CLINICAL REVIEW

Immune, inflammatory and cardiovascular consequences of sleep restriction and recovery

Brice Faraut \textsuperscript{a,b,c}, Karim Zouaoui Boudjeltia \textsuperscript{b}, Luc Vanhamme \textsuperscript{d}, Myriam Kerkhofs \textsuperscript{a,b,}\footnote{Corresponding author. Sleep Laboratory, CHU de Charleroi, A. Vésale Hospital, Montigny-le-Tilleul, Université Libre de Bruxelles, Belgium}  

\textsuperscript{a}Sleep Laboratory, CHU de Charleroi, A. Vésale Hospital, Montigny-le-Tilleul, Université Libre de Bruxelles, Belgium  
\textsuperscript{b}Laboratory of Experimental Medicine (ULB 222 Unit), CHU de Charleroi, A. Vésale Hospital, Montigny-le-Tilleul, Université Libre de Bruxelles, Belgium  
\textsuperscript{c}Université Paris Descartes, APHP, Hôtel Dieu, Centre du Sommeil et de la Vigilance, Paris, France  
\textsuperscript{d}Laboratory of Molecular Parasitology, Institute for Molecular Biology and Medicine, Université Libre de Bruxelles, Gosselies, Belgium

A R T I C L E   I N F O

Article history:
Received 2 February 2011
Received in revised form 4 May 2011
Accepted 4 May 2011
Available online 10 August 2011

Keywords:
Sleep restriction  
Sleep recovery  
Inflammatory marker  
Stress system  
Cardiovascular risk  
Sleep countermeasures

S U M M A R Y

In addition to its effects on cognitive function, compelling evidence links sleep loss to alterations in the neuroendocrine, immune and inflammatory systems with potential negative public-health ramifications. The evidence to suggest that shorter sleep is associated with detrimental health outcomes comes from both epidemiological and experimental sleep deprivation studies. This review will focus on the post-sleep deprivation and recovery changes in immune and inflammatory functions in well-controlled sleep restriction laboratory studies. The data obtained indicate non-specific activation of leukocyte populations and a state of low-level systemic inflammation after sleep loss. Furthermore, one night of recovery sleep does not allow full recovery of a number of these systemic immune and inflammatory markers. We will speculate on the mechanism(s) that link(s) sleep loss to these responses and to the progression of cardiovascular disease. The immune and inflammatory responses to chronic sleep restriction suggest that chronic exposure to reduced sleep (<6 h/day) and insufficient time for recovery sleep could have gradual deleterious effects, over years, on cardiovascular pathogenesis with a heightened risk in women and in night and shift workers. Finally, we will examine countermeasures, e.g., napping or sleep extension, which could improve the recovery processes, in terms of alertness and immune and inflammatory parameters, after sleep restriction.

© 2011 Elsevier Ltd. All rights reserved.

Introduction

There is a clear trend emerging of reduced sleep duration at night leading to a growing sleep debt in the general population in western countries. The proportion of adults who sleep less than 6 h per night in the US is now greater than at any other time on record this past decade. The 2009 National Sleep Foundation survey reported that the percentage of the population sleeping less than 6 h per night on weekdays has almost doubled over the last ten years, increasing from 12% in 1998 to 20% in 2009.\footnote{Some effects are modest and some will argue that adaptive physiological processes and/or sleep recovery could be sufficient to counterbalance these changes. However, chronic exposure to sleep restriction (SR) could have gradual and cumulative deleterious health effects over years as indicated by epidemiological results.}

Increasing numbers of people are becoming chronically sleep deprived because of greater work pressure in urban economies, e.g., extended working hours outside the regular 0800–1700 h working day, shift work, or increased accessibility to media of all sorts.

What are the consequences of sleep loss and lack of time for recovery sleep? It was commonly thought that the most important effect of night time sleep loss was daytime sleepiness resulting in cognitive impairment.\footnote{SD triggers impairment and dysregulation of all these physiological functions. Some effects are modest and some will argue that adaptive physiological processes and/or sleep recovery could be sufficient to counterbalance these changes. However, chronic exposure to sleep restriction (SR) could have gradual and cumulative deleterious health effects over years as indicated by epidemiological results.} However, in addition to cognitive dysfunction, compelling evidence links sleep loss to alterations in the metabolic, endocrine, immune and inflammatory systems with potential clinical relevance and public-health ramifications.

The evidence to suggest that shorter sleep is associated with detrimental health outcomes comes from epidemiological studies and well-controlled sleep deprivation (SD) laboratory studies. Experimental laboratory studies have primarily investigated neurobehavioral performance, metabolism, neuroendocrine stress, immune and inflammatory systems. The data obtained suggest that SD triggers impairment and dysregulation of all these physiological functions. Some effects are modest and some will argue that adaptive physiological processes and/or sleep recovery could be sufficient to counterbalance these changes. However, chronic exposure to sleep restriction (SR) could have gradual and cumulative deleterious health effects over years as indicated by epidemiological results.
The main domains addressed by epidemiological studies related to sleep are mental health, mortality risk, obesity and cardiovascular disease. Epidemiological surveys highlight that night and shift workers (NSWs), a population that is chronically sleep restricted in addition to sleeping and eating at abnormal circadian times, are at an increased risk of diabetes, obesity and cardiovascular pathologies.7,8 Short duration sleep has, by itself, also been found to be associated with a higher risk of obesity, diabetes and hypertension.9,10 Epidemiological surveys relating subjective self-reported sleep duration to health implicate poor sleep as a predictor of cardiovascular risk, and meta-analyses have also been found to be associated with a higher incidence of cardiovascular events.13,11,12

However, the underlying mechanism(s) that link(s) sleep loss to the progression of cardiovascular diseases is poorly understood. In this review, we will focus on post-SD changes in immune and inflammatory functions — possibly mediated via the neuroendocrine system — in well-controlled SR laboratory studies and the links of these changes to cardiovascular pathogenesis. Finally, we will examine countermeasures that may improve the recovery processes of immune and inflammatory parameters after SD.

**Immune consequences of sleep restriction and recovery**

**Introduction**

Most of the current knowledge on the effects of sleep loss in humans comes from controlled studies of total SD applied for 1 or 2 days, acute SR during a single night (25%–50% of a normal 8 h night’s sleep), or chronic SR for several successive nights (50%–75% of a normal 8 h night’s sleep). Most studies have investigated immune and inflammatory changes that occur in response to controlled experimental total SD or SR in rigorously screened healthy men and women who normally sleep approximately 8 h per night. The gold standard to assess the effects of SR is to precisely check sleep duration by continuously monitoring subjects throughout the study and to control dietary intake and light environment. These controlled experimental designs are informative models to begin to improve our understanding of the physiological consequences of sleep loss and the function(s) of sleep.

Activation of the immune and inflammatory systems under conditions of absent or reduced sleep can be detected by changes in a number of systemic markers measured in blood samples. Under regular sleep—wake conditions, a growing body of evidence suggests that human peripheral blood mononuclear cell (PBMC) subsets show circadian rhythms with peak counts at night or during the day, depending on the cell type.13 Therefore, methodological issues related to blood collection, e.g., time point of blood sampling, multiple vs. single blood collection procedures, have to be carefully considered when assessing the effects of SD. For example, one night of total SD may just displace the circadian rhythm of circulating blood immune cells rather than increase or decrease their levels and a single measurement may not reflect this. The biological marker, melatonin, which is under strong circadian control, has its amplitude weakly but significantly reduced (6.7% ± 2.1%) by one night of total SD in healthy young men when comparing melatonin concentrations measured during sleep to those measured at the same time point during constant wakefulness.14 When we look at chronic SR protocols in young men, a phase delay (1.2 ± 0.9 h) in melatonin secretion onset occurred after 10 days of 4.2 h time in bed (TIB).15 Hence, one night of sleep loss or chronic SR can shift the circadian rhythm in young men. Among leukocyte subsets, circadian rhythms of monocytes and lymphocytes have been shown to be significantly displaced by total SD. Hence, when investigating the effects of sleep loss on leukocyte subsets sensitive to circadian rhythm, potential confounding effects resulting from alterations of the circadian phase need to be controlled for.

However, this effect was not significant for neutrophil counts, proportionally the most important leukocyte sub-population.15

**Effects of experimental total sleep deprivation (see Table 1)**

Experimental total SD in healthy volunteers has been reported to alter several immunological markers. For example, components involved in the early host responses to infection, such as certain leukocyte populations, i.e., monocytes, lymphocytes and neutrophils, are affected by total SD. In an early study of total SD, in a small sample of 8 healthy women, in vitro-stimulated lymphocytes had an enhanced ability to produce interferons and neutrophils had a reduced ability to phagocytose.16 A second investigation in 12 healthy young men showed a reduction in blood lymphocyte DNA synthesis in vitro, an effect that persisted for 5 days.17 Leukocytosis was apparent after 64 h of total SD in young men and women with a significant progressive increase in neutrophil and monocyte levels and natural killer (NK) activity during the SD period.18 In addition, following the first night of recovery sleep, the levels of all these leukocyte subsets remained significantly higher than at baseline. Similarly, one night of total SD increased monocyte, NK cell and lymphocyte levels.13

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>GH</td>
<td>growth hormone</td>
</tr>
<tr>
<td>GHRH</td>
<td>GH-releasing hormone</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>high sensitivity CRP</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>IGF</td>
<td>insulin-like growth factor</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>Mox-LDL</td>
<td>MPO-modified LDL</td>
</tr>
<tr>
<td>MPO</td>
<td>myeloperoxidase</td>
</tr>
<tr>
<td>MT1/MT2</td>
<td>melatonin receptor 1/2</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor-kappa B</td>
</tr>
<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>NSW</td>
<td>night and shift worker</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PVT</td>
<td>psychomotor vigilance task</td>
</tr>
<tr>
<td>RAGE</td>
<td>receptor for advanced glycation end products</td>
</tr>
<tr>
<td>RZR</td>
<td>retinoid Z receptor</td>
</tr>
<tr>
<td>SD</td>
<td>sleep deprivation</td>
</tr>
<tr>
<td>SR</td>
<td>sleep restriction</td>
</tr>
<tr>
<td>SWS</td>
<td>slow-wave sleep</td>
</tr>
<tr>
<td>TIB</td>
<td>time in bed</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
</tbody>
</table>

**References**

Effects of experimental acute and chronic sleep restriction (see Table 2)

Sleep curtailment during only part of the night is one of the most common complaints by individuals who experience psychological or environmental stress and work pressure. In the U.S., 20% of the population sleeps less than 6 h per night on weekdays indicating a situation of chronic partial SR on work days.1 Moreover, a single night of acute sleep reduction is similar to the situation of extended work shifts experienced by health workers

Table 1
Consequences of total sleep deprivation on immune and inflammatory changes and effects of recovery and sleep countermeasures. “After 8-h recovery sleep” indicates changes from similar time points measured following the total sleep deprivation intervention. “Sleep countermeasures” indicates the napping period after or during the sleep deprivation interventions. Abbreviations: C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1ra), tumor necrosis factor-alpha receptor (TNF-αr).

<table>
<thead>
<tr>
<th>First author, year of publication, duration of total sleep deprivation (TSD), sample size</th>
<th>Immune changes</th>
<th>Inflammatory changes</th>
<th>After 8 h-recovery sleep</th>
<th>Sleep countermeasures</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 Palmblad, 1976; 77 h TSD, n = 8</td>
<td>↓ Neutrophil phagocytosis ability</td>
<td>↑ Interferons by in-vitro-stimulated lymphocyte</td>
<td>Not reported</td>
<td>?</td>
</tr>
<tr>
<td>17 Dinges, 1994; 64 h TSD, n = 10</td>
<td>↓ Leukocyte neutrophil, monocyte counts and natural killer activity</td>
<td>Leukocyte, neutrophil and monocyte counts &gt; baseline</td>
<td>Lymphocyte and monocyte counts – baseline, natural killer cells &lt; baseline</td>
<td>?</td>
</tr>
<tr>
<td>13 Born, 1997; 64 h TSD, n = 20</td>
<td>↑ Lymphocyte and monocyte counts and natural killer cells</td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>31 Shearer; 2001; 88 h TSD, n = 21</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>30 Frey, 2007; 40 h TSD, n = 19</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>32 Meier-Ewert; 2007; 88 h TSD, n = 10</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>29 Vgontzas, 2007; 40 h TSD, n = 41</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>34 Irwin, 2006, 2010; 1 night of 4 h-sleep</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>39 Haack, 2007; 10 nights of 4 h-sleep</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>38 Vgontzas, 2004; 7 nights of 6 h-sleep (22:30–04:30 h), n = 25</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>35 Irwin, 2010; 1 night of 4 h-sleep (03:00–07:00 h), n = 26</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>19 Faraut, 2011; 1 night of 2 h-sleep (02:00–04:00 h), n = 12</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>33 Zouaoui-Boudjeltia, submitted; 5 nights of 5 h-sleep (01:00–06:00 h), n = 9</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
</tbody>
</table>

Table 2
Consequences of sleep restriction on immune and inflammatory changes and effects of recovery and sleep countermeasures. “After 8-h recovery sleep” indicates changes from similar time points measured following the sleep restriction intervention. “Sleep countermeasures” indicates the napping period during the day following the sleep restriction intervention. Abbreviations: C-reactive protein (CRP), interleukin-1 β (IL-1β); interleukin-6 (IL-6), interleukin-17 (IL-17); myeloperoxidase-modified low-density lipoprotein (Mox-LDL); peripheral blood mononuclear cell (PBMC); tumor necrosis factor-alpha receptor (TNF-αr).

<table>
<thead>
<tr>
<th>Sleep restriction (SR)</th>
<th>Immune changes</th>
<th>Inflammatory changes</th>
<th>After 8 h-recovery sleep</th>
<th>Sleep countermeasures</th>
</tr>
</thead>
<tbody>
<tr>
<td>38 Vgontzas, 2004; 7 nights of 6 h-sleep (22:30–04:30 h), n = 25</td>
<td>↑ IL-6, TNF-α only in d</td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>34 Irwin, 2006, 2010; 1 night of 4 h-sleep (03:00–07:00 h), n = 30</td>
<td>↑ IL-6, TNF-α gene expression and protein by in vitro-stimulated monocytes</td>
<td>Not reported</td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>39 Haack, 2007; 10 nights of 4 h-sleep (23:00–03:00 h), n = 18</td>
<td>↑ Leukocyte and monocyte counts</td>
<td>↑ IL-6, unchanged CRP</td>
<td>Not reported</td>
<td>?</td>
</tr>
<tr>
<td>21 Kerkhofs, 2007; 3 nights of 4 h-sleep (01:00–05:00 h), n = 10</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>32 Meier-Ewert; 2007; 10 nights of 4 h-sleep (01:00–05:00 h), n = 10</td>
<td>↑ Leukocyte and neutrophil counts</td>
<td>↑ CRP</td>
<td>Not reported</td>
<td>?</td>
</tr>
<tr>
<td>20 Boudjeltia, 2008; 3 nights of 4 h-sleep (01:00–05:00 h), n = 8</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>40 van Leeuwen, 2009; 5 nights of 4 h-sleep (03:00–07:00 h), n = 13</td>
<td>↑ CRP, ↑ IL-6, IL-17 and IL-1β gene expression by in vitro-stimulated PBMC Greater ↑ IL-6, TNF-α by in vitro-stimulated monocytes in ? than in d</td>
<td>CRP and IL-17 &gt; baseline</td>
<td>Not reported</td>
<td>?</td>
</tr>
<tr>
<td>35 Irwin, 2010; 1 night of 4 h-sleep (03:00–07:00 h), n = 26</td>
<td>↑ Leukocyte and neutrophil counts</td>
<td>Leukocyte and neutrophil counts &gt; baseline</td>
<td>–30 min nap (13:00–13:30 h) post-SR: ↓ Leukocyte, neutrophil counts; ↓ cortisol–10 h extended recovery sleep (21:00–07:00 h): ↓ Leukocyte, neutrophil counts</td>
<td>?</td>
</tr>
<tr>
<td>19 Faraut, 2011; 1 night of 2 h-sleep (02:00–04:00 h), n = 12</td>
<td>↑ Leukocyte and neutrophil counts</td>
<td>↑ Myeloperoxidase</td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>33 Zouaoui-Boudjeltia, submitted; 5 nights of 5 h-sleep (01:00–06:00 h), n = 9</td>
<td></td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>12 B. Faraut et al. / Sleep Medicine Reviews 16 (2012) 137–149</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
such as interns during residency training who sleep 2–3 h per night when on duty-call.  

Laboratory studies of SR allow the effects of lack of sleep to be investigated under well-controlled conditions (caloric intake, continuous electroencephalogram monitoring). SR appears closer than total SD to the real life situation. The effects of acute SR were examined in healthy young men subjected to one night of sleep restricted to 2 h (between 02:00 h and 04:00 h, a night time period with a high propensity for sleeping in young subjects) followed by a night of 8 h of recovery sleep. An increase in peripheral blood leukocytes, mainly explained by a higher level of neutrophils, was measured the morning after the SR night; the morning after the recovery night, leukocyte and neutrophil counts remained at the same high levels. A chronic SR protocol in healthy young men, in which they were allowed 4 h of sleep (between 01:00 h and 05:00 h) for three consecutive nights also resulted in increased neutrophil counts the morning after the 3rd night of SR. The same SR protocol in postmenopausal women induced an increase in leukocyte counts with a rise in neutrophil and monocyte subsets the morning after the 3rd night of SR.  

Conclusion  

Taken together, these sleep laboratory studies suggest that non-specific immune parameters are activated when sleep is restricted. An important question then is whether and how sleep helps in combating infectious disease? A few studies have examined the consequences of sleep loss on the immune response to vaccination in healthy individuals. The acute inflammatory response starts a few minutes after contact with the injected antigen and, over subsequent days, activation of T and B cells will contribute to form the immunological memory. Sleep deprivation for only one night substantially impaired the antibody response to hepatitis A vaccine. Indeed, healthy subjects with regular sleep after vaccination displayed a nearly two-fold higher hepatitis A virus antibody titer after 4 weeks than subjects subjected to total SD the night after vaccination. Similarly, chronic SR slowed the response to influenza vaccine. These vaccination experiments in sleep deprived humans suggest that sleep improves the formation of antigen-specific immune defense as reflected by antibody production in humans and support the concept that sleep-mediated factors play an important role in the humoral immune response.  

Finally, in animal studies, prolonged total SD led to death in rats with opportunistic infections, related to potential immuno-depression. Another experiment examined the recuperative value of enhanced sleep in rabbits inoculated with Escherichia coli, Staphylococcus aureus, or Candida albicans. A long period of enhanced sleep was associated with a more favorable prognosis and less severe clinical signs than were relatively short periods of enhanced sleep. These data further suggest that neuroendocrine sleep-mediated changes impact specifically on the humoral immune response and cell-mediated immunity.  

Inflammatory consequences of sleep restriction and recovery  

Introduction  

To investigate inflammatory parameters, numerous sleep deprivation studies have assessed blood cytokine levels. The expression of monocytes, the main source of these cytokines, and neutrophils was significantly enhanced during experimental SD in subjects who underwent rigorous medical examinations to demonstrate their healthy status and usual nighttime sleep length of ~8 h. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8, IL-12, IL-17, IL-18, in balance with anti-inflammatory cytokines (interferon [IFN]-α, transforming growth factor [TGF]-β...) are essential mediators of the inflammatory system. Pro-inflammatory cytokines also have specific receptors that regulate the balance of the inflammatory response. The pro-inflammatory cytokines chemoattract and up-regulate mediators of the inflammatory process, act as endogenous pyrogens or stimulate 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, IL-8 or IL-17, has the methodological advantage that it does not display diurnal variation. Under normal physiological conditions, there are low concentrations of cytokines in the blood, except for IL-6, which is a cytokine with hormone-like actions. Hence, some SD studies have assessed cytokine levels in vitro using stimulated PBMC and measured supernatant cytokine levels. Several studies have reported circadian rhythms for various cytokines, including IL-6, IL-12 or TNF-α, similar to the leukocyte subset cells that are responsible for their production. This suggests that the results of in vitro cytokine assays must be adjusted for the time of blood sampling and the level of each leukocyte subset in the stimulated sample to avoid confounding effects of circadian rhythm. In addition, the procedure used for multiple blood collections must be considered; intravenous indwelling catheters can enhance local inflammation and increase cytokine concentrations independent of the effects of SD, which is not the case when blood is sampled by a needle stick.  

Effects of experimental total sleep deprivation (see Table 1)  

The systemic expression of pro-inflammatory cytokines, such as IL-1β and IL-6, was increased in a group of healthy men and women during the day after total SD. IL-6 and TNF-α receptor levels were elevated at the end of an extended 88 h period of total SD in healthy men. Elevated levels of CRP have also been measured after total SD in healthy young men. Nevertheless, a significant decrease in CRP and IL-6 was reported in a group of healthy men and women after one night of total SD. Although these discrepancies remain unclear, the fact that intravenous catheters used for repetitive blood sampling can increase local IL-6 production is a potential contributing factor. These data also suggest potential differential sex effects of SD on inflammatory markers (as discussed below).  

Effects of experimental acute and chronic sleep restriction (see Table 2)  

Several laboratories have reported blood inflammatory cytokine changes after acute SR of a single night and following multiple consecutive nights of SR. When sleep was allowed during only the second part of the night (from 03:00 h to 07:00 h), the normal nocturnal increase in IL-6 levels was delayed until sleep at 03:00 h in healthy male volunteers. The same early-night partial SR protocol in healthy men and women resulted in greater production of IL-6 and TNF-α proteins in stimulated peripheral blood monocytes during the morning after SR, and greater gene expression. A recent article by the same group and using the same early-night partial SR protocol further indicated that women and men both showed a significant increase in the production of IL-6 and TNF-α in the morning immediately after SR whereas production of these cytokines during the early and late evening was increased in women but decreased in men. SR, in which 2 h of sleep was allowed between 02:00 h and 04:00 h, induced an increase in the plasma protein expression of myeloperoxidase (MPO) in healthy
young men; the same pattern was observed for MPO activity.\textsuperscript{19} MPO – mainly released by neutrophils that are also increased after SR – catalyzes the formation of oxidizing agents that can convert low-density lipoprotein (LDL) into an atherogenic form.\textsuperscript{36,37} Levels of the pro-inflammatory cytokine, IL-8, and high sensitivity C-reactive protein (hs-CRP) were unchanged after SR in this study.

A chronic modest reduction of 2 h of sleep per night, i.e., sleeping 6 h/night for 7 consecutive days, was associated with increased 24 h secretion of IL-6 in a group of healthy young men and women at the end of the SR period, whereas early-morning values of TNF-α were only increased in men.\textsuperscript{38} Increased CRP levels were measured after nighttime sleep was restricted to 4 h over 10 days in a mixed group of men and women. Another study in a group of men and women, with a similar design, i.e., 10 days of nighttime sleep restricted to 4 h, reported unchanged TNF-α receptor levels, a significant increase in IL-6 levels and an insignificant CRP increase compared to baseline.\textsuperscript{39} In a study of five SR nights, during which sleep was allowed only between 01:00 h and 06:00 h, and three recovery nights of 8 h (23:00–07:00 h), MPO-modified low-density lipoprotein (Mox-LDL) levels were increased during restricted sleep. Interestingly, MPO concentrations peaked after the first recovery night and significant increases in slow-wave sleep (SWS) and in insulin-like growth factor (IGF)-1 concentrations were observed during the first recovery night (Zouaoui-Boudjeltia et al., submitted). Recently, it was shown that 5 nights of sleep restriction (4 h) increased blood hs-CRP levels and the in vitro PBMC-stimulated production of pro-inflammatory molecule gene expression, including IL-1β, IL-6, and IL-17, in healthy young men.\textsuperscript{40} The levels of IL-17 and CRP remained significantly elevated after 2 nights of recovery sleep.

**Conclusion**

In conclusion, these altered profiles of inflammatory markers suggest that failure to obtain adequate amounts of sleep promotes low-level systemic inflammation. When interpreting data associating sleep restriction and inflammatory markers, experimental factors prior to blood sampling, such as stress and activity levels, time of meal intake, body mass index (BMI) or smoking status, need to be considered. In addition, especially when a small number of subjects is tested, we have to remember that “healthy status” also includes individual differences in basal levels of inflammatory markers. Although the physiological mechanism(s) underlying the links between sleep deprivation and these immune and inflammatory responses remain(s) largely unknown, neuroendocrine, autonomic vascular stress and SWS-hormone dependent changes are likely involved.

**Potential physiological pathway(s) involved in the immune and inflammatory effects of sleep restriction and recovery**

**Introduction**

The precise mechanism(s) that link(s) sleep loss to immune and inflammatory changes is not well understood. We will discuss and speculate on possible physiological mechanisms involved in the observed changes in immune and inflammatory markers after sleep loss (see Fig. 1).

**Slow-wave-sleep – growth hormone – stress axis interactions**

The elevated SWS pressure and extended epochs of SWS within the first hours of the night coincide with peak secretion of growth hormone (GH) and minimum cortisol release.\textsuperscript{41} This nearly complete suppression of cortisol release during the early hours of usual nighttime sleep indicates that the early night period may be associated with a phase of low stress activity. Accordingly, intravenous administration of GH-releasing hormone (GHRH) has been reported to produce a significant increase in plasma GH concentration associated with a blunted nocturnal secretion of cortisol in healthy men.\textsuperscript{42} Additional evidence of an inhibition of hypothalamic-pituitary adrenal activity by GHRH during early sleep is provided by two further studies. Cortisol release was blunted after pulsatile intravenous administration of GHRH during the first few hours of the night in normal young men and after intranasal administration of GHRH prior to nocturnal sleep in aged as well as in young subjects.\textsuperscript{43,44} Hence, sleep and its SWS component contribute to suppress the release of major mediators of the stress systems. SWS can inhibit cortisol release by the hypothalamic-pituitary adrenal axis as well as the elevated release of catecholamines by the sympathetic adrenal system observed following partial SD.\textsuperscript{45,46} Minimal cortisol release in the presence of maximum GH release results in a pattern mainly present during the early hours of regular nocturnal sleep. During recovery sleep after SR, there is a tight association between the amount of GH secreted and the amount of SWS.\textsuperscript{47} Among the large number of effects mediated by the secreted GH is the production of IGF-1, which is able to induce degranulation of azurophilic granules (the MPO content) by PMBCs.\textsuperscript{48} A peak in IGF-1 levels, associated with a rebound in SWS, has been observed after the first recovery night following chronic SR and may, in part, explain the peak in MPO after this first recovery night (Zouaoui-Boudjeltia et al., submitted).

**Neuroendocrine stress activation and leukocyte mobilization**

Circadian oscillations in leukocyte subsets have been reported to take place independently of the sleep–wake cycle. However, in addition to the circadian influences, sleep exerts a suppressing effect on several circulating leukocyte subsets.\textsuperscript{13} Sleep and its SWS component are expected to enforce the low-activity phase of catecholamine and cortisol release observed during nighttime sleep and to contribute to the nocturnal decrease in leukocyte levels. Hence, the stress system mediators that are overexpressed during partial SD certainly play a role in leukocyte mobilization. The most likely candidates for the neutrophil responses to sleep loss are the hormones cortisol and epinephrine. Plasma or saliva cortisol and blood catecholamines are widely used markers of stress in human research and SD studies. Increased cortisol levels during the evening when cortisol is on the descending phase of its circadian rhythm. However, the effect of napping, one could hypothesize that SWS during the nap could inhibit the hypothalamic-pituitary adrenal axis and cortisol release. Accordingly, leukocyte recovery after SD can be improved by having a nap with SWS prior to the recovery sleep. A stress-releasing effect is induced by napping as shown by the decrease in cortisol levels observed immediately after a midday nap half composed of SWS.\textsuperscript{19} It is worth noting that the cortisol drop induced by the nap in this study occurred during the afternoon when cortisol is on the descending phase of its circadian rhythm.
Several studies have assessed the effects of cortisol and catecholamines on the in vitro production of inflammatory cytokines by human monocytes, the main leukocyte subtype that produces inflammatory cytokines. Norepinephrine and epinephrine inhibited, in a concentration-dependent manner, the stimulated IL-6 and TNF-α production by human whole blood and human monocytes. In addition, although in vitro data generally support the hypothesis that cortisol suppresses the production of cytokines, physiological levels of corticosterone (rodent cortisol) have been shown to cause an increase in TNF and IL-6 in isolated perfused rat livers. In the isolated perfused rat liver, norepinephrine also promoted IL-6 secretion from the liver. Under basal conditions, NF-κB is sequestered in the cytoplasm by IkB, an inhibitory molecule that masks the nuclear localization motif of the transcription factor. Inflammatory stimulation results in the downstream signaling events that lead to the activation of IkB kinase. Phosphorylation of IkBa releases NF-κB from the cytoplasm and it is translocated into the nucleus, where it binds to target promoters. Numerous inflammatory genes contain NF-κB-binding sites and are markedly up-regulated in response to pro-inflammatory signals. Of interest, one night of sleep restricted to the second part of the night (from 03:00 h to 07:00 h) has been reported to induce in healthy subjects a rapid increase in activation of the transcription factor NF-κB in PBMC, providing a potential molecular mechanism for the effects of sleep loss on pro-inflammatory gene expression. Although the small size of the sample tested makes further confirmation necessary, this effect was mainly observed in women and could contribute to the sex differences recently reported in the expression of inflammatory

**Cortisol and catecholamine action on inflammatory cytokines**

The catecholamine, norepinephrine, whose release is enhanced by SD, is an inducer of nuclear factor-kappa B (NF-κB) activity, and both these actors participate in the loop of inflammation that has been shown to be crucial in the regulation of inflammatory processes via direct activation of pro-inflammatory cytokines. Under basal conditions, NF-κB is sequestered in the cytoplasm by IkB, an inhibitory molecule that masks the nuclear localization motif of the transcription factor. Inflammatory stimulation results in the downstream signaling events that lead to the activation of IkB kinase. Phosphorylation of IkBa releases NF-κB from the cytoplasm and it is translocated into the nucleus, where it binds to target promoters. Numerous inflammatory genes contain NF-κB-binding sites and are markedly up-regulated in response to pro-inflammatory signals. Of interest, one night of sleep restricted to the second part of the night (from 03:00 h to 07:00 h) has been reported to induce in healthy subjects a rapid increase in activation of the transcription factor NF-κB in PBMC, providing a potential molecular mechanism for the effects of sleep loss on pro-inflammatory gene expression. Although the small size of the sample tested makes further confirmation necessary, this effect was mainly observed in women and could contribute to the sex differences recently reported in the expression of inflammatory

![Potential pathway(s) by which sleep restriction and insufficient recovery sleep lead to cardiovascular pathologies. Sleep restriction coupled to insufficient recovery sleep enhance the activity of the autonomic and stress systems. Vascular shear stress exacerbated by increased blood pressure leads to inflammation in the vascular wall potentially leading to the endothelial production of inflammatory mediators. The stress mediators cortisol/catecholamine can mobilize leukocyte in the blood circulation; among leukocyte subtype neutrophil degranulation can trigger an oxidative burst and the release of oxidative stress markers. Nap and its slow wave sleep (SWS) component can blunt the stress response e.g., reduce cortisol release with subsequent decreased leukocyte mobilization. Catecholamine can enhance the expression of nuclear factor-kappa B (NF-κB), an activator of pro-inflammatory gene expression, e.g., pro-inflammatory cytokines. All these physiopathological altered pathways following SR contribute to a chronic pro-inflammatory status ultimately leading to the development of cardiovascular pathologies. Abbreviations: C-reactive protein (CRP)](image_url)
markers and incidence of cardiovascular pathophysiology after sleep loss. Pharmacological inhibition of NF-κB activity has been shown to reduce atherosclerosis in mice, making it a potential therapeutic target.64

**Enhanced blood pressure and vascular shear stress**

In addition to the hormonal stress response consequent to SD, it has been hypothesized that vascular shear stress exacerbated by increased blood pressure (BP) leads to inflammation in the vascular wall.6 Increased BP associated with SD is a physiological stressor that potentially affects endothelial function.65 The increased endothelial activation has been demonstrated by increased circulating levels of endothelial cell activation markers, such as E-selectin or intercellular adhesion molecule (ICAM)-1, and microvascular dysfunction after one night of total SD.30,66 Increased BP and endothelial activation is a potential mechanism leading to the endothelial production of inflammatory mediators (e.g., IL-6) after SD in healthy subjects.67

**The melatonin hypothesis?**

Melatonin is synthesized in the suprachiasmatic nucleus and secreted by the pineal gland according to a circadian rhythm, with a consequent diurnal oscillation in its blood levels. Melatonin is a strong regulator of circadian rhythms via its action on target cells. Following total SD or chronic SR, melatonin displays a secretion pattern of reduced amplitude or delayed onset, respectively.14,68

These changes observed in healthy young subjects are expected to be enhanced with chronic sleep loss, extended shift work or aging. In addition to its main physiological effects on sleep synchronization, a substantial body of research suggests that melatonin, through its neuroendocrine action, has antioxidant and immunomodulatory properties. Melatonin’s ability to regulate diurnal cytokine production and cells mediating non-specific immunity has been shown in several studies.69,70 Leukocytes are sensitive to melatonin through the presence of specific receptors (Melatonin receptors 1/2 (MT1/MT2) and nuclear retinoid Z receptor (RZR)). Melatonin significantly reduced the increase in leukocyte and lymphocyte count produced directly after exercising. The same study indicated that levels of the inflammatory cytokines, IL-8 and TNF-α, tended to be lower in individuals following exercise.71 Similarly the enhanced cardiac expression of IL-6 and TNF-α after exercise was significantly prevented by melatonin administration in rats.72 In addition, oxidative phenomena play a key role in the early phase of inflammation. Activated neutrophils generate an oxidative burst with the release of potent oxidant agents such as MPO. Melatonin has been described as a powerful antioxidant and as a scavenger of different types of free radicals.73 Moreover, its potent antioxidant ability could reduce oxidative stress in inflamed tissues with subsequent improvement in oxidative-related pathologies such as atherosclerosis.74 Whether these effects have a physiological relevance in SD remains to be investigated. However, these findings support the idea that melatonin could act as a potential modulator of the immune and inflammatory responses induced by sleep loss.

**Conclusion**

In summary, these data suggest that the increase in leukocyte count is secondary to autonomic activation, rather than a direct effect of SR on the immune and inflammatory systems. However, it is difficult to determine experimentally whether the immune alterations are associated with activation of a non-specific immune response or a stress and autonomic response that occurs post-SR. One can speculate that the inhibition of cortisol and catecholamine release during early sleep is critical for the recovery from stress and associated immune alterations. Accordingly, low stress scores were significantly associated with a greater decrease in blood levels of epinephrine and IL-6 after nocturnal sleep in 130 healthy volunteers.75 Finally, sex differences reported in NF-κB activation after sleep loss or in HPA axis responses to different specific stressful conditions may be relevant in the stress response to SD and contribute to the reported differences in markers of inflammation between men and women.33,76,77

**What could be the clinical significance of these altered biomarkers for pathogenesis of cardiovascular disease?**

**Introduction**

The evidence that low-grade local and systemic inflammation occur in all stages of atherogenesis has led to the discovery of a number of novel independent predictors of cardiovascular risk. Among these emerging biomarkers associated with the inflammatory and atherogenesis pathways that lead to cardiovascular disease, leukocyte count, and levels of CRP, IL-6 or MPO are all increased in healthy humans after experimental SD.17,19,32,34 Although these effects are often small, such chronic sub-clinical shifts have been described as contributing to cardiovascular pathogenesis. However, it is not clearly established how post-SR changes in stress, immune and inflammatory functions can affect cardiovascular health.

**Leukocytes**

Immune cells are assigned to adaptive immune functions (e.g., antigen-specific) or to innate non-specific immune functions (e.g., NK-cells, neutrophils, monocytes). In the absence of a foreign antigen, SD, as induced experimentally, triggers a stress-like response which is well characterized immunologically, with increases in leukocyte and neutrophil counts. Earlier studies reported that leukocyte count was a valuable predictor of myocardial infarction.77 Epidemiological studies have identified a link between leukocyte count and an increased risk of cardiovascular disease in primary and secondary prevention. For example, a high leukocyte count has been shown to predict the development of type 2 diabetes suggesting that chronic activation of the immune system may play a role in the pathogenesis of this disease.80

The leukocyte count, mainly determined by neutrophil count in healthy humans, also has to be considered as clinically relevant in the absence of acute medical events. Increased leukocyte counts have long been associated with increased all-cause mortality and considered a biomarker of inflammatory processes that contribute to vascular injury and atherosclerosis.81 Indeed, increased leukocyte and neutrophil counts have been shown to be independent risk factors of cardiovascular mortality in numerous studies and subsequent meta-analyses.82–84 In a prospective study that was conducted over 44 years, higher leukocyte count, even within the normal range (mainly neutrophils), was associated with greater mortality.83 Interestingly, this study found that leukocyte counts (mostly neutrophils) increased progressively in participants who died during the follow-up, starting several years before death, whereas leukocyte counts remained stable over time in those who survived. Hence, similarly, repetitive acute SR, as commonly observed in extended work shifts, could result in progressively elevated leukocyte and neutrophil levels over years with possible long-term effects on survival. The differential leukocyte sub-count in coronary heart disease risk assessment suggests that neutrophil count represents the strongest predictor of incident coronary heart disease.85 Among the leukocyte sub-population, monocytes47 and
neutrophils may influence the development of coronary heart disease through their ability to cause proteolytic and oxidative damage to coronary arteries. Activation of neutrophils has profound systemic effects through the release of enzymes, such as MPO, a heme protein accounting for up to 5% of total cell protein, from the neutrophil’s azurophilic granules.

Oxidative stress markers

Preliminary data reported an increase in MPO after SR, mainly released by neutrophils which are also increased after SR. MPO is a potent pro-atherogenic agent with growing evidence indicating its role, through the generation of Mox-LDL, in the pathways that lead to cardiovascular disease. A peak in MPO concentration was measured after a recovery night following experimental chronic SR (Zouaoui-Boudjelita et al., submitted). Repetition of a chronic sleep restriction and recovery process could ultimately contribute to increased levels of serum MPO, which are known to be associated with cardiovascular events.

Inflammatory cytokines and CRP

Inflammation is involved in cardiovascular pathogenesis and inflammatory markers, such as pro-inflammatory cytokines and CRP, are valuable predictors of cardiovascular events even in healthy, asymptomatic men and women. The pro-inflammatory cytokines, IL-6 and TNF-α, have both been implicated as risk markers for coronary heart disease. IL-6 is a key pro-inflammatory cytokine that mediates expression of several ‘downstream’ inflammatory markers potentially involved in atherogenesis and atherothrombosis. IL-6 has been shown to be valuable for identifying individuals at risk of cardiovascular events. IL-6, by augmenting the expression of matrix-degrading proteases, could participate in the progression of atherogenesis as reported in a mouse study. IL-6 is also a potent inducer of the hepatic acute phase response, which is associated with increased levels of fibrinogen that promote fibrin formation, a risk factor for coronary heart disease. Similarly to IL-6, TNF-α has also been reported to have a predictive value on the incidence of cardiovascular events.

CRP, one of the most suitable markers for use in clinical practice since it does not display a diurnal rhythm, has a long half-life of 19 h and, using hs-CRP assays, can be detected at sub-clinical blood levels in healthy individuals. A direct role for CRP in atherogenesis is supported by a recent report indicating that CRP concentrations known to predict cardiovascular events could impair defenses via modulating the expression of the receptor for advanced glycation end products (RAGE). Finally, serum CRP level has also been independently associated with advanced atherosclerosis in young men and women.

Sex differences for cardiovascular risk markers?

Emerging evidence from epidemiological studies indicates that short sleep duration and subjective symptoms of poor sleep are correlated with a greater risk of cardiovascular disease in women than in men after controlling for potential confounders, such as physical activity or BMI. The relationships between self-reported sleep quality and cardiovascular risk markers were examined in a non-smoking population of 210 healthy men (28 ± 9.7 years) and women (30 ± 9.5 years). The data showed that elevations in systemic CRP, IL-6 and fibrinogen were associated with poor overall sleep quality and more frequent problems falling asleep for women, but not for men. A second report investigated the relationships between self-reported sleep duration and IL-6 (n = 4642) and hs-CRP (n = 4677) in a middle-aged population.

No significant variation in inflammatory markers with sleep duration was observed in men while significantly higher levels of hs-CRP and a trend to increased IL-6 were associated with shorter sleep duration in women.

Similar sex-specific differences have been reported in SD laboratory studies. A chronic SR protocol in young healthy men with three consecutive nights of 4-h TIB (between 01:00 h and 05:00 h) resulted in increased neutrophil counts the morning after the 3rd night of SR but total cholesterol LDL and monocytes were not affected. The same SR protocol in postmenopausal women induced an increase in leukocyte counts the morning after the 3rd night of SR with a rise in neutrophil and monocyte subsets and also total cholesterol and LDL-cholesterol. This further suggests a disparity between the sexes, although the age difference between the two studies could be a potential confounding factor. A recent laboratory study sought to determine sex differences in the effects of one night of partial experimental sleep loss on the inflammatory response in same age populations; interestingly, the production of IL-6 and TNF-α during the early and late evening was increased in women but decreased in men.

Conclusion

Epidemiological evidence suggests that accumulation of a sleep deficit over years could have gradual and cumulative deleterious effects on cardiovascular health. The immune and inflammatory responses to chronic sleep loss are certainly clinically relevant because of the prevalence of these processes in a number of epidemic diseases, e.g., diabetes, obesity, sleep apnea or atherosclerosis. In a healthy middle-aged population of 495 participants, objective sleep duration measured by actimetry was studied in relation to the degree of artery calcification, a marker of the atheromatous plaque, the main process involved in the development of cardiovascular disease. The results showed that objectively measured shorter sleep length was associated with a higher incidence of coronary artery calcification. In addition to alterations in sleep length or quality because of lifestyle factors or sleep disorders, behavioral factors, such as diet, smoking, alcohol or exercise, can influence the expression of inflammatory markers. NSWs very often have chronic SR in addition to several of these behavioral factors with a subsequent negative impact on inflammatory status. Accordingly, there is a greater incidence of cardiovascular pathology, including diabetes, obesity and hypertension, in NSWs than in non-NSW individuals.

Sleep countermeasures to improve the immune and inflammatory recovery functions of sleep

Insufficient recovery sleep

Sleep deprivation and restriction studies have assessed alertness and performance after recovery sleep but immune cells and inflammatory markers have been investigated to a lesser extent. The data obtained on performance indicated that one or even two nights of an 8 h recovery night of sleep is not sufficient to normalize neurobehavioral deficits after SD. The same profiles were also found for immune and inflammatory parameters. The increased levels of the inflammatory marker CRP after total SD remained at the same higher level after the first night of recovery sleep in healthy young men. Similarly after chronic SR, gene expression of the pro-inflammatory cytokines, IL-6 and IL-1β, displayed a trend to higher levels compared to baseline, and IL-17 and CRP proteins were still significantly higher after two 8 h nights of recovery sleep. Neutrophil and monocyte levels, powerful
producers of cytokines, also remained significantly higher than baseline values after 8 h of recovery sleep following one night of total SD or acute SR.\(^{17,19}\)

In a study of five nights of sleep restricted to 5 h, which mimicked the shorter sleep duration of a working week, an enhanced oxidative stress during the restriction period was measured followed by a peak in MPO levels after the first 8 h sleep recovery night (Zouaoui-Boudjeltia et al., submitted). A significant increase in SWS and IGF-1 concentration was also observed during the first recovery night. The SWS rebound was correlated with peak MPO concentrations possibly induced by concomitant SWS-dependent IGF-1 production. All these parameters normalized after the second recovery night. In healthy men, after one night of total SD, Sauvet et al. found an increase in IL-6 and norepinephrine after the first 8 h night of recovery sleep suggesting, as in the previous study, a potential effect of the recovery night per se.\(^{16}\)

In summary, levels of cardiovascular risk markers, such as CRP or pro-inflammatory cytokines, remain increased after the first recovery night. A chronic sleep restriction condition as often occurred in NSWs may contribute to progressive alterations in the cardiovascular risk mediators raising long-term health concerns. An important issue is to look for strategies to improve sleep recovery in terms of alertness, and immune and inflammatory parameters.

**Napping countermeasure**

Napping is an effective strategy to combat fatigue and sleepiness during long working hours. The “post-lunch dip” in the mid-afternoon is characterized by decreased alertness and performance with numerous errors at the workplace. A short nap, especially during the post-noon nap zone, has been shown to restore alertness and promote performance and memory processing without the inconvenience of sleep inertia associated with longer naps.\(^{108–112}\) Sleep inertia is a period after awakening characterized by reduced task performance and a sensation of disorientation, which is usually worse when awoken during SWS. When countermeasures to sleepiness are combined, such as caffeine followed by a nap not longer than 20 min (the time for the caffeine to operate), the benefit on alertness and inertia can be higher than a single countermeasure. Bright light exposure at the end of the nap may also reduce sleep inertia although the optimum wavelength, intensity or exposure duration need to be resolved.\(^{113}\)

The National Sleep Foundation 2008 Sleep in America Poll indicated that nearly one-half of the respondents (46%; \(n = 10000\)) reported having taken two or more naps in the past month (26% had taken 2 to 4 naps/month, 20% had taken 5 or more naps/month) with a length of 15–44 min and 45 min to 1 h 14 min for 36% and 38% of nappers, respectively. Among regular nappers, it is important to discriminate subjects with sleep disorders (e.g., sleep apnea), reduced nighttime sleep duration and sleep fragmentation (e.g., the elderly population) from healthy individuals who are sleep-deprived because of voluntary sleep curtailment or working hour schedules. Both situations result in subsequent daytime sleepiness and sleep episodes. Napping is an efficient countermeasure, especially in young people who are more sensitive to sleep loss and show a greater homeostatic pressure post-SD than do older subjects.\(^{18}\) For example, in resident interns (essentially young physicians) sleeping for 2–3 h during extended work shifts, less overall fatigue was observed when they were assigned to a nap schedule.\(^{18}\)

However, the effects of a short nap following SR, i.e., not longer than 30 min, on leukocyte counts and inflammatory blood markers are largely unknown. Two studies examined the effects of sleep episodes of 2 h on inflammatory cytokines within protocols of total SD. In healthy young women and men, a midday episode of 2 h sleep (with nearly 1 h of SWS) reversed the effect of one night of total SD on the afternoon values of the pro-inflammatory cytokine, IL-6.\(^{29}\) Shearer and collaborators reported that men who were allowed to sleep for a period of 2 h once at night (02:45 h to 04:45 h) and once in the afternoon (14:45 h to 16:45 h) for four days showed no increase in TNF-\(\alpha\) concentrations, whereas IL-6 and TNF-\(\alpha\) receptor levels were significantly increased in men who underwent total SD without the 2 h sleep episodes.\(^{31}\) We recently reported that following a night of acute SR to 2 h, a 30-min (half of which was SWS) midday nap prior to recovery sleep improved alertness and the return of leukocyte – mainly neutrophil counts – to baseline values.\(^{19}\) A nighttime sleep duration of 2 h is close to the situation of extended work shifts experienced by workers such as interns during residency training.\(^{18}\) This further suggests that a recovery night of 8 h following SR is not sufficient to normalize immune alterations to baseline values unless a short midday nap is allowed before the recovery night. Thus, in addition to restoring alertness, napping induces a stress-releasing effect as shown by the decrease in cortisol measured immediately after the nap which could explain the improved neutrophil recovery and why midday napping in healthy individuals has been reported to be inversely associated with coronary mortality among healthy working men after controlling for potential confounders.\(^{114}\) The stronger beneficial effect of midday napping among employees may reflect insufficient nocturnal sleep in this group because of the requirement to wake up early. For the elderly, the benefits of daytime napping are more controversial since some data suggest that napping is a risk factor for myocardial infarct and morbidity.\(^{115–117}\)

In summary, sleep loss produces significant increases in blood levels of neutrophils and IL-6 indicating sleep-dependent interactions between the central nervous system and neuroendocrine and immune functions. These changes may reflect increases in the homeostatic drive for sleep because they occur in sleep-deprived subjects but to a lesser extent when SD is counterbalanced by napping strategies. In addition, neutrophil counts may reflect the sleep debt because only conditions of prolonged sleep (napping or sleep extension) effectively recovered neutrophil counts. An important question is whether a nap without SWS may influence the immune and inflammatory markers to the same extent as a nap rich in SWS.

**Sleep extension countermeasures**

A recent study reported that sleep extension to 10 h TIB compared to 7 h TIB for one week reduced psychomotor vigilance task (PVT) lapses and improved objective alertness during a subsequent SR phase (7 nights of 3 h TIB) and improved PVT speed during the recovery days. This suggests that the speed at which the alertness and performance impaired by chronic SR are subsequently reversed by recovery sleep depends on the amount of sleep obtained during the night prior to the SR period.\(^{118}\) Moreover, when sleep recovery was extended to 9 h compared to 6 h after total SD, subjective sleepiness and PVT performance recovered better.\(^{106}\)

However, although the effects of extended sleep recovery on neurobehavioral performance and alertness have previously been examined after SD, these studies did not simultaneously evaluate the effects on immune parameters. After a single night restricted to 2 h of sleep, additional recovery sleep provided by an extended night of 10 h of sleep instead of the usual 8 h improved alertness and returned neutrophil counts to baseline values.\(^{19}\)

**Implications for extended work hours and night and shift-work**

A dichotomy between physiological rhythms and socio-economic rhythms in NSWs and individuals who work...
extended hours raises possible public-health concerns. For example, physicians on night call have a mean night’s sleep of 2–3 h. Their scores for mental fatigue and feeling well rested were lower after the first recovery night and returned to baseline only after the second recovery night post-call; both post-call nights were assessed by actimetry with a sleep duration of 7 h. A further study reported that, after a 30 h extended work shift, internal medicine residents had increased blood levels of IL-6, CRP and norepinephrine, and decreased flow-mediated vasodilatation compared to the same residents after a non-extended work shift. In addition to the adverse consequences of sleepiness, insufficient recovery sleep after the first recovery night following an experimental night restricted to a similar duration as experienced by physicians on night call is linked to persistently elevated leukocyte counts. NSWs are chronically sleep deprived and experience recovery day sleep of only around 5–6 h after the night shift; objective assessment of sleep indicates that their day sleep is 1–4 h shorter than night sleep. This suggests that the insufficient recovery sleep of NSWs does not allow adequate recovery of the stress and immune parameters altered by sleep deprivation and may contribute to an increased risk of cardiovascular disease. Preliminary reports confirm that NSWs do indeed have higher leukocyte counts than non-NWSs. 122,123 Practical strategies to improve health status and safety in the working environment for NSWs are necessary to improve tolerance to these specific working hour schedules. Further research on the topic is required to elaborate relevant strategies that combine napping facilities, light exposure and educational tools to manage sleepiness and the health consequences faced by NSWs.

**Practice points**

- An increasing number of people are chronically sleep restricted because of working schedules, commuting times, family obligations or increased accessibility to media of all sorts. The proportion of adults who sleep less than 6 h per night is now greater than at any other time on record this last decade in the US.
- In addition to cognitive dysfunction, compelling evidence from epidemiological studies and laboratory studies links sleep loss to alterations in the endocrine, immune and inflammatory systems with potential negative clinical consequences and public-health ramifications.
- The experimental data obtained in well-controlled sleep deprivation protocols indicate a non-specific activation of blood immune parameters, i.e., leukocyte subtypes (neutrophil, monocyte, NK cells, lymphocyte) and a state of low-level systemic inflammation (as indicated by cytokine and CRP levels) after sleep loss.
- Some effects are modest and some will argue that adaptive physiological processes and/or sleep recovery could be sufficient to counterbalance these changes. However, chronic exposure to sleep restriction could have gradual and cumulative deleterious health effects over years as suggested by epidemiological results especially in night and shift workers, a population that is chronically sleep restricted.
- One night of recovery sleep following sleep restriction does not allow full recovery of alertness or of a number of these systemic markers.
- Although the physiological mechanism(s) underlying the links between sleep deprivation and these immune and inflammatory responses remain(s) largely unknown, neuroendocrine, autonomic vascular stress and SWS-hormone dependent changes are likely involved.
- Emerging biomarkers associated with the inflammatory and atherogenesis pathways that lead to cardiovascular disease, e.g., leukocyte count, CRP, IL-6 or MPO, all have their systemic expression elevated in healthy humans after experimental sleep deprivation. Although these effects are often small, such chronic sub-clinical shifts have been described as contributing to cardiovascular pathogenesis and are certainly clinically relevant in terms of their prevalence in a number of epidemic diseases, e.g., diabetes, obesity or atherosclerosis.
- Sleep alterations in addition to behavioral factors, such as diet, smoking, alcohol or exercise, can influence the inflammatory status.
- An investigation of sleep habits should be included in the clinical evaluation of patients with cardiovascular symptoms.
- Sleep countermeasures, such as napping or sleep extension, may improve the recovery processes regarding alertness and immune and inflammatory parameters after sleep restriction.
- Maintaining adequate sleep quality and duration may reduce inflammatory processes and immune dysfunctions associated with aging or inflammatory diseases.
- Practical strategies to improve health status and safety for night and shift workers and those working extended hours are necessary to improve tolerance to these specific conditions. Strategies that combine napping facilities and educational tools to manage sleepiness and the health consequences of night and shift work need to be tested.

**Research agenda**

- Sleep restriction studies should assess immune cells and inflammatory markers also after recovery sleep.
- The molecular pathway(s) by which such sleep loss influences immune and inflammatory system gene expression needs to be investigated.
- Inter-individual vulnerability and sex differences in the adverse impact of sleep loss observed in cognitive performance need to be examined for immune and inflammatory functions.
- A better characterization of the cellular sources and their relative involvement in the release of cytokines after sleep deprivation is of interest.
- Screening is needed for novel pro-inflammatory biomarkers altered specifically by sleep deprivation.
- Non-invasive biological markers need to be developed to identify individuals at increased risk of sleep loss in terms of immune and inflammatory functions.
- The extent to which sleep is recuperative for specific immune defenses and its influence on vaccination delivery needs to be determined.
- Further studying sleep countermeasures, e.g., napping or sleep extension, and the contributing sleep stages that improve the recovery processes regarding both alertness and immune and inflammatory parameters after sleep restriction is needed.
- Further study of practical strategies to improve health status and safety for night and shift workers and those working extended hours is required.
References


* The most important references are denoted by an asterisk.


