RESEARCH ARTICLE

Effect of acute sleep deprivation and recovery on Insulin-like Growth Factor-I responses and inflammatory gene expression in healthy men

Mounir Chennaoui[1,2], Catherine Drogou[1,2], Fabien Sauvet[1,2], Danielle Gomez-Merino[1,2], Denis E. Scofield[3], Bradley C. Nindl[4]

[1] French Armed Forces Biomedical Research Institute (IRBA), Neurosciences and Operational Constraints department, Brétigny-sur-Orge, France
[2] Université Paris Descartes, Sorbonne Paris Cité, EA7330 VIFASOM, équipe d'accueil Vigilance Fatigue et Sommeil, Paris, France
[3] The Military Performance Division, the Unites States Army Research Institute of Environmental Medicine, Natick, MA 01760, United States
[4] The U.S. Army Institute of Public Health, The U.S. Army Public Health Command, Aberdeen Proving Ground, MD 21010

Correspondence
<mounir.chennaoui@irba.fr>

To cite this article: Chennaoui M, Drogou C, Sauvet F, Gomez-Merino D, Scofield DE, Nindl BC. Effect of acute sleep deprivation and recovery on Insulin-like Growth Factor-I responses and inflammatory gene expression in healthy men. *Eur. Cytokine Netw.* 2014; 25(3): 52-7 doi:10.1684/ecn.2014.0356

ABSTRACT. Acute sleep deprivation in humans has been found to increase inflammatory markers and signaling pathways in the periphery through a possible Toll-like receptor 4 (TLR-4). In addition, short duration sleep has been associated with low circulating total Insulin-like Growth Factor-I (IGF-I) concentrations. We aimed to determine whether a total sleep deprivation (TSD) protocol with recovery altered whole-blood gene expression of the proinflammatory cytokines TNF-α and IL-6, as well as TLR-4 expression, and to examine the relationship with circulating concentrations of the IGF-I system. Twelve healthy men participated in a five-day TSD (two control nights followed by one night of sleep deprivation and one night of recovery). Blood was sampled at 0800, before and after sleep deprivation (D2 and D4), and after recovery (D5). It is shown that 25h of sleep deprivation (D4) induced significant increases in mRNA levels of TNF-α and its soluble receptor R1 ($P<0.01$ respectively), as well as TLR-4 ($P<0.05$), while IL-6 mRNA levels remained unchanged. Circulating concentrations of free IGF-I were decreased at D4 ($P<0.001$). One night of recovery was sufficient to restore basal expression levels for TNF-α, sTNF-R1, TLR-4 and circulating IGF-I. Changes in TLR-4 mRNA levels during the protocol correlated positively with those of TNF-α and sTNF-R1 ($r = 0.393$ and $r = 0.490$ respectively), and negatively with circulating free IGF-I ($r = -0.494$). In conclusion, 25h of sleep deprivation in healthy subjects is sufficient to induce transient and reversible genomic expression of the pro-inflammatory cytokine TNF-α and its R1 receptor, and its mediator TLR-4, with a possible link to IGF-I axis inhibition.

Key words: IGF-1 system, proinflammatory cytokines, Toll-like receptor 4, sleep, healthy men

Mounting evidence from both observational and experimental research suggests that sleep disturbance and short sleep duration adversely impact human physical health [1, 2], and mortality risks [3]. The mechanisms by which altered sleep duration affects health are not entirely clear, but experimental studies suggest altered sleep may impact concentrations of inflammatory markers known to be important in regulating inflammation [4]. Experimental sleep deprivation induces increases in circulating concentrations of inflammatory markers such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and C-reactive protein (CRP) [5-8]. In addition, early night partial sleep deprivation (PSD) activates cellular expression of IL-6 and TNF-α, through activation of transcription control pathway nuclear factor kappa-beta (NF)-κB transcription [6, 9] that plays a key role in controlling cellular expression of proinflammatory genes. This response involves the activation of toll-like receptors (TLRs), a class of pattern recognition receptors that may be present on the cell surface or in the endoplasmic reticulum, which, in turn, recognize molecular patterns of self and non-self microorganisms [10]. TLRs and receptor complexes activate the gene transcription that stimulates NF-κB and leads to the production of IL-1β, TNF-α and IL-6.

The roles of the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis, prolactin, cortisol, and catecholamines in the crosstalk between sleep, the circadian system, and the immune system are also important. Indeed, high GH and prolactin concentrations, as well as low cortisol and catecholamine concentrations, were identified as possible mediators of the enhancing and suppressing actions of nocturnal sleep on pro- and anti-inflammatory cytokines, respectively [11]. Moreover, a recent study investigated whether IGF-I was involved in TLR-4-mediated, NF-κB-dependent pro-inflammatory gene expression [12]. The results demonstrated that IGF-I treatment significantly attenuated the endogenous expression of IL-6 and TNF-α, indicating that IGF-I exerts an anti-inflammatory effect on skeletal muscle cells by reducing the expression of pro-inflammatory cytokines under basal conditions through down-regulation of TLR-4 expression. Although the exact mechanism remains

doi: 10.1684/ecn.2014.0356

to be elucidated, the authors speculated that cells with low TLR-4 expression are less sensitive to endogenous inflammation-stimulating ligands such as heat shock proteins, which contributes low basal cytokine expression.

In view of these recent observations and the important negative feedback between the immune and IGF systems [13], we investigated the possible relationship with whole-blood gene expression of the pro-inflammatory cytokine TNF-α and toll-like receptors 2 and 4 (TLR-2 and TLR-4) in order to understand the inflammatory response induced by total sleep deprivation and recovery in healthy young men.

METHODS

Subjects

Twelve healthy men, aged 29.1 ± 3.3 yrs (mean ± SD), with a body mass index (BMI) of 23.4 ± 1.5 kg/m^2, were included in the study after giving written informed consent. The Paris-Cochin ethics committee approved the protocol (N°ID RCB: 2008-A00170-55), which was conducted according to the principles expressed in the Declaration of Helsinki of 1975, as revised in 2001.

All subjects underwent a detailed medical history and examination. Exclusion criteria were: shift-workers, smokers, daily consumption of alcoholic beverages and those consuming more than 400 mg of caffeine per day, subjects with a BMI greater than 30 kg/m^2, and those taking medications. Clinical tests, including electrocardiogram, blood pressure assessment and blood test (glucose, total cholesterol, triglycerides, γGT and transaminases) were negative for abnormal findings. Subjects with excessive daytime somnolence (i.e. Epworth Sleepiness Scales ≥ 9) [14] or sleep complaints (i.e. Pittsburg sleep quality index (PSQI) <31, or > 69) [15], or if they could not be considered as an intermediate chronotype on the Horne and Ostberg questionnaire [16], were also excluded. Sleep/wake patterns were checked using wrist actigraphy (Actiwatch, Cambridge Neurotechnology, Cambridgeshire, UK), one week before the experiment. The mean total sleep duration for the subjects was 8.1 ± 0.6 h (mean ± SD).

Protocol

Subjects were housed individually in a temperature-controlled bedroom (24 ± 1°C) for five days at the Percy Military Hospital (Clamart, France). During the first day (D1), they were familiarized with the laboratory procedures and equipped for continuous polysomnography (electroencephalogram [EEG], electro-oculogram, electromyogram – Embla, Broomfield, CO, USA). During D1 and day two (D2), subjects went to bed at 2300 and woke up at 0700. Total sleep deprivation began on day three (D3) at 0700 and finished on day four (D4) at 2300 (i.e. 40 hours of continuous wakefulness). Subjects left the laboratory on day five (D5) after one night of sleep recovery (2300-0700) (*table 1*).

All measurements were performed in the subject's laboratory bedroom. Blood samples were collected during D2, D4 and D5 at 0800 after a standardized breakfast. Samples were immediately centrifuged at 1,100 G and serum aliquots frozen and stored at -80°C.

During the experiment, apart from when they were showering (between 2200 and 2230 each day), the subjects underwent continuous polysomnography, which was analyzed offline (Embla Somnologica for Windows XP Software). Members of the investigation team were continuously present to monitor wakefulness in order to ensure that subjects did not fall asleep during the sleep deprivation protocol. Core body temperature was measured by means of an ingested wireless capsule (VitalSense, Mini Mitter Company, Inc. Bend, OR, USA). Laboratory illumination was maintained at 150-200 lux during the entire period of sleep deprivation. When not engaged in any specific testing or meal, subjects were allowed to read, watch videos, play games, or converse with the staff or visitors. Subjects were prohibited from exercising, and from using caffeine, tobacco, alcohol, and other psychoactive substances 24 hours before and during the study. Meals and caloric intake were standardized for all subjects (2600 kcal/day). Water was allowed *ad libitum*.

mRNA isolation and reverse transcription

During blood sampling, 2.5 mL of blood were collected in Paxgene Blood RNA tubes (Preanalytix, Switzerland). The tubes were gently inverted before storage at -80°C. The extraction of RNA was carried out on Qiacube automate with a Paxgene blood RNA kit (Preanalytix, Switzerland). An 80 μL final volume of RNA was eluted for each sample. A Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, USA) was used to quantify RNA in extracts. Reverse transcription was immediately performed using the Reverse Transcription Core kit (Eurogentec, Seraing, Belgium). The reaction was carried out using 2 μL of RNA. A calibrator was prepared by pooling cDNA samples. The cDNA was stored at -80°C until use.

Real time PCR

Primer design was performed using MacVector software (Accelrys, San Diego, USA). Oligonucleotide primers were synthesized by Eurogentec (Seraing, Belgium). The selected forward (F) and reverse (R) primers are listed in *table 2*.

Table 1
Sleep deprivation protocol in healthy men

	Days				
	Baseline		Sleep loss	Recovery	
	Day 1 (D1)	Day 2 (D2)	Day 3 (D3)	Day 4 (D4)	Day 5 (D5)
Sleep lab	1	2	3	4	5
Nighttime	x	x		x	x
Blood sampling (0800)		x		x	x

Table 2
List of selected forward (F) and reverse (R) primers used for real time PCR reactions

Gene name		5'-3' primer sequence	Annealing temperature (°C)
TNFα	F	TCTTCTGTCTGCTGCACTTTGGAG	58
	R	GTCTGAGGGTTTGCTACAACATGG	
IL-6	F	CCACTCACCTCTTCAGAACGAATTG	58
	R	TGCCTCTTTGCTGCTTTCACAC	
TLR4	F	TCTCCTGCGTGAGACCAGAAAG	53
	R	GGTCCAGGTTCTTGGTTGAGAAG	
TLR2	F	TTGTGCCCATTGCTCTTTCAC	51
	R	CCTTGGAGAGGCTGATGATGAC	
TNFRSF1A	F	TTGCAGCCTCTGCCTCAATG	54
	R	TGCACACGGTGTTCTGTTTCTC	
Cyclophilin A	F	TATCTGCACTGCCAAGACTGAGTG	58
	R	CTTCTTGCTGGTCTTGCCATTCC	
HPRT	F	CTCATGGACTGATTATGGACAGGAC	58
	R	GCAGGTCAGCAAAGAACTTATAGCC	
RPLP0	F	GCATCTACAACCCTGAAGTGCTTG	56
	R	GCAGACAGACACTGGCAACATTG	

PCR was carried out using the LightCycler Fast DNA master SYBR Green kit (Roche Applied Science, Mannheim, Germany) using 0.2 μL of cDNA, in a final volume of 20 μL, 4 mM $MgCl_2$ and 0.4 μM of each primer (final concentration). Reactions were performed using a LightCycler and the crossing point values were calculated from LightCycler Software v3.5 (Roche Applied Science, Mannheim, Germany). Amplification specificity was checked using a melting curve following the manufacturer's instructions. The relative mRNA values were calculated using the ΔΔCT-method. Normalization was performed by geometric averaging of three housekeeping genes (cyclophilin A, HPRT and RPLP0).

IGF-I system and insulin assays

Blood was allowed to stand for 30 min before being centrifuged at 2000 g and at 4°C for 15 min. Serum was then separated and samples were stored at -80°C until assays were performed.
Total and free serum IGF-I assay kits were purchased from Diagnostic Systems Laboratories (DSL, Webster, TX, USA). The assay procedures were performed using non-competitive, time-resolved immunofluorometric assays (TF-IFMAs) after acid-ethanol extraction of the IGFBPs. Sensitivities for total and free IGF-I were 0.01 ng/mL and 0.015 ng/mL respectively. Both IGF assays had mean intra- and inter-assay coefficients of variation (CVs) of less than 5 and 10%, respectively. IGFBP 1-6 were measured using Luminex xMAP technology (Millipore MILLIPLEX MAP Human IGFBP panel kit-53K). Sensitivities were 0.017 ng/mL, 0.444 ng/mL, 0.178 ng/mL, 0.776 ng/mL, 1.6 ng/mL and 0.093 ng/mL for IGFBP-1, -2, -3, -4, -5, and -6 respectively. Intra-assay CVs for the IGFBPs were less than 10%.
Serum insulin assay kits were purchased from Dia Sorin, Stillwater, MN, USA). The limit of sensitivity was 2 μUI/mL.

Statistical analyses

In this study, we compared the differences between values observed during D2, D4 and D5. Biological parameters were analyzed using a one-way ANOVA, followed by a Tukey, pairwise multiple comparison procedure. Data are presented as means ± SE (standard error). The Pearson test was used for the correlation analysis between changes in biological parameters over the TSD. All statistical analyses were conducted using Statistica 6.0, StatSoft Inc., Maisons-Alfort, France. Unless specified, for all statistics, the significance level was set at $P < 0.05$.

RESULTS

Whole-blood mRNA levels

After 25 hours of continuous wakefulness (D4), a significant increase in the levels of mRNA encoding TNF-α and sTNF-R1 ($P<0.01$ respectively) was observed. On D5, after one night of recovery, these levels returned to their basal concentrations.
At the same time, we observed a significant increase in TLR-4 mRNA levels ($P<0.05$) after 25 hours of continuous wakefulness (D4), followed by a significant decrease on D5 after recovery ($P<0.05$) On D5, TLR-4 mRNA levels were similar to basal levels.
There were no significant changes in TLR-2 and IL-6 mRNA levels over the study period (*table 3*).

Serum IGF-I system assays

After 25 hours of continuous wakefulness (D4 *versus* D2), a significant decrease ($P<0.001$) in free IGF-I concentration was observed. Total IGF-I concentration was significantly increased at D5 *versus* D2 ($P<0.01$). On D5, there was a significant decrease in IGFBP-3 as compared to D2 and D4 ($P<0.05$ respectively). No significant changes were observed for IGFBP -1, -4, or -6, and IGFBP-2 and IGFBP-5 were very close to minimum detectable concentrations.
No significant changes were observed for insulin concentrations (*table 4*).

Correlations

A significant negative correlation was found between changes over the TSD protocol in TLR-4 mRNA levels and free IGF-1 concentrations. Changes in TLR-4 and TLR-2 mRNA levels also correlated positively with those of TNF-α and sTNF-R1 mRNA levels (*table 5*).

Table 3
IL-6, TNF-alpla, sTNF-R1, TLR-4 and TLR-2 mRNA levels before (D2), after (D4) one night of sleep loss, and after (D5) one night of sleep recovery.

mRNA/housekeeping genes mRNA (arbitrary unit)	D2	D4	D5
IL-6	0.611 ± 0.124	0.813 ± 0.210	0.967 ± 0.217
TNF-α	0.876 ± 0.084	1.288 ± 0.096**	1.118 ± 0.071
sTNF-Rl	1.030 ± 0.143	1.624 ± 0.150**	0.951 ± 0.099##
TLR-4	1.512 ± 0.240	1.766 ± 0.292*	1.032 ± 0.092#
TLR-2	1.167 ± 0.110	1.085 ± 0.092	1.016 ± 0.047

* difference vs baseline day (D2),* P<0.05, ** P<0.01
# difference vs after 25h of TSD (D4), # P<0.05, ## P<0.01

Table 4
Changes in serum concentrations of insulin and IGF-I system components before (D2), after (D4) one night of sleep loss, and after (D5) one night of sleep recovery

	D2	D4	D5
Free IGF-1 (ng/mL)	0.35 ± 0.06	0.27 ± 0.05***	0.31 ± 0.06
Total IGF-1 (ng/mL)	285 ± 40	312 ± 38	345 ± 46**
IGFBP-1 (ng/mL)	4.28 ± 0.67	4.26 ± 0.68	4.19 ± 0.61
IGFBP-3 (ng/mL)	1377±51	1380±44	1294 ± 30*#
IGFBP-4 (ng/mL)	27 ± 2	27 ± 2	27 ± 1.4
IGFBP-6 (ng/mL)	133 ± 12	144 ± 13	128 ± 14
Insulin (μUI/mL)	77.0 ± 9.6	89.8 ± 15.3	95.8 ± 12.1

* difference vs baseline day (D2),* P<0.05, ** P<0.01
# difference vs after 25h of TSD (D4), # P<0.05

Table 5
r and p value comparisons between total sleep deprivation-induced changes in TLR4 and TLR-2 mRNA levels, free IGF-1 serum levels, and TNF-alpha and sTNF-R1 mRNA levels

	r	*p*	
TLR-4 mRNA	-0.493	*	Free IGF-1
	0.393	*	TNF-a mRNA
	0.490	*	sTNF-Rl mRNA
TLR-2 mRNA	0.783	*	TNF-a mRNA
	0.717	*	sTNF-Rl mRNA

Pearson rank coefficient ; * p<0.05 (n = 12)

DISCUSSION

Our present study demonstrates that a total sleep deprivation (i.e. 25 h of continuous wakefulness) in healthy young men is transiently associated with a significant decrease in circulating free IGF-I concentrations that are restored after one night of recovery, concomitant with a significant increase in total IGF-I concentrations. In addition, sleep deprivation acutely increases whole-blood gene expression of the pro-inflammatory cytokine TNF-α and its soluble receptor (sTNF)-R1, and also increases TLR-4, but it does not affect IL-6. Interestingly, sleep deprivation and recovery induced changes in whole-blood TLR-4 mRNA levels, a possible mediator of TNF-α secretion, that correlated positively with TNF-α and sTNF-R1 mRNA levels and negatively with serum free IGF-I.

The pro-inflammatory cytokines TNF-α and IL-6 have been suggested to be mediators of excessive sleepiness in humans in pathological conditions, e.g., sleep apnea [17] or in experimentally induced sleepiness (i.e., following sleep deprivation) [5, 8, 18]. As noted, when chronic sleep reduction is investigated in healthy men, TNF-α and IL-6 levels are increased [7, 18, 19], while they remain unchanged when subjects are acutely sleep-deprived [8]. At the mRNA levels, two studies found that sleep restriction increases TNF-α and IL-6, but only on activated peripheral blood monocytes [6, 20]. The increase in TNF-α mRNA levels and unchanged IL-6 mRNA levels that are observed in this study after 25 hours of sleep deprivation could explain the significant increase in TNF-α and the lack of change in IL-6 serum concentrations after 34 and 37 hours of sleep deprivation described previously [5].

It is important to bear in mind that we do not have a precise indication of the relative specific source(s) of the cytokines that we are measuring in peripheral blood. Cytokines can be produced by monocytes, macrophages, neutrophils, endothelial cells, and adipocytes, and their relative contribution to circulating levels, certainly under conditions of sleep deprivation, is unknown. An important contribution to the literature addressing the causes of sleep loss-associated inflammation comes from research by Irwin and his colleagues [6, 9]. They reported that after one night of 4h-sleep restriction, lipopolysaccharide ligation of TLR-4 triggered an increased production of IL-6 and TNF-α in peripheral blood monocytes, and increased monocyte gene expression of IL-6 and TNF-α mRNA under activation of nuclear factor (NF)-kappaB transcription control pathways that play a key role in controlling cellular expression of proinflammatory genes such as TNF-α and IL-6. It is now clear that toll-like receptors (TLRs) on monocytes, macrophages, and dendritic cells contribute significantly to the development of adaptive immune responses. Activation of TLRs on these antigen-presenting cells results in the gene transcription that stimulates nuclear-factor kappa-beta (NF-kB) and leads to the production of inflammatory cytokines, such as IL-1β, TNF-α and IL-6 [10]. In the literature there are few data referring to TLR-4 changes during sleep restriction in

humans, but a recent study demonstrated that a seven-day, multifactorial, high-stress, military ranger training, which included sleep deprivation significantly increased monocyte TLR-4 expression throughout the training course, and transiently increased TNF-α (i.e., on day three) [21]. In our study, TLR-4 expression was increased after 25h of sleep deprivation while the monocyte subset population was unchanged (unpublished results, Chennaoui *et al.*). In addition, changes, over the sleep deprivation protocol, in TLR-4 and TLR-2 mRNA levels correlated positively with those of TNF-α and sTNF-R1 mRNA. Thus, we suggest that the up-regulation of whole-blood gene expression of TNF-α and sTNF-R1 after 25h of sleep deprivation resulted from activation of TLR-4 located on monocyte cells.

Our results demonstrate that the IGF-I system respond to sleep deprivation, as free serum IGF-I concentrations decreased after 25h of sleep deprivation, followed by increased total IGF-I concentrations the day after a night of recovery. This is the first study investigating the effects of sleep deprivation on the IGF-I system in serum, including the determination of free and total IGF-I concentrations and those of the six binding proteins IGFBP-1 to -6. In healthy subjects, we found evidence that only total IGF-I concentrations increase following the first recovery night after chronic sleep restriction [22, 23], and this was concomitant with a significant rebound of slow-wave sleep [22]. In contrast to humans, chronic sleep deprivation in rats results in a decrease in total IGF-I concentrations [24, 25].

The activity of the GH/IGF-I axis is associated with sleep [26-28] and is part of an important negative feedback with the immune system [13]. The IGF system depresses proinflammatory cytokine signaling by increasing IL-10 secretion [29]. Accumulating evidence suggests that it directly depresses proinflammatory cytokine signaling via JNK and NF-κB pathways [13]. Furthermore, it has been recently demonstrated that IGF-I exerts anti-inflammatory effects through down-regulation of TLR-4 signaling and subsequent reduction of NF-κB-target genes such as TNF-α [12]. The down-regulation of TLR-4 has been suggested to represent a beneficial effect on chronic central and whole body inflammation [30, 31]. In our study, changes in TLR-4 mRNA levels correlated negatively with levels of free IGF-I, and positively with levels of TNF-α and sTNF-R1 mRNA. Free IGF-I is universally considered to be the most physiologically relevant component of the IGF-I system, as free IGF-I is not bound to any binding proteins and is therefore bioavailable to cellular receptors [32]. Thus, we can suggest that sleep deprivation led to an alteration in the innate functional cellular immune response, increasing whole-blood gene expression levels of TNF-α, sTNF-R1 through a down-regulation of TLR-4 expression by the IGF-I system, particularly the free circulating component.

The bioavailability of IGF-I is governed by a great deal of regulatory complexity, involving at least the six IGFBPs that differently modulate (i.e., either stimulate or inhibit) IGF-I bioavailability and the proportion of IGF-I that is unbound (i.e., free IGF-I) [32]. Most of the IGF-I in blood is bound to IGFBP-3, and this complex is bound by a third protein, the acid-labile subunit, to form a large, stable 150-kDa complex. IGFBP-3 and the acid-labile subunit are regulated primarily by GH, have a slow clearance from blood, and provide an accessible pool and reservoir of IGF in the blood. In contrast, IGFBP-1 concentrations in blood can change rapidly, are regulated by insulin, and may serve an acute metabolic role in the sequestering of unbound IGF. In our study, we observed decreased IGFBP-3 concentrations after recovery compared with baseline (D2), while no change was observed in the other BPs. Previous research has reported that serum IGFBP-3 concentrations were decreased after eight days of intense military training involving energy deficit, arduous physical activity and sleep deprivation [33, 34]. Thus, we suggest that a lowering of IGFBP-3 levels by proteolysis may represent a compensatory mechanism serving to increase free IGF-I [35]. Our results strengthened the model that sleep deprivation has negative effects on the major components that regulate IGF-I bioavailability, and thus impacts the immune system and inflammatory responses.

In conclusion, our study demonstrated an inhibitory effect of sleep deprivation on the IGF-I axis concomitant to increased whole-blood gene expression of TNF-α, sTNFα-R1 and TLR-4, that were restored to baseline levels after one night of recovery. In addition, we observed that changes in TLR-4 mRNA levels during sleep deprivation and recovery correlated positively to those of TNF-α and sTNF-R1 and negatively to circulating free IGF-I. Therefore, it is possible that inhibition of the IGF-I system by sleep deprivation resulting in the activation of the monocyte TLR-4 expression, may provide a mechanistic basis for pro-inflammatory responses.

Acknowledgments. *This work was supported by the French Délégation Générale pour l'Armement (Contract N° 08co704). We thank Mrs Delor, Bobee Elio and Mr Gourby, Mr Guillard, Mr Lapeyre, Mr Banzet and all personnel from IRBA and the Percy Military Hospital for their technical and logistic contributions to this work. We thank Jeff Staab from the US Army Research Institute of Environmental Medicine for his technical support and laboratory contribution.*

Disclosure. *Financial support: none. Conflict of interest: none.*

REFERENCES

1. Gangwisch JE, Heymsfield SB, Boden-Albala B, *et al*. Sleep duration as a risk factor for diabetes incidence in a large U.S. sample. *Sleep* 2007; 30: 1667-73.

2. Sauvet F, Leftheriotis G, Gomez-Merino D, *et al*. Effect of acute sleep deprivation on vascular function in healthy subjects. *J Appl Physiol* 2010; 108: 68-75.

3. Gangwisch JE, Heymsfield SB, Boden-Albala B, *et al*. Sleep duration associated with mortality in elderly, but not middle-aged adults in a large US sample. *Sleep* 2008; 31: 1087-96.

4. Mullington J. Sleep loss and inflammation. *Best Pract Res Clin Endocrinol Metab* 2010; 24: 775-84.

5. Chennaoui M, Sauvet F, Drogou C, *et al*. Effect of one night of sleep loss on changes in tumor necrosis factor alpha (TNF-α) levels in healthy men. *Cytokine* 2011; 56: 318-24.

6. Irwin MR, Wang M, Campomayor CO, Collado-Hidalgo A, Cole S. Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. *Arch Intern Med* 2006; 166: 1756-62.

7. Meier-Ewert HK, Ridker PM, Rifai N, *et al*. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. *J Am Coll Cardiol* 2004; 43: 678-83.

8. Shearer WT, Reuben JM, Mullington JM, *et al*. Soluble TNF-alpha receptor 1 and IL-6 serum levels in humans subjected to the sleep deprivation model of spaceflight. *J Allergy Clin Immunol* 2001; 107: 165-70.

9. Irwin MR, Wang M, Ribeiro D, *et al*. Sleep loss activates cellular inflammatory signaling. *Biol Psychiatry* 2008; 64: 538-40.

10. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 2010; 11: 373-84.

11. Lange T, Dimitrov S, Born J. Effects of sleep and circadian rhythm on the human immune system. *Ann NY Acad Sci* 2010; 1193: 48-59.

12. Lee WJ. IGF-I exerts an anti-inflammatory effect on skeletal muscle cells through down-regulation of TLR-4 signaling. *Immune Netw* 2011; 11: 223-6.

13. O'Connor JC, McCusker RH, Strle K, Johnson RW, Dantzer R, Kelley KW. Regulation of IGF-I Function by Proinflammatory Cytokines: At the Interface of Immunology and Endocrinology. *Cell Immunol* 2008; 252: 91-110.

14. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 1991; 14: 540-5.

15. Buysse DJ, Reynolds CF, 3rd CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989; 28: 193-213.

16. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol* 1976; 4: 97-110.

17. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of serum cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J Clin Endocrinol Metab* 1997; 82: 1313-6.

18. Vgontzas AN, Zoumakis E, Bixler EO, *et al*. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. *J Clin Endocrinol Metab* 2004; 89: 2119-26.

19. Henning PC, Scofield DE, Spiering BA, *et al*. Recovery of endocrine and inflammatory mediators following an extended energy deficit. *J Clin Endocrinol Metab* 2014; 99: 956-64.

20. van Leeuwen WM, Lehto M, Karisola P, *et al*. Sleep restriction increases the risk of developing cardiovascular diseases by augmenting proinflammatory responses through IL-17 and CRP. *PLoS One* 2009; 4: e4589.

21. Lundeland B, Gundersen Y, Opstad PK, *et al*. One week of multifactorial high-stress military ranger training affects Gram-negative signalling. *Scand J Clin Lab Invest* 2012; 72: 547-54.

22. Boudjeltia KZ, Faraut B, Esposito MJ, *et al*. Temporal dissociation between myeloperoxidase (MPO)-modified LDL and MPO elevations during chronic sleep restriction and recovery in healthy young men. *PLoS One* 2011; 6: e28230.

23. van Leeuwen WM, Hublin C, Sallinen M, Härmä M, Hirvonen A, Porkka-Heiskanen T. Prolonged sleep restriction affects glucose metabolism in healthy young men. *Int J Endocrinol* 2010; 2010: 108641.

24. Everson CA, Crowley WR. Reductions in circulating anabolic hormones induced by sustained sleep deprivation in rats. *Am J Physiol Endocrinol Metab* 2004; 286: E1060.

25. Kim E, Grover LM, Bertolotti D, Green TL. Growth hormone rescues hippocampal synaptic function after sleep deprivation. *Am J Physiol Regul Integr Comp Physiol* 2010; 298: R1588.

26. Veldhuis JD, Iranmanesh A. Physiological regulation of the human growth hormone (GH)-insulin-like growth factor type I (IGF-I) axis: predominant impact of age, obesity, gonadal function, and sleep. *Sleep* 1996; 19: S221.

27. Obál Jr. F, Kapás L, Gardi J, Taishi P, Bodosi B, Krueger JM. Insulin-like growth factor-1 (IGF-1)-induced inhibition of growth hormone secretion is associated with sleep suppression. *Brain Res* 1999; 818: 267-74.

28. Steiger A. Neurochemical regulation of sleep. *J Psychiatr Res* 2007; 41: 537-52.

29. Kooijman R, Coppens A. Insulin-like growth factor-I stimulates IL-10 production in human T cells. *J Leukoc Biol* 2004; 76: 862-7.

30. Ma Y, He M, Qiang L. Exercise therapy downregulates the overexpression of TLR4, TLR2, MyD88 and NF-κB after cerebral ischemia in rats. *Int J Mol Sci* 2013; 14: 3718-33.

31. Gleeson M, McFarlin B, Flynn M. Exercise and Toll-like receptors. *Exerc Immunol Rev* 2006; 12: 34-53.

32. Frystyk J. Free insulin-like growth factors – measurements and relationships to growth hormone secretion and glucose homeostasis. *Growth Horm IGF Res* 2004; 14: 337-75.

33. Gomez-Merino D, Chennaoui M, Drogou C, Guezennec CY. Influence of energy deficiency on the insulin-like growth factor I axis in a military training program. *Horm Metab Res* 2004; 36: 506-11.

34. Nindl BC, Rarick KR, Castellani JW, *et al*. Altered secretion of growth hormone and luteinizing hormone after 84 h of sustained physical exertion superimposed on caloric and sleep restriction. *J Appl Physiol* 2006; 100: 120-8.

35. Rosendal L, Langberg H, Flyvbjerg A, Frystyk J, Ørskov H, Kjaer M. Physical capacity influences the response of insulin-like growth factor and its binding proteins to training. *J Appl Physiol* 2002; 93: 1669-75.