples. The low control levers were 36.4 pmol/L 1.41 nmol/L and 80.0 pmol/L for melatonin, cortisol and testosterone respectively. The high control levels were 108.9 pmol/L, 5.39 nmol/L and 185.7 pmol/L for melatonin, cortisol and testosterone respectively. Westgard control charts (78) were used to document that the results of
the LC-MS/MS method were consistent, comparable, accurate and within specified limits of precision.

**Missing data from saliva samples**

Most of the 73 participants supplied saliva samples for all interventions, except for five who did not supply saliva samples on the 2+2 recovery day, six who did not supply saliva samples on the 2+2 night shift, four who did not supply saliva samples on the 4+4 recovery day, five who did not supply saliva samples on the 4+4 night shift and two who did not supply saliva samples on the 7+7 night shift. For melatonin 183 saliva samples was under the limit of detection (LOD), for cortisol 259 saliva samples were under the LOD and for testosterone eight saliva samples were under the LOD. As a part of the internal quality control in the laboratory all chromatograms were checked by a technician. All samples were reanalyzed if there were problems with internal standard (too high or too low response – approximately no more than 30 % deviation from normal was accepted) or the chromatogram due to matrix issues. The matrix issues typically suppressed the total signal in the chromatogram or gave additional peaks in the chromatogram. If there were problems the samples were reanalyzed and if the same problems remained in the second analysis the sample was not analyzed again and no result was given for that component. It was not possible to analyze all saliva samples due to matrix issues or problems with internal standard and this resulted in the loss of melatonin results from 16 samples, cortisol results from 14 samples and testosterone results from 36 samples. In total 2166 saliva samples from 73 participants were included in the analysis.

**Statistics**

The statistical software SAS 9.3 (SAS Institute, Cary, NC) was used for all statistical analyses. Multilevel regression analyses were performed using the PROC MIXED procedure. Type of day was included as categorical variables in two levels (night shift; recovery days) and interventions were included as categorical variables in three levels (2+2; 4+4; 7+7).

For the HRV analysis in paper II the five 5-minute intervals with the lowest heart rate during each sleep period were extracted for spectral analysis of the heart interbeat interval time series and the following outcomes were analyzed: Total power (ln ms²), RR-mean (ms²), HF (ln ms²), LF (ln ms²) and LF/HF ratio. The time from sleep onset to the first of the five 5 min intervals with lowest heart rate (in minutes and the total sleep duration (in hours) was also recorded and analyzed. HF, LF, LF/HF ratio and total power were on a logarithmic scale and all outcomes were continues. A random intercept was used for each individual with a variance components covariance structure and repeated statement for the five 5-minutes intervals with an autoregressive covariance structure. In the analysis, all six measurement days was included to test for an interaction
between intervention and type of day (night shifts or recovery days). In the second analysis we tested the effects of the interventions by stratifying into night shifts and recovery days.

For the analysis of the rhythms of melatonin, cortisol and testosterone in paper III concentrations of hormones were on a logarithmic scale and all outcomes were continues. A random intercept for each individual was used with a variance components covariance structure and repeated statement for the saliva samples with an autoregressive covariance structure. There were seven different categories for the saliva samples (A-G). A samples were taken 0-60 minutes after awakening, B samples were taken 1-4 hours after awakening, C samples were taken 4-8 hours after awakening, D samples were taken 8-12 hours after awakening, E samples were taken 12-16 hours after awakening, F samples were taken 16-20 hours after awakening and G samples were taken 20-24 hours after awakening. Since there were less than ten samples from each of the six measurement days G samples were excluded from analysis. All analyses were adjusted for time of day.

All six measurement days were included in the first analysis to test for an interaction between intervention and type of day (night shifts or recovery days).

In the second analysis we tested the effects of the interventions by stratifying into night shifts and recovery days. An interaction between intervention and sample category was used to test for difference in the rhythms between the interventions. If we found significant differences in the rhythms between the interventions we included a test of phase shift and amplitude. Test of phase shift was done by testing if the time since awakening for the highest concentration (for melatonin) or the lowest concentration (for cortisol) was different between the three interventions. If there were two samples of equal value we used the time of the first occurring sample for analysis. Test of amplitude changes was done by testing if the highest (for melatonin) or lowest concentration (for cortisol) obtained differed between the three interventions. Both test of phase shift and changes in amplitude were done by use of multilevel regression analyses with the PROC MIXED procedure.

If there were significant effects of intervention on either phase or amplitude, post hoc analysis included test of linearity in relation to number of days by including intervention as a continuous variable in a linear regression model.

All results were considered to be statistically significant at p < .05.
Paper IV: An interlaboratory comparison between similar methods for determination of melatonin, cortisol and testosterone in saliva

Laboratories
Six laboratories were invited to participate in the study. The laboratories were selected because they either had a published method describing the analysis of melatonin, cortisol or testosterone in saliva or we had other information that the laboratory used an analysis of melatonin, cortisol or testosterone in saliva. Five out of six invited laboratories agreed to participate. The laboratories were situated in Belgium, Denmark, England, Finland and Japan. Eight blinded, spiked saliva samples (approximately 1 ml of each) were sent to each participating laboratory. Not all laboratories were able to measure melatonin, cortisol and testosterone. Two laboratories were able to measure melatonin, four were able to measure cortisol (one laboratory used two different methods), and three were able to measure testosterone. The participating laboratories used different analytical strategies for measuring the hormones in saliva. For salivary melatonin; LC-MS/MS (one laboratory) and ELISA (enzyme-linked immunosorbent assay) (one laboratory); for salivary cortisol: RIA, (Coat-A-Count from DPC (one laboratory), ELISA (one laboratory), LC-MS/MS (four laboratories); and for testosterone LC-MS/MS (two laboratories) and ELISA (one laboratory). The laboratories also used different sample purification strategies: liquid-liquid extraction was used for laboratories using LC-MS/MS and no pre-treatment was used before analysis with immune assays (ELISA and RIA). Each participating laboratories gave information on laboratory performance of their method, i.e. limit of detection and reference interval. The analyses of the blinded samples were carried out in March 2011 to June 2011.

Preparation of spiked saliva samples
A pool of natural saliva samples collected from healthy female volunteers late in the afternoon and spiked with cortisol (≥97%), melatonin (≥98%), and testosterone (≥99%) to prepare the eight saliva samples. Female saliva collected late in the afternoon was chosen to ensure a low level of all three hormones in the natural saliva, however, the samples was not analyzed to determine the concentration before they were spiked. Cortisol, melatonin, and testosterone were purchased from Sigma–Aldrich (St. Louis, MO). Stock solutions of all three hormones used to spike the saliva samples were prepared in 20 % MeOH. The final percentage of 20%MeOH in the spiked saliva samples was 5% and the total amount of MeOH in the spiked saliva samples was 1 %. The natural saliva was spiked with the three hormones at seven levels in the following ranges: melatonin: 0.0–579.4 pmol/L, cortisol: 0.0–90.0 nmol/L, and testosterone: 0.0–622.8 pmol/L. The eight’ sample was an unspiked natural saliva sample. The samples were labeled A-H in a random order. All samples were after preparation immediately frozen and sent by mail with freezing elements. Participating laboratories noted the date of delivery. The shipment time of samples was from one
to seven days. The stability of melatonin, cortisol and testosterone has previously been tested (50) and showed that the concentrations of hormones in saliva are stable at room temperature for at least seven days. The concentrations of hormones are also stable at -20ºC for at least three months (50). This means that shipping and potential waiting time for analysis should not have affected the concentration of melatonin, cortisol and testosterone in saliva.

**Statistics**

To compare between laboratories and to establish a method evaluation for each laboratory as well as a method evaluation based on results from all laboratories the software Win AMIQAS was used. The noncommercial program Win AMIQAS was developed for method evaluation and quality control (79;80). In general, the method evaluation function is based on a linear least squares regression analysis of the measured concentration versus the conventional true concentration of a series of method evaluation samples containing the component in the linear range of the method. The slope was used as a measure of the recovery and the content of natural saliva was estimated as the intercept divided by the slope for each laboratory.

**Summary of results**

**Paper I: The effect of the number of consecutive night shifts on diurnal rhythms in cortisol, melatonin and heart rate variability (HRV) – a systematic review of field studies**

The results from paper I investigates research question 1.

Research question 1) *How many consecutive night shifts are required for adaptation of diurnal rhythms in cortisol, melatonin and heart rate variability (HRV) to night work?*

Eighteen papers were included in the review. Cortisol was measured in five studies (of which one also included melatonin and one HRV). Melatonin was measured in 11 studies (of which one also measured cortisol) and HRV was measured in four studies. The level of adaptation for the three chosen diurnal markers is shown in table 1.
<table>
<thead>
<tr>
<th>Study</th>
<th>Diurnal marker</th>
<th>Number of consecutive night shifts</th>
<th>No adaptation</th>
<th>Some signs of adaptation</th>
<th>High degree of adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortisol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costa et al 1994</td>
<td>cortisol</td>
<td>2</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wong et al 2012</td>
<td>cortisol</td>
<td>2</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kudielka et al 2006</td>
<td>cortisol</td>
<td>2</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
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<td>cortisol</td>
<td>2</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Harris et al. 2010</td>
<td>cortisol</td>
<td>7</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td><strong>Melatonin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grundy et al 2011</td>
<td>melatonin</td>
<td>2</td>
<td>x</td>
<td></td>
<td></td>
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<tr>
<td>Costa et al 1994</td>
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<td>2</td>
<td>x</td>
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<td>Hansen et al 2006</td>
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<tr>
<td>Chooosong et al 2006</td>
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<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Borugian et al 2005</td>
<td>melatonin</td>
<td>2</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferguson et al 2012</td>
<td>melatonin</td>
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<td>x</td>
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<td>Hansen et al 2010</td>
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<td>7</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Barnes et al 1998b</td>
<td>melatonin</td>
<td>7</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Gibbs et al 2007</td>
<td>melatonin</td>
<td>7</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Barnes et al 1998a</td>
<td>melatonin</td>
<td>14</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td><strong>HRV</strong></td>
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<td></td>
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<td>Rauchenauner et al 2009</td>
<td>HRV</td>
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<tr>
<td>Kobayashi et al 1997</td>
<td>HRV</td>
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<td>x</td>
<td></td>
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<tr>
<td>Wong et al 2012</td>
<td>HRV</td>
<td>2</td>
<td>x</td>
<td></td>
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<td>Furlan et al 2000</td>
<td>HRV</td>
<td>3</td>
<td>x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The degree of adaptation of the diurnal rhythms of cortisol, melatonin and HRV to night work.

The studies included in the review using melatonin as a diurnal marker found little or no adaptation of the diurnal rhythm after two consecutive night shifts (81-86). However there was a tendency for an increased level of adaptation of the diurnal rhythm after seven consecutive night shifts (87-90).

No adaptation of the rhythm of plasma cortisol after two consecutive night shifts was found in two studies (82;91) and overall there was a tendency for no or little adaptation after two consecutive night shifts in the rhythm of cortisol (92;93). However, there were too few studies to conclude about the effects of more than two consecutive night shifts, although Harris et al. found evidence for full adaptation after seven consecutive night shifts in off-shore workers (14).

The studies that measured HRV only included from one to three consecutive night shifts. Furlan et al found a lowered sympathetic activity after three consecutive night shifts, however both Rauchenauner et al and Wong et al found higher sympathetic balance after one and two night shifts, respectively (91;94;95). Kobayashi et al did find statistically significant differences between night shifts and day shifts after two consecutive shifts, however, there was a tendency for a shift in autonomic balance in direction of higher sympathetic and lower parasympathetic activity during night shifts (96).
In summery the studies indicated that one to three consecutive night shifts does not result in adaptation in the rhythms of melatonin, cortisol and HRV. To make conclusions about how many consecutive are required for full adaptation (and if full adaptation can be archived) in melatonin, cortisol and HRV studies that investigate more consecutive night shifts are needed.

**Papers II and III: In the Middle of the Night**

The results from the “In the Middle of the Night” study investigates research question 2 and 3.

2) **What are the effects of the number of consecutive night shifts on HRV during sleep?**

3) **What are the effects of the number of consecutive night shifts on the rhythm of melatonin, cortisol and testosterone?**

**HRV**

The 17 participants in the HRV study of the “In the Middle of the Night” project were from 28 to 59 years old.

Overall there were no significant differences between the RR-mean values obtained during sleep after night shifts and recovery days indicating that there were no differences in the lowest heart rate obtained during day and night sleep. Since the RR-mean values reported and analyzed is from the five analyzed 5-minute intervals they do not necessarily reflect the average heart rate during the entire sleep.

The analyzed HRV parameters for night shifts and recovery days are shown in Figure 4. There was no effect of the number of consecutive night shifts on RR-mean indicating that the same lowest heart rate was obtained during sleep after the night shifts. With regard to HRV during sleep after night shifts, there was a tendency for the 2+2 and 7+7 night shifts to be similar, and the 4+4 night shift to stand out. This observation was partially confirmed by the statistical analyses. Thus, statistical analysis showed that the total power was highest on the 2+2 night shift compared to the 4+4 and 7+7 night shifts; this indicates an increase in overall HRV on the 2+2 night shift. Total HF was lower for the 4+4 night shift than for the 2+2 and the 7+7 night shifts, indicating less parasympathetic modulation during sleep in this shift system. The LH/HF ratio was lower for the 7+7 night shift than the 2+2 and the 4+4 night shifts. A lower value of the LH/HF ratio indicates decreased sympathetic activity or higher parasympathetic activity. There were no statistically significant differences in LF after night shifts between the three different shift schedules. LF is regarded as a strong indicator of sympathetic activity and generally reflects both sympathetic and parasympathetic activity. However, during sleep and rest high LF values can also indicate an increase in parasympathetic activity (97).
There was no difference in RR-mean between the three different interventions on recovery days showing that the same lowest heart rate was obtained on all three days. The 2+2 recovery day had a significantly higher total power compared with the 4+4 and 7+7 recovery days, indicating overall higher HRV on the 2+2 recovery day. The 2+2 restitution also had a significantly higher HF than the 4+4 and the 7+7 recovery days, indicating lower parasympathetic activity on the 4+4 and 7+7 recovery days. The LF/HF ratio was highest for the 7+7 recovery day compared other two recovery days indicating increased sympathetic activity or reduced parasympathetic activity during sleep on the 7+7 recovery day.

![Graph](image)

**Figure 4.** Estimates of HRV parameters from the separate analysis of night shifts and recovery days. Axis for HF, LF, total power and RR-mean are in ln ms^2. Error bars represent 95% confidence intervals.

In summary the HRV results from the “In the Middle of the Night” showed overall differences in HRV during sleep on days with night shifts and recovery days, primarily in parasympathetic
activity; however, there were no difference in the lowest heart rate obtained during sleep. During sleep after the 2+2 night shift there was the highest overall autonomic activity and this could indicate higher recover than during sleep on the 4+4 and 7+7 night shift. We found that the overall sleep-related autonomic recovery had higher parasympathetic modulation of cardiac rhythm on the 2+2 intervention compared to the 4+4 and 7+7 intervention.

**Hormones**

The 73 participants were included in the hormone analyses from the “In the Middle of the Night” project. The participants were 25 to 62 years old with a mean age of 38 and 40% were very experienced night shift workers with more than 10 years of night work experience, 28% had three to ten years of night work experience and 22% had less than three years of night work experience.

The analysis of the effect of the three interventions was done separately for night shifts and recovery days. All the analyses were done in relation to time since awakening and table 2 describes the number of samples in each category and time of day of each sample category.

<table>
<thead>
<tr>
<th>Time of day</th>
<th>2+2 night shift</th>
<th>4+4 night shift</th>
<th>7+7 night shift</th>
<th>2+2 recovery day</th>
<th>4+4 recovery day</th>
<th>7+7 recovery day</th>
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</thead>
<tbody>
<tr>
<td>Sample A (0-60 min)</td>
<td>14.05 (1:49)</td>
<td>72</td>
<td>14.14 (1:22)</td>
<td>67</td>
<td>14.22 (2:13)</td>
<td>70</td>
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<tr>
<td>Sample B (1-4 hours)</td>
<td>15.49 (2:20)</td>
<td>40</td>
<td>16.40 (1:56)</td>
<td>43</td>
<td>16.48 (2:44)</td>
<td>46</td>
</tr>
<tr>
<td>Sample D (8-12 hours)</td>
<td>23.37 (1:59)</td>
<td>63</td>
<td>23.55 (2:01)</td>
<td>66</td>
<td>00.25 (2:38)</td>
<td>69</td>
</tr>
<tr>
<td>Sample E (12-16 hours)</td>
<td>03.37 (2:17)</td>
<td>66</td>
<td>04.05 (1:54)</td>
<td>59</td>
<td>04.17 (2:19)</td>
<td>68</td>
</tr>
<tr>
<td>Sample F (16-20 hours)</td>
<td>06.59 (1:22)</td>
<td>60</td>
<td>07.14 (0:54)</td>
<td>56</td>
<td>07.21 (1:37)</td>
<td>54</td>
</tr>
</tbody>
</table>

**Table 2.** Time of day for the sample categories in intervention. Standard deviations are shown in brackets.

The melatonin results are shown in figure 5. There was a significant difference between the interventions in the rhythm of melatonin on the days with night shifts (p for interaction =<0.0001). In all of the three interventions the melatonin curve on night shifts showed low concentrations after awakening, a rise and then a drop right before bedtime (around 17-20 hours after awakening or approximately at 07:00). The differences between the interventions were tested for amplitude changes and phase shifts, summarized in table 3. There was a significant change in the amplitude of the melatonin rhythm (measured by the highest concentration obtained): The concentration of melatonin was highest on the 2+2 intervention (87.5 pmol/L) followed by the 4+4 intervention (65.0 pmol/L) and the 7+7 intervention (66.4 pmol/L) (p=.001). The fall in melatonin concentration followed a linear trend: 4.9% per day, 95% CI 1.4–8.2% per day (p=0.0056). No change in the phase of melatonin was fund (measured by the timing of the highest concentration, p=.934). On
all the recovery days the concentration of melatonin was highest after awakening and before bedtime and there was no significant difference in the melatonin rhythm between the three interventions (p for interaction=.510).

**Figure 5.** Melatonin concentration estimates plotted against hours since awakening for night shifts and recovery days. The results are adjusted for time of day. Error bars are 95% confidence intervals.

The cortisol results are shown in figure 6. There was a significant difference between the interventions in the rhythm of cortisol on the days with night shifts (p for interaction =<.001). On all the intervention the cortisol curve on night shifts showed high concentrations after awakening, a drop and a rise again around bedtime. There was a significant shift in phase of the cortisol rhythm on night shifts and the lowest concentration of cortisol was reached 8:50 hours after awakening on the 2+2 intervention, 9 hours and 23min after awakening on the 4+4 intervention,
and 11 hours and 31 min after awakening on the 7+7 intervention. The shift in phase followed a linear trend with a phase delay of 33 minutes/day, 95% CI 18 –48 minutes per day (p= <.001). No difference in the amplitude of the cortisol rhythm was found (measured as the lowest concentration obtained, p=.531). On all the recovery days the concentration of cortisol was highest in the morning after awakening and dropped during the day reaching the lowest levels before bedtime and there was no significant difference in the rhythm of cortisol between the interventions.

Figure 6. Cortisol concentration estimates plotted against hours since awakening for night shifts and recovery days. The results are adjusted for time of day. Error bars are 95% confidence intervals.

The testosterone results are shown in figure 7. On both night shifts and recovery days the highest concentrations were found after awakening and hereafter the concentration fell and reached a relatively constant level until bedtime and there was no significant difference between the
interventions on either the days with night shifts (p for interaction=.898) or on recovery days (p for interaction=.074).

Figure 7. Testosterone concentration estimates plotted against hours since awakening for night shifts and recovery days. The results are adjusted for time of day. Error bars are 95% confidence intervals.
<table>
<thead>
<tr>
<th></th>
<th>2+2 night shift</th>
<th>4+4 night shift</th>
<th>7+7 night shift</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol timing of lowest concentration (hours after awakening – hh:mm)</td>
<td>8:50 (7:55-9:47)</td>
<td>9:23 (8:28-10:19)</td>
<td>11:31 (10:37-12:25)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Amplitude analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level of lowest concentration of cortisol (nmol/L)</td>
<td>1.19 (1.06-1.34)</td>
<td>1.13 (1.00-1.28)</td>
<td>1.12 (0.99-1.25)</td>
<td>.531</td>
</tr>
<tr>
<td>Level of melatonin highest concentration (pmol/L)</td>
<td>87.51 (73.29-104.5)</td>
<td>64.98 (54.47-77.52)</td>
<td>66.42 (55.83-79.02)</td>
<td>.001</td>
</tr>
</tbody>
</table>

Table 3. Analysis of phase and amplitude results for melatonin and cortisol on night shifts. 95% confidence intervals are shown in brackets.

In summery the hormone results from the “In the Middle of the Night” showed that the diurnal rhythms of melatonin, cortisol and testosterone all changed differently to an increasing number of consecutive night shifts: the amplitude of melatonin rhythm was suppressed, but did not show any change in phase. The diurnal rhythm of cortisol phase delayed with an increasing number of night shifts, but did not show any changes in amplitude. The diurnal rhythm of testosterone was not affected by the number of consecutive night shifts and followed the sleep/wake cycle. The results also showed no differences between the interventions in the rhythms of melatonin, cortisol and testosterone on the recovery days.

**Data quality**

Data quality was explored in both a laboratory comparison of the analysis of melatonin, cortisol and testosterone in saliva and in compliance and control in the “In the Middle of the Night” project. This section on data quality summarizes the results in connection with research question 4) Can we compare analysis of hormones in saliva measured in different laboratories? Research question 4 was investigated in paper IV - Paper IV: An interlaboratory comparison between similar methods for determination of melatonin, cortisol and testosterone in saliva.
Laboratory comparison
The interlaboratory comparison for the analysis of melatonin, cortisol and testosterone in saliva included five laboratories. Each of the participating laboratories blindly measured eight samples prepared from natural saliva spiked with melatonin, cortisol and testosterone in the range 0 - 579 pmol/L for melatonin, 0-90 nmol/L for cortisol, and 0-622 pmol/L for testosterone. Figures 8-10 shows the observed concentrations of melatonin, cortisol and testosterone plotted against the spiked concentrations.

**Figure 8.** Observed concentrations of melatonin plotted against the spiked concentrations.

**Figure 9.** Observed concentrations of testosterone plotted against the spiked concentrations.
As seen in figure 8 laboratory V was not able to quantify melatonin concentrations above 76 pmol/L. The recovery of spiked material for melatonin ranged from 91 to 110%, from 83 to 100% for cortisol and from 80 to 94% for testosterone. The content of natural hormone in saliva was estimated to be between 0.278-6.90 pmol/L for melatonin, 0.56-6.72 nmol/L for cortisol and 11.9-73.8 pmol/L for testosterone.

In summary the methods performed optimally within the laboratories own reference intervals, however, there were some difference in the performance of the participating laboratories.

**Compliance and control - “In the Middle of the Night”**

We took, as mentioned in the methods section, several initiatives to help guide the participants through the data collection including: reminder text messages for saliva sampling, phone contact number in case of questions, individual schedules and close collaboration with the police during the data collection. When the participants called the contact number they primary had questions about what to do if they missed the collection of saliva samples, if they could get another saliva sampling kit, if they could change shifts or if they had not received their study material.

**Figure 10.** Observed concentrations of cortisol plotted against the spiked concentrations
Figure 11. Accumulated % of taken 1st saliva sample after awakening according to self-reports.

We asked the participants to take the first saliva sample at awakening and as shown in figure 11 74% had taken their first saliva sample within 15 minutes after awakening according to their self-reports. However we did not use electronic devices to monitor if they indeed did as they described, so the actual times may not be exactly accurate (75).

All participants had to have a period of at least seven days without working night shift before starting each intervention. Since work planning was done by the individual participant in collaboration with the person responsible for personnel-on-duty planning all participants had their actual work schedules controlled and if the schedule was approved it was locked in the electronic work-planning system used by the police (POLVAGT) so they could not change shifts. Optimally the interventions should have a mixture of different days of the week as start day. Figure 12 shows the start day of the intervention according to day of the week. Most of the interventions started on a Monday, especially the 7+7 interventions (58%). The 4+4 intervention started mostly on Mondays (39%) and Thursdays (35%) and the 2+2 interventions on Mondays (32%), Wednesdays (24%) and Fridays (30%). This means that the last night shift in each intervention (the intensive measurement days with saliva samples) was mostly a Sunday for the 7+7 intervention, a Thursday or a Sunday for the 4+4 intervention and a Tuesday or a Saturday for the 2+2 intervention.
Figure 12. Start day of the interventions according to day of the week.

We do know a little about at what time of day and which days were the busiest for the police. To estimate if there were large differences between the different weekdays and different times of day we pulled call records from the call center in Copenhagen; the biggest police district participating in the “In the Middle of the Night” project. When a call is answered in the call center a patrol on the street is contacted, so the data presented in figure 13 reflects activity in both the call center and for officers on the street. The profiles shown in figure 13 are very similar for Monday till Thursday and these weekdays have spikes in activity in the morning around 09:00 and again in the afternoon at 16.00. Friday has a similar profile to Monday-Thursday in the morning and afternoon, but also has a rise in the evening. Saturday has fewer calls in the morning and afternoon than Monday-Thursday and a rise in the evening similar to Friday. Sundays have a similar profile to Saturday in the morning and afternoon, but with fewer calls and a profile similar to Monday-Thursday in the evening and night. The police state that they are more officers on call during the weekends to account for the larger work pressure. However, the busiest time of day was definitely not equal between days, which may be a concern when comparing night shifts.
Discussion

Main findings
The aim of this thesis was to study how the human body reacts to consecutive night shifts and to explore the effects of the number of consecutive night shifts using acute physiological reactions. This thesis also addressed some of the challenges involved when conducting field studies. Current recommendations regarding shift work research (26) recommends more research with intensive measurement during a short period of time in order to study the effects of specific shifts and this is what this thesis aimed to do.

Main findings

1) Research question 1: How many consecutive night shifts are required for adaptation of diurnal rhythms in cortisol, melatonin and heart rate variability (HRV) to night work?

Research question 1 was investigated in a review of field studies – paper I. The diurnal rhythms of cortisol, melatonin and HRV were not adapted to night shifts and hence the new sleep/wake cycle after one to three consecutive night shifts in field studies. The review could not make conclusions on more consecutive night shifts.

2) What are the effects of the number of consecutive night shifts on HRV during sleep?
Research question 2 was investigated as part of the “In the Middle of the Night” project and results were reported in paper II. The results indicated differences in autonomic regulation during sleep between the interventions, but no clear effect of the number of consecutive night shifts. Although we found a slight tendency for overall sleep-related autonomic recovery had higher parasympathetic modulation of cardiac rhythm on the 2+2 intervention compared to the 4+4 and 7+7 intervention.

3) **What are the effects of the number of consecutive night shifts on the rhythm of melatonin, cortisol and testosterone?**

Research question 3 was investigated as part of the “In the Middle of the Night” project and results were reported in paper III. The results showed that the diurnal rhythm of melatonin did not change phase, but that the amplitude was lowered with an increasing number of consecutive night shifts. The amplitude of melatonin rhythm was suppressed 4.9% per day (95% CI 1.4 –8.2% per day; p=.006). The diurnal rhythm of cortisol showed a phase delay, but no change in amplitude with an increasing number of night shifts. The diurnal rhythm of cortisol phase delayed by 33 min/day (95% CI 18 –48 min per day; p< .001). The diurnal rhythm of testosterone completely followed the sleep/wake cycle independently of the number of consecutive night shifts. There was no difference between the diurnal rhythms of the three hormones when measured on the last recovery day in each intervention.

4) **Can we compare analysis of hormones in saliva measured in different laboratories?**

Research question 4 was investigated as part of an interlaboratory comparison of methods for analyzing hormones in saliva. The results of this study were reported in paper IV. The results showed that despite some differences in performance the laboratories performed optimally within their own reference intervals.

**Data quality and the challenges of field studies**

Papers I–III all reported results from field studies. Field studies can be both expensive and time consuming. Therefore it is important to ensure that the data collected is of a sufficient quality.

Paper IV was a comparison of different analytical methods and strategies for analyzing hormones in saliva and a very important part of securing sufficient data quality.

**Strengths and Limitations**

**Paper I:** Low statistical power was a problem in some of the smaller studies and in general most included studies in the review were relatively small in size. The methods used for measuring changes in diurnal rhythms were quite diverse making it difficult to directly compare the effects of
the number of consecutive night shifts. There was also a large diversity of shift schedules investigated. The inclusion criteria was that there had to be at least three samples per day to estimate the diurnal rhythm, as it is extremely difficult to accurately assess a rhythm with such few measurements, however, even with this broad inclusion criteria we were left with a very limited knowledge base.

“In the Middle of the Night” (papers II and III):

The method used to measure the hormones in the “In the Middle of the Night” project participated in the laboratory comparison. The results from the laboratory indicated that there were discrepancies between different analytical methods for determining hormones in saliva, however, the methods performed relatively well within their own analytical range. Thus it is still important to take differences in analytical strategies into account when comparing results from different laboratories. The study clearly emphasizes the importance of external quality control in the analysis of hormones in saliva. Hormones in saliva from the “In the Middle of the Night” project were measured with LC-MS/MS and the method, besides participating in the laboratory comparison, was also under internal quality control.

In 12% of the saliva samples the concentration of cortisol were below the LOD and in 8% of the saliva samples the concentration of melatonin were below the LOD. For both cortisol and melatonin the concentrations of day time samples were expected to be low. We excluded the samples, which may have added uncertainty to the results. This is especially true for the analysis of the amplitude of cortisol were we used the lowest concentration as an estimation and not including samples under the LOD may have caused a too high estimate of the concentration. In the analysis of the phase changes the timing of samples under the detection limit were included in the analysis. Since samples under the LOD still represent a value we also did the analyses were the samples under the LOD were given a random value between 0 and the LOD, with half the LOD as the average. This line of analyses did not change the main conclusions – all tests that showed statistical significant difference also showed statistical significant differences when samples under the LOD were included. The analysis of the amplitude of cortisol (were the lowest concentration obtained was analyzed) obviously showed the biggest changes. Here the mean concentrations changed from 1.19 nmol/L to 0.71 nmol/L for the 2+2 intervention, from 1.13 nmol/L to 0.63 nmol/L for the 4+4 intervention and from 1.12 nmol/L to 0.71 nmol/L for the 7+7 intervention. The p value for this test changed from 0.531 to 0.578.

There were also some problems with matrix effects, were components in the saliva suppresses the signal for the analyzed hormones. This phenomenon is a well described problem with LC-MS/MS
methods (98) and also resulted in loss of data from the project. Again loss of data resulted in lower statistical power, however, we have no reason to suspect that it introduced additional bias, since we do not expect matrix effects to be dependent on intervention type. In the case of the cortisol measurements an estimation of the cortisol awakening response would have made it easier to compare with other field studies using this well establish measure.

For the melatonin measurements it would have been advantageous to have measurements of light intensity, since melatonin production is heavily influenced by light (47). However we were not allowed by the police to have the participant to wear the light monitors visible over their uniforms. Light intensity measures would have allowed us to investigate whether the drop in melatonin seen at approximately 07:00 on night shifts were due to increased light exposure.

All the analysis of the hormones in this thesis uses time since awakening as time reference instead of time of day. Both the rhythm of cortisol and testosterone are influenced by sleep (4;55) so using time since awakening as a time reference allowed us to better describe how the diurnal rhythms of the analyzed hormones changes in relation to the sleep/wake cycle. Night work will necessarily displace the sleep/wake cycle and we wanted to investigate the hormonal responses in melatonin, cortisol and testosterone to this change. All analysis were adjusted for time of day since both sleep and time of day are of critical importance when analyzing diurnal rhythms (99).

The HRV monitors used in the “In the Middle of the Night” project are validated for use in field studies (70), however, technical problems resulted in the loss of 22 recordings. There were also only 17 participants in this study as it was hard to recruit participants from the call centers despite all our efforts and an overall good collaboration with the police. This gave the study low statistical power and added uncertainty to the results. HRV analysis was done using the robust period detection (RPD) algorithm. The RPD method has demonstrated superior performance compared to other commonly used methods such as the Fourier transformation and Lomb method by estimation of power spectral characteristics for HRV analysis. The RPD is less sensitive to artifacts, ectopic beats, missing data, etc. in the recordings (77) and makes it ideal for use in field studies.

In the HRV study we only used the five 5-minute intervals with the lowest heat rate and thus only looked at a short period of the total sleep. Our results are therefore not representative for the entire night’s sleep. One could argue that a sleep period with several minutes of lower heart rate (or parasympathetic activity) is not necessarily more restorative than a sleep period with several minutes of slightly higher heart rate.
The participants were not measured on a neutral reference day to give baseline values of hormones and HRV. A neutral reference day could have been a day off with no night shifts in the previous seven days. The last recovery day in the 7+7 intervention might be used as an estimation of a reference day, however, we have chosen not to use it as such in the analysis, since the main focus was the comparison of the number of consecutive night shifts and not the differences between night and day shifts. This would have been particular helpful information in the case of the HRV measurements, where there were differences between the recovery days of the three interventions. The lack of baseline measurements made it unclear whether the levels of the autonomic nervous system activities increased or decreased by the interventions. For the hormone measurements a reference day would also have been interesting, however, the hormone profiles were all as one would expect for a ‘normal’ day orientation and there were no differences between the recovery days.

We did not succeed in getting the interventions planned with an evenly distribution of days of the week as start days for the interventions. This can be a problem when comparing results from the three interventions, because as seen in figure 13 there were difference in when the busiest times of day occurred. This means that some interventions may have ended with a more demanding shift that others and a more stressful shift could influence especially the HRV measurements (100).

We also don’t know how many night shifts each participant usually work per month and this information might influence the preference for many vs. few consecutive night shifts. The participant’s normal frequency of night shifts may, in turn, also be influenced by their preferences as the participants have the possibility of influencing the scheduling of their own shifts. In any case some studies have found evidence that attitudes towards and satisfaction with the work schedule are important for health and well-being (101-104), so we cannot rule out that the individual preference may have affected the results from the “In the Middle of the Night” project.

Only non-smoking male police officers working night shifts as part of their current work schedule was required and this naturally resulted in a very homogeneous study population. The recruitment strategy enhanced the internal validity of the study, but at the same time reduced the external validity.

**Paper IV:** Both processing of the saliva and shipping may have caused alterations of the saliva in comparison to the natural saliva sample and some of the differences observed between the laboratories could be due to different conditions during shipping. A certified reference standard in saliva for all three hormones would have been preferable, however, no such material was available. A certified reference standard already dissolved in methanol would have been
preferable to weighing of the reference standards and would have eliminated some of the uncertainty (and this procedure was afterwards implemented for reference standards in the laboratory).

**Adaptation of diurnal rhythms**

The “In the Middle of the Night” hormone results presented in paper III showed that the diurnal rhythm of melatonin showed no change in phase, but a suppression of the amplitude in response to an increasing number of consecutive night shifts, but the rhythm of melatonin never returned to the pattern seen on the last recovery days in each intervention or what would be considered a ‘normal’ day oriented rhythm. Both laboratory and field studies have previously found that the diurnal rhythm of melatonin responded to night shifts with a phase delay (89;105;106), so the lack of phase delay in this study is unexpected. The suppression of the amplitude and flattening of the diurnal curve have on the other hand been seen in both field (107) and laboratory studies (108). The diurnal rhythm of cortisol phase delayed, but did not change in amplitude in response to an increasing number of night shifts. As seen for melatonin the rhythm never returned to the pattern seen on the last recovery days in each intervention or what would be considered a ‘normal’ day oriented rhythm. Other studies have also found limited evidence for adaptation of the diurnal rhythm in cortisol (82;91;92), with the exception of Harris at al how found complete adaptation of the cortisol rhythm after seven consecutive night shifts (14). From these results there not evidence for full adaptation to night shifts within seven days for the rhythms of cortisol and melatonin. However the diurnal rhythm of testosterone was completely adapted to night shifts and the new sleep/wake cycle on the second night shift. This demonstrates that testosterone is under control of the sleep/wake cycle and not under direct circadian control. Testosterone has previously been investigated in laboratory studies and has been found to follow sleep episodes with very little circadian effects (109). Of the studies included in the review (paper I) only two found evidence for full adaptation to night shifts, one studies found complete adaptation of the cortisol rhythm after seven consecutive night shifts and one found complete adaptation of the melatonin rhythm after 14 consecutive night shifts, both studies were done in offshore workers (14;89). So also the field studies included in the review supports limited adaptation to night shifts. In a review from 2008, Folkard found that only a very small minority of permanent night workers fully adapt to night shifts evaluated by the rhythm of melatonin and if even permanent night workers do not adapt it may not be expected that night shift workers will. An exception to this might be when people work at remote locations like off shore work, where a full adaptation might be possible (15).
However, the question of whether or not shift workers should adapt their rhythms to night shifts still remains. There might be reasons for night shift to adapt their diurnal rhythms to the sleep-wake cycle rather than the light-dark cycle. One of the most prevalent arguments for more consecutive night shifts and thereby adaptation is safety issues. The first, second and third night shifts have been to affect the number of errors, reaction time, and concentration more that the following nights (110;111). There is also evidence for increasing sleep length with increasing number of consecutive night shifts (8). This could argue for adaptation of the diurnal rhythms. However, there is also good evidence for recommending few consecutive night shifts. In 2012 Bonde et al published a consensus report suggesting minimizing the number of consecutive night shifts in order to prevent circadian disruption and reduce the health consequences of shift work (112).

From acute reactions to disease
The results presented in this thesis are all concerned with acute physiological reactions to night shift work, but what is the evidence that this is linked to disease in the long run?

There is evidence for circadian disruption in both the results from the review and the hormone results from the “In the Middle of the Night” study. The study clearly showed evidence of circadian disruption both in terms of phase shifts of melatonin and cortisol and the acute suppression of nocturnal melatonin production. Circadian disruption is one of the possible mechanisms leading from night work to disease (37).

The “In the Middle of the Night” project used HRV and the level of sympathetic and parasympathetic activity as a proxy measure for recovery during sleep. The HRV results from the “In the Middle of the Night” project showed differences in autonomic recovery during sleep, however, no clear picture could be drawn and both during day time sleep (after night shifts) and during night time sleep (on recovery days) there were differences between the interventions. It is therefore difficult to conclude anything about the possible implications for shift work health.

Both the results from the review and the results from “in the Middle of the Night” suggest that working many consecutive night shifts may suppress the level of melatonin and since melatonin suppression has been linked to cancer (23) this is a possible mechanism leading from night work to disease. The results from the “In the Middle of the Night” showed that there was minimal suppression of melatonin after two consecutive night shifts so only working few consecutive night shift might be better for night shift worker health in terms of melatonin suppression.
The results from the “In the Middle of the Night” projects showed that the rhythms of the measured hormones did not adapt to night shifts with equal speeds. Testosterone levels were tied to the sleep/wake cycle and were in that sense fully adapted to the night shifts already on the second consecutive night shift. Whereas number of consecutive night showed a phase delay of the diurnal rhythm of cortisol and the diurnal rhythm of melatonin showed a suppression of the amplitude. Thus the different physiological systems responded differently to an increasing number of consecutive night shifts. This means that we can prove a lack of coordination of internal rhythms as a result of night shift work in terms of the diurnal rhythms of melatonin, cortisol and testosterone. The mistiming of cortisol and sleep may cause pre-diabetic changes (45) and the imbalance of cortisol and testosterone has an effect on the risk of developing cardiovascular disease and type 2 diabetes (46). The results from the “In the Middle of the Night” project shows that full adaptation is not obtained after seven consecutive night shifts, even if there is a gradual adaptation of both the rhythms of melatonin and cortisol. This means that even if the lack of coordination between internal rhythms is largest after two consecutive night shifts lack of internal synchronization of internal rhythms persists after seven consecutive night shifts.

**Should we recommend 2, 4 or 7 consecutive night shifts?**

The results from paper III, the HRV study of the “In the Middle of the Night” project, indicated differences in autonomic regulation during sleep between the interventions, but no clear effect of the number of consecutive night shifts. Interestingly the same lowest heart rate was reach in all three interventions. However, there was a slight tendency for better autonomic recovery during sleep during the 2+2 night shift compared with the 4+4 and 7+7 night shifts, indicated by higher total power and higher parasympathetic activity. The 2+2 recovery day also had a tendency for better autonomic recovery during sleep compared with the 4+4 and 7+7 recovery day, again indicated by a higher total power and higher parasympathetic activity. The results from the HRV study points, although not very strongly, at the 2+2 intervention as the best in terms of better autonomic recovery during sleep.

The results from the hormones measured in the “In the Middle of the Night” project showed that there were no differences in the level of testosterone between the three interventions. From this result there is no evidence for recommending any of the three shifts systems over the others. There was no phase shift in the diurnal rhythm of melatonin and the amplitude of melatonin was suppressed on the 4+4 and 7+7 night shifts compared to the 2+2 night shift. This indicates that the participants produce less melatonin during the 4+4 and 7+7 interventions. There were no differences in the diurnal rhythm of melatonin between the recovery days. Melatonin suppression is a possible mechanism leading from night shift work to disease, so from the melatonin results
there is evidence for recommending the 2+2 intervention. The curve of cortisol was shifted indicating that adaptation to the night shift occurs gradually, but the participants did not reach full adaptation after seven consecutive night shifts. There were no differences between the recovery days for any of the three measured hormones indicating that the participants were fully back to a normal day oriented diurnal rhythm after the recovery days.

Of course the physiological measures of hormones and HRV are not the only aspects that are important when recommending a specific number of consecutive night shifts or any other shifts system. The number of accidents and the level of performance (113) are also very important aspects to consider. Sleep is a key component of shift worker health and wellbeing sleep (8;10). Sleep was also investigated in the “In the Middle of the Night” projects and showed that early awakening, total sleep time, restless sleep, number of awakenings, sleep latency, sleep efficiency did not depend on number of consecutive night shifts. However, there was adaptation in difficulty falling asleep and ease of awakening after six consecutive night shifts (unpublished results). The results also showed that the participants slept worse on the last day with night shifts in terms of ease of awakening and feeling rested no matter the intervention and more consecutive night shifts would give fewer last nights. From the sleep results result alone there are arguments for recommending more consecutive nights and also arguments that there are no difference between the interventions (results not published). Some studies have also found that attitudes towards and satisfaction with the work schedule are important for health and well-being (101-104). The preferences of the participants in the “In the Middle of the Night” project were also investigated. This study showed that most participating police officers preferred four consecutive night shifts, although some participants have strong preference for fewer or more consecutive shifts. The participant’s preferences reflected considerations with respect to health, sleep, and how demanding night work was perceived (article under review). So to some extend there is evidence for recommending and taking the individual preferences into account when scheduling shifts.

To sum up the HRV and hormone results from the “In the Middle of the Night” project points to some extend to the recommendation of limiting the number of consecutive night shifts. The results from the studies included in the review also supports this in the sense that most of the studies indicated that limiting the number of consecutive night shifts would reduce melatonin suppression and to some extend also circadian disruption.
Conclusion and future perspectives

The results from the review of adaptation of diurnal rhythms in field studies found no evidence for adaptation of the rhythms of melatonin, cortisol or HRV after one to three consecutive night shifts. The results from the “In the Middle of the Night” project showed that the three hormones melatonin, cortisol and testosterone all responded differently to an increasing number of consecutive night shifts. The diurnal rhythm of melatonin did not change phase, but the amplitude was suppressed. The diurnal rhythm of cortisol phase delayed, but did not change amplitude and the diurnal rhythm of testosterone followed the sleep/wake cycle. This demonstrated that different physiological systems react differently to an increasing number of night shifts and that adaptation to the new sleep/wake cycle introduced by working nights occurs with different speeds in a real live setting. The HRV results from the “In the Middle of the Night” project showed that there were differences in autonomic regulation during sleep after two, four and seven consecutive night shifts and also on the last recovery day. Despite a slight tendency for overall sleep-related autonomic recovery had higher parasympathetic modulation of cardiac rhythm on the 2+2 intervention compared to the 4+4 and 7+7 intervention it was not possible to draw any clear conclusions from the HRV study.

The “In the Middle of the Night” demonstrated once again that field studies are associated with a range of challenges, however, it also showed that meaningful results can be archived. Field studies needs to be a part of further shift work research to deliver results that can give us insights into the consequences of night shifts work in a real live setting. The relative few and small studies included in the review also demonstrates that we still need more good quality field studies to explore the effects of night shifts. There is also a need for more studies to include more than one physiological measure, so the results from the “In the Middle of the Night” projects can be challenged in future studies.

The “In the Middle of the Night” only included male night shift workers and a similar study with female participants would be interesting to test if there are differences between men and women in their response to consecutive night shifts. There is also the possibility to investigate coping mechanisms such as napping and meal patterns in response to the number of consecutive night shifts. It would also be interesting to investigate if different chronotypes respond differently to an
increasing number of night shifts. It would also be relevant to look at how sleep quality affects the hormonal response to night shifts: if ‘good’ sleepers adapts faster to night shifts?

Not only do we need more research on how the human body reacts to night shifts and the number of consecutive night shifts, there is still a need for more studies to verify if the acute reactions that we observe leads to future health problems for night shifts workers. The combined results from this thesis point in the direction of fewer consecutive night shifts. However, there is still a need for more research in the long term effects of the number of consecutive night shifts. Thus it is not possible from the results of this thesis alone to recommend a specific number of consecutive night shifts.
Reference List


(96) Kobayashi F. Changes in psychophysiological functions during night shift in nurses. Influence of changing from a full-day to a half-day work shift before night duty. 1997.


Danish summary

Natarbejde er et vilkår inden for mange erhverv, til trods for at nattearbejdere har en forøget risiko for sygdom. Derfor findes en række anbefalinger med hensyn til optimal organisering af arbejdstiden, fx at man maximalt bør arbejde 2-4 nætter i træk for at minimere forskydningen af de biologiske rytmer og søvntab. Selvom disse anbefalinger har været brugt i mange år, er der kun begrænset videnskabelig evidens for, at risikoen for sygdom formindskes, hvis man følger anbefalingerne. Samtidig er der mange eksempler på natarbejdere, der foretrækker at arbejde 7 nætter i træk bl.a. for at undgå hyppige skift i døgnrytmen og for at få flere fridage i træk. Derfor er der behov for viden om, hvorvidt en sådan organisering af arbejdstiden er mere skadelig for helbredet end at arbejde 2-4 nætter i træk. Hvad er fx bedst – mange nætter i træk og dermed få skift i døgnrytmen eller få nætter i træk og dermed mange skift i døgnrytmen?

Det overordnede formål med denne afhandling var at undersøge de akutte fysiologiske effekter af antallet af nattevagter i træk i felt studier.

Det første studie var et review af den eksisterende litteratur om felt studier, der havde målt tilpasning af døgnrytmer målt på kortisol, melatonin eller hjerte rytme variabilitet (HRV). Der blev inkluderet 18 studier i reviewet. Vi fandt at der ikke var evidens for tilpasning af døgnrytmerne målt på kortisol, melatonin og HRV efter en til tre nattevagter i træk, men der var for få studier til at drage konklusioner for graden af tilpasning efter flere en tre nattevagter i træk.

Det andet og tredje studie var en del af projekt ”Midt om Natten” hvor 73 politimen gennem gik tre forskellige interventioner: 2+2 hvor de havde to nattevagter i træk efterfulgt af to dage til restitution, 4+4 hvor de havde fire nattevagter i træk efterfulgt af fire dage til restitution og 7+7 hvor de havde syv nattevagter i træk efterfulgt af syv dage til restitution. Projekt ”Midt om Natten” var designet som et cross-over studie hvor hver deltager gennemførte hver intervention én gang.

I det andet studie blev HRV under søvn undersøgt. De fem 5-minutters intervaller med laveste puls under søvn blev brugt til analysen af HRV. Vi fandt forskelle i HRV mellem de tre interventioner, men ikke med en entydig konklusion. Dog var der en tendens til bedre restitution på 2+2 interventionen.

I det tredje studie blev undersøgt hvilken betydning antallet af nattevagter i træk har for døgnrytmer af hormonerne melatonin, kortisol og testosteron. Vi fandt at de tre hormoner reagerede forskelligt på et stigende antal nattevagter i træk. Melatonin rytmen ændrede ikke timingen af den højeste koncentration, altså fasen af rytmen, men i stedet faldt den højeste
koncentration, altså ændrede amplituden sig. Koncentrationen af melatonin faldt i gennemsnit 4,9% per dag. Kortisolphaseden ændrede ikke amplituden, men forskød i stedet fasen med i gennemsnit 33 minutter per dag. Testosteron fulgte søvnen og der var ikke forskel på rytmen af testosteron på nattevagter mellem de tre interventioner. Der var heller ikke forskel mellem hormonerne på de tre restitutions dage og alle tre hormoner havde på disse dage en rytme, som man ville forvente af normal dagsorientering. Dette betyder at alle tre hormoner var tilbage i normal dagsorientering efter de tre interventioner.

_I det fjerde studie_ sammenlignede vi forskellige laboratorier, der brugte forskellige analysemetoder til måling af koncentrationerne af melatonin, kortisol og testosteron i spyt. Dette blev gjort som en del af kvalitetskontrollen af analysen brugt til at analysere hormoner i spyt i projekt ”Midt om Natten”. Alle laboratorier fik tilsendt otte prøver med ukendt indhold af de tre hormoner i spyt, analyserede dem efter normal procedure i deres laboratorie, og afgav resultat. Der var forskelle mellem de forskellige laboratorier, men generelt var de enkelte laboratorier i stand til at måle koncentrationer i deres eget reference interval.
**English Summary**

Night work common in many professions, despite the fact that night workers have an increased risk of disease. There are a number of recommendations for the optimum organization of working time, such that maximum should work 2-4 nights in a row to minimize the displacement of the biological rhythms and sleep loss. Although these recommendations have been used for many years, there is only limited scientific evidence that they reduce the risk of health problems. There are also many examples of night workers who prefer to work seven nights in a row to avoid frequent changes in the diurnal rhythm and to get more days off in a row. Hence there is a need for knowledge of whether such organization of working time is more damaging to health than working 2-4 nights in a row. What is the best - many nights in a row and thus few changes in diurnal rhythms or few nights in a row and thus many changes in diurnal rhythms?

The overall objective of this thesis was to investigate the acute physiological effects of the number of night shifts in a row in field studies.

The first study was a review of the existing literature on field studies that had measured adaptation of diurnal rhythms measured by cortisol, melatonin, or heart rhythm variability (HRV). There were included 18 studies in the review. We found that there was no evidence of adaptation of diurnal rhythms measured by cortisol, melatonin and HRV after one to three night shifts in a row, but there were too few studies to draw any conclusions on the degree of adaptation after one three night shifts in a row.

The second and the third study was part of the "In the Middle of the Night" project where 73 police officers completed different interventions: 2+2 where they had two night shifts in a row followed by two recovery days, 4+4 where they had four night shifts in a row, followed by four recovery days and 7+7 where they had seven night shifts in a row, followed by seven recovery days. The "In the Middle of the Night" project was designed as a cross-over study in which participants completed each intervention once.

In the second study, the HRV during sleep investigated. The five 5-minute intervals with lowest heart rate during sleep were used for the analysis of HRV. We found differences in HRV between the three interventions, but not a clear conclusion. However, there was a tendency for improved recovery on the 2+2 intervention.
In the third study, the number of consecutive night shifts effects on the diurnal rhythm of the hormones melatonin, cortisol and testosterone were studied. We found that the three hormones reacted differently to an increasing number of consecutive night shifts. Melatonin did not change the timing of the highest concentration, i.e. the phase of the rhythm, but instead suppressed the highest concentration obtained on average by 4.9% per day, thus changing the amplitude of the rhythm. Cortisol did not change the amplitude, but shifted instead phase with an average of 33 minutes per day. Testosterone followed sleep/wake cycle and there was no difference in the rhythm of testosterone on night shifts between the three interventions. There were no differences between the rhythms of melatonin, cortisol and testosterone on the recovery days and all three hormones had on these days a rhythm that one would expect for a normal day orientation. This means that all three hormones returned to a day oriented rhythm at the end of each intervention.

In the fourth study, we compared different laboratories using different analytical methods that all analyzed the concentration of melatonin, cortisol and testosterone in saliva. This was done as part of the quality control of the assay used to analyze hormones in saliva for the "In the Middle of the Night" project. All laboratories were received eight samples with unknown content of the three hormones in saliva, analyzed them on a normal procedure in their lab and gave results. There were differences between the various laboratories, but in general the individual laboratories capable of measuring concentrations in their own reference interval.
Papers I-IV
The effect of the number of consecutive night shifts on diurnal rhythms in cortisol, melatonin and heart rate variability (HRV) – a systematic review of field studies

Marie Aarrebo Jensen, Anne Helene Garde, Jesper Kristiansen, Kirsten Nabe-Nielsen and Åse Marie Hansen
The effect of the number of consecutive night shifts on diurnal rhythms in cortisol, melatonin and heart rate variability (HRV) – a systematic review of field studies

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Abstract:

\textit{Purpose}: The purpose of this review is to summarize the current knowledge from field studies on how many consecutive night shifts are required for adaptation of circadian rhythms in cortisol, melatonin and heart rate variability (HRV) to night work.

\textit{Methods}: A systematic search of the databases PubMed and Web of Science resulted in 18 studies selected for review.

\textit{Results}: Cortisol was measured in five studies, melatonin in 11 studies, and HRV in four studies. Diurnal rhythms were assessed by use of several different measures based on three to eight samples per day for cortisol and melatonin and 24h recordings for HRV. Most of the studies in the review were small studies with less than 30 participants, and most studies evaluated diurnal rhythms after only two consecutive night shifts whereas only six studies used seven or more consecutive night shifts. The majority of studies found that adaptation to night work had not occurred after two consecutive night shifts, whereas a small number found evidence for full adaptation after seven consecutive night shifts based on diurnal rhythms in cortisol and melatonin.

\textit{Conclusion}: There are methodological differences in the field studies analyzing circadian rhythms and large diversity in the occupational fields studied. Nevertheless, we conclude that circadian rhythms in cortisol, melatonin and HRV are not adapted to night work after 1-3 consecutive night shifts. Studies are needed to establish how many consecutive night shifts are needed for full adaptation of diurnal rhythms to night work.
Keywords: circadian disruption, adaptation to night work
Introduction

Night work is a necessity in many occupations. It is estimated that 15-20% of the working population in Europe are involved in night work. Night work is associated with both acute and reversible effects such as poor sleep (Sallinen and Kecklund, 2010), decreased cognitive function (Dula et al., 2001;Griffiths et al., 2006), and gastro-intestinal problems (Knutsson and Bøggild, 2010). Also chronic disorders e.g. peptic ulcer disease (Knutsson and Bøggild, 2010), cardiovascular disease (Bøggild and Knutsson, 1999;Frost et al., 2009), and breast cancer (Costa et al., 2010) are reported in association with night work. In 2007 the Agency for Research on Cancer classified 'shift work that involves circadian disruption' as a probable human carcinogen based on sufficient evidence from animal studies and limited evidence from epidemiological studies (Stevens et al., 2011).

Although unequivocal evidence of a causal effect of night work on e.g. breast cancer and cardiovascular disease is lacking, several mechanisms of disease onset and progression have been suggested (Bonde et al., 2007;Puttonen et al., 2010). One proposed mechanism is related to disruption of circadian rhythms of physiological systems (Costa et al., 2010;Puttonen et al., 2010). Circadian rhythms of physiological systems include sleep/wake cycles, and fluctuations in body temperature, blood pressure, hormone secretion, digestion, metabolism, and cell turnover. They are pivotal for survival and are driven and maintained in a hierarchical manner by a central pacemaker (the biologic master clock) located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Buijs et al., 2003). The SCN receives signals from external Zeitgebers such as light and darkness, social rhythms, timing of meals, and activities such as work and physical activity (Grandin et al., 2006;Zelinski et al., 2014).

Since humans generally are day-oriented, working at night requires a phase change in the sleep/wake cycle and circadian rhythms of other physiological systems. This change occurs gradually with increasing number of consecutive night shifts (Haus and Smolensky, 2006), but it is not clear how many consecutive night shifts are needed for full adaptation to night work in terms of being synchronized relative to the new work schedule and sleep times (and therefore disrupted in relation to the ambient dark/light cycle) Rhythms may be characterized by amplitude, phase and frequency (Hofstra and De Weerd, 2008). Preferably, the adaptation of circadian rhythms is assessed by measurements at several time points in order to assess all the characteristics. However, lack of adaptation may also be approximated by just a few measurements taken at comparable times during night work and day work schedules.
Circadian rhythms and circadian disruption have been investigated in several different ways in laboratory studies, e.g., via dim light melatonin onset (DLMO), body temperature, and sleep-wake logbooks (Figueiro et al., 2014; Mirick and Davis, 2008; Sargent et al., 2012). Laboratory studies have the advantage of a high level of control of the environment, but lack the inherent exposures of the real live setting. Laboratory studies have shown that when working nights in a real life setting, adaptation of the circadian rhythm is complicated by the fact that a night worker continues to be exposed to external Zeitgebers like sunlight and social factors that promote day-orientation. The influence of the external environment and social factors is not necessarily captured in a laboratory setting with simulated night work. Therefore laboratory studies must be supplemented with field studies to evaluate how the human body is affected by night work in real life (Kantermann et al., 2012).

Of the many possible physiological markers of diurnal rhythms that have been studied we chose to focus on two central indicators of physiological adaptation to the environment, that is, cortisol as a measure of the hypothalamus-pituitary-adrenal (HPA) axis (Tsigos and Chrousos, 2002) and heart rate variability (HRV) as a proxy for autonomic regulation (Pagani et al., 1986). Cortisol has a pronounced effect on the immune system and is a vital part of the body’s stress response, thus changes in the rhythmicity of cortisol release may have effects on overall health (McEwen and Karatsoreos, 2015). The autonomic nervous system (ANS) is involved in many physiological processes and is of vital importance of health (Pagani et al., 1986). Moreover, we included melatonin to capture information on the central circadian pacemaker to the external environment (Haus and Smolensky, 2006). Melatonin suppression has also been linked to shift worker health as a possible mechanism leading from shift work to cancer (Stevens and Rea, 2001).

Cortisol is produced in the adrenal gland and is the principal marker of the activation of the hypothalamus-pituitary-adrenal (HPA) axis (Tsigos and Chrousos, 2002). The HPA axis plays a central role in homeostatic processes and it is commonly thought to reflect attempts to adjust to daily pressures and joys (Rosmond and Björntorp, 2000). Cortisol has a pronounced diurnal rhythm and is viewed as a good and robust marker of the overall circadian rhythm (Hofstra and De Weerd, 2008). Cortisol has a characteristic rise 30 min. after awakening and then the concentration falls during the day. This characteristic rise is called the cortisol awakening response (CAR) and is a discrete and distinctive part of the cortisol circadian cycle (Haus and Smolensky, 2006). In healthy individuals, the CAR shows a large degree of variability across days, supporting a regulatory role within the healthy circadian pattern of cortisol secretion (Law et al., 2013). The concentration of cortisol can influenced by awakening time, physical activity and stress (Garde et al., 2008). Cortisol can be measured in blood, urine and saliva (Gatti et al., 2009; Jensen et al., 2011), and there is a good correlation between serum and saliva levels of cortisol (Cadore et al., 2008).
Melatonin is synthesized and secreted primarily by the pineal gland (Arendt, 1995); this molecule contributes to synchronizing the internal hormonal environment to the light-dark cycle of the external environment (Haus and Smolensky, 2006). Melatonin can be measured in blood or saliva and its metabolite 6-sulphatoxymelatonin can be measured in urine. Circulating melatonin level and 6-sulphatoxymelatonin have been shown to be good biomarkers of the circadian rhythm (Arendt, 1986; Jensen et al., 2011). Melatonin production is affected by light intensity. Illumination of sufficient intensity can completely suppress melatonin production. This means that the production of melatonin in night workers can be suppressed by the light in the environment (Mirick and Davis, 2008).

HRV is controlled by the ANS, which oscillates over the 24 h (Pagani et al., 1986; Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). HRV is an indicator of the ANS’s regulation of the cardiac rhythm, which encompasses the influences from the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) activity. The SNS are associated with energy mobilization, and the PNS with vegetative and restorative functions. These two branches of the ANS are in constant dynamic balance (Thayer et al., 2010). Imbalance in autonomic regulation, characterized by a relative dominant SNS hyperactivity over PNS hypoactivity, is a reliable predictor of morbidity and mortality (Dekker et al., 2000; Tsuji et al., 1996). The relative activity in the SNS and PNS can be disentangled by analysing HRV (Kleiger et al., 2005). High frequency (HF) variation (normally calculated for the frequency range 0.15-0.4 Hz) reflects PNS modulation of cardiac rhythm, while low frequency (LF) variation in (0.04-0.15 Hz) reflects SNS modulation with a significant contribution from PNS HRV (Malliani et al., 1998; Montano et al., 1994; Pagani et al., 1986; Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). In addition, the ratio between LF and HF is interpreted as the balance between the sympathetic and parasympathetic modulation of cardiac rhythm (sympathovagal balance) (Malliani et al., 1998; Montano et al., 1994; Pagani et al., 1986). Most measures of HRV show a pronounced circadian profile that is influenced by the sleep-wake cycle (Bonnemeier et al., 2003; Vandewalle et al., 2007) and work versus leisure time and physical activity (Collins et al., 2005; Vrijkotte et al., 2000).

Aim

We aimed at evaluating to what extent night workers adapt to night shifts and how many consecutive shifts are required in real life settings. This was done by reviewing field studies explicitly investigating the effect of the number of consecutive night shifts on diurnal rhythms of cortisol, melatonin and HRV.
Materials and methods

Search strategy

The literature search (Figure 1) was performed during August 2014. We searched the database ‘PubMed’ and ‘Web of Science’. The search strategy was developed by the first author and a search specialist at (workplace is to be revealed after blinded peer-review). The search resulted in a total of 900 articles. All titles and abstracts were read by the first author, and 65 papers were scrutinized further. Of these 65 papers, 15 papers fulfilled the inclusion criteria given below.

The criteria for inclusion in the present review were:

- Original research written in English and published in a peer-reviewed journal
- Data from humans in field studies
- Studies on one or more night shifts, i.e. primary working hours between 23:00 and 07:00
- Results on cortisol, melatonin/6-sulfatoxy-melatonin, and/or HRV
- At least three measurements on the same day to ensure that there was an indication of circadian rhythm
- A comparison of night work with day work or days off

Exclusion criteria were:

- Combined field and laboratory studies
- Intervention studies e.g. of the effect of light therapy and melatonin treatment

In addition to the literature search, we scanned the reference lists of the selected papers for additional studies of relevance revealing two relevant papers that were included in the study, giving a total of 18 papers for the present review.

Evaluation of the degree of adaptation to night work

We classified the degree of adaptation to night work for cortisol, melatonin and HRV in the studies as indicating no degree of adaptation, some signs of adaptation or a high degree of adaptation based on how much the circadian rhythm on night shifts deviated from the circadian rhythm during the day shift in relation to working hours. If there were permanent night workers as part of the study population days off were used for comparison. No adaptation was characterized by no change in the timing of the phase or
amplitude of the circadian rhythm compared to a day shift. Some signs of adaptation were characterized by changes in the timing of the phase or amplitude of the circadian rhythm, but not a full phase shift. A high degree of adaptation was characterized by a rhythm that followed the same pattern as on a day shift only shifted to match a night shift schedule.

Results

In total, 18 papers were included in the present review. Cortisol was measured in five studies (of which one also included melatonin and one HRV, Table 1). Melatonin was measured in 11 studies (of which one also measured cortisol, Table 2) and HRV was measured in four studies (of which one also measured cortisol, Table 3). The 18 studies cover several different occupational fields. Five studies had participants from the offshore oil industry (Barnes et al., 1998a; Barnes et al., 1998b; Gibbs et al., 2007; Hansen et al., 2010; Harris et al., 2010). Six studies had nurses as participants (Anjum et al., 2011; Borugian et al., 2005; Costa et al., 1994; Grundy et al., 2011; Hansen et al., 2006; Kobayashi et al., 1997) and one study had paramedics as participants (Wong et al., 2012). One study had workers from a power station (Vangelova and Dalbokova, 1998). Three studies had participants from other industries such as mining, the glass industry and electronic manufacturing (Choosong et al., 2006; Ferguson et al., 2012; Kudielka et al., 2007). The largest study in the present review consisted of 170 participants (Hansen et al., 2006) and only two additional studies had more than 100 participants: Grundy et al. with 123 participants (Grundy et al., 2011) and Kudielka et al with 102 participants (Kudielka et al., 2007). The rest of the studies had small sample sizes of around 10-30 participants (Anjum et al., 2011; Barnes et al., 1998a; Barnes et al., 1998b; Borugian et al., 2005; Choosong et al., 2006; Ferguson et al., 2012; Furlan et al., 2000; Gibbs et al., 2007; Hansen et al., 2010; Harris et al., 2010; Kobayashi et al., 1997; Rauchenzauner et al., 2009; Vangelova and Dalbokova, 1998; Wong et al., 2012).

The number of consecutive night shifts ranged from a single night shift (Rauchenzauner et al., 2009) to 14 consecutive night shifts (Harris et al., 2010). The frequency of saliva, urine and blood sampling ranged from hourly saliva samples over 24 hours (Ferguson et al., 2012) to blood samples taken at the start, middle and end of each shift (Costa et al., 1994).

Cortisol

We found five relevant papers with cortisol as a circadian marker (Table 1). Four studies used cortisol in saliva and one study used cortisol in blood as the circadian marker.

Of the four studies that used saliva samples, two used the CAR as an indicator of the diurnal rhythm (Harris et al., 2010; Kudielka et al., 2007). Harris et al used saliva taken five times a day to evaluate the level of cortisol decrease during the day and used it as an indicator of the diurnal rhythm (Harris et al., 2010).
Kudielka et al used saliva samples at +4h, +8h, +12h, and +16h after awakening to assess the cortisol level during the day (Kudielka et al., 2007). Wong et al used saliva samples taken 30 min after awakening and samples taken +1h, +6h, and +12h and at bedtime on rest days and at the beginning of the shift, mid shift, end of shift and at bed time on work days. They used this to calculate the slope of the cortisol curve. They also looked at the overall daily production of cortisol (Wong et al., 2012). Anjum et al compared the concentration of cortisol in saliva in evening, night and morning samples during a night shift and during a day shift (Anjum et al., 2011).

One studies used blood samples: Costa et al used three blood samples collected at the start, middle and end of a shift on days with morning, afternoon and night shifts (Costa et al., 1994).

Table 4 shows an overview of the degree of adaptation of the diurnal rhythm of cortisol in relation to the number of consecutive night shifts.

In the study by Harris et al (Harris et al., 2010) off-shore workers tested two types of shifts: fixed shift (14 day shifts or 14 night shifts) and swing shift (seven night shifts followed by seven day shifts). The off-shore workers adapted to night work within seven consecutive night shifts. This is in contrast to the findings by Anjum et al that did not find statistically significant results to indicate adaptation to night work in diurnal rhythms of cortisol after nine night shifts in a study with nurses (Anjum et al., 2011), but the authors did see a tendency towards an alteration in cortisol patterns after two night shifts. Wong et al claimed a tendency towards a flattened diurnal slope for cortisol, but found no statistically significant differences between night and day work after two shifts (Wong et al., 2012). Two studies found no changes in plasma cortisol patterns after two consecutive night shifts (Costa et al., 1994; Kobayashi et al., 1997).

Overall, no or little adaptation after two consecutive night shifts was found. There are very few studies that investigates the effects of more than two consecutive night shifts, although the study by Harris et al. indicate that full adaptation may occur after seven consecutive night shifts in off-shore workers.

**Melatonin**

Of the 11 papers on melatonin as a diurnal marker (Table 2), seven used 6-sulphatxoymelatonin measured in urine as a marker of diurnal rhythm (Barnes et al., 1998a; Barnes et al., 1998b; Costa et al., 1994; Gibbs et al., 2007; Hansen et al., 2006; Hansen et al., 2010; Vangelova and Dalbokova, 1998), 3 studies used salivary melatonin (Borugian et al., 2005; Choosong et al., 2006; Ferguson et al., 2012), and one study used both 6-sulphatxoymelatonin in urine and melatonin in saliva (Grundy et al., 2011). Urine and saliva collection were conducted in slightly different ways. Five studies compared fixed time points (Borugian et al.,
Table 4 shows an overview of the degree of adaptation of the diurnal rhythm of melatonin in relation to the number of consecutive night shifts.

After two consecutive night shifts Grundy et al also did not find any difference in the pattern of melatonin in saliva in a study of rotating shift workers (Grundy et al., 2011). In a study of 10 female shift workers on a glass manufacturing factory Choosong et al found that the older group (age 35-40 years, n=5) adapted to night work after two consecutive night shifts while the younger group (age 20-25) did not (Choosong et al., 2006). Costa at al found the excretion of 6-sulphatoxymelatonin showed a normal diurnal pattern with higher levels at night during two consecutive night shifts compared to day shifts, indicating that the diurnal rhythm did not adapt to night work. The authors found no difference between the first and second night in a row when looking at the excretion of 6-sulphatoxymelatonin (Costa et al., 1994). In a study of both male and female office workers and nurses working either rotating shift or day shifts Borugian et al found that rotating shift workers had abnormally high melatonin levels at arising and during the work day and abnormally low melatonin levels during sleep indicating a disrupted diurnal rhythm after two consecutive night shifts (Borugian et al., 2005).

In the two large studies of female nurses (n=170 and n=123) little evidence for adaptation of the diurnal rhythm of 6-sulphatoxymelatonin after two or more consecutive night shifts was found (Grundy et al., 2011; Hansen et al., 2006). Hansen et al did, however, show that nurses working fixed nights had a lower excretion of 6-sulphatoxymelatonin compared to day shift nurses on both a workday and a day off (Hansen et al., 2006). Vangelova et al also found no adaptation in the rhythm of 6-sulphatoxymelatonin to night work after two consecutive night shift, but significantly lower overall levels of 6-sulphatoxymelatonin on the second night shift compared with the first night shift (Vangelova and Dalbokova, 1998).

Gibbs et al found that 19 of the 23 male off-shore oil workers adapted to night shifts after seven consecutive night shifts by a delay in 6-sulphatoxymelatonin rhythm (Gibbs et al., 2007). Hansen et al also studied offshore workers and found that the rhythm of 6-sulphatoxymelatonin shifted from day one to day seven with a rate of adaptation of 0.84 hour per day (Hansen et al., 2010). In another study of offshore workers Barnes at al showed significant phase delay in 6-sulphatoxymelatonin rhythm in workers working two weeks of night shifts (Barnes et al., 1998a). Ferguson et al used saliva sampling and showed only small
changes in the diurnal rhythm of melatonin and little adaptation to night work after seven days of night shifts in people working in a mining operation (Ferguson et al., 2012). The findings in the included studies suggest little or no adaptation (a high level of circadian disruption) after two consecutive night shifts in diurnal rhythms of melatonin, but also indicate a tendency to increased level of adaptation of diurnal rhythm of melatonin after seven consecutive night shifts.

**HRV**

We found four relevant papers with HRV as a diurnal marker. The results are shown in Table 3.

Fulan et al. and Rauchenzauner et al. used normalized markers of cardiac sympathetic (LF_{nu}) and vagal (HF_{nu}) modulation of cardiac rhythm and of the sympathovagal balance (LF/HF) for one hour periods for a 24 h period (Furlan et al., 2000; Rauchenzauner et al., 2009). Kobayashi et al. used LF and LF/HF for 1 hour periods for 24 h (Kobayashi et al., 1997). Wong et al. used the square root of the mean of the squares of successive NN interval differences (RMSSD), pNN50 (the percentage of intervals >50 ms different from the preceding interval) and HF for 12 h as indicators of parasympathetic activity (Wong et al., 2012).

Table 4 shows an overview of the degree of adaptation of the diurnal rhythm of HRV in relation to the number of consecutive night shifts. Furlan et al. found that the autonomic balance was shifted in direction of lower sympathetic and higher parasympathetic activity during night work compared to day work after an adaptation period of two days (Furlan et al., 2000). In contrast, Kobayashi et al. found a tendency that the autonomic balance was shifted in direction of higher sympathetic and lower parasympathetic activity during night shifts following a half day on day shift compared to a night shift that followed a full or two full day shifts, although the difference between the two conditions was not significant (Kobayashi et al., 1997). Wong et al. did not find statistically significant differences but did report a tendency towards lower parasympathetic cardiac modulation in shift workers compared to day workers after two work days (Wong et al., 2012). Rauchenzauner et al. found higher sympathovagal balance (LF_{nu} and a tendency to higher LF/HF) on a 24 hours on call shift compared to a normal day (Rauchenzauner et al., 2009). In summary one to three consecutive night shifts is not enough for adaptation to work at night, but there is a lack of studies of the effects of more consecutive nights on diurnal rhythms in HRV.

**Discussion**

We aimed at evaluating to what extent night workers adapt to night shifts and how many consecutive shifts are required in real life settings. This was done by reviewing field studies explicitly investigating the effect
of the number of consecutive night shifts on diurnal rhythm of cortisol, melatonin and HRV. The main result is that night work with one to three consecutive night shifts does not result in adaptation to night work by a full shift in diurnal rhythms in salivary melatonin, cortisol and HRV. In total, 18 studies were included in the present review: three measured cortisol, 10 studies measured melatonin, three measured HRV, one studies measured both cortisol and HRV and one study measured both cortisol and melatonin. There were large methodological differences in the field studies. Since we wanted to look at the effects of night shifts in a real live setting we excluded laboratory studies and combined laboratory and field studies as the environmental exposures in these settings (e.g. in terms of light, noise level and meals) are highly controlled. We argue that this is a very different exposure compared to sleeping at home where the participants are in their natural environment which may include high levels of light, noise etc. all of which may contribute to the circadian disruption or degree of adaptation that occur during spells of night work. We also excluded interventions such as light therapy, melatonin supplementation or wearing tinted glasses to limit sun light since we also viewed this as a different exposure than night work in a natural setting.

There was a high degree of diversity with respect to the use of the diurnal markers in the 18 studies in this review. Four studies used cortisol in saliva and two used cortisol in blood. For cortisol, four studies used the CAR and others the cortisol decrease during the day as an indicator of circadian disruption. For the studies using melatonin, four studies looked at melatonin in saliva and eight at 6-sulphatoxyxymelatonin in urine. The HRV studies mostly focused on the balance between sympathetic and parasympathetic activity by analyzing LF/HF, LF$_{nu}$ and HF$_{nu}$. There are also differences in the number of points and timing of measurement and some studies use only very few. The lack of adequate sampling is a major limitation of this review and although it is possible to assess if there is lack of adaptation, this makes it difficult assess what characteristic of the diurnal rhythm is adapted. Another limitation is that it was not always possible to verify if the day shifts analyzed were unaffected by prior night shifts and thus that the timing of the rhythms was 'normal'.

The degree of adaptation to night work varied between the included studies and was studied in different ways and with diverging results. Two of the studies using melatonin that looked at two consecutive nights shift and did not find a significant change in diurnal rhythm (Costa et al., 1994; Grundy et al 2011) indicating that two consecutive night shifts will not cause a shift in the diurnal rhythm at least not in melatonin excretion, whereas two studies found signs of adaptation in the diurnal rhythm of melatonin (Borugian et al., 2005; Choosong et al., 2006). Also the studies using HRV as a diurnal marker found some contradicting results. Furlan et al found a lowered sympathovagal balance, while Wong et al and Rauchenzauner et al found higher sympathovagal balance in response to night work (Furlan et al., 2000; Rauchenzauner et al.,
In a review from 2008 Folkard concluded that even permanent night shift workers rarely fully adapt to their night shift in terms of the diurnal rhythm of melatonin with only one in four permanent night workers adjusting enough to benefit from the adjustment (Folkard, 2008).

Factors influencing the degree of adaptation to night work are mostly related to specific shift characteristics like timing, number of consecutive night shifts, duration and direction of shift rotation as well as factors related to the actual light exposure (intensity, wavelength and timing) (Haus and Smolensky, 2006). In addition, individual factors (e.g., diurnal preference and sleep pattern) and other environmental factors (e.g., eating and lighting when not at work) which have not been specifically assessed in the studies reviewed here may critically influence the circadian adjustment (Haus and Smolensky, 2013). This means that it is difficult to give a specific answer to how many night shifts are required for adaptation in field studies because this to a very high degree depends on both the individual and environmental factors. The behavioral and environmental influences on the evaluated biological markers can also mask some of the circadian effects making it difficult to evaluate whether or not a rhythm has adapted. Only few studies found a high degree of adaptation in diurnal rhythm of melatonin and cortisol to night work after seven consecutive nights (Gibbs et al., 2007; Harris et al., 2010) and the study by Anjum et al indicated that adaptation in diurnal rhythm of cortisol may not be achieved after nine consecutive night shifts (Anjum et al., 2011). The speed of adaptation may differ between different diurnal rhythms, as different physiological systems may adapt to night work in different tempos (Haus and Smolensky, 2006; Turek, 2008).

There may be many reasons for lack of adaptation. Circadian type may be relevant because being predisposed to an earlier circadian phase (‘morningness’) decrease the speed of adaptation to night work (Mirick and Davis, 2008). Further, ‘eveningness’, i.e. being predispose to a later circadian phase, compared to morningness seems to better suited to permanent night work, but not to rotating shift work. (Bonde et al., 2012). Accordingly, future studies should take these factors into account.

The studies of offshore workers generally showed a high degree of adaptation to night work. Thus, Harris et al demonstrated a very fast adaptation to night work with the workers’ cortisol rhythms begin fully adapted to night work within seven days (Harris et al., 2010). In offshore work, the workers are typically on remote locations, isolated from the rest of society and free from commitments to daily family activities. Their meal times and exposure to light are also much more controlled than a typical shift worker onshore. Offshore night workers thereby lack input from conflicting zeitgebers which prevent adaptation in onshore workers. This might be the reason why the studies using offshore shift workers typically show more and faster adaptation to night work (Bjorvatn et al., 2006). Also Studies of night shifts workers in remote Polar Regions...
have also found evidence for a similar fast adaption to night shifts indicating that adaptation to night shifts might occur faster in these remote locations (Midwinter and Arendt, 1991; Ross et al., 1995).

Our aim was to identify and summarize the effects of consecutive night shifts in field studies and we thus excluded both laboratory studies and combined field and laboratory studies. However, combined field and laboratory studies can add some important knowledge to the effects of night work in the field and a laboratory circadian assessment following a period of night or day work is likely to provide an accurate picture of circadian status. A combined field study compared the diurnal rhythm of cortisol and melatonin in permanent night workers (normally 5-6 consecutive night shifts) and controls on a day off in the laboratory. They found that the permanent night workers did not have a permanent shift in their diurnal rhythm of cortisol and melatonin (Roden et al., 1993). This evidence again suggests that even permanent night workers have a normal diurnal rhythm on their days off. Boivin et al conducted a combined field and laboratory study with police officers on seven consecutive night shifts in the field and circadian assessment in the laboratory and found a phase delay of approximately six hours in the rhythm of melatonin (Boivin et al., 2012). This evidence of a significant, but not complete adaptation to night work after seven consecutive night shifts are comparable with the results found in this review.

Even if it is established how many consecutive night shifts are required to adapt diurnal rhythms to work at night it is still questionable what is the optimal way to organize night work. Thus, it is debated whether it is pivotal for health to adapt diurnal rhythms to work at night (by having many consecutive night shifts) or to minimize phase shifts of diurnal rhythms (by having few consecutive night shifts). Complete adaptation of diurnal rhythms may also not be desired, given that individuals would experience circadian misalignment when returning to a day-active schedule. In a study by Merkus et al it was shown that following a two week 12-h night shift periods offshore, recovery was not fully complete up to day 11 (Merkus et al., 2015). In a consensus report published in 2012, Bonde et al. suggest to minimize the number of consecutive night shifts (i.e. one to two consecutive nights), since disruption of the diurnal melatonin secretion pattern can be diminished by restricting the number of consecutive night shifts (Bonde et al., 2012). However, a consequence is that the sleep-wake cycle is affected, because it is out of phase with circadian rhythms of other physiological systems (as well as the light-dark cycle). For this reason it may be argued that diurnal rhythms should be adapted to the sleep-wake cycle rather than the light-dark cycle by having more consecutive night shifts. This may be particularly relevant when focus is on safety rather than health. Studies have shown that both number of errors, reaction time, and concentration are most affected during the first, second and third night shifts, but less so in the following shifts (Lamond et al., 2003; Lamond et al., 2004).
Most of the studies in the present review are small in size and some of the smaller studies did not find statistically significant differences (Anjum et al., 2011; Wong et al., 2012) which could be due to low statistical power. Field studies can be both expensive and time consuming. They also require a company that will allow for its workers to participate in research projects during working hours and that there are participants who will volunteer for the study. However, field studies do provide important knowledge on how people are affected by night work in a real live setting and they can provide insights regarding why and how diseases develop in night workers.

**Conclusion**

There are methodological differences in the field studies analyzing diurnal rhythms and many use only few points of measurement. Although it is possible to assess if there is lack of adaptation, the lack of measuring points and properly timing of the measurements makes it difficult assess which characteristics of the diurnal rhythm that are adapted. For melatonin, cortisol and HRV the results of the studies indicate that night work with one to three consecutive night shifts does not result in adaptation to night work by a full shift in diurnal rhythms. However, there is a need for studies investigating more than three consecutive night shifts to make conclusions on how many number on consecutive night shifts are required for adaptation in diurnal rhythms of cortisol, melatonin and HRV. There is also a need for larger field studies in order to secure statistical significant results.

**Acknowledgments**

Elizabeth Bengtsen is acknowledged for her help in constructing the search strategy. The study was funded by The Danish Working Environment Research Fund (10-2011-09) and a PhD scholarship from the University of Copenhagen.

The authors declare no conflicts of interest.
Figure 1: Search strategy. Initially individual search strategies were made for diurnal rhythms (#1), biomarkers (#2) and shift work (#9). These were combined in the final search strategy (#10).
Table 1. Summary of the six papers with cortisol as a diurnal marker. The papers are listed according to year of publication.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population and sample size</th>
<th>Exposure</th>
<th>Outcome / methods</th>
<th>Reported results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kudielka et al 2007</td>
<td>102 shift workers working in the electronic manufacturing sector. 63% male. Mean age: 40 years +/- 7</td>
<td>Implementation of a new rotating shift system for a part of both the permanent day and night shift workers. Some stayed on permanent night or day shift others shifted to fast-forward rotation including morning, evening and night shifts. Shift length: 8 hours.</td>
<td>• Saliva were collected on the second day of each shift (day, evening, night) during first and second shift cycles and three days off work. • Saliva samples at awakening, +30, +45, and +60 min. after awakening to assess the CAR and +4, +8, +12, and +16 h after awakening to assess cortisol level during the day.</td>
<td>• The CAR was observed on night and day shifts • In the former permanent night workers cortisol profile appeared to be blunted during night work and days off</td>
</tr>
<tr>
<td>Harris et al. 2010</td>
<td>19 offshore workers 6 women 13 men Age: 44 years (28-60)</td>
<td>All participants completed: Fixed shift: 14 day shifts (0700-1900) or 14 night shifts (1900-0700) followed by 4 weeks off (fixed shift) and Swing shift: 7 night shifts (1900-0700) + 7 day shifts (0700-1900) followed by 4 weeks off Working 12 hours.</td>
<td>Saliva was sampled 5 times a day: at awakening, +30 min to assess the CAR, and + 6h, +12h and at bedtime. Saliva were collected: • On two consecutive days off work before the work period, and one week before and after the work period. • Fixed shift: On the second day or night shift offshore, on the middle of the offshore tour and on the last day or night shift offshore. • Swing shift: On the second shift offshore, the last night before shifting to day shifts, the day after shifting to day shifts and the last day offshore</td>
<td>• The cortisol rhythm adapted to night work within a week regardless work schedule indicated by the adaptation of the CAR • During swing shift the cortisol went back towards a normal rhythm in the second week, but was not returned completely when they finished their offshore tour</td>
</tr>
<tr>
<td>Anjum et al.</td>
<td>16 nurses Continuous 9 days night shifts with</td>
<td></td>
<td>Samples collected in the</td>
<td>No statistical significant differences on</td>
</tr>
<tr>
<td>Reference</td>
<td>Study</td>
<td>Exposure</td>
<td>Outcome / methods</td>
<td>Reported results</td>
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<tr>
<td>al 2011</td>
<td>8 men 8 women 20-40 years of age</td>
<td>alternate day shifts. It is not clear how many days saliva were collected Working hours: not given but assume 8 hours.</td>
<td>morning (0500-0800), afternoon (1300-1500) and night (2200-0100) on night shifts • Samples were collected in the morning (0500-0600), afternoon (1400-1500) and evening (2100-2200) on day shifts</td>
<td>night and day shifts • A tendency for alteration in cortisol levels was found during night shifts and that cortisol pattern was reversed slightly during day shifts.</td>
</tr>
<tr>
<td>Wong et al 2012</td>
<td>21 paramedics 7 day workers and 14 rotating shift workers (day shifts followed by night shifts) 14 men and 7 women Mean age: 38-43 years</td>
<td>One rest day and two work days (0600-1800) for both shift and day workers. Working 12 hours.</td>
<td>Saliva samples on the rest day and the first two work days • Rest day: Saliva were collected +30 min after awakening, +1h, + 6h, +12h and at bedtime • Work days: Saliva were collected +30 min after awakening, beginning of shift, mid shift, end of shift (1800) and at bedtime</td>
<td>The authors conclude that shift workers showed a tendency towards a flattened diurnal slope. However, the CI’s of the estimated effects are large and do not show a difference.</td>
</tr>
<tr>
<td>Costa et al 1994</td>
<td>15 female nurses Age: 21-29 years</td>
<td>Continuous rapidly rotating shift: A-M-M-A-N-N-R-R. M: 0700-1400, A:1400-2100, N: 2100-0700, R: rest day Working day and evening 7 hours and at night 10 hours.</td>
<td>• Blood samples were collected at the start, middle and end of each shift</td>
<td>Plasma cortisol did not deviate from a normal diurnal pattern during the night shifts</td>
</tr>
</tbody>
</table>

**Table 2.** Summary of the eleven papers with melatonin as a diurnal marker. The papers are listed according to year of publication.
<table>
<thead>
<tr>
<th>Study</th>
<th>Population and Sample Size</th>
<th>Shift Patterns</th>
<th>Collection Methods</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Costa et al 1994</td>
<td>15 female nurses 21-29 yrs.</td>
<td>Continuous rapidly rotating shift: A-M-M-A-N-N-R-R. M: 0700-1400, A:1400-2100, N: 2100-0700, R: rest day</td>
<td>Urine samples were collected at the start, middle and end of each shift and analyzed for 6-sulfatoxymelatonin</td>
<td>The excretion of 6-sulfatoxymelatonin showed a normal diurnal pattern with higher levels at night during the night shifts compared to the nights in between day shifts. There was no difference between the two night shifts.</td>
</tr>
<tr>
<td>Barnes et al 1998a</td>
<td>11 male offshore workers aged 35-56</td>
<td>2 weeks day shift (0600-1800) and 2 weeks night shifts (1800-0600)</td>
<td>Urine samples collected every 3 hours and overnight collection analyzed for 6-sulfatoxymelatonin</td>
<td>The participants showed adaptation to night shifts within one week.</td>
</tr>
<tr>
<td>Barnes et al 1998b</td>
<td>18 male offshore workers 23-56 yrs.</td>
<td>One week day (1200-0000 h) or one week night (0000-1200)</td>
<td>Urine samples collected every 2-3 hour and overnight collection analyzed for 6-sulfatoxymelatonin on all days.</td>
<td>The crews working day showed no change in their 6-sulfatoxymelatonin rhythm during their shifts. The crews working night (except 1 person) studied in March showed a significant phase delay in 6-sulfatoxymelatonin rhythm.</td>
</tr>
<tr>
<td>Vangelova et al 1998</td>
<td>23 male operators on a thermoelectric power station Age: 35.7+/−8.5 years.</td>
<td>Two day shifts (0700-1900) followed by two night shifts (1900-0700).</td>
<td>Urine samples were collected at the second day shift and both night shifts. Urine samples were collected at 0700, 1100, 1500, 1800 during the day shift and at 1900, 2300, 0300, 0600 during the night shift. Urine samples were analyzed for 6-sulfatoxymelatonin (μmol/hour).</td>
<td>The normal diurnal rhythm of excretion of 6-sulfatoxymelatonin. The second night had a significantly lower levels of 6-sulfatoxymelatonin than the first night.</td>
</tr>
<tr>
<td>Borugian et al 2005</td>
<td>5 office workers with day (0700-1900) shifts (2 men and 3</td>
<td>Seven day period including days off and work days.</td>
<td>Saliva samples analyzed for melatonin. Samples were collected on 3 time points (on arising, mid-day).</td>
<td>Rotating shift workers had abnormally high melatonin levels a arising and during the work day and abnormally low melatonin levels during sleep indicating a disrupted</td>
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<tr>
<td>Study</td>
<td>N (nurses)</td>
<td>Shifts</td>
<td>Period</td>
<td>Saliva Samples</td>
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<tr>
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<tr>
<td>Choosong et al 2006</td>
<td>10</td>
<td>2</td>
<td>Any 24h</td>
<td>Night, Day</td>
</tr>
<tr>
<td>Hansen et al 2006</td>
<td>170</td>
<td>2</td>
<td>Any 7d</td>
<td>Night, Day</td>
</tr>
<tr>
<td>Hansen et al 7 offshore fleet</td>
<td>7</td>
<td>1</td>
<td>Any 7d</td>
<td>Day, Night</td>
</tr>
</tbody>
</table>

- Women: 3 female nurses working day (0700-1900) shifts
- 14 female nurses working rotating night (two or more; 12 hrs) and day shifts
- Period included both night and day shifts.
- Five 24 hour period during the test week.
- For shift workers saliva samples were taken on either a night shift, day shift or a day off.
- Eight saliva samples a day (0700, 1000, 1300, 1600, 1900, 2200, 0100, 0400) at a day with morning shifts and a day with night shifts.
- Peak melatonin production at 2200 for the night shift
- Peak melatonin production during the morning shifts at 0100 and 0400, for the young and the old group, respectively
- All urine samples were collected over 24 hours (5-8 samples per participant)
- Analyzed for 6-sulfatoxymelatonin (μmol/mol creatinine), adjusted for creatinine and time of sampling
- Nurses working fixed night shift had a lower excretion of 6-sulfatoxymelatonin compared to day shift nurses on both the work day and the day off
- 19 of the 23 participants adapted to night shifts by a delay in 6-sulfatoxymelatonin rhythm.
- After the night shifts, some phase delayed (n=6), some advanced (n=6) and some did not re-adapt to day-time orientation (n=7) within 7 days.
- The rhythm of 6-sulfatoxymelatonin shifted
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population and sample size</th>
<th>Working time schedules studied</th>
<th>Main HRV Outcome/ methods</th>
<th>Reported results</th>
</tr>
</thead>
</table>
| al 2010              | workers 6 men and 1 woman Age: 34+/-12 years | consecutive nights (1800-0600)                                                                 | seven days starting at 1200 the 1. day and ending at 1200 the 7. day and analyzed for 6-sulfatoxymelatonin  
  • Every 3-4 hour with longer intervals during night. | from day 1 to day 7.  
  • The rate of acrophase adaptation was 0.84 hour per day |
| Grundy et al 2011    | 123 female nurses                | Two 12 hour days followed by two 12 hours night shifts, five days off in the summer and winter | Two urine samples over 48 hours analyzed for 6-sulfatoxymelatonin  
  • 2x4 saliva samples taken over 48 hours (at awakening, mid-shift, before sleep and at awakening on a day and a night shift) were analyzed for melatonin. | The pattern of melatonin production did not differ between night and day shifts |
| Ferguson et al 2012  | 24 individuals working at a live-in mining operation 27.4±6.8 yrs. | Seven day shifts, seven night shift and seven days off work | Saliva samples analyzed for melatonin  
  Saliva was collected hourly each day from 1900 to 2300 | The time of onset of melatonin secretion changed significantly during both the week with day shifts and the week with night shifts but the effects were small (from 2104 to 2130 hours)  
  • The small changes indicate a lack of true adaptation of the diurnal rhythm. |
| Table 3. Summary of the four papers with HRV as a diurnal marker. The papers are listed according to year of publication |

Reference: Kobayashi et al 1997  
Study population and sample size: 12 female nurses  
Working time schedules studied: Each participant completed three shift patterns: Two consecutive day shifts (D-D), a day shift followed by a night shift (D-N) and a half day followed by a night shift (HD-N)  
Main HRV Outcome/ methods: LF and LF/HF for 1 h periods for 24 h  
Reported results: Comparing night shifts: There was a tendency that the autonomic balance was shifted in direction of higher sympathetic and lower parasympathetic activity in the HD-N condition compared to the D-N condition during the
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Study Design</th>
<th>Data Collection</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furlan et al. 2000</td>
<td>22 males</td>
<td>24h oscillations in HRV reflected sleeping and working (wake) patterns.</td>
<td>LF, LF_nu, HF, HF_nu and LF/HF for 1h periods for 24h</td>
<td>Night shift: HR was higher, HF lower, and LF/HF higher. The</td>
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<td></td>
<td>blue-collar</td>
<td>Comparing work periods: The autonomic balance was shifted in direction of</td>
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<td>difference between the two conditions was not significant, however.</td>
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<td></td>
<td>workers</td>
<td>lower sympathetic and higher parasympathetic activity during the morning</td>
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<td>shift compared to the other shifts: LF_nu and LF/HF was lower (M compared</td>
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<td></td>
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<td>to N and A), while HF_nu was higher (M compared to N).</td>
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<td></td>
<td></td>
<td>Comparing sleeping periods: There were no significant differences.</td>
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<tr>
<td>Rauchenzauner et al. 2009</td>
<td>30 physicians</td>
<td>Comparing OCD with NOC: Higher LF_nu and a tendency to higher LF/HF on the OCD day compared to the NOC day. Also more ventricular premature beats, higher 24 systolic blood pressure, and higher night-time diastolic blood pressure was observed during OCD compared to NOC.</td>
<td>LF_nu, HF_nu, LF/HF for a 24h period</td>
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<td></td>
<td>21 men</td>
<td>Each participant completed on-call duty (OCD) comprising day work 8 AM to</td>
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<td>4:30 PM, followed by OCD for 16 h, and one regular day (not on-call, NOC)</td>
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<td>comprising day work 8 AM to 4:30 PM, followed by staying and sleeping at</td>
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<td></td>
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<td>home until next morning</td>
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<tr>
<td>Wong et al. 2012</td>
<td>21 paramedics</td>
<td>Comparing shift workers with day-time only workers: Non-significant tendency</td>
<td>RMSSD, pNN50 and HF for 12 h period (0600 to 1800)</td>
<td>Night shift: HR was higher, HF lower, and LF/HF higher. The</td>
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<tr>
<td></td>
<td>(7 daytime</td>
<td>to lower RMSSD in shift workers.</td>
<td></td>
<td>difference between the two conditions was not significant, however.</td>
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<td>only workers and 14 rotating shift workers (day shifts followed by night shifts)</td>
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<td>14 men and 7 women</td>
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</tbody>
</table>
Table 4. Overview of the degree of adaptation of the diurnal rhythm to night work

<table>
<thead>
<tr>
<th>Study</th>
<th>Diurnal marker</th>
<th>Number of consecutive night shifts</th>
<th>No adaptation</th>
<th>Some signs of adaptation</th>
<th>High degree of adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortisol</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Costa et al 1994</td>
<td>cortisol</td>
<td>2</td>
<td>x</td>
<td></td>
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<tr>
<td>Wong et al 2012</td>
<td>cortisol</td>
<td>2</td>
<td>x</td>
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<tr>
<td>Kudielka et al 2006</td>
<td>cortisol</td>
<td>2</td>
<td></td>
<td>x</td>
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<tr>
<td>Anjum et al 2011</td>
<td>cortisol</td>
<td>2</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Harris et al. 2010</td>
<td>cortisol</td>
<td>7</td>
<td></td>
<td></td>
<td>x</td>
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<tr>
<td><strong>Melatonin</strong></td>
<td></td>
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<td>Grundy et al 2011</td>
<td>melatonin</td>
<td>2</td>
<td>x</td>
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<tr>
<td>Costa et al 1994</td>
<td>melatonin</td>
<td>2</td>
<td>x</td>
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<tr>
<td>Vangelova et al 1998</td>
<td>melatonin</td>
<td>2</td>
<td>x</td>
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<tr>
<td>Hansen et al 2006</td>
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<td>2</td>
<td>x</td>
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<tr>
<td>Choosong et al 2006</td>
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<td>2</td>
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<td>x</td>
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<tr>
<td>Borugian et al 2005</td>
<td>melatonin</td>
<td>2</td>
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<tr>
<td>Ferguson et al 2012</td>
<td>melatonin</td>
<td>7</td>
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<td>x</td>
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<tr>
<td>Hansen et al 2010</td>
<td>melatonin</td>
<td>7</td>
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<td>x</td>
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<tr>
<td>Gibbs et al 2007 (+Gibbs et al 2002)</td>
<td>melatonin</td>
<td>7</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Barnes et al 1998b</td>
<td>melatonin</td>
<td>7</td>
<td>x</td>
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<tr>
<td>Barnes et al 1998a</td>
<td>melatonin</td>
<td>14</td>
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<td><strong>HRV</strong></td>
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<td>HRV</td>
<td>3</td>
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</table>
References Cited


Heart rate variability during sleep after 2, 4 and 7 consecutive night shifts and recovery days - a cross-over intervention study

Marie Aarrebo Jensen, Åse Marie Hansen, Kirsten Nabe-Nielsen, Anne Helene Garde and Jesper Kristiansen
Heart rate variability during sleep after 2, 4 and 7 consecutive night shifts and recovery days - a cross-over intervention study

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Abstract

Night work has been associated with a large range of diseases, and when work at night is inevitable the question is whether there is an optimal way to organize it to reduce potential health problems. Is it preferable to work many or few consecutive night shifts? Recovery after shift work is an important part reducing the health problems related to shift work. Heart rate variability (HRV) is an indicator of the balance between sympathetic and parasympathetic activity in the autonomic nervous system (ANS) and can be used as a measure of recovery after night shifts.

The overall aim of the present paper is to investigate autonomic recovery during sleep in response to night work in a crossover intervention study among Danish male police officers when they work two, four and seven consecutive night shifts.

The study is a part of the “In the Middle of the Night” project. Seventeen male police officers working in call centers in five different police district participated in the HRV measurements. The participants were exposed to three interventions: 2+2: two consecutive night shifts followed by two consecutive day recovery days (with either day work shifts or off work); 4+4: four consecutive night shifts followed by four consecutive recovery days; 7+7: seven consecutive night shifts followed by seven consecutive recovery days. On the last day with night shift and the last recovery day in each intervention the participants underwent 24h HRV recordings. We analyzed HRV during sleep. The five 5-minute intervals with the lowest heart rate during each sleep period were chosen for spectral analysis of the heart interbeat interval time series. Periods with lowest heart rate reflects relatively dominant parasympathetic modulation of the heart rate. It is assumed to correspond to peak activity in restorative biological systems and hence the time of maximal physiological restitution during sleep.

There were overall differences in HRV during sleep on days with night shifts and recovery days, primarily in parasympathetic activity; however, there were no difference in the lowest heart rate obtained during sleep. Over all autonomic activity was highest on the 2+2 night shift and this could indicate less recovery during sleep after the 4+4 and 7+7 night shifts. In addition, parasympathetic activity was lowest on the 4+4 night shift with the 2+2 and 7+7 night shift at the same level. The 2+2 recovery day had a significantly higher overall autonomic activity and parasympathetic activity than the 4+4 and the 7+7 recovery days, indicating better sleep related autonomic recovery. Overall sleep-related autonomic recovery had higher parasympathetic modulation of cardiac rhythm on the 2+2 shift system compared to the 4+4 and 7+7 shift system.

Key words: Autonomic nervous system, shift work, recovery
**Introduction**

Night work is common in our increasingly 24/7 society. It is estimated that 15-20% of the working population in Europe are involved in night work (Costa et al., 2010) and night workers have been suggested to have a higher risk of a large range of diseases including cardiovascular disease (Frost et al., 2009). So when working at night the inevitable question is whether there is an optimal way to organize night work to reduce potential health problems? One aspect is whether it is optimal to have many or few consecutive night shifts?

The ability to recover after night work is important for employee health (Harma, 2006). During recovery, psycho-physiological systems that have been activated during work unwind to and stabilize at a baseline level of activation (Geurts et al., 2006). Sleep is an important part of recovery after night work (Åkerstedt et al., 2003). The autonomic nervous system (ANS) plays a key role in the physiology of sleep. The ANS' regulation modulates cardiovascular functions during sleep onset and the transition to different sleep stages (Tobaldini et al., 2013). HRV is the physiological phenomenon of variation in the time interval between heartbeats. It is measured by the variation in the beat-to-beat interval (Kristal-Boneh et al., 1995). HRV is controlled by the ANS (Pagani et al., 1986; Task Force of the European Society of Cardiology et al., 1996) and is an indicator of the ANS's regulation of cardiac rhythm, which encompasses the influences from the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). High frequency (HF) variation of the HRV reflects PNS modulation of cardiac rhythm, while low frequency (LF) variation reflects SNS modulation with a significant contribution from PNS HRV. The cardiac sympathetic/parasympathetic balance is reflected in the ratio of the low frequency and high frequency (LF/HF) components of the HRV (Malliani et al., 1998; Montano et al., 1994; Pagani et al., 1986; Task Force of the European Society of Cardiology et al., 1996). Compared to wakefulness there is an overall reduction in heart rate and blood pressure during non-rapid eye movement (REM) sleep and sleep is characterized by a predominantly parasympathetic activity (Tobaldini et al., 2013). Most measures of HRV show a pronounced circadian profile that is influenced by the sleep-wake cycle (Bonnemeier et al., 2003; Vandewalle et al., 2007) and work versus leisure time (Collins et al., 2005; Vrijkotte et al., 2000).
Wehrens et al. have shown that a tendency for higher LF/HF ratio and lower HRV in male shift workers compared to non-shift workers (Wehrens et al., 2012), reflecting an overall tendency for higher sympathetic activity in shift workers.

Some previous studies have investigated the adaption of HRV in relation to the number of consecutive night shifts although none of them compared difference number of consecutive nights. Furlan et al. found that the autonomic balance was shifted in direction of lower sympathetic and higher parasympathetic activity during night work compared to day work after an adaption period of two days (Furlan, 2000). In contrast, Kobayashi et al. found a tendency that the autonomic balance was shifted in direction of higher sympathetic and lower parasympathetic activity during night shifts following a half day on day shift compared to a night shift that followed one or two full day shifts, although the difference between the two conditions was not significant (Kobayashi, 1997). Wong et al. did not find statistically significant differences between night and day shifts but did report a tendency towards lower parasympathetic cardiac modulation in shift workers compared to day workers after two work days (Wong, 2012). Rauchenzauner et al found higher sympathetic activity on a 24h on call shift compared to a normal work day (Rauchenzauner, 2009). Chung et al. found that the LF/HF ratio was higher after two consecutive night shifts than on days off, indicating higher sympathetic activity (Chung et al., 2012) Combined, the studies do not show full adaption of the ANS after one or two consecutive night shifts and a tendency towards either higher sympathetic activity during shift work, although one study showed the opposite tendency. However, to our knowledge there are no studies that investigate the effect of more than three consecutive night shifts on HRV and that directly compare the effect of the number of consecutive night shifts.

**Aim**

The overall aim of the present paper is to investigate how the cardiovascular autonomic regulation responds to night work in a crossover intervention study among Danish male police officers when they work two, four and seven consecutive night shifts. Specific questions that are investigated are: Do any of the three shift systems stand out with regard to recovery in the ANS during sleep? Are there any differences between HRV measured during sleep between the three interventions?

**Methods**
This paper presents results from the project “In the Middle of the Night”. The overall aim of the project was to compare the effects of two, four and seven consecutive night shifts on a range of outcomes related to the risk of shift work-related diseases. The present paper focuses on HRV. By investigating few participants working specific types of shifts with intensive measurement during a short period of time, the project complies with current recommendations regarding shift work research (Stevens et al., 2010). The study was approved by The National Committee on Health Research Ethics in Denmark (protocol number H-4-2012-155) and complies with the ethical principles and standards for the conduct of human and animal biological rhythm research (Touitou et al., 2004).

Recruitment procedure

The Danish police volunteered to participate in the study and the labor union approved the participation in the study. For logistic reason we only recruited participants from the five police districts at Zeeland, Denmark. The inclusion criteria for the study where that the participants had to be non-smoking male police officers and that they had to work night shifts as part of their normal shift schedule. Throughout all phases of the study there was intensive collaboration and strong support from both management and employee representatives. Before recruitment began, information meetings were held for leaders, the person in charge of the personnel-on-duty planning, and employee representatives, and all districts were offered an initial information meeting for potential participants, however, this offer were only accepted by two police districts.

An e-mail was sent to all potential participants in all five participating districts with an invitation to participate in the study. A total of 121 police officers returned an email showing interest in participating in the study. Among these, 99 police officers received individual, detailed information about the project either face-to-face or on the phone. For 84 police officers, the three intervention conditions were planned as part of their work schedule. Among these, 73 police officers filled in the baseline questionnaire. For safety reasons, the Danish Police Force did not want officers on patrol to wear the HRV monitors, so only police officers from the call centers were included in the HRV measurements. Of the 73 participants who volunteered, 18 worked at call centers and were invited to participate in the HRV measurements. One declined and 17 participants were included in this study.
The intervention conditions: Three different shift systems

The study was designed as a crossover intervention study exposing the participants to three different interventions: 2 night shifts followed by 2 recovery days (“the 2+2 intervention”), 4 night shifts followed by 4 recovery days (“the 4+4 intervention”), and 7 night shifts followed by 7 recovery days (“the 7+7 intervention”). Recovery days were defined as day shifts or days off (Figure 1). Day shifts started between 05.45 and 10.00 and ended between 13.45 and 17.00 and night shifts started between 21.15 and 23.00 and ended between 05.45 and 07.00.

The three interventions lasted 26 days in total and they could be distributed over no longer than three months. The study period was April-June 2013 and September-November 2013. The order of the three interventions was neither fixed nor random, but could occur in the order that suited the person in charge of the personnel-on-duty planning. This person was, however, instructed to mix the interventions so they occurred in different orders and to let the intervention conditions begin at different weekdays. The work schedule was planned so that it also suited the individual employee and thereby mimicking the usual way of scheduling the shifts. Before the beginning of an intervention condition the participant was not allowed to work any night shifts during the 7 preceding days. The participants filled out a background questionnaire before starting their first intervention. During all 26 days of the intervention the participants filled in a sleep questionnaire with questions on sleep time e.g. time in bed and time of awakening.

HRV

HRV measurements were done by Actiheart (CamNtech Ltd U.K.) Actiheart is a small lightweight system that has previously been applied for monitoring HRV (Ivarsson et al., 2009) and has been validated against standard clinical measurements (Holter recordings) (Kristiansen et al., 2011). The participants were carefully instructed how to put on and where to place the device by a member of the project group. They also received written instructions and were given a cell phone number they could contact in case they had any questions. The following measurement days the participant put on the device themselves. We analyzed HRV during primary sleep. Primary sleep was defined as the sleep during the night for the recovery days and as the first sleep after work for the night shifts. Wake and sleep times were noted by the participants in sleep questionnaires and these times were used to determine time of sleep. Five 5-minute intervals with the lowest heart
rate during each sleep period were extracted for spectral analysis of the heart interbeat interval time series. Periods with lowest heart rate reflects relatively dominant parasympathetic activity in the ANS and it is assumed to correspond to peak activity in restorative biological systems and hence the time of maximal physiological restitution during sleep (Stein et al., 2012). We used a sampling frequency of 4 Hz. Analyses of the data were done using a robust period detection (RPD) algorithm (Skotte et al., 2014). The RPD method has demonstrated superior performance compared to the Fourier transformation and Lomb method by estimation of power spectral characteristics for HRV analysis and is less sensitive to artifacts, ectopic beats, missing data, etc. in the recordings (Skotte et al., 2014). For each 5-minute interval an average of the following variables were extracted: RR mean (ms²), HF (ms²), LF (ms²), LF/HF (ms²) and total power (ms²). HF was 0.15-0.4 Hz and LF was 0.04-0.15 Hz.

**Missing data**

All participants completed all three interventions, except one who did not complete his 2+2 intervention due to problems with planning of his schedule, this resulted in loss of data from one 2+2 night shift and one 2+2 recovery day. Due to technical a technical problem, 22 recordings were not initiated properly and no data was recorded. This resulted in the loss of data from two 2+2 night shifts, two 4+4 night shifts, and four 7+7 night shifts, three 2+2 recovery days, five 4+4 recovery days and six 7+7 recovery days. 12 recordings were lost because there were not readable data during sleep. This resulted in the loss of data from one 2+2 night shift, one 4+4 night shift, four 2+2 recovery days, two 4+4 recovery days and four 7+7 recovery days. This means that there was a total of 40 recordings from the night shifts and a total of 26 recordings from the recovery days.

**Statistics**

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. Interventions were included as categorical variables in three levels (2+2; 4+4; 7+7) and type of day in two levels (night shift; recovery days). Five 5-minute intervals with the lowest heart rate during each sleep period were extracted for spectral analysis of the heart interbeat interval time series. The following outcomes were analyzed: RR mean (ms²), HF (ln ms²), LF (ln ms²), LF/HF ratio (ln ms²) and total power (ln
We also did analysis on time from sleep onset to the 5 minute interval with lowest heart rate and sleep duration. All outcomes were continuous and HF, LF, LF/HF ratio and total power were on a logarithmic scale. We used a random intercept for each individual with a variance components covariance structure and repeated statement for the five 5-minutes intervals with an autoregressive covariance structure. In the first step, we included all six measurement days to test for an interaction between intervention and type of day (night shifts or recovery days). In the second step, we stratified into night shifts and recovery days to test the effect of the interventions. All results were considered to be statistically significant at \( p < 0.05 \).

**Results**

The participants were from 28 to 59 years old and were all satisfied or very satisfied with their job (table 1). Five were morning or more morning that evening types and 12 were evening or more evening than morning types. On average they had 16 years of experience with night work and they preferred to work on average 4.6 consecutive night shifts.

Table 2 describes the participants’ sleep and HRV parameters after night shifts and recovery days. Table 2 also presents the p-values for the test of interaction between intervention and type of day (night shifts or recovery days). On average the participants slept between 4 hours and 18 minutes and 5 hours and 6 minutes after night shifts and between 6 hours and 18 minutes and 8 hours and 6 minutes on recovery days. The lowest heart rate after night shifts occurred 112 (SD 79) minutes, 174 (SD 115) minutes and 135 (SD 94) minutes after sleep onset for the 2+2 night shift, the 4+4 night shift and the 7+7 night shift, respectively. On the recovery days the lowest heart rate occurred 376 (SD 100) minutes, 322 (SD 110) minutes and 268 (SD 99) minutes after sleep onset for the 2+2 recovery day, the 4+4 recovery day and the 7+7 recovery day, respectively.

There was a statistically significant interaction between intervention and type of day (night shifts or recovery days) in HF, LF/HF ratio and total power. The analysis of the effects of the interventions was therefore stratified for night shifts and recovery days. RR mean and heart rate have a direct inverse relationship and an increase in RR mean equals a decrease in heart rate. There were no overall significant differences between the RR mean values obtained during sleep after night shifts and recovery days indicating that there were no differences in the lowest heart rate obtained during day and night sleep. The RR mean values reported and analyzed is from the
five analyzed 5-minute intervals and does not necessarily reflect the average heart rate during sleep.

The analyzed HRV parameters for night shifts and recovery days are shown in Figure 2. There were no statistically significant differences in LF after night shifts between the three different shift schedules. Generally LF reflects both sympathetic and parasympathetic activity and is regarded as a strong indicator of sympathetic activity. However, under sleep and rest high LF values can indicate an increase in parasympathetic activity (Tobaldini et al., 2013). There was no effect of the number of consecutive night shifts on RR mean indicating that the same lowest heart rate was obtained during sleep after the night shifts. Total power was highest on the 2+2 night shift compared to the 4+4 and 7+7 night shifts, indicating a decrease in overall HRV on the 4+4 and 7+7 night shifts. Total HF was lower for the 4+4 night shift than for the 2+2 and the 7+7 night shifts, indicating less parasympathetic modulation during sleep in this shift system. The LH/HF ratio was lower for the 7+7 night shift than the 2+2 and the 4+4 night shifts. A lower value of the LH/HF ratio indicates decreased sympathetic activity or higher parasympathetic activity.

We found no difference in RR mean for the three different interventions on recovery days showing that the same lowest heart rate was obtained on all three days. The 2+2 recovery day had a significantly higher total power compared with the 4+4 and 7+7 recovery days. Decreased total power indicates an overall lower HRV. The 2+2 restitution also had a significantly higher HF than the 4+4 and the 7+7 recovery days, indicating higher parasympathetic activity on the 2+2 recovery day. There was a tendency for lower LF on the 4+4 recovery day. The LF/HF ratio were highest for the 7+7 recovery day compared with the 2+2 and 4+4 recovery days indicating increased sympathetic activity or reduced parasympathetic activity during sleep on the 7+7 recovery day.

**Discussion**

There was a significant interaction between intervention and type of day (night shifts or recovery days) for HF, LF/HF ratio and total power and the analysis of the effect of the interventions were stratified for night shifts and recovery days.

With regard to the influence of the number of night shifts on HRV, there were signs that the 2+2 night shift was associated with largest physiological recovery (highest parasympathetic modulation
of cardiac rhythm), followed by the 7+7 night shift and the 4+4 night shift. Total power was highest on the 2+2 night shift. Total power reflects over all autonomic activity and this could indicate less recovery sleep after the 4+4 and 7+7 night shifts. In addition, HF was lowest on the 4+4 night shift (with the 2+2 and 7+7 night shift at the same level), and the LH/HF ratio lowest on the 7+7 night shift. Thus, the data indicate an apparent non-linear association between physical recovery and the number of consecutive night shifts, which is observed most clearly seen for the HF results. At present, we have no explanation for this possible non-linear relation. However, it may be speculated that it arises due to a balance between a “homeostatic drive” (need) for recovery, which presumably is highest early in the night shift period when the shift has just changed, and a “circadian drive” associated with internal biological rhythms closely linked to the circadian master clock, which presumably is low early in the night shift period (day orientation) but increases with the number of night shifts. The HRV during the recovery days after different consecutive night shifts had a similar complex pattern, but overall the 2+2 system was characterized by greater physiological recovery in terms of higher parasympathetic activation than the 4+4 and 7+7 system. For example, the 2+2 recovery day had a significantly higher total power and HF than the 4+4 and the 7+7 recovery days, indicating higher parasympathetic activity. This could indicate that the participants were not fully recovered on the 4+4 and the 7+7 recovery days. This could mean that in the 2+2 shift system the number of consecutive night shifts does not induce major disturbances in the circadian rhythm and that in the 7+7 shift system the number of consecutive night shifts might allow time for the circadian system to adapt to night work. Chung et al. suggested that two days off were sufficient to recover sleep-related autonomic functions after two consecutive night shifts in rotating shift nurses (Chung et al., 2012). Our results show that the same might also be true for male police officers in terms of recovery in the ANS.

The participants reached the lowest heart rate closer to the start of sleep after night shifts compared to the recovery days. This might reflect a circadian effect on heart rate. The level of physical activity is mainly responsible for the circadian variations of heart rate when awake, however, the endogenous circadian variation of heart rate during sleep results in the lowest heart rate in the last part of a night’s sleep (Deguette et al., 1991). There were a significant difference in sleep length between night shifts and recovery days. The participants slept shorter after night
shifts. A shortened sleep length after night shifts is already described (Åkerstedt, 2003) and corresponds well with the average sleep length of the participants in this study.

In a consensus report published in 2012, Bonde et al. suggest to minimize the number of consecutive night shifts (i.e. 1-2 consecutive nights) in order to prevent the negative health effects of night work (Bonde et al., 2012). Sleep loss has also been shown to be accumulated across night shifts (Pilcher et al., 2000) and in order to reduce sleep loss it may be preferable to work as few night shift in a row as possible. However there may also be reasons for working more consecutive night shifts. Another study showed that sleep length between night shifts tend to increase with the number of consecutive nights (Barton et al., 1995), thus recovery after a night shift may be better after an adaption period. There are also security reasons for preferring more consecutive night shifts. Negative effects related to number of mistakes, reaction time and concentration are most pronounced in relation to the 1st to 3rd night shift (Lamond et al., 2003; Lamond et al., 2004). From this study alone it is not possible to recommend more or fewer consecutive night shifts. However, in combination the results from night shifts and recovery days do indicate that the participants had an overall better recovery in terms of higher parasympathetic HRV on the 2+2 shift system.

This was a small study with 17 participants and data were lost due to technical problems. Especially on recovery days there was a reduced number of useable recordings and this may have affected the validity of the results, however, the problems with the measuring device was random and not systematic. The participants in this study are all male non-smoking police officers working in call centers and they all have night work experience. This makes it difficult to directly compare this study with studies of other occupations and with studies of females; however, it strengthens the internal validity of the study. The strength of the study is the cross-over design where the participants are their own controls. It is to our knowledge the first field study to directly compare the effects of two, four and seven consecutive night shifts on HRV.

**Conclusion**

There were overall differences in HRV during sleep on days with night shifts and recovery days primary in HF; however, there were no difference in the lowest heart rate obtained during sleep.
Overall sleep-related autonomic functions were better on the 2+2 shift system compared to the 4+4 and 7+7 shift system.

Acknowledgments

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The authors declare no conflicts of interest.


Furlan R (2000). Modifications of cardiac autonomic profile associated with a shift schedule of work.


Kobayashi F (1997). Changes in psychophysiological functions during night shift in nurses. Influence of changing from a full-day to a half-day work shift before night duty.


Table 1: Background information on participants, (n=17)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>17</td>
<td></td>
<td>44 (9)</td>
<td>26-57</td>
</tr>
<tr>
<td>BMI</td>
<td>17</td>
<td></td>
<td>26 (2)</td>
<td>21-30</td>
</tr>
<tr>
<td>Tenure within the police force (years)</td>
<td>17</td>
<td></td>
<td>16 (9)</td>
<td>2-31</td>
</tr>
<tr>
<td>Night work experience (years)</td>
<td>17</td>
<td></td>
<td>16 (9)</td>
<td>4-30</td>
</tr>
<tr>
<td>How many consecutive night shifts do you prefer?</td>
<td>17</td>
<td></td>
<td>4.6 (2)</td>
<td>2-10</td>
</tr>
<tr>
<td>How demanding is night shift work?</td>
<td>17</td>
<td></td>
<td>3.6(1)</td>
<td>1-5</td>
</tr>
</tbody>
</table>

General job satisfaction
- Very dissatisfied: 0 (0 %)
- Dissatisfied: 0 (0 %)
- Satisfied: 8 (47 %)
- Very satisfied: 9 (53 %)

Diurnal type
- Morning: 3 (18 %)
- More morning than evening: 2 (12 %)
- More evening than morning: 8 (47 %)
- Evening: 4 (24 %)

Table 2. Description of sleep and HRV parameters. P-values are from the test of interaction between type of day (night shifts and recovery days) and intervention.

<table>
<thead>
<tr>
<th></th>
<th>2+2 night shift</th>
<th>4+4 night shift</th>
<th>7+7 night shift</th>
<th>2+2 recovery day</th>
<th>4+4 recovery day</th>
<th>7+7 recovery day</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>14</td>
<td>13</td>
<td>9</td>
<td>10</td>
<td>7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sleep length hours (SD)</td>
<td>5.0 (1.0)</td>
<td>5.1 (1.6)</td>
<td>4.3 (1.6)</td>
<td>8.0 (1.2)</td>
<td>7.9 (1.2)</td>
<td>6.3 (1.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time from sleep onset to the analyzed 5 minutes intervals in minutes (SD)</td>
<td>112 (79)</td>
<td>174 (115)</td>
<td>135 (94)</td>
<td>376 (100)</td>
<td>322 (110)</td>
<td>268 (99)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Average HRV parameters from the five 5-minute intervals

<table>
<thead>
<tr>
<th></th>
<th>RR mean ms² (SD)</th>
<th>HF ln ms² (SD)</th>
<th>LF ln ms² (SD)</th>
<th>LF/HF ln ms² (SD)</th>
<th>Total power ln ms² (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2+2 night shift</td>
<td>1188.6 (132.4)</td>
<td>5.236 (1.354)</td>
<td>5.778 (1.135)</td>
<td>0.542 (1.039)</td>
<td>6.917 (1.182)</td>
</tr>
<tr>
<td>4+4 night shift</td>
<td>1167.4 (174.1)</td>
<td>5.087 (1.088)</td>
<td>5.581 (0.831)</td>
<td>0.494 (0.927)</td>
<td>6.706 (0.859)</td>
</tr>
<tr>
<td>7+7 night shift</td>
<td>1177.9 (173.1)</td>
<td>5.380 (1.004)</td>
<td>5.726 (0.897)</td>
<td>0.346 (1.079)</td>
<td>6.899 (0.872)</td>
</tr>
<tr>
<td>2+2 recovery day</td>
<td>1177.9 (106.2)</td>
<td>5.129 (0.908)</td>
<td>5.971 (0.962)</td>
<td>0.842 (0.678)</td>
<td>7.079 (0.958)</td>
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<td>4+4 recovery day</td>
<td>1164.9 (116.8)</td>
<td>5.165 (0.908)</td>
<td>5.485 (0.995)</td>
<td>0.320 (1.117)</td>
<td>6.753 (0.929)</td>
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<td>7+7 recovery day</td>
<td>1096.7 (128.6)</td>
<td>5.129 (0.908)</td>
<td>5.884 (0.732)</td>
<td>1.007 (0.566)</td>
<td>6.763 (0.778)</td>
</tr>
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P values: 0.1335, 0.0177, 0.0623, 0.0010, 0.0391
Figure 1: Overview of the three intervention conditions. Grey color = night shift; no color = recovery days. X = 24 hour HRV measurements. The three intervention conditions were not planned in a fixed order.

<table>
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<tr>
<th>Intervention condition</th>
<th>1</th>
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<td>&quot;7+7&quot;</td>
<td></td>
<td></td>
<td>X</td>
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<td></td>
<td></td>
<td>X</td>
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<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>&quot;4+4&quot;</td>
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Figure 2. HRV estimates from the separate analysis of night shifts and recovery days. All axis are in ln ms\(^2\).
Changes in the diurnal rhythms of cortisol, melatonin and testosterone after 2, 4 and 7 consecutive night shifts in male police officers

Marie Aarrebo Jensen, Åse Marie Hansen, Jesper Kristiansen, Kirsten Nabe-Nielsen and Anne Helene Garde
Changes in the diurnal rhythms of cortisol, melatonin and testosterone after 2, 4 and 7 consecutive night shifts in male police officers

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Abstract

Night work is associated with a large range of both acute health problems and possible also health consequences in the long run. Yet, only very few studies specifically studies the effects of consecutive night shifts in field studies.

In this study we investigated the effects of consecutive night shifts on the three hormones: melatonin, cortisol and testosterone in a field study. The aim was to investigate how the diurnal rhythm of melatonin, cortisol and testosterone respond to two, four and seven consecutive night shifts and a corresponding number of days for recovery.

The study is a part of the “In the Middle of the Night” project and includes 73 male police officers from five different police districts. The participants were exposed to three intervention conditions: ‘2+2’: two consecutive night shifts followed by two consecutive day recovery days; ‘4+4’: four consecutive night shifts followed by four consecutive recovery days; ‘7+7’: seven consecutive night shifts followed by seven consecutive recovery days. On the last day with night shift and the last recovery day in each intervention the participants collected saliva samples every 4th hour when awake. Changes in diurnal rhythms were analyzed with respect to time since awakening.

The diurnal rhythms of melatonin, cortisol and testosterone all changed differently in response to an increasing number of consecutive night shifts in this field study: the amplitude of melatonin rhythm was suppressed 4.9% per day (95% CI 1.4 –8.2% per day; p= .006), but did not show any change in phase. The diurnal rhythm of cortisol phase delayed with increasing number of night shifts by 33 min/day (95% CI 18 –48 min per day; p= <.001), but did not show any changes in amplitude. For the diurnal rhythm of testosterone there was no effect of the number of consecutive night shifts and the diurnal rhythm completely followed the sleep/wake cycle. We found that there were no differences in the rhythms of melatonin, cortisol and testosterone after 2, 4, and 7 recovery days, respectively.

In conclusion we found lack of synchronization in terms of suppressed amplitude of melatonin and phase delay of salivary cortisol as a consequence of the increasing number of consecutive night shifts, but not in recovery days. Lack synchronization has been suggested as a possible mechanism linking night work to disease, but this remains to be determined.

Key words: shift work, circadian disruption
**Introduction**

The rotation of the Earth around its central axis causes a daily rhythm in environmental factors, including light intensity and temperature. In order to anticipate these 24 hour changes in the environment, many physiological systems fluctuate within 24 hours. Diurnal rhythms of physiological systems include sleep/wake cycles, and fluctuations in body temperature, blood pressure, hormone secretion, digestion, metabolism, and cell turnover. They are pivotal for survival and are driven and maintained in a hierarchical manner by a central pacemaker (the biologic master clock) located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Buijs et al., 2003). The SCN receives signals from external Zeitgebers such as light and darkness, social rhythms, timing of meals, and activities such as work and physical activity (Zelinski et al., 2014).

Night work disrupts these physiological rhythms leading to circadian disruption (Haus et al., 2006). Circadian disruption of physiological systems in relation to the light/dark cycle is one of the possible mechanisms leading from night work to disease (Haus et al., 2006). Another mechanism is lack of coordination between internal circadian rhythms (Zelinski et al., 2014). Lack of coordination develops because different biological rhythms change their rhythm with different speed (Zelinski et al., 2014).

Diurnal rhythms and circadian disruption have been studied in both field and laboratory studies. Both study approaches are needed to fully understand the effects of night shifts; laboratory studies are important for investigating underlying mechanism and field studies is the only way of testing the effects of the of the real world exposure (Kantermann et al., 2012). In this field study we investigate the effects of consecutive night shifts on the three hormones: melatonin, cortisol and testosterone.

*Melatonin* synchronizes the internal hormonal environment to the light–dark cycle of the external environment. Melatonin is synthesized and primarily secreted by the pineal gland (Arendt, 1995). Melatonin is often used in both field and laboratory studies to evaluate the level of circadian disruption as a result of night or shift work and is regarded as a good biomarker of circadian dysregulation (Mirick et al., 2008). Melatonin production is suppressed by light, and illumination of sufficient intensity can completely suppress melatonin production. Correspondingly, melatonin reaches the highest level during the night and is low during the day (Arendt, 1995). *Cortisol* is
produced in the adrenal gland and is the principal marker for the activation of the hypothalamus–pituitary–adrenal (HPA) axis (Tsigos et al., 2002). Cortisol has a characteristic and stable diurnal rhythm. Under the influence of the SCN, HPA axis activity gradually increases toward the end of sleep and reaches the highest level after awakening and gradually falls during the day reaching the lowest level in the early hours of sleep (Clow et al., 2010; Haus et al., 2006). Testosterone is an anabolic steroid produced in the testes in men. There is a slight diurnal variation in the level of testosterone and testosterone is released during deep sleep (Luboshitzky et al., 2003; Wittert, 2014).

It is estimated that 5-20% of the working population in Europe are involved in night work (Costa et al., 2010) and the International Agency for Research on Cancer has classified “shift work that involves circadian disruption” as a probable human carcinogen based on sufficient evidence from animal studies and limited evidence from epidemiological studies (Stevens et al., 2011). How different physiological systems react to the number of consecutive night shifts have implications for possible recommendation for scheduling night shift work. There is currently very limited evidence to support a recommendation of a specific number of night shifts, although, Bonde et al suggested minimizing the number of consecutive night shifts in order to prevent circadian disruption and melatonin suppression based on both experimental and epidemiological evidence (Bonde et al., 2012). Thus, there is a need for better understanding of the consequences of night shift work and better evidence and understanding of how to plan night work to minimize the health consequences of night work when it is inevitable (Stevens et al., 2011).

The overall aim of the present paper is to investigate how the diurnal rhythms of melatonin, cortisol and testosterone respond to different numbers of consecutive night shifts in a crossover intervention study among Danish male police officers. More specifically, the participants worked two, four and seven consecutive night shifts with a corresponding number of days for recovery. The research questions were: (1) Do the diurnal rhythm of melatonin, cortisol and testosterone differ in response to 2, 4 and 7 consecutive night shifts? (2) Are there differences in the diurnal rhythm of melatonin, cortisol and testosterone on the last of the recovery days? (3) If there are differences in the rhythm of melatonin, cortisol and testosterone between the three interventions is it then a difference in amplitude or phase of the rhythm? By testing three different hormones in
a field study we can gain insights into how different physiological systems are affected by consecutive night shifts in a real live setting.

Methods

This paper presents results from the project “In the Middle of the Night”. The overall aim of the project was to compare the effect of two, four and seven consecutive night shifts on a range of outcomes related to the risk of heart disease and metabolic disorders. The present paper focuses on melatonin, cortisol and testosterone. The study was approved by The National Committee on Health Research Ethics in Denmark (protocol number H-4-2012-155).

Recruitment procedure

Danish police volunteered to participate in the study and the labor union approved the participation in the study. We recruited participants from the five police districts at Zeeland, 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. Throughout all phases of the study there was intensive collaboration and strong support from both management and employee representatives. Before recruitment began, information meetings were held for leader, the people responsible for personnel-on-duty planning, and employee representatives. All districts were also offered an initial information meeting for potential participants, which were accepted by two police districts.

An e-mail was sent to all potential participants in all districts with an invitation to participate in the study. 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 either face-to-face or on the phone. For 84 police officers, the three intervention conditions were planned as part of their work schedule. Among these, 73 police officers filled in the baseline questionnaire and provided a total of 2166 saliva samples.

Participants
Table 1 describes the participants in the study. The participants were between 25 and 62 years old with a mean age of 38 years and 22% had less than three years of night shift experience 28% had three to ten years of night work experience and 40% had more than ten years of night work experience. Most rated their health to be excellent or very good and they were generally physically active in their free time.

The intervention conditions: Three different shift systems

The study was designed as a crossover intervention study exposing the participants to three different interventions: 2 night shifts followed by 2 restitution days (‘2+2’), 4 night shifts followed by 4 restitution days (‘4+4’), and 7 night shifts followed by 7 restitution days (‘7+7’). Restitution days were defined as day shifts or days off (Figure 1). Day shifts were typically from 7.00 to 15.00 (we allowed day shifts to end as late as 18.00) and night shifts were from typically from 23.00 to 7.00 (we allowed the start of the shift to be from 22.00 to 00.00).

The data collection period was April-June 2013 and September-November 2013. The three interventions lasted 26 days in total and they could be distributed over no longer than three months and each individual participant only participated in either the spring or the fall. The order of the three interventions was neither fixed nor random but could occur in the order that suited the person in charge of the personnel-on-duty planning. This person was, however, instructed to mix the interventions so they occurred in different orders and to let the intervention conditions begin at different weekdays. To mimic the usual way of scheduling the shifts the schedule was planned so that it also suited the individual employee. Before the beginning of an intervention condition the participant was not allowed to work any night shifts during the seven preceding days.

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Figure 1: Overview of the three intervention conditions. Grey color = night shift; no color = recovery days. X = intensive measurement days: saliva samples every 4 hours when awake. The three intervention conditions were not planned in a fixed order.
The participants filled out a background questionnaire before starting their first intervention.

**Saliva sampling**

On the last day with night shift and on the last recovery day (marked X in Figure 1) the participants gave saliva samples. Participants were instructed to make the first sample upon awakening and the last sample right before bedtime and the rest of the day participants were instructed to collect samples at 07.00, 11.00, 15.00, 19.00, 23.00, and 03.00 only if they were awake. So if a participant woke at 07.30 and went to bed at 23.45 he collected 6 saliva samples that day: 07.30 (awakening), 11.00, 15.00, 19.00, 23.00 and 23.45 (bedtime). The participants were given verbal and written instructions on how to collect the saliva samples and a cell phone number to contact in case of questions. The participants received a text message the day before saliva sampling as a reminder. Saliva samples were collected by drooling directly into tubes. The participants were instructed to keep the samples in the refrigerator or freezer until they had completed each intervention and then mail the samples to (institution name will be added after blinded peer-review). Melatonin, cortisol and testosterone in saliva are stable at room temperature for at least seven days (Jensen et al., 2011) and all samples were received within seven days.

**Analyses of hormones**

Analysis of hormones in saliva was done using liquid chromatography tandem mass spectrometry (LC-MS/MS). The analysis is described in detail by Jensen et al. (Jensen et al., 2011). 25 µL was injected into an Agilent 1200 HPLC (Agilent technologies, Santa Clara, CA, USA) equipped with a C18 2.1 mm × 50 mm 2.6 µm Kinetex column (Phenomenex, Torrance, CA). A linear gradient was run over 3 min from 10% to 100% MeOH and maintained at 100% MeOH for 2.5 min, followed by 1 min of equilibration at 10% MeOH. The mass spectrometer, an Agilent 6460 QQQ (Agilent technologies, Santa Clara, CA) equipped with a jet stream ESI ion source, was operated in the positive ion mode. The quantification was achieved by using the mass spectrometer in multiple reaction monitoring mode. A single precursor ion-product ion transition was measured for each hormone and its internal standard. The transitions were: m/z 233.2 → m/z 174.1 for melatonin; m/z 237.2 → m/z 178.1 for d-4- melatonin; m/z 363.2 → m/z 121.1 for cortisol; m/z 367.2 → m/z 121.2 for d-4-cortisol m/z 289.2 → m/z 97 for testosterone; m/z 291.2 → m/z 97 for d-3-
testsosterone. The detection limit was 3.73 pmol/L, 0.26 nmol/L and 6.63 pmol/L for melatonin, cortisol and testosterone respectively. To test equivalence between analysis, reference samples at two levels were analyzed with every 14 samples. Westgard control charts (Westgard et al., 1981) were used to document that the LC-MS/MS method remained under statistical and analytical control.

Statistics

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. Interventions were included as categorical variables in three levels (‘2+2’; ‘4+4’; ‘7+7’) and type of day in two levels (night shift; recovery days). All concentrations of hormones were on a logarithmic scale and treated as continuous variables. We used a random intercept for each individual with a variance component covariance structure and repeated statement for the saliva samples with an autoregressive covariance structure. The saliva samples were categorized in to seven categories (A-G): 0-60 minutes after awakening (A), 1-4 hours after awakening (B), 4-8 hours after awakening (C), 8-12 hours after awakening (D), 12-16 hours after awakening (E), 16-20 hours after awakening (F) and 20-24 hours after awakening (G). G samples were excluded from analysis since there were less than ten samples from each of the six measurement days. We adjusted for time of day in all analyses. All results were considered to be statistically significant at p < .05.

In Model 1 we included all six measurement days to test for an interaction between intervention and type of day (night shifts or recovery days) to test if the effect of the interventions differed between night shifts and recovery days.

In Model 2 we stratified into night shifts and recovery days to test the effect of the interventions. We included an interaction between intervention and sample category to test for difference in the rhythms between the interventions.

If differences in Model 2 were significant we used Model 3 to test for phase shift by testing if the time since awakening for the highest concentration (for melatonin) or the lowest concentration (for cortisol) was different between the three interventions. Analysis on amplitude changes were done by testing if the highest (for melatonin) or lowest concentration (for cortisol) obtained
differed between the three interventions by use of multilevel regression analyses with the PROC MIXED procedure. Where significant effects of intervention were observed on either phase or amplitude, post hoc analysis included test of linearity in relation to number of days by including intervention as a continuous variable in a linear regression model.

Results

Table 2 shows the average concentrations from the measured saliva samples at each time interval (A-F) from all the measurement days. Table 2 also describes on what time of day the samples A-F were taken in each intervention. We found a significant interaction between the interventions and type of day (night shifts and recovery days) for melatonin (p value for interaction = <.001), cortisol (p for interaction =<.001) and testosterone (p value for interaction = 0.003) and therefore stratified our analysis into night shifts and recovery days.

Melatonin

Estimates from the analysis of the effects of the interventions on the rhythm of melatonin are shown in figure 2. Days with night shifts are shown in figure 2A and recovery days in figure 2B. On days with night shifts, there was a significant difference between the interventions in the rhythm of melatonin (p value for interaction =<.001). All three melatonin curves on night shifts (Figure 2A) curves show low concentrations after awakening, a rise and then a drop right before bedtime (around 17-20 hours after awakening or approximately at 07.00). The results of the phase and amplitude tests are summarized in table 4. The was a change in amplitude (measured by the highest concentration obtained) for the melatonin rhythm: The concentration of melatonin was highest on the 2+2 intervention (87.5 pmol/L) followed by the 4+4 intervention (65.0 pmol/L) and the 7+7 intervention (66.4 pmol/L) (p=0.001). There was a linear trend for the fall in melatonin concentrations (4.9% per day, 95% CI 1.4–8.2% per day; p= .006). However, there was no change in the phase (measured by the timing of the highest concentration) of the melatonin rhythm (p= .934). On recovery days, there was no significant difference in the melatonin rhythm between the three interventions (p value for interaction=.510). The melatonin rhythm on the recovery days showed that melatonin was highest after awakening and before bedtime.
Cortisol

Estimates from the analysis of the effects of the interventions on the rhythm of cortisol are shown in figure 3. Days with night shifts are shown in figure 3A and recovery days in figure 3B. There was a significant difference between the interventions in the rhythm of cortisol on days with night shifts (p value for interaction = <.001). All three cortisol curves on night shifts (Figure 3A) showed high concentrations after awakening, a drop and a rise again around bedtime. The results of the phase and amplitude tests are summarized in table 4. This analysis showed a phase delay in the diurnal rhythm of cortisol (measured by the timing of the lowest concentration of cortisol). The lowest concentration of cortisol was reached 8:50 hours after awakening on the 2+2 intervention, 9:23 hours after awakening on the 4+4 intervention, and 11:31 hours after awakening on the 7+7 intervention. There was a linear change in the phase delay (33 minutes/day, 95% CI 18 –48 minutes per day; p= <.001). There was no difference in amplitude (measured as the lowest concentration obtained) between the interventions in the rhythm of cortisol. There was no significant difference between the interventions on recovery days in the rhythm of cortisol (p value for interaction=.338). On all the recovery days the rhythm of cortisol showed the highest concentration after awakening and dropped during the day reaching the lowest concentrations before bedtime.

Testosterone

Estimates from the analysis of the effects of the interventions on the rhythm of testosterone are shown in figure 4. Days with night shifts are shown in figure 4A and recovery days in figure 4B. There was no significant difference between the interventions on the days with night shifts (p value for interaction=.898) or on recovery days (p value for interaction=.074). On all days the highest concentration were found after awakening and hereafter the concentrations fell and reached a level that was relatively constant until bedtime.
Discussion

In this cross-over intervention study we have investigated the effect of two, four and seven consecutive night shifts and a corresponding number of recovery days on the diurnal rhythms of melatonin, cortisol and testosterone. For the amplitude of melatonin, we found a progressive suppression with increasing number of consecutive night shifts but no phase shift based on the timing of the highest concentration. The suppression of melatonin was 4.9% per day (95% CI 1.4 – 8.2% per day). For the amplitude of cortisol, we found no difference in the lowest concentration between interventions, however in contrast to melatonin, the phase of the cortisol rhythm was delayed 33 min/day (95% CI 18 – 48 min/day). For testosterone, the diurnal rhythm showed no difference between the interventions on night shifts. On all the last recovery days in each intervention we found no significant difference in the rhythms of melatonin, cortisol and testosterone between the interventions.

The observed a change in amplitude for the diurnal rhythm in melatonin may be explained both by suppression of melatonin by light and by adaption of circadian rhythm. Exposure to light at night can cause suppression of melatonin (Arendt, 2010), however, the progressive decrease in melatonin with increasing number of consecutive night shifts suggests that this is also a results of adaptation to night shifts and the corresponding new sleep/wake cycle. In a study from 2014 Dumont and Paquet also showed a progressive suppression of melatonin in a laboratory study with three consecutive simulated night shifts. The study was done under constant light conditions so light at night was probably not the direct cause of the suppression of melatonin production. Instead, the authors suggested that the suppression of melatonin production was a result of circadian disruption (Dumont et al., 2014). In a study of female nurses, Hansen et al also found a lower excretion of melatonin in urine from nurses after at least two consecutive night shifts compared to day shifts and days off (Hansen et al., 2006). A field study of truck driver showed that night shift melatonin levels significantly decreased across four consecutive weeks in both a control group and an intervention group being treated with bright light implying that a general adaptation to night work occurred, however, it was slower in the control group (Lowden et al., 2004).

Our finding that there is no phase delay in melatonin is somewhat surprising, since other studies, both laboratory and field studies have found that the diurnal rhythm of melatonin responded to
night shifts with a phase delay (Barnes et al., 1998; Boivin et al., 2012; Gibbs et al., 2007). We did not see a phase delay in the diurnal melatonin rhythm in this study; however, we did see a flattening of the curve and this could mask a possible phase delay as it is very difficult to accurately assess the timing of the highest concentration on a flat curve. We found a drop in melatonin around 17-20 hours after awakening on night shifts (approximately at 07.00) in all three interventions and this corresponds to the end of the night shifts or when the participants return home from work. We speculate that the drop in melatonin that occurs in the diurnal rhythm of melatonin on night shifts in this study is due to the exposure to light. At 07.00 the participants would have been exposed to sunlight which would suppress their production of melatonin more efficiently than electric light (Arendt, 2010), however, we do not have light measurements to support this explanation.

The diurnal rhythm of cortisol showed an increasing phase delay with increasing number of night shifts with 33 minutes per day, however, the curve of cortisol did not show the same diurnal rhythm in relation to sleep as on the recovery days. This indicates that the diurnal rhythm of cortisol did not fully adapt to the night shifts and the new/sleep wake cycle within seven consecutive night shifts. Full adaptation to night shifts in the diurnal rhythm of cortisol has previously been demonstrated in a field study and Harris et al found full adaption of the diurnal rhythm of cortisol within seven days in study with offshore workers (Harris et al., 2010). However, offshore workers have been shown to adapt faster to night shifts than onshore workers probably due to their isolated working conditions (Bjorvatn et al., 2006). Other field studies have found very limited evidence for changes to night shifts in the rhythm of cortisol after two consecutive night shifts (Costa et al., 1994; Kudielka et al., 2007; Wong et al., 2012) these results are in line with the results from this study where we do not see adaptation of the diurnal rhythm of cortisol to the sleep/wake cycle after two consecutive night shifts.

We found that the diurnal rhythm of testosterone completely adapt to the sleep/wake cycle and was highest upon awakening regardless of intervention and type of day (night shifts or recovery days). This fits well with a study by Axelsson et al, who investigated the effects of night sleep and day sleep on the level of testosterone in healthy young men in a laboratory study and found that testosterone increased during sleep and fell during waking regardless of the time of day of the
Sleep, whereas circadian effects seemed marginal (Axelsson et al., 2005). This result is in line with the finding in this study were we also find that the rhythm of testosterone follows the sleep/wake cycle and thereby replicating the laboratory results in a real live setting.

There were no differences in the diurnal rhythms for melatonin, cortisol and testosterone on the recovery days across the three interventions and the diurnal rhythms were all as expected for a normal day-orientation. This indicates that the participants re-adapted to day-orientation and a normal sleep/wake cycle in all three interventions. In a study of offshore workers it was shown that following a two week 12-h night shift periods recovery of the cortisol diurnal rhythm was not fully complete up to day 11 (Merkus et al., 2015). However, as mentioned earlier offshore workers have been shown to change faster to night shifts than onshore workers and after two weeks offshore the rhythm of cortisol were expected to be fully adapted to night shifts after two weeks offshore (Merkus et al., 2015). The participants in this study never fully adapted to night shifts in their diurnal rhythm of cortisol and a re-adaption to day orientation may therefore be faster.

This study showed that the diurnal rhythms of melatonin, cortisol and testosterone all responded differently to an increasing number of consecutive night shifts. As a consequence there is a lack of synchronization of internal diurnal rhythms of melatonin, cortisol and testosterone even after 7 consecutive night shifts. Lack of synchronization has been suggested as a mechanism for linking night work to disease. In animal experiments, the lack of coordination of internal rhythms has been shown to cause cardiovascular disease and renal failure (Martino et al., 2008). Laboratory studies of humans have shown that lack of synchronization of diurnal rhythms, measured by sleep and cortisol release may cause pre-diabetic changes (Scheer et al., 2009). The internal balance of testosterone and cortisol has an effect on the risk of developing cardiovascular disease and type 2 diabetes (Rosmond et al., 2003).

Our results suggest lack of synchronization between diurnal rhythms of melatonin, cortisol and testosterone even after seven consecutive night shifts. However, it remains to be settled if this is associated with increased risk of disease and what types of desynchronization are the most adverse. From the results of this study alone it is not possible to recommend a specific number of consecutive night shifts, however, the amount of both melatonin suppression and lack of synchronization of internal diurnal rhythms is more pronounced during the 7+7 intervention. This
supports the recommendations of minimizing the number of consecutive night shifts as proposed by Bonde et al (Bonde et al., 2012). Of course other factor such as sleep (Barton et al., 1995; Pilcher et al., 2000), preferences (Kecklund et al., 2008), accidents and performance (Lamond et al., 2004) are also very important aspects to consider for a recommendation of a specific number of consecutive night shifts. There is also a need to study the long-term effects of consecutive night shifts before recommending a specific number of night shifts.

**Strengths and limitations**

The strength of the present study is that it is designed as a crossover intervention study in which all participants were exposed to all three intervention conditions. Saliva samples collected repeatedly during a total of six day giving us the possibility to evaluate the diurnal rhythms and their changes in response to the number of consecutive night shifts. The study also analyzed three different hormones in the same population making it possible to gain information on more than one physiological system. By investigating few participants working specific types of shifts with intensive measurement during a short period of time, the project complies with current recommendations regarding shift work research (Stevens et al., 2010).

We did not include a baseline day and this makes it impossible to accurately assess if the participants had a “normal” rhythm on the recovery days. However, all recovery days had identical rhythms and they all had diurnal profiles as expected from normal day-orientation for all three hormones. The police officers that participated were a rather homogenous group of male physical active non-smoking police officers with high job-satisfaction. This fact can limit the external validity of the study, however, at the same time enhance the internal validity. Since melatonin is so influenced by light intensity a measure of light exposure it would have been relevant to study to what extend the suppression of melatonin was influenced by light exposure. However, light exposure measurements were not possible to obtain due to restrictions from the police force who could not guarantee that officers on patrol could wear visible light monitors over their uniform. We used the timing of the highest (for melatonin) and lowest (for cortisol) concentration to estimate changes in the phase of the rhythms, however, with saliva samples every 4th hour when the participants were awake we did not necessarily capture the true extremes in concentration.
giving an added insecurity to the results. Using the lowest concentration of cortisol to assess changes in amplitude may not be optimal, but the best we could do since the maximum is occurring during the primary sleep where the participants did not collect saliva.

The sleep/wake cycle changes when humans are awake during a night shift. All the results presented in this paper uses time since awakening as time reference instead of time of day. This was done to better describe how the diurnal rhythms of the analyzed hormones changes in relation to the sleep/wake cycle and because the rhythm of both cortisol and testosterone are influenced by sleep (Haus et al., 2006; Wittert, 2014). During night shifts the sleep/wake cycle is forced into a new timing and we wanted to investigate the hormonal response to this change. Since both sleep time and time of day is of critical importance when analyzing diurnal rhythms (Kantermann et al., 2012) all analyses are adjusted for time of day.

Conclusion

We found that the diurnal rhythms of melatonin, cortisol and testosterone all changed differently to an increasing number of consecutive night shifts: the amplitude of melatonin rhythm was suppressed 4.9% per day (95% CI 1.4 – 8.2% per day), but did not show any changes in phase. The diurnal rhythm of cortisol phase delayed with increasing number of night shifts by 33 min/day (95% CI 18 – 48 min per day; p < .0001), but did not show any changes in amplitude. For the diurnal rhythm of testosterone there was no effect of the number of consecutive night shifts and the diurnal rhythm completely followed the sleep/wake cycle. We found that there were no differences in the diurnal rhythms of melatonin, cortisol and testosterone after 2, 4, and 7 recovery days, respectively. As a consequence of the differences in diurnal rhythms of melatonin, cortisol and testosterone in response to an increasing number of consecutive night shifts there is a lack of synchronization of the three hormones even after seven consecutive night shifts. Lack of synchronization has been suggested as a possible mechanism linking night work to disease, but this remains to be determined.
Acknowledgments

We would like to thank all the participating police officers and the Danish police force and police union for all their help. Ulla Tegner, Dorrit Meincke, Anne Abildtrup and Inge Christiansen are thanked for their help with data collection. The study was funded by The Danish Working Environment Research Fund (10-2011-09) and a PhD grant from the University of Copenhagen.

The authors declare no conflicts of interest.
Reference List


Table 1: Information on age, job satisfaction, night work experience, health, physical activity and diurnal type.

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</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical inactive</td>
<td>3</td>
<td>4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light physical activity</td>
<td>13</td>
<td>18%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate physical activity</td>
<td>34</td>
<td>47%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High physical activity</td>
<td>22</td>
<td>31%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-rated overall health</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>22</td>
<td>31%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quite good</td>
<td>34</td>
<td>47%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>14</td>
<td>19%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less good</td>
<td>2</td>
<td>3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bad</td>
<td>0</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General job satisfaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very dissatisfied</td>
<td>3</td>
<td>4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissatisfied</td>
<td>0</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Satisfied</td>
<td>29</td>
<td>40%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very satisfied</td>
<td>41</td>
<td>56%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diurnal type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>10</td>
<td>14%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More morning than evening</td>
<td>10</td>
<td>14%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More evening than morning</td>
<td>38</td>
<td>52%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening</td>
<td>15</td>
<td>21%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 2. Averages form hormone measurements from the six different intervention days and the number of saliva samples included in the average. Standard deviations are shown in brackets.

<table>
<thead>
<tr>
<th>Sample A (0-60 min)</th>
<th>2+2 night shift N</th>
<th>4+4 night shift N</th>
<th>7+7 night shift N</th>
<th>2+2 recovery day N</th>
<th>4+4 recovery day N</th>
<th>7+7 recovery day N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin pmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample A (0-60 min)</td>
<td>15.7 (20.9)</td>
<td>51.0 (18.0)</td>
<td>18.0 (22.5)</td>
<td>67</td>
<td>20.7 (15.9)</td>
<td>70</td>
</tr>
<tr>
<td>Sample B (1-4 hours)</td>
<td>14.5 (17.9)</td>
<td>50.0 (16.7)</td>
<td>16.7 (16.1)</td>
<td>43</td>
<td>15.1 (13.9)</td>
<td>46</td>
</tr>
<tr>
<td>Sample C (4-8 hours)</td>
<td>25.6 (72.7)</td>
<td>63.0 (20.5)</td>
<td>20.5 (30.5)</td>
<td>62</td>
<td>27.2 (36.9)</td>
<td>67</td>
</tr>
<tr>
<td>Sample D (8-12 hours)</td>
<td>58.0 (57.0)</td>
<td>63.0 (32.1)</td>
<td>32.1 (34.5)</td>
<td>65</td>
<td>31.5 (37.5)</td>
<td>69</td>
</tr>
<tr>
<td>Sample E (12-16 hours)</td>
<td>90.0 (105.3)</td>
<td>64.0 (69.4)</td>
<td>69.4 (56.7)</td>
<td>59</td>
<td>57.1 (50.8)</td>
<td>68</td>
</tr>
<tr>
<td>Sample F (16-20 hours)</td>
<td>47.5 (36.0)</td>
<td>60.0 (47.8)</td>
<td>47.8 (37.0)</td>
<td>56</td>
<td>51.1 (50.8)</td>
<td>54</td>
</tr>
<tr>
<td>Cortisol nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample A (0-60 min)</td>
<td>4.0 (2.0)</td>
<td>72</td>
<td>4.2 (2.1)</td>
<td>67</td>
<td>5.2 (4.1)</td>
<td>67</td>
</tr>
<tr>
<td>Sample B (1-4 hours)</td>
<td>2.4 (1.2)</td>
<td>36</td>
<td>3.3 (2.6)</td>
<td>42</td>
<td>3.1 (2.1)</td>
<td>45</td>
</tr>
<tr>
<td>Sample C (4-8 hours)</td>
<td>1.9 (1.3)</td>
<td>53</td>
<td>2.4 (2.2)</td>
<td>55</td>
<td>3.1 (6.3)</td>
<td>59</td>
</tr>
<tr>
<td>Sample D (8-12 hours)</td>
<td>2.1 (1.8)</td>
<td>49</td>
<td>1.7 (1.2)</td>
<td>43</td>
<td>2.1 (2.2)</td>
<td>57</td>
</tr>
<tr>
<td>Sample E (12-16 hours)</td>
<td>2.6 (2.1)</td>
<td>56</td>
<td>1.7 (0.9)</td>
<td>44</td>
<td>1.7 (1.1)</td>
<td>51</td>
</tr>
<tr>
<td>Sample F (16-20 hours)</td>
<td>4.2 (3.2)</td>
<td>58</td>
<td>3.1 (2.1)</td>
<td>52</td>
<td>2.5 (1.8)</td>
<td>44</td>
</tr>
<tr>
<td>Testosterone pmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample A (0-60 min)</td>
<td>252.8 (102.4)</td>
<td>72</td>
<td>297.3 (174.6)</td>
<td>67</td>
<td>318.3 (348.3)</td>
<td>70</td>
</tr>
<tr>
<td>Sample B (1-4 hours)</td>
<td>156.8 (67.7)</td>
<td>40</td>
<td>173.1 (127.7)</td>
<td>43</td>
<td>181.2 (98.9)</td>
<td>45</td>
</tr>
<tr>
<td>Sample C (4-8 hours)</td>
<td>154.0 (75.1)</td>
<td>63</td>
<td>193.8 (215.1)</td>
<td>62</td>
<td>191.4 (202.8)</td>
<td>64</td>
</tr>
<tr>
<td>Sample D (8-12 hours)</td>
<td>140.3 (71.8)</td>
<td>63</td>
<td>154.3 (130.4)</td>
<td>66</td>
<td>147.0 (73.6)</td>
<td>69</td>
</tr>
<tr>
<td>Sample E (12-16 hours)</td>
<td>142.8 (93.4)</td>
<td>64</td>
<td>130.2 (64.5)</td>
<td>59</td>
<td>137.3 (82.0)</td>
<td>67</td>
</tr>
<tr>
<td>Sample F (16-20 hours)</td>
<td>149.0 (65.6)</td>
<td>60</td>
<td>150.4 (56.0)</td>
<td>55</td>
<td>235.4 (532.5)</td>
<td>54</td>
</tr>
<tr>
<td>Time of day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample A (0-60 min)</td>
<td>14.05 (1.49)</td>
<td>72</td>
<td>14.14 (1.22)</td>
<td>67</td>
<td>14.22 (2.13)</td>
<td>70</td>
</tr>
<tr>
<td>Sample B (1-4 hours)</td>
<td>15.40 (2.20)</td>
<td>40</td>
<td>16.40 (1.56)</td>
<td>43</td>
<td>16.48 (2.44)</td>
<td>46</td>
</tr>
<tr>
<td>Sample C (4-8 hours)</td>
<td>19.44 (2.05)</td>
<td>63</td>
<td>19.58 (1.55)</td>
<td>62</td>
<td>20.19 (2.28)</td>
<td>67</td>
</tr>
<tr>
<td>Sample D (8-12 hours)</td>
<td>23.37 (1.59)</td>
<td>63</td>
<td>23.55 (2.01)</td>
<td>66</td>
<td>0.25 (2.38)</td>
<td>69</td>
</tr>
<tr>
<td>Sample E (12-16 hours)</td>
<td>03.37 (2.17)</td>
<td>66</td>
<td>0.45 (1.54)</td>
<td>59</td>
<td>0.47 (2.19)</td>
<td>68</td>
</tr>
<tr>
<td>Sample F (16-20 hours)</td>
<td>06.59 (1.22)</td>
<td>60</td>
<td>0.71 (0.54)</td>
<td>56</td>
<td>0.71 (1.37)</td>
<td>54</td>
</tr>
</tbody>
</table>
Table 3. Results from the analysis of phase and amplitude for melatonin and cortisol on night shifts.

<table>
<thead>
<tr>
<th></th>
<th>2+2 night shift</th>
<th>4+4 night shift</th>
<th>7+7 night shift</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol timing of lowest concentration (hours after awakening – hh:mm)</td>
<td>8:50 (7:55-9:47)</td>
<td>9:23 (8:28-10:19)</td>
<td>11:31 (10:37-12:25)</td>
<td>&gt; 0.0001</td>
</tr>
<tr>
<td><strong>Amplitude analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level of lowest concentration of cortisol (nmol/L)</td>
<td>1.19 (1.06-1.34)</td>
<td>1.13 (1.00-1.28)</td>
<td>1.12 (0.99-1.25)</td>
<td>0.5312</td>
</tr>
<tr>
<td>Level of melatonin highest concentration (pmol/L)</td>
<td>87.51 (73.29-104.5)</td>
<td>64.98 (54.47-77.52)</td>
<td>66.42 (55.83-79.02)</td>
<td>0.0012</td>
</tr>
</tbody>
</table>
Figure 2. Estimates of melatonin concentrations plotted against hours since awakening for night shifts and recovery days. The results are adjusted for time of day.
Figure 3. Estimates of cortisol concentrations plotted against hours since awakening for night shifts and recovery days. The results are adjusted for time of day.
Figure 4. Estimates of testosterone concentrations plotted against hours since awakening for night shifts and recovery days. The results are adjusted for time of day.
An interlaboratory comparison between similar methods for
determination of melatonin, cortisol and testosterone in saliva

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Hansen
TECHNICAL NOTE

An interlaboratory comparison between similar methods for determination of melatonin, cortisol and testosterone in saliva

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Abstract
An interlaboratory comparison study for melatonin, cortisol and testosterone in saliva in which five laboratories participated is reported in this study. Each laboratory blindly measured eight samples prepared from natural saliva spiked with melatonin, cortisol and testosterone in the range 0–579 pmol/L for melatonin, 0–90 nmol/L for cortisol, and 0–622 pmol/L for testosterone. The recovery of spiked material for melatonin ranged from 91–110%, from 83–100% for cortisol and from 80–94% for testosterone. The content of natural hormone in saliva was estimated to be between 0.278 and 6.90 pmol/L for melatonin, 0.56 and 6.72 nmol/L for cortisol and 11.9 and 73.8 pmol/L for testosterone. This indicates a large interlaboratory variation. The present study emphasizes the importance of external quality control for the analysis of melatonin, cortisol and testosterone in saliva.

Key Words: Saliva, quality control, melatonin, cortisol, testosterone

Introduction
Saliva samples have become the sampling procedure of choice for many studies involving measurement of melatonin, cortisol and testosterone [1–3]. Saliva sampling is preferred since it has several advantages: It is noninvasive, painless and easy to perform. Salivary levels of steroid hormones and other analytes that are protein bound in serum reflect the unbound and active concentration of the hormone [4]. Several different analytical methods are presently employed for the measurement of melatonin, cortisol and testosterone in saliva. The most used techniques to analyse melatonin, cortisol and testosterone in saliva are immunochemistry-based methods [5–7] but in the last few years several mass spectrometry (MS)-based techniques have become increasingly popular [8–12]. The immunochemistry-based techniques, such as radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) are highly sensitive and simple to use, but suffer from a potential risk of cross-reactivity from structurally similar compounds [13,14]. In addition, RIA has been shown to be sensitive to both collection device and type of antibody used; making it difficult to compare results obtained with different RIA kits [15]. MS-based methods also have potential analytical problems, such as matrix effect and ion suppression [16].

It has been stated that ‘there is likely to be a high degree of overlap between what good practice in a routine situation is, and what good practice in a non-routine situation is’ [17]. Hence, we suggest that methods used in research and development fulfill the requirements for routine analysis stated in the guide on accreditation [18]: The analytical method should be described clearly and in sufficient detail in order to allow other laboratories to repeat the measurements. Precision, range, robustness and other relevant performance parameters are requisite. Statistical control of measurement results and possible bias must be documented by method evaluation, internal quality control (e.g. use of control charts), analysis of certified reference materials and external quality
control (e.g. participation in proficiency-testing schemes) [19,20]. Finally, measurement results should be reported together with an uncertainty, which allows the user to evaluate the reliability of the results [18]. This is accommodated by following the recommendations given in [19,21]. Most of the statements are performance parameters that can be achieved by the individual laboratory, while external quality control requires a proficiency-testing scheme, preferably international. At present no such commercial proficiency-testing scheme is available for melatonin, cortisol, and testosterone in saliva. So in order to compare results from different laboratories we initiated a non-commercial comparison of analysis of melatonin, cortisol and testosterone in saliva.

The purpose of the present study was to perform a laboratory comparison of measurements of melatonin, cortisol and testosterone in saliva using both immunochemistry-based techniques and liquid chromatography tandem mass spectrometry (LC-MS/MS) methods among non-commercial laboratories.

**Materials and methods**

**Laboratories**

We invited six laboratories to participate in the study. Five out of six invited laboratories agreed to participate in the study. Each laboratory received eight blinded, spiked saliva samples (approximately 1 ml of each). The laboratories were situated in Finland, Belgium, England, Japan and Denmark. Not all laboratories were able to measure all three components. Of the five participating laboratories two were able to measure melatonin, four measured cortisol (one laboratory used two different methods), and three measured testosterone. Different methods were used by the participating laboratories for salivary melatonin; LC-MS/MS (one laboratory) and ELISA (one laboratory); for salivary cortisol: RIA, (Coat-A-Count from DPC (one laboratory), ELISA (one laboratory), LC-MS/MS (four laboratories); and for salivary testosterone LC-MS/MS (two laboratories) and ELISA (one laboratory). The participating laboratories were asked to give information on laboratory performance of their method, i.e. limit of detection, reference interval and whether the method has been published. Sample purification before analyses were liquid-liquid extraction for laboratories using LC-MS/MS. No pre-treatment was used before analysis with ELISA and RIA. Table I gives an overview of pre-treatment methods for the different methods.

The study was carried out between March 2011 and June 2011.

**Preparation of spiked saliva samples**

The eight saliva samples were prepared from a pool of natural saliva samples collected from healthy female volunteers late in the afternoon and spiked with cortisol (purity ≥ 97%), melatonin (purity ≥ 98%), and testosterone (purity ≥ 99%). Cortisol, melatonin, and testosterone were purchased from Sigma–Aldrich (St Louis, MO). Stock solutions of hormones were prepared in 20% methanol and used to spike the saliva. Final percentage of stock solution (20% methanol) in the spiked saliva samples was 5% and the total amount of methanol in the spiked saliva samples was 1%. Female saliva collected late in the afternoon was chosen to obtain a low level of all three hormones. Saliva was collected drooling directly into tubes. No swabs or tampons were used. The natural saliva was spiked with the three hormones at seven levels in the following ranges: melatonin: 0.0–579.4 pmol/L, cortisol: 0.0–90.0 nmol/L, and testosterone: 0.0–622.8 pmol/L. The eighth sample was an unspiked natural saliva sample. The levels of spiked hormone were chosen to include the expected level of hormones in saliva. The samples were labelled A–H. Samples were frozen immediately after pre-preservation and sent by mail with freezing elements. Participating laboratories were instructed to note the date of delivery. The shipment time of samples to the participating laboratories were from 1–7 days. We have previously conducted a stability test [10]. For long-term stability, samples were measured by LC-MS/MS immediately after production and then kept at −20°C until measured again 3 months later. To test short-term stability samples were left at room temperature and measured on day 1, 3 and 7. Samples were found to be stable for 3 months at −20°C, with a CV of 11.1%, 9.6% and 13.1% for melatonin, cortisol and testosterone, respectively. Further the samples were found to be stable in saliva at room temperature for at least 7 days, with a CV of 6.0%, 8.8% and 8.4% for melatonin, cortisol and testosterone, respectively. The used LC-MS/MS method had an inter-day precision of 17.5%, 14.3% and 15.2% for melatonin, cortisol and testosterone, respectively [10]. The determined CV% for long-term and short-term stabilities were below the interassay variation of the analysis for melatonin, cortisol and testosterone in saliva. Hence we conclude that storing the saliva samples 7 days at room temperature and 3 months at −20°C did not affect the measurement of melatonin, cortisol and testosterone [10].

**Statistics**

Win AMIQAS was used to establish a method evaluation for each laboratory as well as a method evaluation based on results from all laboratories [22,23]. Win AMIQAS is a non-commercial programme developed for method evaluation and quality control. The method evaluation function is based on a linear least squares regression analysis of the measured concentration versus the conventional true concentration of a series of method evaluation samples containing the component in the linear range of the
method. Significant deviations from the ideal slope ($\beta = 1.00$) and intercept ($\alpha = 0.00$) are expressions of the systematic effects. The content of natural saliva was estimated for each laboratory as the intercept in the un-weighted method evaluation divided by the slope. The slope was estimated by using the weighted method evaluation function and used as a measure of the recovery. We used a pure error lack of fit test for linearity. It was only possible to perform the test for linearity on the combined method evaluations.

Table I. Preparation of saliva samples before analysis of the five participating laboratories.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Method</th>
<th>Components</th>
<th>Sample pre-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory I</td>
<td>RIA</td>
<td>Cortisol</td>
<td>50 µL saliva, no further pre-treatment of samples</td>
</tr>
<tr>
<td>Laboratory I</td>
<td>LC-MS/MS</td>
<td>Melatonin, cortisol and testosterone</td>
<td>200 µL saliva</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LLE was carried out by adding 50 µL D$_4$-ISs and 1.0 mL ethyl acetate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Shaken for 45 min and centrifuged for 5 min at 3500 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frozen at –20°C for approximately 30 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The ethyl acetate layer was poured off and evaporated to dryness under nitrogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The residues were dissolved in 200 µL 10% methanol</td>
</tr>
<tr>
<td>Laboratory II</td>
<td>LC-MS/MS</td>
<td>Cortisol and testosterone</td>
<td>500 µL saliva</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LLE by adding to acetonitrile (1 mL) containing D$_4$-ISs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vortex-mixed for 30 s and centrifuged at 1000 g (4°C, 5min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The supernatant was diluted with water (1.5 mL)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Soli phase extraction (Strata-X cartridge), washing with water (2 mL) and methanol-water (7:3, v/v) (2 mL), the steroids were eluted with ethyl acetate (2 mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Evaporation to dryness and dissolved in ethanol (30 µl), added to a freshly prepared solution of 2-hydrazino-1-methylpyridine (10 µg) in ethanol (50 µL) containing 25 µg of trifluoroacetic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frozen at –60°C for 1 h. After removal of the solvent, the products were dissolved in the methanol–10 mM ammoniumformate (1:1, v/v, 30 µL)</td>
</tr>
<tr>
<td>Laboratory III</td>
<td>LC-MS/MS</td>
<td>Cortisol</td>
<td>200 µL saliva</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Add 200 µL internal standard (cortisol-D4) in acetonitrile; centrifugation</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Add of 2 mL CH$_2$Cl$_2$ to perform a liquid-liquid extraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Evaporation of the organic layer at 45°C under nitrogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Redissolution in 80 µL methanol:water:50/50</td>
</tr>
<tr>
<td>Laboratory IV</td>
<td>LC-MS/MS</td>
<td>Cortisol</td>
<td>200 µL saliva</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pipetted directly to the well of a 96-deep-well block (Abgene, Epsom UK). To this, 25 µL of D$_4$-ISs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The block was thermosealed (Abgene, Epsom UK) and vortexed for 1 min, then centrifuged at 1500 g for 10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Following centrifugation, the block was transferred directly to the autosampler for analysis; 50 µL of sample was injected into the liquid chromatography (LC) system</td>
</tr>
<tr>
<td>Laboratory V</td>
<td>ELISA</td>
<td>Melatonin</td>
<td>200 µL saliva add 25 µL pre-treatment solution</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vortexed and left for 10 min at room temperature, add 25 µL neutralizing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Centrifugation 5 min at 10,000 rpm</td>
</tr>
<tr>
<td>Laboratory V</td>
<td>ELISA</td>
<td>Cortisol and testosterone</td>
<td>20 µL saliva for cortisol and 50 µL for testosterone, no pre-treatment of samples</td>
</tr>
</tbody>
</table>

*All laboratories froze and centrifuged the saliva before analysis; LLE, Liquid-liquid-extraction; D$_4$-ISs, D$_4$-Internal standard of the component.
Tests for normal distribution of the standardized residuals were done by the N-score test. The test for normal distribution was done on both combined and individual method evaluations. The weighting used was $1/\mu$. No results were treated as outliers. The analytical methods were compared by calculating the slope and intercept.

**Results**

Figures 1, 2 and 3 show the observed concentrations of melatonin (Figure 1), cortisol (Figure 2) and testosterone (Figure 3) plotted against the concentration of spiked hormone. Laboratory V was unable to quantify melatonin concentrations above 76 pmol/L and therefore higher concentrations were excluded in the combined method evaluation for the two laboratories measuring salivary melatonin (Figure 1).

Tables II, III and IV present results from the method evaluation from each laboratory. The laboratories measuring salivary melatonin showed a recovery of 96% [CI: 91–100%] for the LC-MS/MS method and 97% [CI: 40–150] for the ELISA method. Confidence intervals were given for the recovery and the
intercept from the method evaluation. The confidence interval of recovery for both laboratories included 100, indicating that the recovery was not significantly different from 100%. The confidence intervals indicated that there were statistically significant differences between performances for individual laboratories. The largest variation was observed for the ELISA method. The recovery of melatonin in saliva was observed to be between 91 and 110% for the combined comparison of two laboratories. The laboratories estimated, based on linear regression intercept/slope, the natural content in the pool of natural saliva to be 0.278 pmol/L (Laboratory I) and 6.90 pmol/L (Laboratory V). The intercept of the methods evaluation showed the content to be 2.09 pmol/L and 6.71 pmol/L respectively indicating that the used method were able to measure low natural sample concentrations.

The recovery of cortisol varied between 83 and 100% for the combined comparison of six analyses, with the highest recovery for the laboratory using the ELISA method (130% [CI: 110–150%]). The confidence interval of recovery at Laboratory I (LC-MS/MS and RIA) included 100, indicating that the recovery was not significantly different from 100%. The natural content of cortisol in saliva was estimated to be between 0.56 and 6.72 nmol/L indicating a large variation in detecting a low concentration of cortisol in saliva. The content of natural cortisol estimated by the combined methods evaluation was 3.02 nmol/L. The intercept of the methods evaluation showed the content to be from 0.49–4.67 nmol/L. Two methods, Laboratory II (LC-MS/MS) and Laboratory V (ELISA) measured higher natural content of cortisol (6.72 and 3.53 nmol/L).

The recovery of testosterone was between 80 and 94% for the combined comparison of three laboratories, with the lowest recovery in one of the laboratories using an LC-MS/MS method (60% [CI: –1.89–120%]). The confidence interval of recovery at Laboratories I, II and V included 100, indicating that the recovery was not significantly different from 100%. The content of salivary testosterone was measured to be 11.9–73.8 pmol/L in natural saliva, indicating a large variation in detecting a low concentration of testosterone in saliva. The intercept of the methods evaluation showed the content to be from 11.7–44.5 pmol/L.

**Discussion**

The present study reports results from an interlaboratory comparison for salivary melatonin, cortisol and
testosterone. The samples used were prepared from a pool of natural saliva spiked with melatonin, cortisol and testosterone. The recovery of the spiked material ranged from 91–110%, 83–100% and 80–94% for melatonin, cortisol and testosterone, respectively. No matrix certified reference materials are available for melatonin, cortisol or testosterone in saliva. Hence, due to lack of traceability, i.e. that the result of the measurements can be tracked back, through an unbroken chain of comparisons, to a National or international standard [24], it is not possible to establish the true concentration in the samples. The content of hormones in the natural saliva were estimated by the combined method evaluation for all laboratories and were found to be 2.18 pmol/L for melatonin, 3.02 nmol/L for cortisol and 37.9 pmol/L for testosterone. The evaluation of the natural content of hormones in saliva was based on the combined method evaluation without any judgement of the individual laboratories results. Therefore the natural content of hormones in saliva estimated by this combined method has a high level of uncertainty.

The confidence intervals for the ELISA analysis of melatonin were from 40–150% indicating that the recovery was not significantly different from 100%; however the results show a large dispersion of values indicating some problems with calibration. All steps in an analytical procedure may introduce uncertainty to the final measured concentration [25]. The ELISA method included sample purification and concentrating step and the LC-MS/MS method included a purification step with an internal standard. Using an internal standard for the purification step in the LC-MS/MS method may have diminished the added variance in the LC-MS/MS method. However, without an external reference method or certified reference materials it is not possible to determine if the LC-MS/MS method yield more true results.

The recovery of the spiked cortisol was between 69 and 130%. The lowest recovery was obtained by a LC-MS/MS method and the largest was obtained by the laboratory using an ELISA. Three LC-MS/MS methods and the ELISA method had recoveries where the confidence intervals that did not cover 100%, whereas recoveries in the two other methods assay did include 100%. The differences may originate from calibration, as calibration make up a basic link in the traceability chain [26,27]. However, it can also be due to the different clean-up procedures. LC-MS/MS needs a pre-treatment before analysis, which was not the case for the immune assays. On the other hand immunochemistry-based methods are highly sensitive, but may suffer inherently from a potential risk of cross-reactivity to structurally similar compounds [13].

The recovery of testosterone was between 60 and 98%. There were substantial differences between the two laboratories using LC-MS/MS (Laboratories I and II) for the analysis of salivary testosterone. This is somewhat surprising since they used similar methods. This indicates calibration issues and possibly interference or other analytical errors. Again we do not have matrix certified reference materials available for testosterone in saliva and therefore we cannot establish the true concentration in saliva. The discrepancy may be due to traceability, clean-up procedures or problems with calibration.

In the absence of matrix certified reference materials, a laboratory comparison is an alternative when you wish to compare results obtained from different laboratories.

Table III. Method performance parameters for the measurement of cortisol (concentration range 0–90 nmol/L). Slope and intercept with confidence intervals were computed by linear regression. The natural content of cortisol in saliva was computed using the formula intercept/slope.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Method</th>
<th>Recovery (%)</th>
<th>95% Confidence interval (%)</th>
<th>Intercept (nmol/L)</th>
<th>95% Confidence interval (%)</th>
<th>Natural content of saliva (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>LC-MS/MS</td>
<td>100</td>
<td>[95–110]</td>
<td>0.56</td>
<td>[–1.09–2.21]</td>
<td>0.56</td>
</tr>
<tr>
<td>I</td>
<td>RIA</td>
<td>88</td>
<td>[74–100]</td>
<td>2.76</td>
<td>[–2.98–8.45]</td>
<td>3.13</td>
</tr>
<tr>
<td>II</td>
<td>LC-MS/MS</td>
<td>69</td>
<td>[60–78]</td>
<td>4.66</td>
<td>[0.80–8.52]</td>
<td>6.72</td>
</tr>
<tr>
<td>III</td>
<td>LC-MS/MS</td>
<td>77</td>
<td>[72–82]</td>
<td>0.49</td>
<td>[–1.40–2.38]</td>
<td>0.63</td>
</tr>
<tr>
<td>IV</td>
<td>LC-MS/MS</td>
<td>82</td>
<td>[66–99]</td>
<td>2.95</td>
<td>[–6.36–12.3]</td>
<td>3.58</td>
</tr>
<tr>
<td>All laboratories</td>
<td></td>
<td>92</td>
<td>[83–100]</td>
<td>2.77</td>
<td>[–1.70–27.24]</td>
<td>3.02</td>
</tr>
</tbody>
</table>

Table IV. Method performance parameters for the measurement of testosterone (concentration range 0–600 pmol/L). Slope and intercept with confidence intervals were computed by linear regression. The natural content of testosterone in saliva was computed using the formula intercept/slope.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Method</th>
<th>Recovery (%)</th>
<th>95% Confidence interval (%)</th>
<th>Intercept (pmol/L)</th>
<th>95% Confidence interval (%)</th>
<th>Natural content of saliva (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>LC-MS/MS</td>
<td>98</td>
<td>[93–100]</td>
<td>11.7</td>
<td>[–3.35–26.7]</td>
<td>11.9</td>
</tr>
<tr>
<td>II</td>
<td>LC-MS/MS</td>
<td>60</td>
<td>[–1.89–120]</td>
<td>44.5</td>
<td>[18.9–70.2]</td>
<td>73.8</td>
</tr>
<tr>
<td>V</td>
<td>ELISA</td>
<td>87</td>
<td>[68–110]</td>
<td>42.9</td>
<td>[–9.3–95.0]</td>
<td>49.5</td>
</tr>
<tr>
<td>All laboratories</td>
<td></td>
<td>87</td>
<td>[80–94]</td>
<td>33.0</td>
<td>[11.9–54.2]</td>
<td>37.9</td>
</tr>
</tbody>
</table>
laboratories. However this study was not able to establish a target value for the natural content of melatonin, cortisol and testosterone in saliva. This emphasizes the need for a commercial proficiency-testing scheme for melatonin, cortisol, and testosterone in saliva. The natural samples were prepared from a pool of saliva collected in the late afternoon from healthy female volunteers. This should ensure that the natural content of all three hormones is relative low [28–30].

The concentration levels of spiked hormones in this study is many times higher that the natural content in the saliva. This high level of added hormone in relation to matrix could have made it difficult to check the specificity of the procedures.

Processing of saliva (e.g. pooling, spiking, shipment, etc.) may alter the matrix or cause ‘artificial’ matrix effects in comparison to the natural saliva sample. Although no reference saliva is available it would have been an advantage to use cortisol and testosterone dissolved in methanol in which levels are certified and traceable to reference standards. This would have eliminated the added uncertainty of weighing the reference standards. It is almost impossible to evaluate the combined effect of these different processing steps and the induced uncertainty may be so large that it overrules the effects of the different methods used. Accordingly processing has been kept to a minimum in this study, however it cannot be excluded that processing of the saliva could have affected the results. The stability of spiked melatonin, cortisol and testosterone in saliva has previously been studied and it was shown that all three components are stable in saliva at room temperature for at least 7 days [10]. None of the participating laboratories reported a shipment period longer than a week. However, samples may have been exposed to more extreme temperatures during shipment by mail.

In this study, all the saliva used was collected by drooling directly into a tube. However many laboratories use different techniques for collecting saliva such as cotton or synthetic swabs, some also use procedures for stimulating saliva flow. It is likely the discrepant results between various methods would have been even larger if the preferred saliva collection devices for each laboratory had been used. Hence collection of saliva should be taken into consideration when comparing results from different laboratories.

Saliva is becoming increasingly popular in both research and as a tool for diagnosing endocrine disorders, such as Cushing’s syndrome and hyperandrogenism in females. However careful consideration needs to be taken when comparing results from different laboratories.

In conclusion the methods generally perform optimally within laboratories as long as each laboratory establishes their own reference interval. Inclusion of interlaboratory variation adds considerably to the analytical variation. Again, this shows the need for establishing traceability of the results. The present study emphasizes the need for a proficiency testing scheme and a certified reference material for melatonin, testosterone and cortisol in saliva.

Acknowledgements
None.

Declaration of interest: The authors have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References
An interlaboratory comparison


Appendix I

Material given to participants in the “In the Middle of the Night” project
### Materiale til projekt MIDT OM NATTEN

**Deltagerens navn:** Xxxxx Yyyyyy  
**Deltagerens løbenummer:** 20001  
**Deltagerens Actigraph nummer:** H001

**Deltagerens pose skal indeholde følgende:**
- Samtykkeerklæring
- Forsøgspersonens rettigheder i et biomedicinsk forskningsprojekt
- Deltagerinstruktion
- Eksempel på intensiv måledag
- Personlig forsøgsplan
- Baggrundsspørgeskema
- Afsluttende spørgeskema

**Intervention 2+2**
- Svarkonvolut
- Søvndagbog
- 2 x Logbog
- Spytprøvemateriale
- Labels
- Urinprøvemateriale

**Intervention 4+4**
- Svarkonvolut
- Søvndagbog
- 2 x Logbog
- Spytprøvemateriale
- Labels
- Urinprøvemateriale

**Intervention 7+7**
- Svarkonvolut
- Søvndagbog
- 2 x Logbog
- Spytprøvemateriale
- Labels
- Urinprøvemateriale
**Deltagerinstruktion**

Tak fordi du vil være med i projekt MIDT OM NATTEN

Som deltager i projektet arbejder du i tre forskellige skift (interventioner):

- 2 nattevagter efterfulgt af 2 fridage/dagvagter
- 4 nattevagter efterfulgt af 4 fridage/dagvagter
- 7 nattevagter efterfulgt af 2 fridage/dagvagter

Som deltager beder vi dig om at udfylde nogle spørgeskemaer og afgive spyt og urinprøver, samt at bære nogle måleinstrumenter. I dette dokument kan du læse om de forskellige dele af projektet. Se også oversigten over de tre interventioner og eksemplerne på de intensive måledage.

**Baggrundsspørgeskema**


**Søvndagbog**


**Søvnåmåling**


Actigraph registrerer din arms bevægelse:

- Actigraph bæres på den **ikke-dominante arm**. Det vil sige højre arm, hvis du er venstreåndet og venstre arm, hvis du er højreåndet.
- Actigraph tages kun af **når du går i bad**, da det er stænktaet, men ikke vandtæt.

Actigraph-målingerne bruges til at vurdere både, hvornår og hvor længe du sover. Der sidder et nummer på siden af Actigraph, fx H183, det skal skrives på forsiden af din søvndagbog.

**Spytprøve**

Sådan tages spytprøven:

- **Knæk** proppen af røret.
- **Fyld** røret med ca. 1 ml spyt (til mærket) – det tager ca. to minutter. Lad det tynde spyt løbe ned i røret eller skub det ned i røret med tungen. Tænk på en citron eller lækkere mad, hvis du er tør i munden.
- **Udfyld** en etikette og sæt den på røret.
- **Læg** prøven i den medfølgende pose og i et køleskab eller en fryser.


**Logbog**


**Urinprøver**

Når du udfylder den sidste side i søvndagbøgerne (i alt 3 dage), beder vi dig afgive en morgenurinprøve.

- Vask hænder umiddelbart inden urinprøven tages.
- Tis ned i koppen uden brug af andre beholdere.
- Hæld fra koblen over i to rør til mærket, ca. 10 ml.
- Skru lågane fast på rørene.
- Udfyld 2 etiketter og sæt dem på rørene. Det er de samme etiketter, som bruges til spytprøverne.
- Læg rørene i den medfølgende pose og læg dem i køleskabet.

Urinprøverne analyseres for melatonin. Denne måling bruges til at undersøge, hvor meget melatonin du udskiller, mens du sover.

**Afsluttende spørgeskema**

Når du har gennemført alle tre interventioner, beder vi dig oplyse, hvilken intervention du foretræk. Dette spørgeskema sendes ind sammen med materialet fra din sidste intervention.

**Indsendelse af materiale**

det også dit baggrundsspørgeskema og efter sidste intervention også det afsluttende spørgeskema. Der følger en svarkuvert med til hver intervention.

Måling af hjerterytme – kun for deltagere på vagtcentral!

På dagen for sidste nattevagt og den sidste dag i hver intervention (i alt 6 dage) beder vi deltagere fra vagtcentralerne om at få målt deres hjerterytme. Dette gøres ved hjælp af Actiheart.

- Actiheart består af to elektroder, som sættes på brystkassen
- Første gang sætter en medarbejder fra MIDT OM NATTEN elektroderne på
- Det gør vi aftenen inden din nattevagt
- De næste gange sætter du selv elektroderne på. Det gøres om morgenen inden din sidste nattevagt eller den sidste dag i hver intervention.


Hvis du har spørgsmål er du velkommen til at kontakte os.

Med venlig hilsen

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Jesper Kristiansen, seniorforsker
Marie Aarrebo Jensen, ph.d. studerende

Åse Marie Hansen, professor
Kirsten Nabe-Nielsen, post doc
<table>
<thead>
<tr>
<th>Dato</th>
<th>Aktion</th>
</tr>
</thead>
<tbody>
<tr>
<td>22. april</td>
<td><strong>Start på intervention 4 + 4</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Dag 1 nattevagt</strong></td>
</tr>
<tr>
<td></td>
<td>• Tag Actigraph på når du vågner</td>
</tr>
<tr>
<td></td>
<td>• Mød ind på din første nattevagt</td>
</tr>
<tr>
<td></td>
<td>• Udfyld baggrundsspørgeskema</td>
</tr>
<tr>
<td>23. april</td>
<td><strong>Dag 2 nattevagt</strong></td>
</tr>
<tr>
<td></td>
<td>• Udfyld søvndagbogen</td>
</tr>
<tr>
<td>24. april</td>
<td><strong>Dag 3 nattevagt</strong></td>
</tr>
<tr>
<td></td>
<td>• Udfyld søvndagbogen</td>
</tr>
<tr>
<td></td>
<td>• Påsætning af Actiheart af en fra projekt Midt Om Natten – vi ringer og aftaler tid</td>
</tr>
<tr>
<td>25. april</td>
<td><strong>Dag 4 nattevagt</strong></td>
</tr>
<tr>
<td></td>
<td>• Tag spytprøver hver 4. time – husk første spytprøve når du vågner</td>
</tr>
<tr>
<td></td>
<td>• Udfyld søvndagbogen</td>
</tr>
<tr>
<td></td>
<td>• Udfyld logbogen samtidig med spytprøverne</td>
</tr>
<tr>
<td>26. april</td>
<td><strong>Dag 5 fri/formiddagsvagt</strong></td>
</tr>
<tr>
<td></td>
<td>• Tag dit Actiheart af når du vågner</td>
</tr>
<tr>
<td></td>
<td>• Udfyld søvndagbogen</td>
</tr>
<tr>
<td></td>
<td><strong>Når denne intervention er slut, sender du begge Actiheart med politiets interne post.</strong></td>
</tr>
<tr>
<td>27. – 28. april</td>
<td><strong>Dag 6-7 fri/formiddagsvagt</strong></td>
</tr>
<tr>
<td></td>
<td>• Udfyld søvndagbogen</td>
</tr>
<tr>
<td>29. april</td>
<td><strong>Dag 8 fri/formiddagsvagt</strong></td>
</tr>
<tr>
<td></td>
<td>• Tag spytprøver hver 4. time – husk første spytprøve når du vågner</td>
</tr>
<tr>
<td></td>
<td>• Når du vågner, sætter du selv det andet Actiheart på brystet</td>
</tr>
<tr>
<td></td>
<td>• Udfyld søvndagbogen</td>
</tr>
<tr>
<td></td>
<td>• Udfyld logbogen samtidig med spytprøverne</td>
</tr>
<tr>
<td>30. april</td>
<td><strong>Dag 9</strong></td>
</tr>
<tr>
<td></td>
<td>• Tag morgen urinprøve.</td>
</tr>
<tr>
<td></td>
<td>• Tag dit Actiheart af når du vågner</td>
</tr>
<tr>
<td></td>
<td>• Udfyld de sidste sider i søvndagbogen</td>
</tr>
<tr>
<td></td>
<td>• Pak søvndagbog, logbøger, Actigraph, baggrundsspørgeskema, spytprøver og urinprøver i svarkuvert og send med posten</td>
</tr>
<tr>
<td></td>
<td>• Pak begge Actiheart i svarkuverten mærket “Actiheart” og send med intern post.</td>
</tr>
</tbody>
</table>

**Du vil modtage en ny Actigraph til næste intervention.**

| 27. maj   | **Start på intervention 7 + 7**                                       |
|           | **Dag 1 nattevagt**                                                    |
|           | • Tag Actigraph på når du vågner                                        |
|           | • Mød ind på din første nattevagt                                       |
| 28. maj – 1. juni | **Dag 2-6 nattevagt**                                                 |
|           | • Udfyld søvndagbogen                                                  |
| 2. juni   | **Dag 7 nattevagt**                                                    |
|           | • Tag spytprøver hver 4. time – husk første spytprøve når du vågner      |
|           | • Når du vågner, sætter du selv dit Actiheart på brystet                |
|           | • Udfyld søvndagbogen                                                  |
|           | • Udfyld logbogen samtidig med spytprøverne                            |

VEND
**Kære Xxxxx Yyyyyy**  
**Her er dit forløb i Projekt MIDT OM NATTEN**  

**Deltager 20001**

**Dato** | **Aktion**
---|---
3. juni | **Dag 8 fri/formiddagsvagt**  
- Tag dit Actiheart af når du vågner.  
- Du får besked, når der ligger et nyt Actiheart til dag 14  
- Udfyld søvndagbogen  
> Når denne intervention er slut, sender du begge Actiheart med politiets interne post.

4. – 8. juni | **Dag 9-13 fri/formiddagsvagt**  
- Udfyld søvndagbogen

9. juni | **Dag 14 fri/formiddagsvagt**  
- Tag spytprøver hver 4. time – husk første spytprøve når du vågner  
- Når du vågner, sætter du selv dit Actiheart på brystet  
- Udfyld søvndagbogen  
- Udfyld logbogen samtidig med spytprøverne

10. juni | **Dag 15**  
- Tag morgen urinprøve  
- Tag dit Actiheart af når du vågner.  
- Udfyld de sidste sider i søvndagbogen  
- Udfyld det afsluttende spørgeskema  
- Pak søvndagbog, logbøger, Actigraph, spytprøver og urinprøver i svarkuvert og send med posten  
> Pak begge Actiheart i svarkuverten mærket ”Actiheart” og send med intern post.

24. juni | **Start på intervention 2 + 2**  
**Dag 1 nattevagt**  
- Tag Actigraph på når du vågner  
- Mød ind på din første nattevagt

25. juni | **Dag 2 nattevagt**  
- Tag spytprøver hver 4. time – husk første spytprøve når du vågner  
- Når du vågner, sætter du selv dit Actiheart på brystet  
- Udfyld søvndagbogen  
- Udfyld logbogen samtidig med spytprøverne

26. juni | **Dag 3 fri/formiddagsvagt**  
- Udfyld søvndagbogen  
- Tag dit Actiheart af når du vågner.  
> Når denne intervention er slut, sender du begge Actiheart med politiets interne post.

27. juni | **Dag 4 fri/formiddagsvagt**  
- Tag spytprøver hver 4. time – husk første spytprøve når du vågner  
- Når du vågner, sætter du selv dit Actiheart på brystet  
- Udfyld søvndagbogen  
- Udfyld logbogen samtidig med spytprøverne

28. juni | **Dag 5 fri/formiddagsvagt**  
- Tag morgen urinprøve.  
- Tag dit Actiheart af når du vågner.  
- Udfyld de sidste sider i søvndagbogen  
- Pak søvndagbog, logbøger, spytprøver, urinprøver, Actigraph og afsluttende spørgeskema i svarkuvert og send med posten  
> Pak begge Actiheart i svarkuverten mærket ”Actiheart” og send med intern post.

**Projekt MIDT OM NATTEN takker for din deltagelse.**
Eksempel på hvordan en intensiv måledag med nattevagt kan se ud

<table>
<thead>
<tr>
<th>Tid</th>
<th>Aktivitet</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.30</td>
<td>Du spiser og noterer tidspunktet i logbogen.</td>
</tr>
<tr>
<td>15.00</td>
<td>Du indsamler spyt og udfylder logbogen.</td>
</tr>
<tr>
<td>19.00</td>
<td>Du indsamler spyt og udfylder logbogen.</td>
</tr>
<tr>
<td>20.00</td>
<td>Du spiser aftensmad og noterer tidspunktet i logbogen.</td>
</tr>
<tr>
<td>21.00</td>
<td>Du tager en lur og noterer tidspunktet i logbogen.</td>
</tr>
<tr>
<td>23.00</td>
<td>Du møder ind på nattevagt. Du indsamler spyt og udfylder logbogen.</td>
</tr>
<tr>
<td>02.00</td>
<td>Du spiser en snack og noterer tidspunktet i logbogen.</td>
</tr>
<tr>
<td>03.00</td>
<td>Du afgiver spytprøve og udfylder logbogen.</td>
</tr>
<tr>
<td>07.00</td>
<td>Din vagt slutter. Du indsamler spyt og udfylder logbogen.</td>
</tr>
<tr>
<td>07.45</td>
<td>Du indsamler spyt og går i seng. Sov godt.</td>
</tr>
</tbody>
</table>
Eksempel på hvordan en intensiv måledag med formiddagsvagt kan se ud

06.00  Du vågner og tager din første spytprøve med det samme. Du tager Actiheart på. Herefter udfylder du søvndagbogen.
06.30  Du spiser morgenmad og noterer tidspunktet i logbogen.
07.00  Du møder ind på din vagt. Du indsamler spyt og udfylder logbogen.
10.00  Du spiser en snack og noterer tidspunktet i logbogen.
11.00  Du indsamler spyt og udfylder logbogen.
12.00  Du spiser frokost og noterer tidspunktet i logbogen.
15.00  Din vagt slutter. Du indsamler spyt og udfylder logbogen.
19.00  Du indsamler spyt og udfylder logbogen.
19.30  Du spiser aftensmad og noterer tidspunktet i logbogen.
22.30  Du tager en spytprøve og går i seng. Sov godt.
Baggrundsspørgeskema
Kære deltager i projekt MIDT OM NATTEN.

Spørgsmålene i spørgeskemaet handler om arbejdstider, søvn, helbred og trivsel. Dine svar er vigtige for os og for Politiet. Du skal ikke bruge for lang tid på spørgsmålene, men svare det, der først falder dig ind - der er ingen rigtige eller forkerte svar!

Vi behandler din besvarelse strengt fortroligt.

Det tager ca. 20 min at besvare spørgeskemaet. Du svarer på de fleste spørgsmål ved at sætte et kryds. Ved nogle spørgsmål skal du skrive et tal eller få ord.

Eksempel på talbesvarelse


Eksempel på afkrydsning

3. På hvilke tider af døgnet arbejder din ægtefælle/samleven primært?
   (Sæt kun ét krys)

   Døgningssagter ............................................................... [1]
   Aftensagter ..................................................................... [2]
   Nattetid ........................................................................... [3]
   Skiftende arbejdstider ...................................................... [4]
   Ikke relevant .................................................................... [5]

Kommer du til at sætte krydset i en forkert boks, så streg hele boksen ud og sæt kryds i den rigtige boks.

Hvis du har spørgsmål eller brug for hjælp til at besvare spørgeskemaet, er du altid velkommen til at kontakte os. Tak fordi du vil deltage i undersøgelsen. Vi glæder os til at modtage din besvarelse.

Med venlig hilsen

Marie Aarrebo Jensen, tlf.: 40 83 21 63, e-mail: maa@arbejdsmiljoforskning.dk

Anne Helene Garde, tlf.: 39 16 52 58, e-mail: ahg@arbejdsmiljoforskning.dk

Projektgruppen MIDT OM NATTEN består af:

Anne Helene Garde, seniorforsker
Jesper Kristiansen, seniorforsker
Marie Aarrebo Jensen, ph.d.-studerende
Åse Marie Hansen, professor
Kirsten Nabe-Nielsen, post doc
Baggrund

1. Hvilket år er du født? .................................................................
2. Bor du sammen med ægtefælle/samlever? (Sæt kun ét kryds)
   Ja ............................................  □ 1
   Nej .......................................... □ 2
3. På hvilke tider af døgnet arbejder din ægtefælle/samlever primært?
   (Sæt kun ét kryds)
   Dagarbejde ......................... □ 1
   Aftenarbejde ......................... □ 2
   Natarbejde ............................ □ 3
   Skiftende arbejdstider ............. □ 4
   Ikke relevant .......................... □ 5
4. Hvor mange børn er der i din husstand? .................................
   (Medtag bonus og delebørn. Skriv 0 hvis du ikke har børn og gå til spørgsmål 5)
   4.1 Hvor mange af dine børn er under 7 år? ..............................
5. Hvor mange år har du været ansat indenfor politiet? ....................
6. Hvor mange år af dit liv har du haft nattevagter? ........................

Motivation

7. Hvor tilfreds er du med dit job som helhed - alt taget i betragtning?
   (Sæt kun ét kryds)
   Meget utilfreds ....................... □ 1
   Utilfreds .................................. □ 2
   Tilfreds ................................... □ 3
   Meget tilfreds .......................... □ 4
8. Hvis du helt frit kunne bestemme dine vagter, hvor ofte vil du så arbejde ...
(Sæt kun ét kryds i hver linje)

<table>
<thead>
<tr>
<th></th>
<th>Aldrig</th>
<th>Sjældent</th>
<th>En gang imellem</th>
<th>Ofte</th>
<th>Altid</th>
</tr>
</thead>
<tbody>
<tr>
<td>om dagen?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
<td></td>
</tr>
<tr>
<td>om aftenen?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
<td></td>
</tr>
<tr>
<td>om natten?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
<td></td>
</tr>
</tbody>
</table>

9. Hvor mange nattevagter kan du bestå lide at have i træk? .................
(Skriv antal, skriv kun ét tal)

Hvorfor?

________________________________________________________________________
________________________________________________________________________

10. Alt taget i betragtning hvor belastende oplever du det at have nattevagter?

<table>
<thead>
<tr>
<th>Slet ikke belastende</th>
<th>Særdeles belastende</th>
</tr>
</thead>
<tbody>
<tr>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

11. Hvilke fordele oplever du ved at arbejde om natten?
(Sæt gerne mere end ét kryds)

<table>
<thead>
<tr>
<th>Ingen fordele</th>
<th>□ 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Det giver afveksling i hverdagen</td>
<td>□ 2</td>
</tr>
<tr>
<td>Det passer godt sammen med mit privatliv/familieliv</td>
<td>□ 3</td>
</tr>
<tr>
<td>Jeg kan godt lide at have fri fra arbejde i dagtimerne</td>
<td>□ 4</td>
</tr>
<tr>
<td>Det er en økonomisk fordel</td>
<td>□ 5</td>
</tr>
<tr>
<td>Der er mere spændende arbejdsopgaver</td>
<td>□ 6</td>
</tr>
<tr>
<td>Der er en bedre stemning på arbejdet om natten</td>
<td>□ 7</td>
</tr>
</tbody>
</table>

Andet? Skriv:
### Arbejde og privatliv

12. Sker det, at der er konflikt mellem dit arbejde og privatliv, sådan at du helst vil være begge steder på én gang?

(Sæt kun ét kryds)

- Ja, ofte ........................................... 1
- Ja, jævnligt ...................................... 2
- Sjældent ......................................... 3
- Nej, aldrig ..................................... 4


(Sæt kun ét kryds i hver linje)

<table>
<thead>
<tr>
<th></th>
<th>Ja, helt sikkert</th>
<th>Ja, til en vis grad</th>
<th>Ja, en men lidt</th>
<th>Nej</th>
<th>Slet ikke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Føler du, at dit arbejde tager så meget af din tid, at det går ud over dit privatliv?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Føler du, at dit arbejde tager så meget af din energi, at det går ud over dit privatliv?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

### Søvn


(Sæt kun ét kryds i hver linje. Medregn ikke ferie)

<table>
<thead>
<tr>
<th></th>
<th>Aldrig</th>
<th>Sjældent</th>
<th>En gang imellem (en eller flere gange om måneden)</th>
<th>For det meste (en eller flere gange om ugen)</th>
<th>Altid (stort set hver dag)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Har du haft svært ved at falde i søvn?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Har du haft svært ved at vågne?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Er du vågnet for tidligt uden at kunne falde i søvn igen?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Har du følt, at du ikke var udhvilet, når du vågnede?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Er du vågnet flere gange og har haft svært ved at falde i søvn igen?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Har du følt dig udmattet, når du vågnede?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
15. Hvordan vurderer du din samlede søvnkvalitet?

(Sæt kun ét kryds)

Fremragende .........................  □ 1
Vældig god ..........................  □ 2
God .......................................  □ 3
Mindre god ..........................  □ 4
Dårlig ...................................  □ 5

16. Hvor mange timer har du sovet gennemsnitligt i døgnet inden for de sidste 4 uger?

(Sæt kun ét kryds)

5 timer eller mindre .................  □ 1
6 timer ..................................  □ 2
7 timer ..................................  □ 3
8 timer ..................................  □ 4
9 timer ..................................  □ 5
10 timer eller mere ..................  □ 6

17. Hvis du skal beskrive dig selv som morgenmenneske eller aftenmenneske, hvad er du så?

(Sæt kun ét kryds)

Helt sikkert morgenmenneske ......  □ 1
Mere morgenmenneske end aftenmenneske ..........................  □ 2
Mere aftenmenneske end morgenmenneske ..........................  □ 3
Helt sikkert aftenmenneske ........  □ 4
18. De næste spørgsmål handler om din evne til at sove og arbejde på forskellige tider af døgnet.

Svar på hvad du er i stand til og foretrækker, og ikke hvad du kan blive tvunget til i en arbejdssituation.

(Sæt kun ét kryds i hver linje)

<table>
<thead>
<tr>
<th>Spørgsmål</th>
<th>Aldrig</th>
<th>Sjældent</th>
<th>Nogle gange</th>
<th>Som regel</th>
<th>Altid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plejer du at have brug for mere søvn end andre mennesker? ..................</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Har du nemt ved at sove på forskellige tidspunkter af døgnet? ..............</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Er det svært for dig at vågne, hvis du bliver vækket på et usædvanligt tidspunkt? ..........</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Har du nemt ved at arbejde på forskellige tidspunkter af døgnet? ..........</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Føler du dig søvnig et stykke tid efter at være vågnet om morgenen? ........</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

19. De næste spørgsmål handler om dine vaner efter en nattevagt.

(Sæt kun ét kryds i hver linje)

<table>
<thead>
<tr>
<th>Spørgsmål</th>
<th>Aldrig</th>
<th>Sjældent</th>
<th>Nogle gange</th>
<th>Som regel</th>
<th>Altid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sover du i et mørkt rum? ............................................</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bruger du solbriller på vej hjem? ....................................</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sørger du for at sove uforstyrret? ...................................</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bruger du ørepropper for at minimere støj? ..</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tager du sovemedicin? ..................................................</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andet? Skriv hvad:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Vaner og livsstil

20. Hvor mange genstande drikker du sædvanligvis om ugen?
(1 genstand = 1 flaske øl eller 1 glas vin eller 2 cl. spiritus)

Sæt kun ét kryds

0 genstande ............................  □ 1
1-7 genstande .........................  □ 2
8-14 genstande .......................  □ 3
15-21 genstande .....................  □ 4
22-28 genstande .....................  □ 5
>28 genstande .......................  □ 6

21. Hvis du skal anføre dine fysiske aktiviteter i fritiden, herunder transport til og fra arbejde, inden for det sidste år, hvilken gruppe passer så på dig?

(Sæt kun ét kryds)

Næsten helt fysisk passiv eller let fysisk aktiv i mindre end 2 timer pr. uge .............................................  □ 1
Let fysisk aktivitet i 2-4 timer pr. uge ..........................  □ 2
Let fysisk aktivitet i mere end 4 timer pr. uge eller mere anstrengende fysisk aktivitet i 2-4 timer pr. uge ...............  □ 3
Mere anstrengende fysisk aktivitet i mere end 4 timer eller regelmæssig hård træning og evt. konkurrencer flere gange om ugen ............................................................  □ 4

Almen helbredstilstand/velbefindende

22. Hvor høj er du? ................................................................. cm

23. Hvad vejer du? ............................................................... kg
24. Hvordan, synes du, dit helbred er, alt i alt?
(Sæt kun ét kryds)

Fremragende ............................  □ 1
Vældig godt ..............................  □ 2
Godt ........................................  □ 3
Mindre godt ..............................  □ 4
Dårligt .................................  □ 5

25. Lider du af en eller flere kronisk(e) sygdom(me)? (Sæt kun ét kryds)

Ja ............................................  □ 1
Nej ..........................................  □ 2

Hvis ja, skriv hvilke(n):

26. I løbet af de sidste 4 uger hvor meget har du været generet af ...
(Sæt kun ét kryds i hver linje)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Slet ikke</th>
<th>Lidt</th>
<th>Noget</th>
<th>En hel del</th>
<th>Virkelig meget</th>
</tr>
</thead>
<tbody>
<tr>
<td>hovedpine?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>svimmelhed eller tilløb til at besvime?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>smert i hjerte eller bryst?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>hurtig hjertebanken?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>lavtsiddende rygsmerten?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>uro i maven eller kvalme?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>muskelsmerter eller -spændinger?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>appetitløshed?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>løs afføring eller forstoppelse?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>oppustethed?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>at du føler dig svag i kroppen?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
<tr>
<td>at dine arme og ben føles tunge?</td>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>
27. I løbet af de sidste 4 uger hvor tit ...
(Sæt kun ét kryds i hver linje)

<table>
<thead>
<tr>
<th></th>
<th>Slet ikke</th>
<th>Lidt</th>
<th>Noget</th>
<th>En hel del</th>
<th>Virkelig meget</th>
</tr>
</thead>
<tbody>
<tr>
<td>har du været trist til mode?</td>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
<tr>
<td>har du manglet selvtillid?</td>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
<tr>
<td>har du haft dårlig samvittighed eller skyldfølelse?</td>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
<tr>
<td>har du manglet interesse for de ting, du foretager dig i dagligdagen?</td>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
<tr>
<td>har du haft problemer med at slappe af?</td>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
<tr>
<td>har du været irritabel?</td>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
<tr>
<td>har du været anspændt?</td>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
<tr>
<td>har du været stresset?</td>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
</tbody>
</table>
# Personlighed

**28. Hvordan er du som person?**

*(Sæt kun ét kryds i hver linje)*

<table>
<thead>
<tr>
<th></th>
<th>Ja</th>
<th>Nej</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Går dit humør ofte op og ned?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>2. Er du snakkesaglig?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>3. Er du ret livlig?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>4. Føler du dig ofte led og ked af det hele?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>5. Vil du betegne dig selv som en nervøs person?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>6. Kan du nemt sætte fut i et temmelig kedeligt selskab?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>7. Gør du dig mange bekymringer?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>8. Plejer du at holde dig i baggrunden ved selskabelige lejligheder?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>9. Lider du af &quot;nerver&quot;?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>10. Er du for det meste tavs, når du er sammen med andre?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>11. Føler du dig ofte ensom?</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>12. Anser andre mennesker dig for at være livlig?</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

---

**Tak fordi du vil være forsøgsdeltager i projekt MIDT OM NATTEN. Vi glæder os til at modtage din besvarelse!**

---

Side 11
Actiwatch nr: ____________________________

SØVNDARBOG
Intervention 2+2
Udfyldes én gang i døgnet
- efter primærsøvn
Kære deltager i projekt MIDT OM NATTEN.


Vi behandler din besvarelse strengt fortroligt.


<table>
<thead>
<tr>
<th>Eksempel på talbesvarelse</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.1 Hvornår startede og sluttede din vagt?</td>
</tr>
<tr>
<td>Vagt start: 08:15</td>
</tr>
<tr>
<td>Vagt slut: 15:45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eksempel på afkrydsning</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.2 Er du tilfreds med kvaliteten af dit arbejde på din sidste vagt?</td>
</tr>
<tr>
<td>(Sæt kun ét kryds)</td>
</tr>
<tr>
<td>Meget utilfreds</td>
</tr>
<tr>
<td>☐ 1</td>
</tr>
</tbody>
</table>

Kommer du til at sætte krydset i en forkert boks, så streg hele boksen ud og sæt kryds i den rigtige boks.

Hvis du har spørgsmål eller brug for hjælp til at besvare spørgeskemaet, er du altid velkommen til at kontakte os. Tak fordi du vil deltage i undersøgelsen. Vi glæder os til at modtage din besvarelse.

Med venlig hilsen

Marie Aarrebo Jensen, tlf.: 40 83 21 63, e-mail: maa@arbejdsmiljoforskning.dk

Anne Helene Garde, tlf.: 39 16 52 58, e-mail: ahg@arbejdsmiljoforskning.dk

**Projektgruppen MIDT OM NATTEN består af:**

Anne Helene Garde, seniorforsker
Jesper Kristiansen, seniorforsker
Marie Aarrebo Jensen, ph.d.-studerende

Åse Marie Hansen, professor
Kirsten Nabe-Nielsen, post doc
Intervention 2+2
NAT 1

1. Dato og klokkeslæt .........................  
   Dag Måned År Time Minut

   Time Minut

3. Hvor lang tid var du om at falde i søvn? .................................
   Antal minutter

   Time Minut

5. Er du i løbet af din søvn blevet forstyrret af ...
   (Sæt ét kryds i hver linje)
   
   - lys? ...................................................
     □ 1 □ 2 □ 3 □ 4 □ 5
   - familiemedlemmer? ..............................
     □ 1 □ 2 □ 3 □ 4 □ 5
   - larm fra nabo/gade ..............................
     □ 1 □ 2 □ 3 □ 4 □ 5
   - temperatur - for varmt eller for koldt? ....
     □ 1 □ 2 □ 3 □ 4 □ 5
   - andet, skriv hvad: ________________________

6. Var det svært at falde i søvn?
   (Sæt kun ét kryds)
   
   Overhovedet ikke Meget lidt Noget Ganske meget Meget
   □ 1 □ 2 □ 3 □ 4 □ 5

7. Hvordan var søvnen?
   (Sæt kun ét kryds)
   
   Særdeles god Ganske god Hverken eller Ganske dårlig Særdeles dårlig
   □ 1 □ 2 □ 3 □ 4 □ 5
8. Sov du uroligt?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Slet ikke</th>
<th>Meget lidt</th>
<th>Noget</th>
<th>Ganske meget</th>
<th>Meget</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

9. Vågnede du for tidligt uden at kunne sove videre?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Slet ikke</th>
<th>Meget lidt</th>
<th>Noget</th>
<th>Ganske meget</th>
<th>Meget</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

10. Hvor mange gange vågnede du?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>0 gange</th>
<th>1 gang</th>
<th>2 gange</th>
<th>3 gange</th>
<th>4 gange eller flere</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

11. Var det let at stå op?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Meget let</th>
<th>Let eller Hverken</th>
<th>Svært</th>
<th>Meget svært</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

12. Hvor udhvilet er du?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Helt udhvilet</th>
<th>Meget udhvilet</th>
<th>Ganske udhvilet</th>
<th>Lidt udhvilet</th>
<th>Slet ikke udhvilet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

13. Indtog du alkohol eller medicin, inden du gik i seng?
(Sæt evt. flere krydser)

<table>
<thead>
<tr>
<th>Nej</th>
<th>Ja, sovemiddel</th>
<th>Ja, alkohol</th>
<th>Ja, smertestillende</th>
<th>Ja, andet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Andet, skriv hvad: ____________________________

14. Har du været på arbejde det sidste døgn (inden du lagde dig til at sove)?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Ja</th>
<th>Nej</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Hvis nej, gå til spørgsmål 15
14.1 Hvornår startede og sluttede din vagt?

Vagt start: ______:______

Vagt slut: ______:______

14.2 Er du tilfreds med kvaliteten af dit arbejde på din sidste vagt?

(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Meget utilfreds</th>
<th>Utilfreds</th>
<th>Hverken eller</th>
<th>Tilfreds</th>
<th>Meget tilfreds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

14.3 Hvordan var din vagt?

(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Meget travl</th>
<th>Hverken stil-le eller travl (normal)</th>
<th>Stille</th>
<th>Meget stille</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

15. Hvor anstrengende har det sidste døgn været?

Medtag både fritid og arbejde.

(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Overhovedet ikke anstrengende</th>
<th>Ikke anstrengende</th>
<th>Delvist anstrengende</th>
<th>Meget anstrengende</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

16. Har du, inden for det sidste døgn, en eller flere gange taget en lur eller powernap?

Medtag både fritid og arbejde.

(Sæt kun ét kryds)

Ja Nej

16.1 Hvis ja, skriv hvornår du faldt i søvn, og hvornår du vågnede for hver lur eller powernap.

Faldt i søvn kl: ______:______

Vågnde kl: ______:______

Faldt i søvn kl: ______:______

Vågnde kl: ______:______

Faldt i søvn kl: ______:______

Vågnde kl: ______:______
**Intervention 2+2**

**NAT 2**

### 1. Dato og klokkeslæt

- **Dag**: ____________
- **Måned**: ____________
- **År**: ____________
- **Time**: ____________
- **Minut**: ____________

### 2. På hvilket tidspunkt lagde du dig til at sove?

- **Time**: ____________
- **Minut**: ____________

### 3. Hvor lang tid var du om at falde i søvn?

- **Antal minutter**: ____________

### 4. På hvilket tidspunkt vågnede du?

- **Time**: ____________
- **Minut**: ____________

### 5. Er du i løbet af din søvn blevet forstyrret af ...

(Sæt ét kryds i hver linje)

- **ly?**
  - Slet ikke: □ 1
  - Meget lidt: □ 2
  - Noget: □ 3
  - Ganske meget: □ 4
  - Meget: □ 5

- **Familiemedlemmer?**
  - Slet ikke: □ 1
  - Meget lidt: □ 2
  - Noget: □ 3
  - Ganske meget: □ 4
  - Meget: □ 5

- **Larm fra nabo/gade**
  - Slet ikke: □ 1
  - Meget lidt: □ 2
  - Noget: □ 3
  - Ganske meget: □ 4
  - Meget: □ 5

- **Temperatur - for varmt eller for koldt?**
  - Slet ikke: □ 1
  - Meget lidt: □ 2
  - Noget: □ 3
  - Ganske meget: □ 4
  - Meget: □ 5

- **Andet, skriv hvad:** ____________________________

### 6. Var det svært at falde i søvn?

(Sæt kun ét kryds)

- **Overhovedet ikke**
  - □ 1
  - □ 2
  - □ 3
  - □ 4
  - □ 5

### 7. Hvordan var søvnen?

(Sæt kun ét kryds)

- **Særdeles god**
  - □ 1
  - □ 2
  - □ 3
  - □ 4
  - □ 5

- **Ganske god**
  - □ 1
  - □ 2
  - □ 3
  - □ 4
  - □ 5

- **Hverken eller**

- **Ganske dårlig**

- **Særdeles dårlig**

---

**Side 6**
8. Sov du uroligt?  
(Sæt kun ét kryds)  

<table>
<thead>
<tr>
<th>Slet ikke</th>
<th>Meget lidt</th>
<th>Noget</th>
<th>Ganske meget</th>
<th>Meget</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
</tbody>
</table>

9. Vågnede du for tidligt uden at kunne sove videre?  
(Sæt kun ét kryds)  

<table>
<thead>
<tr>
<th>Slet ikke</th>
<th>Meget lidt</th>
<th>Noget</th>
<th>Ganske meget</th>
<th>Meget</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
</tbody>
</table>

10. Hvor mange gange vågnede du?  
(Sæt kun ét kryds)  

<table>
<thead>
<tr>
<th>0 gange</th>
<th>1 gang</th>
<th>2 gange</th>
<th>3 gange</th>
<th>4 gange eller flere</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
</tbody>
</table>

11. Var det let at stå op?  
(Sæt kun ét kryds)  

<table>
<thead>
<tr>
<th>Meget let</th>
<th>Let</th>
<th>Hverken eller</th>
<th>Svært</th>
<th>Meget svært</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
</tbody>
</table>

12. Hvor udhvilet er du?  
(Sæt kun ét kryds)  

<table>
<thead>
<tr>
<th>Helt udhvilet</th>
<th>Meget udhvilet</th>
<th>Ganske udhvilet</th>
<th>Lidt udhvilet</th>
<th>Slet ikke udhvilet</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
</tbody>
</table>

13. Indtog du alkohol eller medicin, inden du gik i seng?  
(Sæt evt. flere krydser)  

<table>
<thead>
<tr>
<th>Nej</th>
<th>Ja, sovemiddel</th>
<th>Ja, alkohol</th>
<th>Ja, smertenstillende</th>
<th>Ja, andet</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1</td>
<td>☐ 2</td>
<td>☐ 3</td>
<td>☐ 4</td>
<td>☐ 5</td>
</tr>
</tbody>
</table>

Andet, skriv hvad: ____________________________

14. Har du været på arbejde det sidste døgn (inden du lagde dig til at sove)?  
(Sæt kun ét kryds)  

<table>
<thead>
<tr>
<th>Ja</th>
<th>Nej</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1</td>
<td>☐ 2</td>
</tr>
</tbody>
</table>

Hvis nej, gå til spørgsmål 15
14.1 Hvornår startede og sluttede din vagt?

Vagt start: ______:______
Time  Minut

Vagt slut: ______:______
Time  Minut

14.2 Er du tilfreds med kvaliteten af dit arbejde på din sidste vagt?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Meget utilfreds</th>
<th>Utilfreds</th>
<th>Hverken eller</th>
<th>Tilfreds</th>
<th>Meget tilfreds</th>
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<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

14.3 Hvordan var din vagt?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Meget travl</th>
<th>Hverken stille eller travl (normal)</th>
<th>Stille</th>
<th>Meget stille</th>
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</thead>
<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
</tr>
</tbody>
</table>

15. Hvor anstrengende har det sidste døgn været?

Medtag både fritid og arbejde.
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Overhovedet ikke anstrengende</th>
<th>Ikke anstrengende</th>
<th>Delvist</th>
<th>Anstrengende</th>
<th>Meget anstrengende</th>
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<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

16. Har du, inden for det sidste døgn, en eller flere gange taget en lur eller powernap?

Medtag både fritid og arbejde.
(Sæt kun ét kryds)

Ja  Nej

Ja, gå til spørgsmål 16.1

16.1 Hvis ja, skriv hvornår du faldt i søvn, og hvornår du vågnede for hver lur eller powernap.

<table>
<thead>
<tr>
<th>Faldt i søvn kl:</th>
<th>Vågnede kl:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time  Minut</td>
<td>Time  Minut</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Faldt i søvn kl:</th>
<th>Vågnede kl:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time  Minut</td>
<td>Time  Minut</td>
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</table>

<table>
<thead>
<tr>
<th>Faldt i søvn kl:</th>
<th>Vågnede kl:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time  Minut</td>
<td>Time  Minut</td>
</tr>
</tbody>
</table>
Intervention 2+2
DAG 1

1. Dato og klokkeslæt ...........................................  
   Dag  |  Måned  |  År  
   Time  |  Minut  

   Time  |  Minut  

3. Hvor lang tid var du om at falde i søvn? ................................. 
   Antal minutter 

   Time  |  Minut  

5. Er du i løbet af din søvn blevet forstyrret af ...  
   (Sæt ét kryds i hver linje) 
   Slet ikke  |  Meget lidt  |  Noget  |  Ganske meget  |  Meget 
   □ 1  |  □ 2  |  □ 3  |  □ 4  |  □ 5 
   □ 1  |  □ 2  |  □ 3  |  □ 4  |  □ 5 
   □ 1  |  □ 2  |  □ 3  |  □ 4  |  □ 5 
   □ 1  |  □ 2  |  □ 3  |  □ 4  |  □ 5 
   □ 1  |  □ 2  |  □ 3  |  □ 4  |  □ 5 

   andet, skriv hvad: ________________________________________________

6. Var det svært at falde i søvn?  
   (Sæt kun ét kryds) 
   Overhovedet ikke  |  Meget lidt  |  Noget  |  Ganske meget  |  Meget 
   □ 1  |  □ 2  |  □ 3  |  □ 4  |  □ 5 

7. Hvordan var søvnen?  
   (Sæt kun ét kryds) 
   Sædeles god  |  Ganske god  |  Hverken eller  |  Ganske dårlig  |  Sædeles dårlig 
   □ 1  |  □ 2  |  □ 3  |  □ 4  |  □ 5
8. Sov du uroligt?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Slet ikke</th>
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<th>Noget</th>
<th>Ganske meget</th>
<th>Meget</th>
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<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

9. Vågnede du for tidligt uden at kunne sove videre?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Slet ikke</th>
<th>Meget lidt</th>
<th>Noget</th>
<th>Ganske meget</th>
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<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

10. Hvor mange gange vågnede du?
(Sæt kun ét kryds)

0 gange | 1 gang | 2 gange | 3 gange | 4 gange eller flere

□ 1 □ 2 □ 3 □ 4 □ 5

11. Var det let at stå op?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Meget let</th>
<th>Let</th>
<th>Hverken eller</th>
<th>Svært</th>
<th>Meget svært</th>
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<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

12. Hvor udhvilet er du?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Helt udhvilet</th>
<th>Meget udhvilet</th>
<th>Ganske udhvilet</th>
<th>Lidt udhvilet</th>
<th>Slet ikke udhvilet</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

13. Indtog du alkohol eller medicin, inden du gik i seng?
(Sæt evt. flere krydser)

<table>
<thead>
<tr>
<th>Nej</th>
<th>Ja, sovemiddel</th>
<th>Ja, alkohol</th>
<th>Ja, smertestillende</th>
<th>Ja, andet</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

Andet, skriv hvad:

14. Har du været på arbejde det sidste døgn (inden du lagde dig til at sove)?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Ja</th>
<th>Nej</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
</tr>
</tbody>
</table>

Hvis nej, gå til spørgsmål 15
14.1 Hvornår startede og sluttede din vagt?
Vagt start: _______:______  Vagt slut: _______:______

14.2 Er du tilfreds med kvaliteten af dit arbejde på din sidste vagt?
(Sæt kun ét kryds)

14.3 Hvordan var din vagt?
(Sæt kun ét kryds)

15. Hvor anstrengende har det sidste døgn været?
Medtag både fritid og arbejde.
(Sæt kun ét kryds)

16. Har du, inden for det sidste døgn, en eller flere gange taget en lur eller powernap?
Medtag både fritid og arbejde.
(Sæt kun ét kryds)

16.1 Hvis ja, skriv hvornår du faldt i søvn, og hvornår du vågnede for hver lur eller powernap.

Faldt i søvn kl: _______:______  Vågnede kl: _______:______
Faldt i søvn kl: _______:______  Vågnede kl: _______:______
Faldt i søvn kl: _______:______  Vågnede kl: _______:______
**Intervention 2+2**

**DAG 2**

1. **Dato og klokkeslæt** ............................

2. **På hvilket tidspunkt lagde du dig til at sove?** ............................ kl.

3. **Hvor lang tid var du om at falde i søvn?** ............................

4. **På hvilket tidspunkt vågnede du?** ............................ kl.

5. **Er du i løbet af din søvn blevet forstyrret af ...**
   
   (Sæt ét kryds i hver linje)

   lys? .............................................................

   familiemedlemmer? ........................................

   larm fra nabo/gade ........................................

   temperatur - for varmt eller for koldt? ....

   andet, skriv hvad: ........................................

6. **Var det svært at falde i søvn?**
   
   (Sæt kun ét kryds)

7. **Hvordan var søvnen?**
   
   (Sæt kun ét kryds)
8. Sov du uroligt?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th></th>
<th>Slet ikke</th>
<th>Meget lidt</th>
<th>Noget</th>
<th>Ganske meget</th>
<th>Meget</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td></td>
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</table>

9. Vågnede du for tidligt uden at kunne sove videre?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th></th>
<th>Slet ikke</th>
<th>Meget lidt</th>
<th>Noget</th>
<th>Ganske meget</th>
<th>Meget</th>
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<td>1</td>
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</tbody>
</table>

10. Hvor mange gange vågnede du?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th></th>
<th>0 gange</th>
<th>1 gang</th>
<th>2 gange</th>
<th>3 gange</th>
<th>4 gange eller flere</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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</tbody>
</table>

11. Var det let at stå op?
(Sæt kun ét kryds)

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<thead>
<tr>
<th></th>
<th>Meget let</th>
<th>Let</th>
<th>Hverken eller</th>
<th>Svaert</th>
<th>Meget svært</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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</tbody>
</table>

12. Hvor udhvilet er du?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th></th>
<th>Helt udhvilet</th>
<th>Meget udhvilet</th>
<th>Ganske udhvilet</th>
<th>Lidt udhvilet</th>
<th>Slet ikke udhvilet</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td></td>
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</tr>
</tbody>
</table>

13. Indtog du alkohol eller medicin, inden du gik i seng?
(Sæt evt. flere kryds)

<table>
<thead>
<tr>
<th></th>
<th>Nej</th>
<th>Ja, sove-middel</th>
<th>Ja, alkohol</th>
<th>Ja, smerte-stillende</th>
<th>Ja, andet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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</tbody>
</table>

Andet, skriv hvad:

14. Har du været på arbejde det sidste døgn (inden du lagde dig til at sove)?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th></th>
<th>Ja</th>
<th>Nej</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
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</tbody>
</table>

Hvis nej, gå til spørgsmål 15
14.1 Hvornår startede og sluttede din vagt?
Vagt start: ______:______
Vagt slut: ______:______

14.2 Er du tilfreds med kvaliteten af dit arbejde på din sidste vagt?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Meget utilfreds</th>
<th>Utilfreds</th>
<th>Hverken eller</th>
<th>Tilfreds</th>
<th>Meget tilfreds</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

14.3 Hvordan var din vagt?
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Meget travl</th>
<th>Travl (normal)</th>
<th>Stille</th>
<th>Meget stille</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
</tr>
</tbody>
</table>

15. Hvor anstrengende har det sidste døgn været?
Medtag både fritid og arbejde.
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Overhovedet ikke anstrengende</th>
<th>Ikke anstrengende</th>
<th>Delvist</th>
<th>Anstrengende</th>
<th>Meget anstrengende</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

16. Har du, inden for det sidste døgn, en eller flere gange taget en lur eller powernap?
Medtag både fritid og arbejde.
(Sæt kun ét kryds)

<table>
<thead>
<tr>
<th>Ja</th>
<th>Nej</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1</td>
<td>□ 2</td>
</tr>
</tbody>
</table>

Hvis ja, gå til spørgsmål 16.1

16.1 Hvis ja, skriv hvornår du faldt i søvn, og hvornår du vågnede for hver lur eller powernap.

Faldt i søvn kl: ______:______
Vågnede kl: ______:______

Faldt i søvn kl: ______:______
Vågnede kl: ______:______

Faldt i søvn kl: ______:______
Vågnede kl: ______:______
Måltider og snacks

Udfyld tidspunkter for hovedmåltider (fx morgenmad, frokost, aftenmad)

<table>
<thead>
<tr>
<th>1. hovedmåltid kl.</th>
<th>2. hovedmåltid kl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Minut</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. hovedmåltid kl.</th>
<th>4. hovedmåltid kl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Minut</td>
</tr>
</tbody>
</table>

Udfyld tidspunkter for snacks (fx frugt, slik, mellemmåltider)

<table>
<thead>
<tr>
<th>Snack kl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
</tr>
</tbody>
</table>

Dato og starttidspunkt

Arbejder du dag eller nat og i hvilken intervention?

Sæt et kryds i det felt, der passer

Intervention 2+2

Nat □ Dag □

Intervention 4+4

Nat □ Dag □

Intervention 7+7

Nat □ Dag □

Fysisk aktivitet

Hvor meget har du siddet ned de sidste 4 timer? (Sæt ét kryds hver 4. time)

<table>
<thead>
<tr>
<th>Kl. 7</th>
<th>Kl. 11</th>
<th>Kl. 15</th>
<th>Kl. 19</th>
<th>Kl. 23</th>
<th>Kl. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 time</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
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<tr>
<td>1-2 timer</td>
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<td>□</td>
<td>□</td>
</tr>
<tr>
<td>2-3 timer</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>3-4 timer</td>
<td>□</td>
<td>□</td>
<td>□</td>
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</tbody>
</table>

Evt. Actiheart nr: ____________________________

Udfyldes på sidste nattevagt og sidste dagvagt i hver intervention. Startes samtidig med søvndagbogen, altså efter primært søvn. Udfyld kun de tidspunkter, hvor du er vågen.

LOGBOG

MÅLTIDER OG SNACKS

1. Hovedmåltid kl.: Time Minut

2. Hovedmåltid kl.: Time Minut

3. Hovedmåltid kl.: Time Minut

4. Hovedmåltid kl.: Time Minut

Sæt et kryds i det felt, der passer
<table>
<thead>
<tr>
<th>Træthed</th>
<th>Kl. 7</th>
<th>Kl. 11</th>
<th>Kl. 15</th>
<th>Kl. 19</th>
<th>Kl. 23</th>
<th>Kl. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hvordan har du haft det den sidste time?</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1 meget vågen</td>
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<tr>
<td>2</td>
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<tr>
<td>3 vågen</td>
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<td>4</td>
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<td></td>
</tr>
<tr>
<td>5 hverken vågen eller søvnig</td>
<td></td>
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<tr>
<td>6</td>
<td></td>
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<tr>
<td>7 søvnig, men ikke anstrengende at holde sig vågen</td>
<td></td>
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<td>8</td>
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<tr>
<td>9 meget søvnig, anstrengende at være vågen, kæmper med søvnen</td>
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<tr>
<td>2 i meget ringe grad</td>
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<tr>
<td>3 delvist</td>
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<tr>
<td>4 meget</td>
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<tr>
<td>5 særdeles meget</td>
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<table>
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<tr>
<th>Jeg har følt mig energiforladt?</th>
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<td>2 i meget ringe grad</td>
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<td>5 særdeles meget</td>
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<table>
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<td>5 særdeles meget</td>
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<tr>
<th>Jeg har følt mig afslappet?</th>
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<th>Jeg har følt mig presset?</th>
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<td>4 meget</td>
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<tr>
<td>5 særdeles meget</td>
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</tbody>
</table>
Mange tak fordi du deltog i projekt MIDT OM NATTEN!
De følgende spørgsmål handler om, hvordan du har oplevet de forskellige typer af natarbejde.

1. Dato: .......................... 2013
   Dag  Måned  År

2. Hvilken type af natarbejde var bedst i forhold til dit privatliv? .........................................................

3. Hvilken type af natarbejde var bedst i forhold til søvn? ..........................................................

4. På hvilken type af natarbejde var du mest tilfreds med kvaliteten af dit arbejde? .............................

5. Hvilken type af natarbejde, mener du, var bedst for dit helbred? ......................................................

6. Hvilken type af natarbejde foretræk du alt taget i betragtning? ............................................................

Andre uddybende kommentarer?

______________________________________________________________________________________________

______________________________________________________________________________________________

______________________________________________________________________________________________

______________________________________________________________________________________________