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Eur J Appl Physiol
DOI 10.1007/s00421-008-0931-3

ORIGINAL ARTICLE

No effect of a 30-h period of sleep deprivation on leukocyte trafficking, neutrophil degranulation and saliva IgA responses to exercise

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Accepted: 30 October 2008

Abstract A one night period without sleep is not uncommon amongst athletes travelling across time zones and military personnel during training and operations. However, the effect of one night without sleep on immune indices in response to strenuous exercise remains unknown. The objective was to determine the effect of one night without sleep on immune indices in response to subsequent strenuous exercise. Using a repeated measures cross-over design, on one occasion eleven male participants slept normally (CON) and on another they were sleep deprived for 30 h (SDEP). After 30 h participants performed 30 min steady state (SS) treadmill exercise at 60% $\dot{V}O_{2\,max}$ followed by a 30 min treadmill time trial (TT). Blood and saliva samples were collected at 0 h, 30 h, post-SS, post-TT, 2 h post-TT and 18 h post-TT. Circulating leukocyte and T-lymphocyte subset counts, bacterially-stimulated neutrophil degranulation, saliva secretory immunoglobulin A (S-IgA) and plasma cortisol were determined. No trial × time interactions were observed for immune indices and plasma cortisol. A leukocytosis, neutrophilia, and lymphocytosis was observed post-TT compared with 30 h ($P < 0.01$). Also, at post-TT compared with 30 h an increase in circulating T-lymphocyte CD3 + (55%) and CD8 + (67%) counts ($P < 0.05$), a decrease in neutrophil degranulation (20%; $P < 0.05$) and an increase in S-IgA concentration (83%) was observed ($P < 0.01$). Plasma cortisol concentration increased post-TT (62%) compared with post-SS ($P < 0.01$). In conclusion, a 30 h period of sleep deprivation does not alter leukocyte trafficking, neutrophil degranulation or S-IgA responses either at rest or after submaximal and strenuous exercise.

Keywords Stress · Immune · Phagocyte · Cortisol · Running

Introduction

Missing one night of sleep is not uncommon amongst athletes travelling across time zones, recreational athletes with nocturnal work habits and military personnel during training and operations. In a similar manner to the acute immune response to strenuous exercise (Gleeson 2007) two to three nights of sleep deprivation has been shown to elicit a circulating leukocytosis (Dinges et al. 1994) and a decrease in neutrophil and lymphocyte function (Moldofsky et al. 1989; Palmblad et al. 1976, 1979). Although the clinical significance of a decrease in immune function with prolonged sleep deprivation in healthy individuals remains unclear, decreases in neutrophil function for example have been implicated in increased infection incidence in clinical populations (Ellis et al. 1988; Smitherman and Peacock 1995). A stimulatory effect of sleep deprivation on sleep-regulating cytokines (e.g. IL-1β, TNFα and IFNγ) and stress

All procedures accord with current local guidelines and the declaration of Helsinki.

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Published online: 19 November 2008

hormones (Dickstein and Moldofsky 1999; Hu et al. 2003; Radomski et al. 1992) may mediate the altered immune response which in turn might increase susceptibility to infection (Dinges et al. 1995; Irwin 2002).

The effect of one night of sleep deprivation on immune function remains unclear due to limited and conflicting evidence and a lack of experimental control in previous studies. For example, a one night period of sleep deprivation increased circulating leukocyte counts in one study (Born et al. 1997) but had no effect in another (Heiser et al. 2000). In addition, one study showed that only one night of a three night period of sleep deprivation was required to decrease neutrophil phagocytosis (Palmblad et al. 1976). However, the female participants were exposed to additional stressors that could account, at least in part, for the observed decrease in neutrophil function; these stressors were incorporated to simulate a battlefield environment and included a continuous 95 dB noise and a rifle shooting task. It is plausible that exercise stress after one night of sleep deprivation, as sometimes practiced by travelling athletes and military personnel during training and operations, may amplify stress hormone responses and associated exercise-induced immune perturbations (Gleeson 2007). However, to our knowledge the influence of sleep deprivation on immune indices in response to subsequent exercise has not been investigated.

Therefore, the aim of the present study was to determine the effects of one night of sleep deprivation on selected immune indices at rest and following submaximal and strenuous exercise. We hypothesised that performing exercise after sleep deprivation would evoke greater immune disturbances compared with performing exercise after one night of normal sleep.

Methods

Participants

Eleven healthy recreationally active male students (mean (SD): age 20 (3) years; nude body mass 77.6 (7.8) kg; height 1.80 (0.05) m; BMI 23.3 (2.1) kg m^2; body fat 13.2 (4.5) %; $\dot{V}O_{2\,max}$ 55.5 (5.6) ml kg^{-1} min^{-1}) volunteered to participate in the study. All participants gave written informed consent before the study, which received local Ethics Committee approval. Participants reported no symptoms of infection or illness, and no medications or supplements were taken in the twelve weeks prior or during the study.

Preliminary measurements

Prior to the first experimental trial, and after a 24-h period without training, participants were asked to report to the laboratory where height and nude body mass were recorded. Body composition was determined by whole body dual-energy X-ray absorptiometry (Oliver et al. 2007b). Maximal oxygen uptake $\left(\dot{V}O_{2\,max}\right)$ was estimated by means of a continuous incremental exercise test to volitional exhaustion on a motorised treadmill (Oliver et al. 2007b) and the treadmill speed that elicited 60% $\dot{V}O_{2\,max}$ on a 1% gradient was extrapolated.

Experimental trials

Using a randomised cross-over design, participants completed two trials separated by seven days. On one occasion participants were allowed normal nocturnal sleep [CON: 496 (18) min] and on another occasion participants were sleep deprived for 30 h (SDEP). To control dietary and fluid intake prior to and during each experimental trial, participants were provided with food and fluid which catered for their estimated daily energy [3280 (209) kcal day^{-1}] (Cunningham 1980) and fluid (35 ml kg BM day^{-1}) requirements. Participants were required to abstain from caffeine containing products and alcohol prior to and during the experimental trials. For the experimental trials participants reported to the laboratory at 0600 h after a night of normal sleep [CON: 517 (19) min and SDEP: 513 (21) min] and resided, under supervision, in laboratory accommodation until 1800 hours the following day. Nocturnal movements were monitored by an accelerometer (GT1 M, ActiGraph LLC, Florida, USA). During SDEP, the laboratory accommodation was brightly lit at all times. At 0800 hours on the second day of each trial participants were provided with a standard breakfast and at 1200 hours participants began the exercise protocol. The exercise protocol was performed on a motorised treadmill and consisted of a 30-min steady state (SS) run at 60% $\dot{V}O_{2\,max}$, followed by 15 min seated rest and then a 30-min time trial (TT), whereby, participants ran as far as possible in 30 min (data presented elsewhere (Oliver et al. 2007a). Participants wore athletic shorts, socks and trainers and the ambient conditions were 20°C and 59% relative humidity. Heart rate (HR), measured by short-range radiotelemetry monitor (Polar Electro, Kempele, Finland) was recorded every 5 min during SS and TT. During the SS and TT exercise bout participants were not permitted to consume any food or fluids. Both exercise bouts were performed with two fans placed 1 m from the treadmill at a speed of 2.3 m s^{-1}.

Saliva collection and analysis

Unstimulated whole saliva samples were collected by dribble for 4 min as previously described (Oliver et al. 2007b), at 0800 hour on day one, 30 h, post-SS, post-TT,

2 h and 18 h post-TT. Saliva samples were handled and stored as previously described (Oliver et al. 2007b) and S-IgA concentration ($n = 8$) was determined by polymeric-IgA-directed ELISA (Immundiagnostik, Bensheim, Germany). The intra-assay CV for S-IgA concentration was 2.1%.

Blood collection and analysis

Whole blood samples were collected by venepuncture from an antecubital vein at 0800 hour on day one, 30 h, post-SS, post-TT, 2 h and 18 h post-TT into two K_3EDTA vacutainer tubes (4 ml, 1.6 mgEDTA ml^{-1}; Becton Dickinson, Oxford, UK) and two lithium heparin vacutainer tubes (4 ml, 1.5 IU heparin ml^{-1}; Becton Dickinson, Oxford, UK). Haematocrit (heparin) was determined by capillary method and haemoglobin concentration (EDTA) using an automated cell counter (Gen-S, Beckman Coulter, High Wycombe, UK). Relative changes in plasma volume were calculated and blood parameters corrected accordingly (Dill and Costill 1974). Lithium heparin samples were centrifuged at 1,500*g* for 10 min at 4°C within 15 min of sample collection and plasma was stored at −80°C for further analysis. One K_3EDTA vacutainer tube was stored at room temperature prior to haematological analysis within 6 h of collection. Haematological analysis included circulating total and differential leukocyte counts performed using an automated cell counter (Gen-S, Beckman Coulter, High Wycombe, UK). Standard flow cytometric techniques were used to determine T-lymphocytes (CD3+), helper/inducer T-lymphocytes (CD4+) and cytotoxic/suppressor T-lymphocytes (CD8+) as described previously (Laing et al. 2008b).

Plasma elastase concentration, a marker of neutrophil degranulation, was determined in unstimulated and bacterially-stimulated heparinised plasma using ELISA as described (Laing et al. 2008a). Cortisol concentration was determined on heparinised plasma using ELISA (DRG Diagnostics, Marburg, Germany). The intra-assay CV for elastase and cortisol concentration was 4.4 and 3.5% respectively.

Statistical analysis

Data in the text and tables are presented as mean and standard error of the mean (SEM). The data were examined using a 2-way repeated measures ANOVA. Assumptions of homogeneity and sphericity in data were checked and, where appropriate, adjustments to the degrees of freedom were made using the Greenhouse-Geisser correction method. Significant differences were analysed using post hoc Tukey's HSD test. The required sample size was estimated to be between four and ten participants (www.dssresearch.com/toolkit/sscalc) using previous data examining the effects of exercise on selected immune indices (Laing et al. 2008a, b; Oliver et al. 2007b). Alpha and beta levels were set at 0.05 and 0.8 respectively, both of which are standard estimates. To allow for dropout eleven participants were recruited. Significance was set at $P < 0.05$.

Results

There were no trial × time interactions, but there was a significant main effect of time (MEOT) for exercising HR (SS: $F(6, 60) = 262.4$, $P < 0.01$; TT: $F(6, 60) = 232.0$, $P < 0.01$), plasma volume change ($F(5, 50) = 20.0$, $P < 0.01$) and plasma cortisol concentration ($F(5, 50) = 12.7$, $P < 0.01$). Mean exercising HR was similar during both SS (CON: 143 (4) and SDEP: 143 (5) beats $\cdot min^{-1}$) and TT (CON: 183 (3) and SDEP: 181 (3) beats min^{-1}). We have previously presented a small, albeit significant ($P < 0.05$), 2.9% decrease in the total distance covered during the TT on SDEP (Oliver et al. 2007a). Plasma volume change was −8.8 (1.8) % post-TT compared with 0 h. Plasma cortisol concentration decreased after 30 h [348 (39) nmol L^{-1}] compared with 0 h [500 (47) nmol L^{-1}: $P < 0.01$] and increased after the TT [440 (45) nmol L^{-1}] compared with post-SS [271 (16) nmol L^{-1}: $P < 0.01$].

Circulating leukocyte, neutrophil and lymphocyte/T-lymphocyte subset counts

There were no trial × time interactions, but there was a significant MEOT for circulating leukocyte ($F(5, 50) = 6.5$, $P < 0.01$), neutrophil ($F(5, 50) = 35.4$, $P < 0.01$) and lymphocyte counts ($F(5, 50) = 19.4$, $P < 0.01$) and T-lymphocyte (CD3+, CD4+, and CD8+) counts (Table 1). A leukocytosis, neutrophilia and lymphocytosis was observed after the TT ($P < 0.01$). However, lower circulating lymphocyte/T-lymphocyte subset counts were observed after 30 h, post-SS and 2 h post-TT compared with 0 h ($P < 0.01$). Circulating T-lymphocyte CD4 + :CD8 + ratio was lower post-TT (1.9 (0.3)) compared with 0 h (2.6 (0.3): $P < 0.05$) but returned to resting levels by 18 h post.

Neutrophil degranulation

There were no trial × time interactions, but there was a significant MEOT for unstimulated plasma elastase concentration ($F(4, 28) = 15.2$, $P < 0.01$), bacterially-stimulated elastase concentration ($F(4, 28) = 61.4$,

Table 1 Circulating leukocyte, differential leukocyte ($n = 11$) and T-lymphocyte subset (T-lymphocyte CD3+, T-lymphocyte helper CD4+, T-lymphocyte suppressor CD8+: $n = 6$) response to 30-min steady state (SS) treadmill exercise at 60% $\dot{V}O_{2\,max}$ followed by a 30 min time trial (TT) after sleep (CON) and 30 h sleep deprivation (SDEP)

	0 h	30 h (Pre-SS)	Post SS	Post TT	2 h post TT	18 h post TT
Leukocyte ($\times 10^9$ l^{-1})						
				##**	##**	
CON	6.5 (0.6)	5.6 (0.6)	6.9 (0.8)	10.4 (1.3)	9.8 (1.1)	6.0 (0.6)
SDEP	5.9 (0.4)	6.0 (0.5)	7.1 (0.6)	10.7 (0.9)	10.1 (0.7)	5.8 (0.4)
Neutrophil ($\times 10^9$ l^{-1})						
				##*	##**	
CON	3.6 (0.5)	3.7 (0.6)	5.1 (0.8)	6.7 (1.2)	8.1 (1.1)	3.7 (0.5)
SDEP	3.0 (0.3)	4.1 (0.4)	5.1 (0.5)	7.6 (0.8)	8.3 (0.7)	3.6 (0.3)
Lymphocyte ($\times 10^9$ l^{-1})						
		##	##	**	##	
CON	2.1 (0.2)	1.3 (0.1)	1.3 (0.2)	2.4 (0.3)	1.1 (0.1)	1.5 (0.1)
SDEP	2.1 (0.1)	1.4 (0.1)	1.4 (0.2)	2.4 (0.2)	1.2 (0.1)	1.5 (0.1)
CD3 + ($\times 10^9$ l^{-1})						
		##	##	*	##	#
CON	1.6 (0.2)	1.0 (0.2)	1.0 (0.2)	1.6 (0.3)	0.9 (0.2)	1.1 (0.1)
SDEP	1.7 (0.2)	1.0 (0.1)	1.0 (0.2)	1.5 (0.2)	0.9 (0.1)	1.2 (0.1)
CD4 + ($\times 10^9$ l^{-1})						
		##	##		##	
CON	1.1 (0.1)	0.5 (0.1)	0.6 (0.1)	0.8 (0.1)	0.6 (0.1)	0.7 (0.1)
SDEP	1.1 (0.1)	0.6 (0.1)	0.6 (0.1)	0.8 (0.1)	0.6 (0.1)	0.7 (0.1)
CD8 + ($\times 10^9$ l^{-1})						
		##	##	*	##	#
CON	0.4 (0.1)	0.3 (0.1)	0.3 (0.1)	0.5 (0.1)	0.2 (0.0)	0.3 (0.0)
SDEP	0.5 (0.1)	0.3 (0.0)	0.3 (0.0)	0.5 (0.1)	0.2 (0.0)	0.3 (0.0)

Mean (SEM). MEOT # $P < 0.05$ and ## $P < 0.01$ versus 0 h; * $P < 0.05$ and ** $P < 0.01$ vs. 30 h

$P < 0.01$) and bacterially-stimulated elastase release per neutrophil ($F(4, 28) = 6.3$, $P < 0.01$: Table 2). Unstimulated elastase concentration increased post-TT vs. 0 h ($P < 0.01$). Bacterially-stimulated elastase concentration was higher than 0 h from 30 h onwards ($P < 0.05$). Bacterially-stimulated elastase release per neutrophil was lower post-TT (20%) and 2 h post-TT (17%) compared with 30 h but was not significantly lower than 0 h during recovery.

S-IgA

There were no trial × time interactions or main effects for saliva flow rate (280 (34) µl min^{-1} at 0 h and 375 (47) µl min^{-1} at 30 h) or S-IgA secretion rate (77 (12) µg min^{-1} at 0 h and 82 (14) µg min^{-1} at 30 h). However, a significant MEOT was observed for S-IgA concentration ($F(5, 35) = 4.1$, $P < 0.01$). A significant increase in S-IgA concentration (83%) was observed post-TT (483 (72) µg ml^{-1}) compared with 30 h (264 (19) µg ml^{-1}: $P < 0.05$).

Discussion

To the best of our knowledge no study has investigated the effects of sleep deprivation on immune function in response to subsequent strenuous exercise. Also, from a practical standpoint, the one night duration of sleep deprivation in the present study is similar to that often encountered by athletes travelling across time zones, recreational athletes with nocturnal work habits and military personnel during training and operations. However, the current data do not support our hypothesis as they show that a 30 h period of sleep deprivation does not alter leukocyte trafficking, neutrophil degranulation or S-IgA responses either at rest or after subsequent strenuous exercise.

The observed alterations in leukocyte trafficking, neutrophil degranulation and S-IgA in the present study most likely reflect circadian variations (Shephard and Shek 1997) and the widely reported acute effects of strenuous exercise (Gleeson 2007). Our results agree with one study showing no change in circulating leukocyte counts after

Table 2 Neutrophil degranulation response to 30-min steady state (SS) treadmill exercise at 60% $\dot{V}O_{2\,max}$ followed by a 30 min time trial (TT) after sleep (CON) and 30 h sleep deprivation (SDEP)

	0 h	30 h (Pre-SS)	Post SS	Post TT	2 h post TT
Unstimulated plasma elastase (ng ml^{-1})					
				##**	
CON	40 (5)	43 (5)	85 (14)	272 (56)	93 (10)
SDEP	36 (3)	53 (12)	74 (11)	209 (42)	70 (9)
Stimulated plasma elastase (ng ml^{-1})					
		#	##*	##**	##**
CON	2529 (379)	3705 (480)	4704 (575)	5463 (554)	5759 (370)
SDEP	2506 (332)	2791 (240)	3476 (495)	5027 (497)	5714 (310)
Stimulated elastase per cell (fg cell^{-1})					
				**	**
CON	416 (33)	496 (46)	453 (44)	358 (40)	367 (44)
SDEP	428 (35)	414 (36)	399 (22)	372 (39)	389 (38)

Mean (SEM) ($n = 8$). MEOT # $P < 0.05$ and ## $P < 0.01$ versus 0 h; MEOT * $P < 0.05$ and ** $P < 0.01$ versus 30 h

one night of sleep deprivation (Heiser et al. 2000) but disagree with others showing an increase in circulating leukocyte count (Born et al. 1997) and a decrease in neutrophil phagocytosis (Palmblad et al. 1976) after a night without sleep. It is conceivable though that the decrease in neutrophil phagocytosis after one night of sleep deprivation shown previously (Palmblad et al. 1976) was due to the additional stressors that participants were exposed to; these included battlefield noise and a rifle shooting task. Typically, longer periods of sleep deprivation ranging from 40 to 77 h have been shown to elicit a circulating leukocytosis (Dinges et al. 1994) and a decrease in lymphocyte and phagocyte function (Moldofsky et al. 1989; Palmblad et al. 1976, 1979) so it is possible that the relatively short period of sleep deprivation in the present study was not long enough to alter stress hormone and immune responses. Nevertheless, our results have practical relevance as they clearly demonstrate that one night of sleep deprivation, as commonly experienced by athletes and military personnel, does not amplify stress hormone and associated immune responses to subsequent strenuous exercise. It remains to be shown if more prolonged sleep deprivation or more prolonged exercise following a similar period of sleep deprivation to that used here alters immune function or infection incidence. For example, it is very difficult to isolate an effect of sleep deprivation, if any, in the decrease in immune function (e.g. decreased saliva S-IgA) observed in military personnel during more prolonged 5–7 day combat training exercises (Boyum et al. 1996; Gomez-Merino et al. 2003). In these studies, participants were exposed to a combination of stressors that could account for decreased immune function including sleep deprivation and prolonged physical exertion (Gleeson 2007) but also including fluid and energy restriction (Laing et al. 2008b; Oliver et al. 2007b), psychological stress (Keller et al. 1981) and exposure to extreme environments (Walsh and Whitham 2006).

In conclusion, these results show that a 30 h period of sleep deprivation does not alter leukocyte trafficking, neutrophil degranulation, S-IgA responses or plasma cortisol concentration either at rest or following submaximal or strenuous exercise.

Acknowledgments The authors acknowledge the Headquarters Army Recruiting and Training Division, Upavon, UK for supporting the study. The authors also acknowledge the Sports Council for Wales for partly funding Dr. S. Laing's Ph.D. studentship.

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