Sleep restriction increases white blood cells, mainly neutrophil count, in young healthy men: A pilot study

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Objectives: This study examines the effects of sleep restricted to four hours for three consecutive nights on blood parameters, known to be associated with cardiovascular risk, in young healthy men.

Material and methods: Eight young healthy men (age 24.5 ± 3.3 years) were studied in the sleep restricted group. Nine young healthy men (age 24 ± 2 years) were included in the control group and spent the days and nights in the sleep lab, while sleeping eight hours/night. One baseline night was followed by three nights of sleep restriction to four hours and by one recovery night of eight hours. Blood samplings were performed after the baseline night and after the third night of sleep restriction or without restriction for the control group.

Results: A significant increase in white blood cells (WBC) (5.79 ± 1.05 vs. 6.89 ± 1.31 10^3 cell/µl, $p = 0.03$), and neutrophils (3.17 ± 0.69 vs 4.24 ± 0.97 10^3 cell/µl, $p = 0.01$) was observed after the third night of sleep restriction. Other blood parameters were not affected. No significant variation was observed in the control group.

Conclusion: Sleep restriction affected WBC count, mainly neutrophils, considered as risk factor for cardiovascular disease. Stress induced by the short term sleep restriction could be involved in this observation.

Keywords: sleep restriction, men, cardiovascular risk, cholesterol, neutrophils

Introduction

Accumulating evidence indicates that atherosclerosis is an inflammatory process involving a network of vascular cells, leukocytes, proinflammatory cytokines, chemoattractant cytokines (chemokines), and growth factors (Lind 2003).

In our society, many people choose to prioritise social life, leisure, professional activities to the detriment of sleep and voluntary reduce their sleep duration. Several reports indicate that a shortened sleep may be associated with an increased risk of morbidity, mortality (Heslop et al 2002) and hypertension (Gangwisch et al 2006). The mechanisms underlying these associations are not yet elucidated. A study performed in healthy adult subjects showed an increase in high-sensitivity C-reactive-protein (hs-CRP), a stable marker of inflammation, during acute total and short term partial sleep deprivation (Meier-Ewert et al 2004). Recently, we observed that in postmenopausal women, treated with hormonal replacement therapy, a sleep restriction to four hours of sleep for three consecutive nights increased white blood cells (WBC), monocytes, neutrophils, total cholesterol and low density lipoprotein – cholesterol (LDL-C) (Kerkhofs et al 2007).

Over the last several decades, an inversing number of prospective studies conducted in coronary heart disease (CHD) free populations have shown a clear and positive correlation between the leukocyte count and risk of CHD (Zalokar et al 1981;

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Grimm et al 1985; Phillips et al 1992; Kannel et al 1992; Gillum et al 1993). The correlation appears to persist even after adjustment for other risk factors (Folsom et al 1997; Lee et al 2001; Brown et al 2001). Other studies reported also correlations between other leukocytes subtypes such as monocyte count (Olivares et al 1993; Sweetnam et al 1997) and neutrophil count (Horne et al 2005).

We hypothesized that sleep restriction could activate inflammatory processes and secondarily affect blood variables known to be associated with cardiovascular events and that a similar process could occur in young men. The current study examined the effect of sleep restriction to four hours for three consecutive nights on blood variables (inflammatory markers and lipid profile) known to be associated with an increased cardiovascular risk.

Material and methods

Eight young healthy nonsmoker men (age 24.5 ± 3.3 years) free of neurological, psychiatric, cardiac and endocrine disease participated in the partial sleep deprivation experiment and nine nonsmoker young healthy men aged 22–29 years (age 24 ± 2 years) were included in the control group. They had no sleep complaints and were not snoring. The presence of a sleep disorder was excluded on the basis of an interview and one night of polysomnography before inclusion in the study. All volunteers were not drinking alcohol on a regular basis and were drug-free. Their consumption of xanthine beverages had to be less than five units/day.

The protocol was approved by the Ethics Committee at the Vésale Hospital. The volunteers gave written informed consent and received a financial compensation for their participation in the study.

After two weeks of a regular sleep–wake schedule of eight hours of sleep per night documented by actigraphic recordings and sleep diaries, the volunteers were admitted to the Sleep Laboratory of the Vésale Hospital, for five days and nights. The first night was a baseline night during which the subjects were allowed to sleep from 11 pm to 7 am. The following three nights were the restriction nights during which sleep was allowed only between 1 am and 5 am. The last night was a recovery night of 8 hours (from 11 pm to 7 am). The control group slept three consecutive nights of 8 hours (from 11 pm to 7 am). Both groups were studied in similar conditions.

During the study, the subjects were under close supervision of the staff and performed several neurobehavioral tests. Continuous EEG recordings were performed with an ambulatory device (Medatec Pamela®) during the day (except between 9 am and 1 pm to allow the subjects to have a shower and to perform cognitive tests) and night, in order to control the vigilance state of the subjects and to avoid sleep episodes outside the permitted hours. Sleep EEG recordings were scored according to standard criteria (Iber et al 2007). The subjects received standard hospital meals of a maximum of 2500 calories/day with a comparable proportion of nutrients (protein, fat, carbohydrate) across days and sessions. Controlled drinks and snacks were available during the restriction nights. Alcohol and xanthine derivatives (coffee, tea, chocolate, colas) were prohibited during the study.

The effectiveness of the sleep restriction procedure was confirmed by the continuous EEG recordings.

Blood samples were obtained from an antecubital vein at 7 am after the baseline night and after the third night of sleep with or without restriction. Serum samples were collected in vacuum tubes without anticoagulant. Plasma samples were harvested in citrated vacuum tubes. Whole blood was collected on EDTA-treated tubes. Lipids (total cholesterol, HDL-c, triglycerides) were measured by standardized laboratory techniques on a SYNCHRON LX® automate (Beckman Coulter), LDL cholesterol was calculated as LDL-C = total cholesterol – HDL cholesterol – triglycerides/5 (in mg/dl). Hs-CRP, ApoA and ApoB were evaluated on an Immage® device (Beckman). Fibrinogen was determined by the Clauss method on a STA® automate (Stago). Leucocytes and subsets were determined on a CELL-DYN4000® hemocytometer (Abbott).

Wilcoxon paired tests were used to compare baseline values to the values obtained after the third night of sleep restriction.

Results and discussion

Table 1 represents the values of the different blood parameters obtained after the baseline night and after the third night of sleep restriction.

A significant increase in WBC and neutrophils was observed after the third night of sleep restriction. The other blood parameters were not affected. Table 2 represents blood parameters in the control group. No modifications were observed.

Polysomnography recordings indicated a significant raise in the percentage of slow wave sleep during the third night of sleep restriction as compared to the baseline night in the sleep restricted group ($p = 0.008$) while no significant variation was found in the control group.

Our data indicate that a decrease in sleep duration to four hours for three consecutive nights significantly affected WBC

Table 1 Inflammatory markers and lipid profiles (mean ± SD) at baseline and after the third night of sleep restriction

Variables	Baseline	3rd Restriction Night	Wilcoxon p values
WBC 10^3 cells/μl	5.79 ± 1.04	6.88 ± 1.31	0.03
Lymphocytes 10^3 cells/μl	2.06 ± 0.41	2.01 ± 0.31	1
Monocytes 10^3 cells/μl	0.51 ± 0.15	0.61 ± 0.17	0.20
Neutrophils 10^3 cells/μl	3.17 ± 0.69	4.24 ± 0.97	0.01
RBC 10^6 cells/μl	5.09 ± 0.34	5.19 ± 0.28	0.78
Fibrinogen mg/dl	2.84 ± 0.27	2.71 ± 0.12	0.46
Hs-CRP mg/dl	0.21 ± 0.24	0.20 ± 0.24	1
Cholesterol mg/dl	171.5 ± 17.64	163.24 ± 25.84	0.23
Triglycerides mg/dl	58.5 ± 17.52	68.0 ± 26.59	0.29
HDL-C mg/dl	47.5 ± 10.97	46.25 ± 10.95	0.53
LDL-C mg/dl	112.3 ± 19.47	103.4 ± 22.44	0.16
ApoA mg/dl	117.5 ± 15.63	120.13 ± 20.61	0.46
ApoB mg/dl	94.45 +12.07	89.52 ± 23.79	0.34

Abbreviations: WBC, white blood cells; RBC, red blood cells; Hs-CRP, high sensitivity C-reactive protein; LDL, low density lipoprotein; HDL, high density lipoprotein.
Notes: Conversion for lipids; Total cholesterol, HDL-C, LDL-C: 1mol/L = 38.67 mg/dl; Triglycerides: 1 mol/L = 88.57 mg/dl.

counts and mainly neutrophil counts in young healthy men. In contrast to our previous work in postmenopausal women, we did not find any significant modifications in monocyte counts or the lipid profile (total cholesterol, LDL). This difference could be attributed to the age and gender.

In addition, contrary to the study of Meier-Ewert and colleagues (2004), we did not observe any change in hs-CRP levels. This could be attributed to the shorter duration of sleep restriction in our study (three nights vs 10).

Interestingly, Gomez-Merino and colleagues (2005) reported that, in young men, three weeks of physical conditioning followed by five days of combat course, sleep deprivation and physiological stress increased leukocyte and neutrophil counts while total lymphocytes were unchanged. Dinges and colleagues (1994) also observed that 64 hours of sleep deprivation increased neutrophil and monocyte counts. Another work showed that after one partial night of sleep deprivation, norepinephrine and epinephrine blood concentration were

Table 2 Inflammatory markers and lipid profiles (mean ± SD) at baseline and after the third night of sleep without restriction. Control group

Variables	Baseline	3rd Night without Restriction	Wilcoxon p values
WBC 10^3 cells/μl	6.71 ± 0.43	6.88 ± 0.42	0.82
Lymphocytes 10^3 cells/μl	2.48 ± 0.41	2.47 ± 0.14	0.73
Monocytes 10^3 cells/μl	0.58 ± 0.03	0.53 ± 0.04	0.3
Neutrophils 10^3 cells/μl	3.40 ± 0.32	3.65 ± 0.35	0.1
RBC 10^6 cells/μl	5.31 ± 0.29	5.28 ± 0.33	0.68
Fibrinogen mg/dl	2.79 ± 0.13	2.86 ± 0.15	0.25
Hs-CRP mg/dl	0.13 ± 0.04	0.10 ± 0.02	0.25
Cholesterol mg/dl	163 ± 8.3	165 ± 8.8	0.65
Triglycerides mg/dl	68 ± 37	60 ± 29	0.54
HDL-C mg/dl	46.1 ± 2.5	45 ± 2.4	0.19
LDL-C mg/dl	103 ± 6.7	107 ± 7.9	0.49
ApoA mg/dl	124 ± 8	118 ± 8.7	0.17
ApoB mg/dl	79 ± 5.3	83 ± 5.5	0.42

Abbreviations: WBC, white blood cells; RBC, red blood cells; Hs-CRP, high sensitivity C-reactive protein; LDL, low density lipoprotein; HDL, high density lipoprotein.
Notes: Conversion for lipids; Total cholesterol, HDL-C, LDL-C: 1mol/L = 38.67 mg/dl; Triglycerides: 1 mol/L = 88.57 mg/dl.

raised (Irwin et al 1999). Interestingly, shifts in leukocyte have already been shown in healthy subjects after administration of epinephrine and hydrocortisone (Brohee et al 1990). Considering our data and all these findings, we surmise that the stress induced by the sleep restriction could be involved in the observed modifications.

Leukocytes may influence the development of coronary heart disease through their ability to cause proteolytic and oxidative damage to coronary arteries. Indeed, stimulated neutrophils are known to: 1, secrete proteolytic proteases that promote the detachment of endothelial cells from vessel walls and the adherence of paltelets to subendothelial collagen and fibronectin (Harlan et al 1981); 2, release large amounts of the chemotactic agent leukotriene B4 in patients with stable angina (Mehta et al 1989); 3, secrete large amounts of inflammatory mediators (Weissman et al 1980); 4, release superoxide anions in hyperlipidemic patients Ludwig et al 1982).

Furthermore, Elkind and colleagues (2005) reported that the leukocyte count was associated with a reduced endothelial reactivity, furnishing a possible link with a higher cardiovascular risk.

Conclusion

Sleep restriction to four hours of sleep during three consecutive nights induced an increase in WBC counts, mainly neutrophils in young healthy subjects. The stress induced by the sleep restriction could be one mechanism involved. Further studies are needed to confirm these findings and to explore the supposed mechanisms.

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