

Contents lists available at [SciVerse ScienceDirect](#)

Sleep Medicine Reviews

journal homepage: www.elsevier.com/locate/smr

CLINICAL REVIEW

Neuroendocrine, immune and oxidative stress in shift workers

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ARTICLE INFO

Article history:

Received 5 April 2012

Received in revised form

11 December 2012

Accepted 20 December 2012

Available online xxx

Keywords:

Shift work

Neuroendocrine stress

Immunity

Oxidative stress

Circadian desynchronization

Sleep restriction

Nap countermeasure

SUMMARY

Shift work is commonly associated with disturbed life rhythms, resulting in chronic exposure to circadian desynchronization and sleep restriction. Epidemiological data have shown that shift workers are at an increased risk of cardiovascular disease and breast cancer. In this review, we will explore how observed increases in neuroendocrine stress, non-specific immune responses and pro-oxidative status could act as biological mediators for these damaging health risks in shift workers. To explain these risks, compelling evidence from laboratory studies links circadian misalignment but also sleep restriction to disruptions in the neuroendocrine, immune and oxidative stress systems. Assessment of neuroendocrine, oxidative and immune stress in the shift worker population is still a limited and novel field, which may have considerable clinical relevance. Finally, we will consider the potential benefits of a countermeasure, such as napping, in minimizing the neuroendocrine and immune stress and cardiovascular risk imposed by shift work.

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Introduction

Shift work usually involves between 20 and 25% of the work force in industrialized countries.¹ The percentage of shift workers is generally increasing around the world because of the developing world economy and improved worldwide communications. The prevalence of shift work is greatest in critical services and public utilities, such as healthcare, transportation, electrical power, police and fire protection, or in industries with production processes exceeding 8 h, e.g., chemical industries.²

The definition of shift work is not the same in all countries because of different legal rules and social obligations. However, according to the International Labour Office (1990), shift work is “a method of organization of working time in which workers succeed one another at the workplace so that the establishment can operate longer than the hours of work of individual workers.”

In addition to this definition, in the scientific literature, the term “shift work” has been widely used and generally includes any arrangement of daily working hours other than during standard daylight hours (07:00/08:00 h–17:00/18:00 h). In most cases, shift work is synonymous with irregular, odd, flexible, variable, unusual, non-standard working hours.

Several types of shift work exist and can be described as follows:

- permanent – people work regularly on one shift only, i.e., morning or afternoon or night; or rotating – people alternate more or less periodically on different shifts;
- continuous – all days of the week are covered; or discontinuous – interruption at weekends or on Sundays;
- with or without night work – the work time can be extended to all or part of the night, and the number of nights worked per week/month/year can vary.

In the daily lives of workers and their families, shift work is associated with irregular life rhythms, which expose the worker to sleep restriction, circadian desynchronization, and often psychosocial stress. Although there is clear individual variability in shift work adaptation, a large number of shift workers do not adapt their biological rhythms to these shifted working rhythms.³ The consequences of shift work fluctuate from sleep debt, sleepiness and fatigue, which are thought to be reversible symptoms to a certain extent, to major deleterious effects on cardiovascular and cancer pathogenesis, and traffic and work accidents because of sleepiness.^{4–7}

Numerous data have demonstrated a role of desynchronization between the biological clock and working rhythms in the development of medical disorders in shift workers. Neuroendocrine and immune functions may be regulated by both circadian and sleep homeostatic systems via hormones and neural innervations. The

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Abbreviations

CRP	C-reactive protein
DNA	deoxyribonucleic acid
HPA	hypothalamic-pituitary adrenal
IL	interleukin
LDL	Low-density lipoprotein
NK cells	natural-killer cells
PBMC	peripheral blood mononuclear cell
SWS	slow-wave sleep
SOD	superoxide dismutase
TNF	tumor necrosis factor

potential detrimental health effects of circadian rhythm desynchronization and sleep restriction (partial loss of sleep for one night or more) related to shift work, therefore, need to be further explored. Compelling evidence from well-controlled laboratory studies links circadian misalignment and sleep restriction to deregulation of the immune, inflammatory and cardiovascular systems.^{8–10}

Throughout the present review, we will use for effects of shift work more generally as a result of circadian phase shifts the term “circadian desynchronization” and the term “circadian misalignment” for laboratory-based forced desynchrony protocols studies only.

Growing epidemiological evidence also indicates that short duration sleep, by itself, is associated with a higher incidence of cardiovascular risk and an increased risk of diabetes, obesity, cardiovascular disease and mortality.^{11–14}

Assessments of neuroendocrine, oxidative and immune stress in the shift worker population are, however, still limited, and we believe it is important to review this novel field.

We will first provide an overview of the available studies related to these topics and explain how these studies may have clinical relevance. We will then attempt to explore how neuroendocrine, immune and oxidative stress could act as biological mediators for these damaging health effects imposed by shift work exposure. We will close with some potential countermeasures, such as napping, that may result in improvements in neuroendocrine stress and immune recovery.

Overview on studies on neuroendocrine, immune and oxidative stress in shift workers

Neuroendocrine stress in shift workers

Introduction

Cortisol and catecholamines are major mediators released by the stress system via the hypothalamic–pituitary adrenal (HPA) axis and the sympatho-adrenal system, respectively. Cortisol and catecholamines (epinephrine and norepinephrine) are widely used stress markers in human research and are measured in blood, saliva or urine. Cortisol, a robust and widely used biological marker of circadian rhythm, follows a strict diurnal rhythm with high levels in the early morning (acrophase around 08:00 h in usual sleep–wake conditions) and low levels in the evening and night periods. Diurnal variations in norepinephrine and epinephrine at rest are also observed with maximum release during the day and minimum release during the night.⁹

Several studies have been performed to assess the impact of shift work schedules on stress hormone rhythms and levels. Cortisol is not only regulated by circadian control but is also affected by the sleep/wake cycle, light conditions, the pattern of activity and exercise. Shift work, especially during the night, triggers

a desynchronization of natural biological rhythms, shifts the sleep–wake cycle, the activity pattern and the light exposure period. Hence, we will briefly describe the consequences and the time-dependant effects of circadian misalignment, exercise and light on cortisol and catecholamine secretions investigated in laboratory-based research. We will then examine the studies that have assessed these neuroendocrine stress markers in shift workers.

Neuroendocrine stress marker responses to circadian misalignment, exercise and light in laboratory protocols, and to shift work in field studies

A first investigation in young healthy male subjects reported that from the second day of change in the sleep–wake cycle (sleep time from 07:00 h to 15:00 h during two consecutive days), a phase shift was observed in cortisol secretion with a new acrophase at 14:21 h \pm 67 min as compared to 07:54 h \pm 14 min in normal sleep conditions (sleep time from 23:00 h to 07:00 h).¹⁵ In a second study, an abrupt 8-h advance of the sleep–wake, dark–light, rest-activity, and feeding cycles (sleep time from 15:00 h to 23:00 h) resulted, within 6–9 h, in a 3- to 4-h advance in the timing of the nadir and end of the quiescent period in cortisol secretion in young healthy men and women.¹⁶ A further study focused on the effects of the direction of shift rotation on cortisol levels: 28 participants were assigned to a clockwise or a counterclockwise shift rotation for two weeks.¹⁷ This report indicated no significant effect of rotation direction on cortisol profile variations. However, for an additional circadian marker, i.e., the rectal temperature, there was a lower amplitude and a delay of the acrophase in the counter clockwise group.

Exercise-induced cortisol response appears to be modulated by the time of day. Effects of moderate-intensity exercise on plasma cortisol were clearly detected during the afternoon session (i.e., during the declining phase of cortisol secretion) rather than during early morning and midnight sessions in young healthy men.¹⁸ Another study found that higher intensity physical activity, such as acute exercise, triggered the greatest increase in plasma cortisol levels at midnight compared to control day levels in healthy young men.¹⁹ Exercise, consisting of 15 min cycling on an ergometer at 60% maximum heart rate, resulted in a two and a half fold increase in epinephrine and norepinephrine levels in healthy subjects in all circadian phases.⁹

Bright light acts mainly on the circadian component, although the optimum wavelength, intensity or duration of exposure as well as the relationship to neuroendocrine variations in cortisol and alertness need to be further resolved.²⁰ The response to photic information is mediated by the adrenal glands, which produce cortisol and catecholamines.²¹ The effects of light on cortisol have been reported to be circadian phase-dependent. Exposure to bright light in the early morning has been mainly described to induce an increase in cortisol levels during light exposure, whereas later exposure during the afternoon was not associated with significant effects.^{22–24} Results regarding the effects of bright light exposure in the evening and during nighttime when cortisol production is usually low are inconclusive.

Rüger and collaborators²⁴ reported no effect of bright light exposure (5000 lux between midnight and 04:00 h) on cortisol levels whereas Kostoglou-Athanassiou et al.²⁵ (5500 lux from 20:00 h to 02:00 h) and Jung et al.²⁶ (6.7-h exposure to bright light at 10,000 lux after sunrise) found that exposure of their subjects to a longer duration of bright light had suppressive effects. The inconsistent findings among these studies may be related to discrepancies in light intensity or exposure durations.

Investigations performed in shift workers have mainly been cross-sectional studies comparing the values of neuroendocrine stress markers in shift workers and non-shift workers. Increased

urinary levels of norepinephrine (noradrenaline) were reported in transportation industry drivers who worked for extended shift hours and lacked sleep recovery.²⁷ In this study, 10 male long-distance coach drivers (age 47 ± 7 y with 11 ± 9 y of experience) were investigated during a 49-h driving shift. Urine samples were collected every 4 h (mean sleep duration of 5.4 h during a 49-h driving episode). Although, the consumption of coffee, nicotine, and alcohol was relatively high during the trip (averaging 11 cups, 16 cigarettes, and four glasses of alcohol a day), it did not vary significantly within the three working days and the first day of recovery or *versus* baseline consumption. The mean urinary excretion rates of epinephrine (adrenaline) on the first working day and cortisol excretion in most samples on all working days (especially the 07:00 h and 11:00 h time points) were higher than the baseline levels. During the first day of recovery, however, the mean excretion rates of both epinephrine and norepinephrine were lower than the baseline values and there was evidence of disruption of their circadian rhythms.

A large number of reports suggest that shift rotation in a clockwise direction produces less disruption of circadian rhythms than rotation in a counter clockwise direction. Vangelova et al. investigated the effects of shift rotation on variations in salivary cortisol levels sampled at several time points and on fatigue and subjective sleep in a sample of 25 sound engineers (12 working very fast backward-rotating and 13 working very fast forward-rotating shifts). The results indicated that backward rotation was associated with higher cortisol values during morning and night shifts and worse sleep quality compared to forward rotation.²⁸

Several studies have been performed in internal medicine residents working at night with a mean night's sleep of 2–3 h.²⁹ Zheng et al. reported that, after a 30-h extended work shift, internal medicine female and male residents ($n = 22$, mean age 29 y) had increased blood levels of norepinephrine, and decreased flow-mediated vasodilatation compared to the same residents after a non-extended work shift.³⁰ These findings indicate that the sympatho-adrenal axis is particularly solicited during extended work shifts.

An additional report also assessed the recovery dynamics of cortisol during the period following shift work in a group of 19 healthy offshore workers on a Norwegian oil rig with a fixed night shift schedule. Interestingly, the employees returning home after 14 consecutive night shifts needed more than one week at home to reset their cortisol rhythm compared to the same workers during a day shift.³¹ This last result suggests that the environmental effects of being at home for one week are not sufficient to re-synchronize these workers after disruption of cortisol circadian rhythms by such a shift schedule. Regarding this issue, a recent report detailed a method to quantify long-term cortisol levels in the scalp, with 1 cm of hair representing a period of approximately one month.³² This new methodological approach is suitable for evaluating cortisol alterations in shift workers over a prolonged period of time and for assessing the potential negative long-time health effects of these alterations. Shift workers ($n = 33$, median age 41 y [27–62]) from a Dutch textile factory working in a fast-forward rotating shift were recruited for a study that involved measuring their hair cortisol levels. The authors reported that long-term cortisol levels (measured in scalp hair) were significantly increased in shiftworkers.³³ This effect was particularly evident in younger shift workers (<40 y) for which the protein concentration of cortisol per mg of hair was almost doubled, i.e., 48.53 pg/mg hair (95% CI, 36.56–64.29) *versus* 26.42 pg/mg hair (95% CI, 22.91–30.55) in day workers ($n = 89$, median age 33 y [19–63]). Because of the relatively small number of shift workers included and the lack of control for potential confounders (e.g., the direction of the rotation, exercise status), further shift work studies using similar approaches are needed.

Conclusions

Shift workers who are affected by sleep debt or insomnia often display an elevated response to stress and a deregulation of the HPA axis as well as its mediator, cortisol.³⁴ Experimental investigations have not been performed under constant routine conditions, but were conducted in the presence of at least one uncontrolled external rhythmic influence (light/dark, temperature, posture, mealtimes, activity, social factors), which could have acted as a masking influence. Hence, the phase or amplitude variations observed can only suggest that the temporal regulation and the endogenous pacemaker are affected. Although deregulation of the stress system associated specifically with shift work requires further confirmation with longitudinal studies and control of confounding parameters, such as physical activity, body posture or light environment, this deregulation must be considered as a contributing factor in the development of cardiovascular risk in shift workers.

Immune and inflammatory biomarkers in shift workers

Introduction

The circadian sleep–wake rhythm plays a critical role in the homeostatic regulation of the activity and expression of numerous immune cells. Under regular circadian and sleep–wake conditions, human peripheral blood mononuclear cell (PBMC) subsets display diurnal rhythms. Depending on the cell type, levels peak at night, e.g., monocytes, or during the day, e.g., natural-killer (NK) cells or neutrophils, which have a major role in phagocytosis.³⁵

Under normal physiological conditions, there are low concentrations of cytokines in the blood, except for interleukin (IL)-6, which has hormone-like actions. Several human studies have reported diurnal rhythms for various cytokines, including IL-6, IL-12 or tumor necrosis factor (TNF)- α , with higher values during nocturnal sleep, as well as for the leukocyte subset cells (e.g., monocytes) that produce them.^{35–39} Pro-inflammatory cytokines, such as IL-6, IL-17 or TNF- α , counteracted by anti-inflammatory cytokines (interferon- α , transforming growth factor- β) are essential mediators of the inflammatory response. Pro-inflammatory cytokines act as endogenous pyrogens, stimulating and chemically attracting mediators of the inflammatory process, and enhancing the production of acute phase proteins, such as C-reactive protein (CRP). CRP, a hepatic protein stimulated by pro-inflammatory cytokines, such as IL-6 and IL-17, has a key methodological advantage in that it does not display diurnal variation.⁴⁰ Numerous sleep manipulation studies performed in non-constant routine conditions have assessed blood levels of cytokines and monocytes (a major source of cytokines) among other PBMCs, and demonstrated that the diurnal rhythms are suppressed or more or less unchanged during one night of total sleep deprivation (i.e., continuous wakefulness) depending on the specific cytokine and leukocyte subtype measured in blood.^{10,35,36} Numerous changes in the amplitude and backward or forward shifts in the nadirs and peaks of the immune cell rhythms observed indicate strong regulation of these cells by sleep.

Circadian phase alterations in levels of PBMC and cytokines are possible confounding factors and need to be controlled for. Time points for blood sampling need to be carefully chosen in laboratory-based circadian or sleep manipulation studies and, as far as possible, in shift work setting studies.

In light of the previous remarks, we will now discuss the immune and inflammatory changes measured in shift workers, which remains a relatively recent research field.

Immune and inflammatory biomarker changes in shift work studies

In an early study, Nakano et al. investigated the effects of shift work on cellular immune function and reported that lymphocyte

proliferation capability was reduced in shift workers compared to daytime workers.⁴¹ More recently, the activity of NK cells (non-specific immune cells that recognize and lyse foreign bodies or material, such as viruses) was assessed in 89 Japanese emergency physicians. NK cell activity was lower at the beginning of the night shift in the night work group (age 34.4 ± 6.3 y, sleeping hours at home 6.4 ± 1.3) compared to the levels measured at the start of work in the day work group (age 41.3 ± 5.8 y, sleeping hours at home 6.9 ± 0.8).⁴² A major limitation of this study is that levels and activity of NK cells vary according to a diurnal rhythm, which offers a possible explanation for the differences observed between the day workers (morning blood values) and the night workers (evening blood values). These were both cross-sectional studies and do not establish a direct causal relationship between shift work and immune response changes.

NK cytotoxicity and levels of several cytokines (IL-1 β , TNF- α and IL-6) were also longitudinally assessed in a limited sample of 68 shift working (median age [25th–75th percentile], 35 y [30–40]; job seniority 6 [3–14] y) and 28 daytime (40 y [34–43.5]) nurses at baseline and at 12 mo. The results showed no significant effect of shift work on these immune variables.⁴³ However, a recent before–after study investigated the effects of shift work and associated fatigue on immune function in 57 middle-aged nurses (42.1 ± 8.4 y) working in a shift format over a long period of time (19 ± 8.4 y). In nurses who worked a daytime shift (08:30–17:15 h) immediately followed by a night shift (00:30–09:15 h), NK cell activity was reduced the next morning compared to values on the morning at the beginning of the day shift.⁴⁴ The decrease in NK cell activity was more pronounced among subjects reporting greater fatigue, drowsiness and local pain. These data were adjusted for several potential confounders but not for menstrual cycles, which may influence the immune response.

Regarding blood leukocyte levels, several cross-sectional reports have recently indicated that shift workers have higher leukocyte counts than do day workers.^{45–47} A first study involved 208 male Japanese workers (107 daytime workers and 101 shift workers [aged 33.7 ± 12.3 y]) with no history of inflammatory disease.⁴⁵ Interestingly, a higher rate of subjective poor sleep was associated with increased leukocyte counts. As expected, smoking status was positively associated with leukocyte counts. Smoking is, indeed, a partial confounder in the relationship between leukocyte counts and shift work since smoking status is strongly associated with increased leukocyte counts.⁴⁸ A further investigation compared 877 male day workers with 474 male rotating shift workers (aged 34.4 ± 8.4 y), using multiple regression analysis. Leukocyte count was correlated with rotating shift work independently of smoking, age, education and physical activity.⁴⁶

Another interesting study was conducted in 1877 airline-company employees. After controlling for confounders, including smoking, education, alcohol consumption, physical activity, and body mass index, the results showed that shift work was associated with higher leukocyte counts in both women and men.⁴⁹ Previous studies all sampled blood in the morning (e.g., Puttonen et al. between 07:00 h and 10:00 h⁴⁹; Sookoian et al. and BURGUEÑO et al. between 06:00 h and 09:00 h^{46,47}) in fasting conditions, at a period corresponding to the nadir levels of leukocyte subsets.³⁵

Blood leukocyte count has been described as a powerful predictor of resistin levels, an adipokine that exerts direct effects to promote endothelial cell activation and is an inflammatory marker for atherosclerosis in humans.⁵⁰ Both resistin and leukocyte blood levels were elevated in a sample of 439 young adult men (34.4 ± 8.6 y) exposed to a rotating shift work schedule compared to day workers.⁴⁷ Assessing inflammatory cytokines, one study reported no significant differences in IL-6 or TNF- α serum concentrations between shift workers (mean age 36.4 ± 9.34 y, 86.9%

men) and day workers (mean age 40.1 ± 7.7 y, 75.7% of men) who did not differ in body mass index.⁵¹ These data suggest that chronic sleep debt and circadian shift may not always trigger activation of immune and inflammatory responses. However this study mainly included men and several recent reports suggest a higher inflammatory response in women than in men when subjected to sleep debt.^{52,53} Also, levels of IL-6 were measured in the morning at a single point of blood sampling during the nadir level of IL-6 in day workers and shift workers in whom the circadian rhythm was potentially shifted. These results would, therefore, ideally need confirmation with multiple blood samplings in order to measure the 24-h pattern. Indeed, Vgontzas et al. previously reported in a laboratory study with 24-h blood sampling performed serially every 30 min that one night of total sleep deprivation was sufficient to change the diurnal pattern of IL-6 secretion.³⁹

CRP, a more common systemic marker of inflammation, which in contrast to IL-6 or TNF- α , does not display diurnal variation, was evaluated after a 30-h extended work shift in a small sample of young internal medicine male and female residents ($n = 22$, mean age 29 y). Compared to the same residents after a non-extended work day, physicians following extended work shifts had 5-fold higher CRP concentrations.³⁰ This study also measured the pro-inflammatory cytokine, IL-6, but used only a single blood sampling time point, and showed higher levels after extended work shifts. Similarly, in 1877 airline-company employees, significantly higher levels of CRP were measured in shift workers compared to day workers. These differences remained significant, especially in men, after controlling for numerous potential confounding factors.⁴⁹

Conclusions

The changes observed in shift workers could be part of an immune defensive stress-response to circadian shift and sleep restriction. Shifted circadian patterns, as indicated by altered endocrine biomarkers, such as melatonin, are observed in shift workers. Day workers, therefore, often have a different circadian pattern to that of shift workers and the same time point may correspond to different phases of the circadian rhythm for blood parameters that display 24-h variations. Using biomarkers that do not display 24-h rhythms or performing repeated measures over the 24-h period may, therefore, represent a more valid methodological approach to assess the effects of shift work on immune biomarker levels.

There are insufficient reliable, longitudinal shift work studies to establish causative links for the immune and inflammatory biomarkers that display diurnal rhythm. The long-term consequences of these immune modulations remain uncertain in terms of physiological adaptation, but may have clinically relevant effects in shift worker populations.

Oxidative stress in shift workers

Introduction

Oxygen-based free radicals are produced as derived products of normal metabolism as well as by inflammatory cells in response to external agents triggering an immune reaction. Diurnal rhythms of oxidative and anti-oxidative enzyme activities have been described in various phylogenetically distant organisms, including mammals.⁵⁴ Significant amplitudes have been detected in numerous mammal species suggesting the potential relevance of oxidative and anti-oxidative rhythmicity in reducing excessive oxidative stress. Superoxide dismutase (SOD) is an enzyme that catalyzes the formation of hydrogen peroxide from the pro-oxidative superoxide radicals. Hydrogen peroxides are responsible for the formation of toxic hydroxyl radicals but can be removed by a reaction catalyzed by glutathione peroxidase and catalase. In rats, daily rhythms of SOD gene expression, antioxidant defenses (glutathione peroxidase

activity), lipid peroxidation and glutathione levels have often been observed with oxidative stress, with levels increasing progressively from the early stages of the dark phase, corresponding to the start of the motor activity phase.⁵⁵

Similar observations have been made in humans; for example, diurnal variation in glutathione and cysteine redox levels in human plasma with values peaking during the day period.⁵⁶ *In vivo*, the presence of oxidative stress products can be quantified in urine; for example, 8-hydroxydeoxyguanosine (an oxidative DNA (deoxyribonucleic acid) damage product) and two lipid peroxidation products, malondialdehyde and 8-isoprostane, were measured in the urine of healthy males. There was a significant 24-h variation in the concentrations of these markers of oxidative stress with peak urine levels occurring early in the evening.⁵⁷

All these data suggest that these time-dependent variations in pro- and anti-oxidant components could modulate sensitivity to oxidative stress over a course of hours.

Although investigations into the effects of shift work on oxidative stress are still an emerging field, the available data suggest that shift work alters the pro-oxidant/anti-oxidant balance in favor of oxidative stress.

Oxidative stress in shift work studies

The oxidative stress index (ratio of total oxidant status to total anti-oxidant status) was assessed after 16-h extended shift work in 115 young medical male and female residents, and compared to 30 young non-healthcare staff at the same hospital with a 8-h non-extended morning or afternoon shift work schedule. All participants were healthy non-smokers and had not recently used any drug with antioxidant properties, such as vitamin E or C or acetylcysteine. Compared to their 08:00 h baseline blood values, after 16 h of continuous shift work, the total oxidant status was increased, whereas the total anti-oxidant status was reduced, resulting in an almost two-fold increase in the oxidative stress index.⁵⁸ In contrast, in the non-extended work group, there were no differences between the two same time points blood samplings for oxidative stress measurements. Limitations of the study can be that blood samples were collected at 08:00 h for baseline values and at 00:00 h for post-shift work values and that these data compared extended work shift not to regular day workers but to non-extended work shift.

Two further studies reported the effects of night and evening shift work in hospital workers in palliative and intensive care units, assessing the relationship between occupational stress and increases in oxidative stress levels. The participants in the studies had not used any substance that could potentially interfere with anti-oxidant status. Elevated malondialdehyde levels (an indicator of lipid peroxidation) were measured in the red blood cells of 32 nurses in a public health service in Madrid (Spain) and 52 staff at a palliative care unit (23 men and 29 women) working night and evening shifts. These data were compared to the values obtained in 85 age-matched healthy control individuals of both sexes.^{59,60} Erythrocyte levels of SOD were higher in both groups of evening and night shift workers than in the controls. A limitation of these studies was that the authors did not provide details about the timing of the blood sampling. Another study evaluated the changes in oxidative stress variables in two groups of nurses after a day shift ($n = 60$) or a night shift ($n = 60$) and reported that oxidative stress parameters were increased in nurses at the end of the day and after the night shifts.⁶¹ A pilot study examined 90 middle-aged healthy male shift-workers who worked for a long period in Alaska. The authors reported accumulation of the lipid peroxidation product, malondialdehyde, and a decrease in glutathione reductase activity (reduces glutathione disulfide to the sulfhydryl form, a major antioxidant).⁶²

Regarding the effects of oxidative stress on DNA, increased levels of the marker of oxidative DNA damage, product 8-OH-dG, were measured in nurses engaged in shift work compared to those who were engaged in part-time work.⁶³

Finally, a study assessed total plasma antioxidant capacity in 44 shift-workers (mean age 36.6 y [SD: 10.2] and mean body mass index of 26.1 kg/m² [SD: 4.4]) at the end of the work day as compared to values obtained at a different time point, i.e., at the end of night work. The authors observed a mean reduction in total plasma antioxidant capacity after night shifts.⁶⁴

Conclusions

A potentially key mediator in the relationship between circadian disruption and oxidative stress is the pineal hormone, melatonin. Melatonin production times adjust to the light/dark cycle thus acting as an endogenous synchronizer that stabilizes and reinforces circadian rhythms, but melatonin also displays anti-oxidative properties. Indeed, melatonin is a powerful scavenger of reactive oxygen species, e.g., toxic hydroxyl radical, and is more effective than the majority of its analogs and other typical free radical scavengers such as vitamin E.^{65,66}

The multilevel endocrine changes caused by circadian disruption in shift workers, with melatonin suppression through exposure to light at night, are hypothesized to lead to chronically reduced levels of melatonin. A number of studies indicate that many shift workers do not adapt and retain the timings of melatonin secretion that are typical in day-oriented people.^{67–71} As a result, numerous night shift workers exhibit altered melatonin profiles and reduced melatonin production. The degree of melatonin suppression reported in night and shift work settings ranges upwards from 20%, depending on the study.^{69,72,73}

In summary, shift work can act as an oxidative stressor and may favor the development of medical disorders, in particular the development of cardiovascular disease. Aging, the potential reduction in the anti-oxidative protective effects of melatonin, and the higher prevalence of cardiovascular comorbidities (obesity, diabetes, atherosclerosis) may act together in shift workers to make them more sensitive to the harmful effects of oxidative stress.

Potential physiopathological mechanisms and clinical relevance

Introduction

Shift work triggers reductions in the quality and length of sleep and a desynchronization in circadian regulation, which may lead to immune profile alterations in shift workers. Circadian signals and sleep have strong regulatory effects on the immune and oxidative stress systems. Sleep restriction and circadian shift have complex, intimate interactions and consequences, and it is difficult to discriminate their effects. Numerous laboratory studies investigating the effects of sleep restriction on the immune system and oxidative stress levels have not simultaneously controlled for a robust marker of circadian rhythm. On the other hand, forced desynchronization and circadian misalignment protocols often result in reducing sleep efficiency and total sleep time, which again make it difficult to differentiate between the respective effects of sleep restriction and circadian shift. Further investigations under constant routine conditions are required in which the sleep parameter and the circadian parameter would be manipulated in isolation to confirm the respective roles of sleep restriction and circadian desynchronization on immune and oxidative stress.

Our intention here is not to present the epidemiological evidence for an increased risk of female breast and male prostate

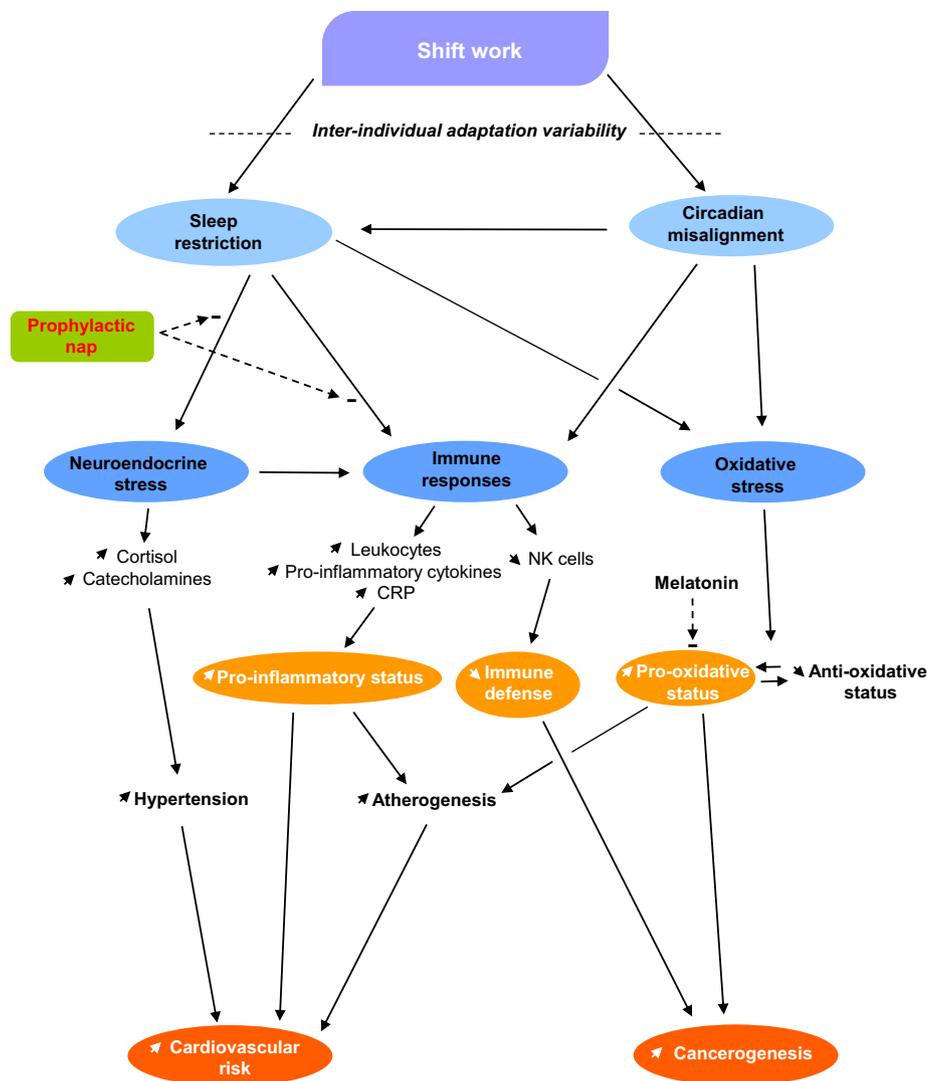


Fig. 1. Potential physiopathological pathway(s) by which shift work may lead to cardiovascular disease and cancer genesis. Experimental circadian misalignment and sleep restriction protocols disrupt and enhance the activity of neuroendocrine stress systems,^{9,34} reduce immune defense (NK cells¹¹³), and increase pro-inflammatory (leukocyte,^{86,87} IL-6³⁹ and C-reactive protein¹⁴² levels) and pro-oxidative (reduced anti-oxidative glutathione, catalase and superoxide dismutase levels^{95,103} and increased pro-oxidative modified oxidized myeloperoxidase-LDL concentrations¹⁰⁵) status. Similar alterations in the levels of these biomarkers are also reported in shift workers: cortisol and catecholamines,^{28,30,31,33} NK cells,^{43,44} glutathione⁶² and leukocyte,^{45–47,49} IL-6³⁰ and C-reactive protein.^{30,49} Inter-individual vulnerability to the adverse effects of sleep restriction and circadian misalignment contribute to a heterogeneous tolerance to shift work.³ However, chronic elevations in stress mediators (cortisol and catecholamines), pro-inflammatory and pro-oxidative status elicit hypertension and atherogenesis and increase global cardiovascular risk in shift workers.^{5,6,133,134} Reduced immune (NK cells) and anti-oxidative (melatonin) defense abilities coupled to increased reactive oxygen species may also contribute to the development of cancer pathologies.^{65,103,111,113,115} Prophylactic naps could blunt the stress response (cortisol^{39,87}) and possibly correct stress-dependent immune changes and improve the recovery of immune homeostasis (neutrophil counts⁸⁷ and IL-6³⁹). CRP: C-reactive protein; IL: interleukin; NK: natural killer; LDL: low-density lipoprotein.

cancers and cumulative cardiovascular risk factors, e.g., obesity, atherosclerosis, hypertension, when exposed to shift work. We will rather examine how oxidative stress and neuroendocrine, immune and inflammatory deregulations may be some of the first steps to oncogenesis and cardiovascular pathogenesis (Fig. 1).

Potential effects of circadian desynchronization on immune function in shift workers

When looking at a validated marker of circadian phase, the time of onset of melatonin secretion varies amongst night shift workers (from 21:45 h to 05:05 h).⁷⁴ Melatonin also has well-known effects on the immune system, and may contribute to the control of diurnal cytokine release and activity. Melatonin's ability to regulate diurnal production of cytokines and cells mediating non-specific immunity – possibly via specific receptors present on immune

cells – and to display anti-inflammatory effects has been demonstrated in several mouse and human studies.^{75–77}

When using global gene transcription measurements with Affymetrix chips in the critical immune cell macrophage, more than 8% (1403 among the 17308 transcripts detected) of the total transcriptome displayed circadian patterns, including immune pathogen mediators for cytokine secretion and pathogen recognition.⁷⁸ Growing evidence, especially from animal studies, indicates a strong association between circadian timing and the immune system, with immune response deregulation resulting from circadian disruption. Genetic manipulation of circadian timing in mice resulted in alteration or suppression of numerous cells and mediators of the immune system. Among the mammalian clock genes that regulate circadian rhythm, disruption of the circadian clock gene, *Bmal-1* (brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1), in knockout mice resulted in chronic inflammation, reduced

lymphocyte count, and increased neutrophil count.⁷⁹ Cry1 and Cry2 knockout mice had increased TNF- α production from spleen cells and developed more atherosclerosis in an experimental model of induced arthritis.⁸⁰ Endotoxemic shock induced by lipopolysaccharide resulted in hypothermia and death after chronic circadian rhythm disruption in mice (four consecutive weekly 6-h phase advances of the light/dark schedule): there was a mortality rate of 89% in shifted mice versus 21% in non-shifted control mice.⁸¹ In shifted mice, elevated levels of pro-inflammatory cytokines released in response to lipopolysaccharide treatment were measured in blood and in isolated peritoneal macrophages, a major target cell of circadian disruption. Polysomnographic recordings in shifted mice revealed there were no significant changes in sleep loss and stress measures. The authors, therefore, suggested that circadian disruption, rather than sleep loss or stress, could be the major cause of the observed immune alterations. However, these data showed that desynchronized mice did not show altered sleep duration in shift work conditions highlighting the fact that rodent shift-work models (changing light cycles) are not entirely satisfactory. Additional information could be obtained from the study of non-mammal species, such as flies, in which inverting the light–dark cycle and preventing efficient sleep periods do not non-specifically activate stress responses.⁸²

Potential neuroendocrine stress effects on immunity in shift workers

Chronic and long-term stress (week to months) is believed to suppress immunity by decreasing baseline immune cell numbers and deregulating pro-inflammatory responses, e.g., increased CRP and IL-6 levels.^{83,84}

In a 10-day confinement study – a prolonged stressful condition – performed in young healthy volunteers, numbers of innate immune cells, such as NK cells and neutrophils, all increased consecutively over the days of confinement.⁸⁵ Similarly, an acute stress situation, such as acute sleep restriction or total sleep deprivation (i.e., continuous wakefulness), increased plasma neutrophil and NK cell measurements.^{86,87} These leukocyte “stress-sensitive” cells displayed increased expression of β -adrenoreceptors and vascular mobilization in response to norepinephrine or cortisol.^{88–91} As the stress response continues, these stress-sensitive leukocytes exit the blood and show a high potential to migrate to key immune organs (e.g., lymph nodes) for further immune challenges or to sites of action, such as the skin.⁹⁰ This redistribution of leukocytes results in a decrease in blood leukocyte levels, which could be a factor mediating the immunosuppressive effect of chronic stress.

We previously mentioned that shift workers, a chronically sleep-deprived population, had reduced measurements of NK cells and activity, as well as enhanced pro-inflammatory biomarkers. Partial sleep loss, over more than one night, or a long-lasting period of sleep restriction for several consecutive days as observed in shift workers, may correspond to a stressful condition associated with immune deregulation.³⁴

Components involved in the early host responses to infection, such as certain leukocyte populations (i.e., monocytes, lymphocytes and neutrophils), are affected by experimental sleep loss with increased blood levels of these immune cells regardless of gender and age.¹⁰

The inflammatory responses observed after chronic sleep restriction do not seem to be counterbalanced by anti-inflammatory cytokines or through significantly enhanced levels of the anti-inflammatory hormone, cortisol. The inflammatory signaling pathways that underlie the responses to sleep loss are poorly described. However, sleep restriction has been reported to induce, in healthy subjects, a rapid increase in activation of the transcription factor, nuclear factor-kappa B, a well-described activator of pro-inflammatory gene expression, in PBMC.^{92,93} These laboratory studies can be considered as a relatively stress-free environment, because,

although they are sleep deprived, subjects are aware of the schedule of the day. This setting cannot, however, be compared to everyday life with non-scheduled events in real-life shift-work conditions. Shift times are also associated with disturbed social interactions with friends and family,⁹⁴ which may contribute to the development of chronic psychosocial stress. This observation suggests that the physiological changes observed in laboratory-based studies may underestimate values found in real-life conditions.

Potential oxidative stress genesis pathway(s) in shift workers

We will briefly describe the effects of circadian manipulation and sleep restriction on oxidative state in mammal species.

Regarding circadian effects: the impact of constant light exposure on oxidative damage, age related-damage and survival was assessed in rats from the age of one month and for 13 consecutive months. In male and female rats under a constant light regimen (24 h light on at 750 lux), the activity of catalase and SOD in various organs was reduced and life span was shorter compared to rats maintained at a standard alternating light regimen (12 h light at 750 lux/12 h dark).⁹⁵

Continuous light exposure has been previously reported to affect melatonin level and disrupt endogenous circadian rhythms. In rats, constant illumination triggers a strong reduction in melatonin level, and free-running circadian rhythms of temperature and locomotor activity and sleep–wake rhythms are observed until about the second month and then these rhythms progressively disintegrated.^{96–98} Prolonged exposure of rats to continuous light for 17 wk has also been reported to suppress blood pressure and heart rate circadian rhythms.⁹⁹

The disruption of all these circadian rhythms coupled to lower level of melatonin and its anti-oxidative abilities contribute to reduce antioxidant defense as measured by the decreased activity of catalase and SOD reported after 13 mo of chronic light exposure as reported above.⁹⁵

Regarding the effects of sleep restriction: sleep restriction has been shown to increase oxidative stress levels in many regions of the brain in rodents, including the brainstem and the hippocampus.^{100,101} A possible hypothesis is that hippocampal oxidative stress, induced by sleep restriction, is one of the contributing mechanisms to memory deficit.¹⁰² Everson et al. assessed anti-oxidant responses in peripheral tissues of rats during 10 d of sleep restriction and of sleep recovery.¹⁰³ Glutathione content (a major free radical scavenger indicating the intensity to which a tissue has been oxidatively challenged), catalase and glutathione peroxidase levels were measured in the liver, lung and heart. Catalase activity and glutathione content were reduced in the liver by 23–36% by days 5 and 10, respectively, of sleep restriction without a compensatory increase in glutathione peroxidase. Although recovery sleep was associated with increased anti-oxidant enzymatic responses in the heart and the liver, these data suggest that during the prolonged sleep restriction period (10 d), uncompensated oxidative stress occurred. Interestingly, a recent study in mice suggests a potential involvement of a GABA (gamma-aminobutyric acid) ergic mechanism in the protective effect of melatonin treatment against sleep restriction-induced oxidative damage.¹⁰⁴

In healthy humans, acute and chronic sleep restriction and recovery were associated with enhanced levels of the pro-oxidative myeloperoxidase, an enzyme mainly released during neutrophil degranulation and the subsequent oxidative burst.^{87,105} In addition, during a period of chronic mild sleep restriction over five consecutive days, oxidative stress was increased as indicated by increasing blood levels of myeloperoxidase-modified oxidized LDL (low-density lipoprotein).¹⁰⁵

These data indicate a significant role for sleep in antioxidant detoxification.

Oxidative and immune stress-dependent oncogenesis in shift workers?

In 2007, the International Agency for Research on Cancer stated that shift work was a probable carcinogen although this is still a topic of debate.¹⁰⁶ In 2009, the Danish government decided to compensate shift workers who develop breast cancer. The mechanisms by which circadian disruption may favor the development of malignant tumors are certainly complex and multi-factorial and require further epidemiological study and mechanistic investigations (Fig. 1).

The multilevel endocrine changes caused by circadian disruption and melatonin suppression through light exposure at night suggest that the anti-oxidative abilities of melatonin should be considered as having possible oncostatic activity.¹⁰⁷ Reactive oxygen species are well described carcinogenic agents, sources of genotoxicity that cause oxidative DNA lesions.¹⁰⁸ To date, the most convincing data result from demonstrating that continuous light exposure or forced desynchronization protocols in animals lead to increased oncogenesis.¹⁰⁹ Repeated phase shifting with endogenous desynchronization could result in alterations in the regulation of the circadian cell cycle, thus contributing to uncontrolled cell division and growth. Furthermore, emerging evidence indicates a link between immunity, inflammation and cancer development.¹¹⁰ NK cells can exert cytotoxicity against cancer cells, whereas lower NK activity and levels, as observed in shift workers, may have long-term consequences on natural immune host defense mechanisms against the development of cancer.^{111,112} Of interest, a recent study reported that chronic shift-lag alters the circadian clock of NK cells and promotes lung cancer growth in rats. Indeed, following intravenous injection of tumor cells, chronic shift-lag attenuated NK cell cytolytic activity and promoted lung tumor growth.¹¹³

Numerous data indicate that a state of inflammation is critical in terms of tumor progression. Inflammatory conditions increase the risk of oncogenesis particularly in anatomical sites such as the colon or prostate. A pro-inflammatory state and increased leukocyte counts have been found to be more prevalent in shift workers. The increase in leukocyte levels most likely results from enhanced levels of neutrophils, hypothesized to be an immune sensor of sleep debt.¹¹⁴ Hypochlorous acid, a reactive oxygen species generated by myeloperoxidase, which is mainly released by neutrophils, is a potential source of genotoxicity as it inhibits base-excision repair.¹¹⁵

Neuroendocrine, oxidative and immune stress and cardiovascular pathogenesis in shift workers

Chronically elevated oxidative and neuroendocrine stress associated with increased levels in markers of future cardiovascular events in humans, such as CRP, IL-6, resistin or leukocyte count, contribute to a cumulative increase in cardiovascular risk in shift workers (Fig. 1).^{49,116,117}

Low-grade local and subclinical systemic inflammation occur in all stages of atherogenesis and are expected to increase in proportion to the number of years an employee is exposed to shift work. Accordingly, the longer employees work in night and shift systems, the more likely they are to develop heart disease.⁵ Oxidative stress *per se* is a known contributor to the onset and development of several cardiovascular diseases, including atherosclerosis and diabetes.¹¹⁸ Endothelial dysfunction, which promotes the progression of atherosclerosis, is also affected after a night shift, e.g., decreased flow-mediated dilation of the brachial artery and coronary microcirculation.^{119,120}

Adverse cardiovascular outcomes include several parameters, such as cardiovascular risk factors, inter-individual vulnerability, but also circadian control of cardiovascular activities. The occurrences of presyncope events, ischemic strokes and myocardial

infarctions peak in the morning.^{121,122} Similarly, sympathetic nervous system activity, release of the stress mediators, cortisol and catecholamines, and platelet activation involved in thromboses are all elevated in the morning. These factors may, therefore, promote and trigger cardiovascular events in the period from 06:00 h to 12:00 h.¹²³ However, to our knowledge, specific timings associated with an increased incidence of adverse cardiovascular events have not been precisely described in shift workers.

During nocturnal sleep, there is a 10–20% decrease in mean nocturnal blood pressure compared to daytime values. This “nocturnal dipping” is partly mediated by decreases in sympathetic output. The absence of, or reduced, nocturnal dipping of blood pressure is considered as a strong, independent predictor of cardiovascular risk.¹²⁴ These data suggest that processes such as reduced sleep duration and circadian shift, in addition to elevated neuroendocrine stress in shift workers could affect the 24 h-blood pressure profile and potentially predispose to hypertension.

Dietary intake at abnormal circadian phases also certainly has an impact on cardiovascular health status. Postprandial increases in lipid, glucose and protein levels can lead to increased oxidative stress, which has been associated with a higher risk of atherosclerosis.^{125–127} Postprandial oxidative stress results from an increased susceptibility of the organism to oxidative damage after consumption of a meal rich in lipids and carbohydrates and consecutive sustained postprandial hyperglycemia and hyperlipidemia. Hyperlipidemia and hyperglycemia have been associated with increased oxidative damage affecting lipoproteins and the antioxidant status.¹²⁸

Most studies in shift workers indicate a circadian redistribution of food intake over 24 h. Study of simulated shifts in the laboratory and of real shift workers indicated relatively impaired lipid and glucose tolerance after meals consumed during the night shift compared with a day meal.¹²⁹ As previously mentioned, carbohydrate and lipid intakes have been reported as enhancers of reactive oxygen species production. Conversely, the antioxidant capacity of fruit and vegetables seems to play a relevant role in the protective effects of these foods.¹³⁰ Low intake of nutritional antioxidants, including carotenoids, and polyphenol vitamins C and E, may contribute to the reduced antioxidant status in shift workers.

Workers engaged in night shifts as compared to workers engaged in morning shifts displayed significantly higher carbohydrate intakes.¹³¹ Specifically, this study also indicated that the consumption of sweets was greater in workers engaged in night shifts *versus* workers engaged in morning shifts.¹³¹ In contrast, intake of vegetables with high anti-oxidant capacity tended to be lower in shift workers at night compared to day workers.¹³²

It thus appears reasonable to assume that shift workers at night should be encouraged to eat fruits and vegetables rather than meals that are too rich in lipids and proteins. This is clearly relevant when considering the fact that during the biological night, it is hypothesized that sleep reduces the oxidative stress accumulated during the activity of the day.

Finally, increased cardiovascular risk has been observed in young shift workers although they have only had short-term exposure to shift work and are expected to have a greater capacity of adaptation.^{3,133,134}

Conclusions

The concept of the “healthy worker” suggests that former shift workers, after leaving their jobs, are not considered in the shift worker group. This could be an important confounder in the assessment of the long-term effects of shift work on health. Another important factor, with major implications on everyday functioning, is that good quality and duration of sleep help combat

infectious diseases. In support of this idea, laboratory-based studies have indicated that sleep improves the formation of antigen-specific immune defenses and that sleep-mediated factors play an important role in the formation of the immunological memory.¹³⁵ Studies carried out on larger sample populations also reported that subjects with shorter sleep durations were more vulnerable to rhinovirus.¹³⁶ Shift workers are chronically sleep-deprived and experience recovery day sleep averaging 5–6 h after their night shift; objective assessment of sleep indicates that their day sleep is 1–4 h shorter than night sleep.^{4,137} These data suggest that chronic, insufficient sleep recovery in shift workers does not allow for adequate recovery of immune function and may progressively contribute to an increased risk of infection. This immunodeficiency, coupled to low-grade systemic inflammation, certainly suggests an increased risk for various diseases.

Potential countermeasures

Introduction

Napping has been proposed as a powerful countermeasure to sleep restriction in shift workers.^{4,138–140} It is not the goal of this review to provide evidence on the impact of naps in shift workers but to discuss how napping may counteract the neuroendocrine and immune stress in shift workers (Fig. 1).

Napping as a countermeasure to sleep restriction: improvement in neuroendocrine stress and immune recovery?

Previous reports have mainly studied the countermeasure aspect of napping on alertness, and to a lesser extent its effects on stress, immune and cardiovascular risk markers. Interestingly, recent studies have observed stress and immune responses following napping although these effects and the nature of the stage(s) of sleep involved remain to be further detailed, and the effects of napping on neuroendocrine stress and immune systems are still largely unknown.^{39,87}

Experimental data have clearly indicated that one night of sleep recovery is not sufficient to restore alertness and psychomotor vigilance test performance following acute and chronic sleep restriction.^{87,141} Similarly, laboratory data suggest that one standard night of recovery sleep after sleep restriction is not sufficient to recover basal pro-inflammatory and immune conditions and could be associated with enhanced cardiovascular risk if chronic.^{87,105,142} Sleep loss produces significant increases in stress and immune markers, indicating sleep-dependent interactions between the central nervous and the neuroendocrine and immune systems. These changes may reflect increases in the homeostatic drive for sleep because they occur in sleep-deprived subjects but to a lesser extent when sleep loss is counterbalanced with napping strategies. Minimal cortisol release, in the presence of maximal growth hormone release, results in a pattern mainly present during the early hours of slow-wave sleep (SWS) during the night. It suggests that sleep, and its SWS component, contribute to suppressing the release of major mediators of the stress response.¹⁴³ To support this physiological pattern, evidence has shown that SWS oscillates in phase opposition with cortisol secretion in healthy humans.¹⁴⁴

A nap with SWS could inhibit the HPA axis, cortisol release and catecholamine production from the sympatho-adrenal system. Accordingly, napping has a stress releasing effect, as shown by the decrease in cortisol levels observed during long 2-h midday naps, or immediately after a shorter 30-min midday nap where half the duration of the naps consisted of SWS.^{39,87} The cortisol drop induced by 'napping' in both studies was measured during the afternoon when cortisol is in the descending phase of its circadian

rhythm. Furthermore, following a night of acute sleep restriction to 2 h, characteristic of the work shifts experienced by, for example, interns during residency training,²⁹ a 30-min midday nap improved alertness and the return of leukocyte – mainly neutrophil – counts to baseline values after a night of recovery sleep.⁸⁷ This further suggests that a standard recovery night of 8 h following sleep restriction is not sufficient to normalize immune alterations to baseline values unless a midday nap is taken before the recovery night. Thus, in addition to restoring alertness, napping induces a stress-releasing effect and down-regulates cortisol (also a potent enhancer of vascular neutrophil mobilization) measured immediately after the nap, which could explain the improved neutrophil recovery. Finally, following the dissipation of sleep inertia, better performance and alertness recovery were associated with more sustained cortisol levels during the first hours following the nap.³⁹

A field study investigated middle-aged nurses (42.1 ± 8.4 y) involved in shift work over a long period of time (19 ± 8.4 y).⁴⁴ The authors showed that, after a daytime shift (08:30–17:15 h), when nurses were sleeping 2.5–4.0 h before immediately continuing with a night shift (00:30–09:15 h), the blood levels of CD3⁺ lymphocytes were significantly reduced the next morning compared with nurses sleeping less (0.0–2.0 h). This is consistent with the laboratory study from Born et al.³⁵ showing that one night of total sleep deprivation enhanced the levels of CD3⁺ lymphocytes, and illustrates again the sensitivity of the immune system to sleep length variations. The sleep stage composition present in the nap is certainly a contributing factor that requires better understanding for its distinct effects on alertness, stress-releasing, immune and cardiovascular benefits.

Last but not least, an investigation conducted in 23,681 individuals reported that midday napping in healthy working men was inversely associated with coronary mortality after controlling for potential confounders.¹⁴⁵

Practice points

- 1) The desynchronization between the biological clock and working patterns in shift workers is involved in gradual and cumulative deleterious health effects over the years, raising public health concerns.
- 2) Some shift workers may have individual genetic and physiological predisposition and personal preferences that may, in part, help adapt to shift work, although a large number of these workers never really get used to these working schedules.
- 3) Epidemiological studies have shown that the consequences in shift workers range from sleep debt and sleepiness, which are thought to be to a certain degree reversible symptoms, to major deleterious effects on cardiovascular disease and cancer pathogenesis.
- 4) Neuroendocrine, immune and oxidative stress could act as biological mediators of the damaging health effects imposed by shift work.
- 5) Compelling evidence from laboratory studies shows that circadian misalignment but also sleep restriction can disrupt and enhance the activity of neuroendocrine stress systems, reduce immune defenses and increase the pro-oxidative status.
- 6) The assessment of neuroendocrine, oxidative and immune stress in the shift worker population is still a limited and novel field, which may have clinical relevance.
- 7) Napping could be a countermeasure to the enhanced neuroendocrine stress, reduced immune defense and cardiovascular risks imposed by shift work.

Research agenda

- 1) The molecular pathway(s) by which circadian misalignment and sleep restriction influence immune and oxidative stress gene expression needs to be further investigated.
- 2) Inter-individual vulnerability to the adverse impact of circadian disruption, sleep restriction and light at night should be investigated to detect relevant immune and oxidative stress markers.
- 3) Non-invasive biological markers need to be developed to identify individuals at increased risk of sleep debt and circadian disruption in terms of immune functions.
- 4) More longitudinal and prospective epidemiological investigations are needed to accurately evaluate the health consequences of chronic exposure to shift work schedules, including the health status of subjects after leaving shift work.
- 5) Practical napping strategies in the working environment would help improve health status and safety for shift workers.
- 6) The preventive and long-term health effects of melatonin administration need to be further documented before it can be widely used in shift workers.
- 7) Instituting programs to improve worker awareness about the physiological effects of shift work is needed.

Acknowledgments

We would like to thank the members of the consensus group on shift work: « Recommandations pour la surveillance médico-professionnelle des travailleurs postés et/ou de nuit », Haute Autorité de Santé (HAS): Catherine Didier Marsac, Arnaud Metlaine, Patrick Rodriguez, Claude Gronfier, Nicolas Hommey, Bernard Laumon, Valérie Jeunet, Marie-Christine Soula, Emmanuel Legoix, Etienne Larger, Jean-Daniel Lalau, Jean-Jacques Monsuez, Véronique Viot-Blanc, Francis Levi, Carine Davitian and also Karine Petitprez (HAS), Caroline Gauriau (Centre du Sommeil et de la Vigilance) and Karen Pickett for English-language editing. This review was supported by a grant of the Direction Générale du Travail (DGT), Ministère du Travail, de l'Emploi et de la Santé auprès de la Société Française de Médecine du Travail (SFMT).

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