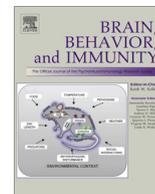




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Sleep disruption and its effect on lymphocyte redeployment following an acute bout of exercise

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ABSTRACT

Sleep disruption and deprivation are common in contemporary society and have been linked with poor health, decreased job performance and increased life-stress. The rapid redeployment of lymphocytes between the blood and tissues is an archetypal feature of the acute stress response, but it is not known if short-term perturbations in sleep architecture affect lymphocyte redeployment. We examined the effects of a disrupted night sleep on the exercise-induced redeployment of lymphocytes and their subtypes. 10 healthy male cyclists performed 1 h of cycling at a fixed power output on an indoor cycle ergometer, following a night of undisrupted sleep (US) or a night of disrupted sleep (DS). Blood was collected before, immediately after and 1 h after exercise completion. Lymphocytes and their subtypes were enumerated using direct immunofluorescence assays and 4-colour flow cytometry. DS was associated with elevated concentrations of total lymphocytes and CD3⁺/CD56⁺ NK-cells. Although not affecting baseline levels, DS augmented the exercise-induced redeployment of CD8⁺ T-cells, with the naïve/early differentiated subtypes (KLRG1⁻/CD45RA⁺) being affected most. While the mobilisation of cytotoxic lymphocyte subsets (NK cells, CD8⁺ T-cells $\gamma\delta$ T-cells), tended to be larger in response to exercise following DS, their enhanced egress at 1 h post-exercise was more marked. This occurred despite similar serum cortisol and catecholamine levels between the US and DS trials. NK-cells redeployed with exercise after DS retained their expression of perforin and Granzyme-B indicating that DS did not affect NK-cell 'arming'. Our findings indicate that short-term changes in sleep architecture may 'prime' the immune system and cause minor enhancements in lymphocyte trafficking in response to acute dynamic exercise.

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1. Introduction

Sleep disruption and deprivation are common in contemporary society and have been linked with poor health, decreased job performance and increased life-stress (Mosendane et al., 2008; Coffey et al., 2006). This problem is exacerbated by the demands placed on many professions that require employees to carry out shift work, and to travel across numerous time zones that often result in circadian disruption and further alterations to sleep architecture. Increasing evidence has associated sleep impairments with disruptions in the normal functioning of the immune system (Bryant et al., 2004) and elevated disease risk (Gottlieb et al., 2005; Meier-Ewert et al., 2004). Furthermore, circadian disruption

is known to cause profound changes in hypothalamic–pituitary–adrenal axis (HPA) and sympathetic nervous system (SNS) activity and the resulting secretion of cortisol and catecholamines, which, in turn, are known to have marked effects on the immune system (Anane et al., 2009; Atanackovic et al., 2006).

A single bout of dynamic exercise causes a rapid increase in the blood lymphocyte count (lymphocytosis) (Gleeson, 2007; Simpson et al., 2007). Increased SNS activity and the release of catecholamines that bind to high affinity β_2 adrenergic-receptors on lymphocytes are largely responsible for this exercise-induced lymphocytosis (Anane et al., 2009; Atanackovic et al., 2006). Those lymphocyte subsets that exhibit phenotypes associated with enhanced cytotoxic and tissue-migrating potential (i.e. NK^{dim} cells, CD8⁺ T-cells and $\gamma\delta$ T-cells) are preferentially mobilised to the peripheral blood with exercise (Anane et al., 2009; Krüger and Mooren, 2007). Moreover, antigen experienced highly differentiated subsets of CD8⁺ T-cells (i.e. KLRG1⁺) are redeployed in relatively greater numbers to stress and exercise compared to naïve

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and “early” differentiated CD8⁺ T-cells (Simpson et al., 2007; Campbell et al., 2009; Turner et al., 2010). Upon exercise cessation, there is a rapid egress of these same lymphocyte subsets resulting in a transient lymphocytopenia (Walsh et al., 2011; Simpson et al., 2007, 2006). During this time, lymphocytes may traffic to the spleen, lung, bone marrow and lymph nodes, which is important during times of immune activation and ensures regulation of the immune response (Dhabhar et al., 2012). This rapid redeployment of cytotoxic cells with exercise is believed to be an evolutionary conserved mechanism to protect the host during periods of acute stress when injury/infection is more likely to occur (Dhabhar et al., 2012).

Studies that have examined the effects of sleep deprivation on the immune system are scarce and largely conflicting, perhaps due to disparate methodologies (partial sleep deprivation of a few hours to complete sleep deprivation for up to 48 h) and heterogeneous outcome measures. Blood leukocyte numbers have been reported to increase following chronic sleep restriction (4 h for 3 days) (Kerhofs et al., 2007; Boudjeltia et al., 2008) and 24 h (Born et al., 1997), whilst others report no effect (Ricardo et al., 2009). While sleep deprivation studies provide useful information on the effects of lack of sleep, in this contemporary society, it is perhaps disrupted sleep that is more commonly experienced. Indeed, the effects of sleep disruption on immunity could have important implications for the health of athletes and those working in professions that perturb the circadian system (i.e. the military, fire service and police force), especially when physically demanding tasks are performed. That is, infection susceptibility may be greater if sleep disruption is found to impinge normal lymphocyte redeployment and immuno-surveillance following acute bouts of physical stress. No study, to our knowledge, has examined the immune response to an acute stressor following a period of sleep disruption.

The aim of this study was to determine if a single night of sleep disruption alters blood lymphocyte redeployment following a single bout of strenuous exercise. We hypothesised that acute exercise performed the day after a night of disrupted sleep would amplify the redeployment of blood lymphocytes when compared to exercise performed after an undisturbed night of sleep. We also hypothesised that the effects of sleep disruption would be more prominent among the cytotoxic cells (CD8⁺ T-cells, $\gamma\delta$ T-cells and NK-cells) that are typically redeployed in relatively greater numbers with exercise (Anane et al., 2009; Kruger et al., 2008).

2. Methodology

2.1. Participants

Ten healthy adult males (mean \pm SD) age: 27 \pm 8 years; height: 176 \pm 7 centimetres (cm); mass: 73.9 \pm 8 kilograms (kg) participated in the study. Participants were non-smokers who were taking no medication or supplementation, familiar with cycling time trials (TT), had abstained from alcohol and caffeine orientated beverages 24 h before participation and had been free from illness at least 2 weeks prior to the first trial. After receiving both oral and written information pertaining to the risks and demands associated with the study, each participant gave written informed consent. Ethical approval for the study was granted from the ethics committee at Edinburgh Napier University.

2.2. Experimental design

All participants completed a 40 km TT on a cycle ergometer (Kingcycle Trainer Tester Rig, Kingcycle, Buckinghamshire, UK). Heart rate (Polar S610, Finland) was monitored at 5 s intervals

throughout the test. After completion of the TT all participants were provided with an actiwatch (AW, Cambridge Neurotechnology Ltd.), a small wristwatch unit used to measure behaviours associated with sleep. Prior to participation in the study, participants were screened for a 7 day period in order to monitor their regular sleep–wake cycle. If individuals consistently slept for less than 6 h a night they were excluded from the study.

2.3. Trial conditions

Participants were instructed to attend the laboratory on 2 further occasions interspersed by 1 week in a randomised counter-balanced order. The exercise protocol consisted of 1 h of exercise on a cycle ergometer (Kingcycle Trainer Tester Rig, Kingcycle, Buckinghamshire, UK) at a fixed wattage (90% mean wattage (W) \pm 10 W obtained from the 40 km TT). Data from the 40 km TT (mean \pm SD): 55:12 \pm 2:18 min; average power (mean \pm SD) 265 \pm 27 watts (W). Heart rate (HR) was recorded at 5 s intervals during the trial (Polar, Finland, S610) and rate of perceived exertion (RPE, Borg, 1970) was recorded at 5 km intervals. Before each trial participants also completed the Epworth Sleepiness Scale (ESS, Johns, 1991). Experimental trials occurred after a night of undisturbed sleep (US; 8 h) and a night of disrupted sleep (DS; woken every hour during an 8 h period). For both trials participants were instructed to go to bed at 2300 h and remain there until 700 h. During the DS trial an alarm clock was set to go off 1 h after entering bed. Upon awakening from the alarm participants were required to open an envelope that contained a set of instructions (see below):

- Push the button on your actiwatch.
- Reset the alarm to go off 1 h from now.
- Stand up and switch on the light.
- Complete the arousal and alertness questionnaire and also the profile of mood states (BRUMS, Terry et al., 1999).
- Switch off the light.
- Return to bed.

2.4. Questionnaires

The ESS contains 8 situations which the participant has to score related to the chance of falling asleep at that precise moment if they were to partake in the situations. A Likert scale of 0–3 is used (0 = no chance of dozing, 3 = high chance of dozing). The total score of all situations is then used to calculate sleepiness. RPE comprises of a scale from 6 to 20 where participants rate how difficult they find the exercise bout (6 = no exertion at all, 20 = maximal exertion).

2.5. Sleep measures

An actiwatch was worn on the non-dominant wrist and used to measure sleep behaviours the night prior to each trial. The actiwatch records movement in any of 3-dimensions using an accelerometer that has a sensitivity of 0.01 g. Data was logged continuously in 1 min epochs. When participants entered bed at night and once they were awake in the morning they were instructed to push the button on the front of the actiwatch enabling sleep onset and awakening times to be determined. During the DS trial, each disruption during the night was recorded by the participant pushing the button on the actiwatch. This allowed recording and confirmation of the sleep disruption events. Adherence during the night DS was confirmed at 100% by the actiwatch trace, with each participant waking every hour of the night.

2.6. Blood sampling and separation

Intravenous blood samples were collected before (pre), immediately after (post) and one hour after (1 h post) completing the 1 h cycling challenge in 6 ml vacutainer tubes that contained EDTA or serum specific blood tubes (Becton–Dickinson, Oxford, UK). Total blood leukocyte and differential counts were determined using an automated haematology analyser (Sysmex, XS 1000i, UK). Serum was removed and frozen at -80°C until analysis for cortisol. Serum cortisol (R&Dsystems, UK) and plasma catecholamine (IBL international, UK) levels were measured using a commercially available enzyme linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions. Plates were read on a plate reader at a wavelength of 450 nm for cortisol and catecholamines (Labtech LW5000, UK).

2.7. Lymphocyte phenotyping

Blood samples were mixed with an equal volume of 0.9% NaCl (Baxter, UK) and 6 ml of the diluted blood sample was carefully layered over 3 ml of lymphoprep solution (Axis-Shield, Oslo, Norway) for the separation of peripheral blood mononuclear cells (PBMCs) following procedures previously described by Simpson et al. (2006). Aliquots of 1.0×10^6 isolated PBMCs were labelled with an APC conjugated anti-CD3 (MEM57, IgG_{2a}), an ALEXA Fluor 488-conjugated anti KLRG1 (clone 13A2) or a PE-CY5-conjugated anti CD4 (RPA-T4, IgG₁) or an anti CD8 (HIT8a, IgG₁) and one PE-conjugated anti CD45RA (HI100, IgG_{2B}), or anti CD45RO (UCHL1, IgG_{2a}) or an anti $\gamma\delta$ TCR (B1.1, IgG₁) for T cell populations or a PE CY7 anti CD56 (B159, IgG₁) or a PE conjugated anti Perforin (HC4 IgG₁) or anti Granzyme B (HC4, IgG₁) monoclonal antibodies (mAb) for NK cells. PBMCs were incubated with 100 μl of each pre-diluted (1/100) mAb solution for 45 min at room temperature. The anti CD3, CD4, CD8, CD45RA, CD45RO and CD56 mAbs were purchased from BD Biosciences (Oxford, UK), anti $\gamma\delta$ TCR from ebioscience (San Diego, CA, USA) with the anti-perforin and Granzyme B purchased from Immunotools (Friesoythe, Germany).

After labelling cell surface antigens for NK cell markers, 0.5 ml phosphate buffered saline + 1% bovine serum albumin + 0.02% sodium azide (PBS–BSA) was added to the samples. Cells were centrifuged at room temperature for 5 min at 250 G. Supernatant was removed and cells were re-suspended in 250 μl of BD Cytofix and incubated for 20 min at 4°C . Cells were washed twice in BD Permawash for 5 min, 250 G, 4°C . Anti-Perforin (HC4, IgG₁) mAbs or anti-Granzyme B (HC4, IgG₁) mAbs were incubated with the cells for 60 min at 4°C . Cells were washed twice in 0.5 ml of BD Permawash for 5 min, 250 G, 4°C in preparation for analysis by flow cytometry.

2.8. Flow cytometry

Cell phenotype data was attained using CELLQuest Pro software (BD Biosciences, San Jose, CA, USA) on a FACSCalibur flow cytometer equipped with a 15 mW argon ion laser emitting light at a fixed wave length of 488 nm. An electronic gate was placed around the lymphocyte population in the flow cytometry forward and side scatter mode. Side scatter against APC fluorescence was used to identify and gate CD3⁺ cells (T-cells) and CD3⁻ cells (NK-cells). The expression of CD4, CD8 and $\gamma\delta$ TCR was determined on the CD3⁺ gate, and CD56 expression was determined on the CD3⁻ gate. Flow cytometry dot plots were used to identify CD3⁺ CD4⁺ and CD3⁺ CD8⁺ and CD3⁺ $\gamma\delta$ populations and their co-expression of (KLRG1, CD45RA, CD45RO). 10,000 gated lymphocytes were acquired for analysis for all the samples with the exception $\gamma\delta$ T-cell samples where 50,000 events were acquired to account for the low circulating numbers. Total numbers of lymphocyte subtypes were obtained by multiplying the percentage values

obtained from the flow cytometer by the corresponding total lymphocyte counts.

2.9. Statistical analysis

All results are presented as mean \pm SE unless otherwise stated. Statistical analysis was conducted in the SPSS software version 20 (Chicago, IL). Prior to statistical analysis all data was assessed for normal distribution. The effect time (i.e. pre, post and 1 h post exercise) and trial (undisrupted sleep, disrupted sleep) on the expression of various cell surface markers on lymphocyte populations were analysed using repeated measures analyses of variance (ANOVA). Only significant interactions (time \times trial) are presented unless indicated otherwise. When significant differences were detected Bonferroni corrected paired *t*-tests were utilised to determine the trial differences, significance was set at $p < 0.01$ to adjust for multiple testing. Assumptions of sphericity in data were checked for each ANOVA, and, where appropriate, adjustments were made to the degrees of freedom. Effect size was calculated using Pearson's correlation coefficient (Fields, 2009). The ingress and egress of lymphocytes values are expressed as change in cell number (Δ) from pre to post exercise (ingress) and post to 1 h post exercise (egress) were analysed using paired sample *t*-tests. Statistical significance was recorded as $p < 0.05$.

3. Results

All participants completed the study successfully. Statistically significant differences between US and DS trials were observed for the mean heart rate during the exercise bout ($t_{(9)} = 4.80$, $p < 0.01$, $r = 0.85$), ESS ($t_{(9)} = 3.25$, $p < 0.05$, $r = 0.73$) and RPE ($t_{(9)} = 3.86$, $p < 0.01$, $r = 0.79$). In comparison to the US trial, participants exhibited lower average heart rates during exercise (160 bpm US, 152 bpm DS), reported feeling sleepier (moderate sleep debt US, heavy sleep debt DS) and perceived the bout of exercise to be more difficult.

3.1. Lymphocyte response to exercise and sleep

Table 1 illustrates summary data for total lymphocytes and subsets following a night of US and DS. Exercise was associated with an acute lymphocytosis during both trials, whereby lymphocytes (+36%), CD8⁺ T-cells (+31%), NK cells (+107%) and $\gamma\delta$ T-cells (+66%) were significantly elevated from baseline (pre) during the US trial. Exercise-induced changes following a night of DS also demonstrated a significant elevation in lymphocytes and subsets from baseline values, with lymphocytes (+58%), CD4⁺ T-cells (+29%), CD8⁺ T-cells (+50%), NK cells (+147%) and $\gamma\delta$ T-cells (+117%) also increasing significantly from baseline. At 1 h into exercise recovery, circulating levels of lymphocytes and lymphocyte subsets returned to near baseline values under both trial conditions, while NK cells were significant lower than baseline values (pre) by -74% and -81% for the US and DS trials respectively.

Repeated measures ANOVA yielded a significant trial (US/DS) \times interaction with time (pre, post, 1 h post) for total lymphocytes ($F_{(2/18)} = 5.84$, $p < 0.05$, $r = 0.5$) suggesting lymphocytes were significantly altered at with exercise depending on whether the participants had endured a night of DS or US. Bonferroni post hoc analysis revealed this was driven by the larger increase in lymphocytes at the post time point between US and DS trials ($t_{(9)} = 2.88$, $p < 0.01$, $r = 0.69$). CD8⁺ T-cells similarly demonstrated a trial \times time interaction ($F_{(2/18)} = 4.12$; $p < 0.03$, $r = 0.43$) with a larger increase in cell number apparent during the DS trial, post hoc analysis revealed this was driven by a larger increase in CD8⁺ T-cells immediately post exercise during the DS trial ($t_{(9)} = 2.69$,

Table 1
Total cell number of peripheral blood lymphocytes and subsets in response to an acute bout of exercise following a night of US or DS (mean ± SE).

Cell subset	Trial	Pre (cells/μl)	Post (cells/μl)	1 h Post (cells/μl)	Main effects of sleep	Main effects of exercise	Sleep × exercise interaction
Lymphocytes	US DS	1701 ± 141 1828 ± 114	2314 ± 169 2871 ± 291	1491 ± 91 1574 ± 85	$F_{(1/9)} = 5.21; p = 0.04$	$F_{(2/15)} = 29.2; p < 0.01$	$F_{(2/14)} = 5.84; p = 0.01$
CD4 ⁺ T-cells	US DS	619 ± 80 651 ± 69	713 ± 73 838 ± 97	624 ± 57 664 ± 58	$F_{(1/9)} = 2.28; NS$	$F_{(2/18)} = 6.21; p = 0.009$	$F_{(2/18)} = 3.35; NS$
CD8 ⁺ T-cells	US DS	363 ± 64 401 ± 62	474 ± 75 595 ± 110	303 ± 44 324 ± 47	$F_{(1/9)} = 4.12; p = NS$	$F_{(2/18)} = 12.27; p < 0.01$	$F_{(2/18)} = 4.12; p = 0.03$
NK cells	US DS	258 ± 42 291 ± 38	535 ± 88 720 ± 129	139 ± 30 140 ± 27	$F_{(1/9)} = 7.67; p = 0.02$	$F_{(2/18)} = 26.76; p < 0.01$	$F_{(2/18)} = 3.44; NS$
γδ T-cells	US DS	37 ± 9 36 ± 9	62 ± 13 77 ± 17	32 ± 6 32 ± 4	$F_{(1/9)} = 0.63; NS$	$F_{(2/18)} = 8.64; p = 0.01$	$F_{(2/18)} = 2.71; NS$

NS = Not significant.

$p < 0.05, r = 0.67$). There was no time × trial interaction observed for CD4⁺ T-cells, NK cells or γδ T-cells between US and DS conditions (see Table 1).

3.2. Ingress and egress of lymphocytes and subpopulations

Alterations in the ingress and egress of lymphocytes and lymphocyte subsets in response to exercise after US and DS are depicted in Fig. 1A and B respectively. Despite all lymphocyte populations demonstrating a greater mobilisation following DS, no

significant differences were observed between the US and DS trials for the mobilisation of total lymphocytes, CD8⁺ T-cells, CD4⁺ T-cells, γδ T-cells or NK cells (Fig. 1A). The egress of total lymphocytes ($t_{(9)} = 3.06, p < 0.05, r = 0.71$), CD8⁺ T-cells ($t_{(9)} = 2.92, p < 0.05, r = 0.7$), NK cells ($t_{(9)} = 2.32, p < 0.05, r = 0.61$) and γδ T-cells ($t_{(9)} = 2.52, p < 0.05, r = 0.64$) was significantly greater after DS compared to US, whereas CD4⁺ T-cells ($t_{(9)} = 2.02, p > 0.05$) egress was not significantly different (Fig. 1B).

3.3. NK cell subset response

Fig. 2A demonstrates the pattern of mobilisation within NK cell subsets (CD56^{dim} and CD56^{bright}). No significant interaction between trial × time was demonstrated for CD56^{dim} NK cells ($F_{(2/18)} = 4.44, p > 0.05$) or CD56^{bright} NK cells ($F_{(2/18)} = 1.22, p > 0.05$). Fig. 2B displays the response of intracellular perforin and Granzyme B within the CD56^{dim} population. No significant interaction occurred between time × trial for CD56^{dim} perforin⁺ NK cells ($F_{(2/18)} = 2.40, p > 0.05$) or CD56^{dim} Granzyme B⁺ NK cells ($F_{(2/18)} = 3.29, p > 0.05$). Similarly CD56^{bright} perforin⁺ ($F_{(2/18)} = 0.18, p > 0.05$) and Granzyme B⁺ ($F_{(2/18)} = 1.39, p > 0.05$) NK cells did not demonstrate a significant time × trial interaction (data not shown).

3.4. Sleep disruption and CD8⁺/CD4⁺ T-cell subset response to exercise

Fig. 3 displays the response of CD8⁺ T-cells subsets identified by the expression of CD45RA⁺ KLRG1⁻ (naïve); CD45RO⁺ (memory); CD45RA⁺ KLRG1⁺ (senescent). A significant time × trial interaction occurred in CD8⁺ CD45RA⁺/KLRG1⁻ ($F_{(2/18)} = 4.91, p < 0.05, r = 0.46$) subset, suggesting that the increase observed in CD8⁺ T-cells is largely driven by the naïve subset. Post hoc analyses revealed that this was driven by a larger increase in CD45RA⁺/KLRG1⁻ ($t_{(9)} = 2.68, p < 0.05, r = 0.67$) immediately post exercise (see Fig. 3). There was no time × trial interaction observed for CD45RA⁺ KLRG1⁻ (naïve); CD45RO⁺ (memory); CD45RA⁺ KLRG1⁺ (senescent) CD4⁺ T-cells (data not shown).

3.5. Cortisol and catecholamine response to sleep disruption

Fig. 4 illustrates the response of serum concentrations of cortisol to DS, with no time × trial interaction ($F_{(2/18)} = 1.24, p > 0.05$) observed. Plasma concentrations of adrenaline and nor-adrenaline after the US and DS exercise trials are illustrated in Fig. 5 A and B. There was no significant time × trial interaction observed for either adrenaline ($F_{(2/18)} = 1.50, p > 0.05$) or noradrenaline

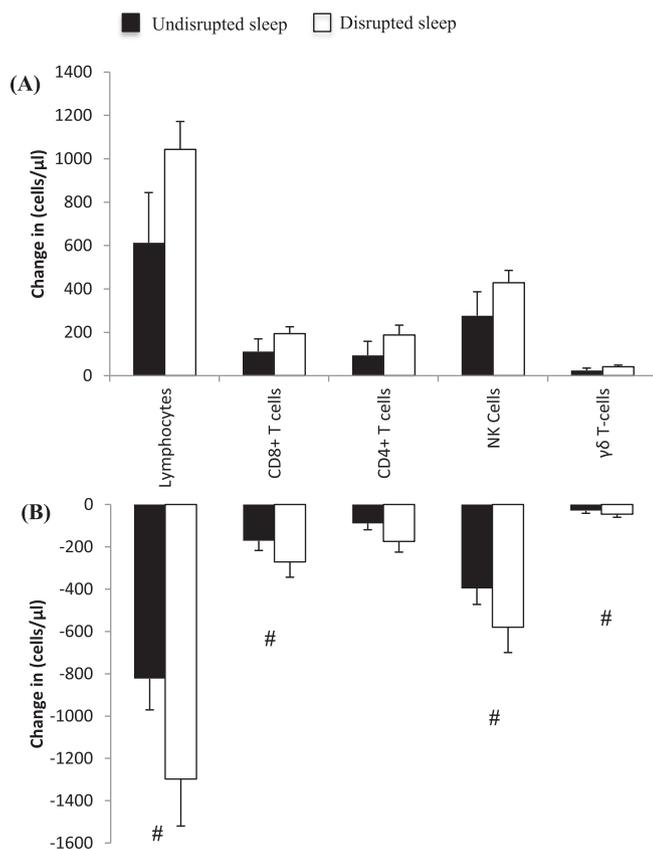


Fig. 1. The impact of disrupted sleep on the (A) ingress and (B) egress of lymphocytes, CD8⁺ T-cells, CD4⁺ T-cells, NK cells and γδ T-cells within the peripheral blood compartment following a bout of acute exercise. Values are expressed as change in cell number (Δ) from (A) pre to post exercise and (B) post to 1 h post exercise. Statistical significant between trials are indicated by # $p < 0.05$.

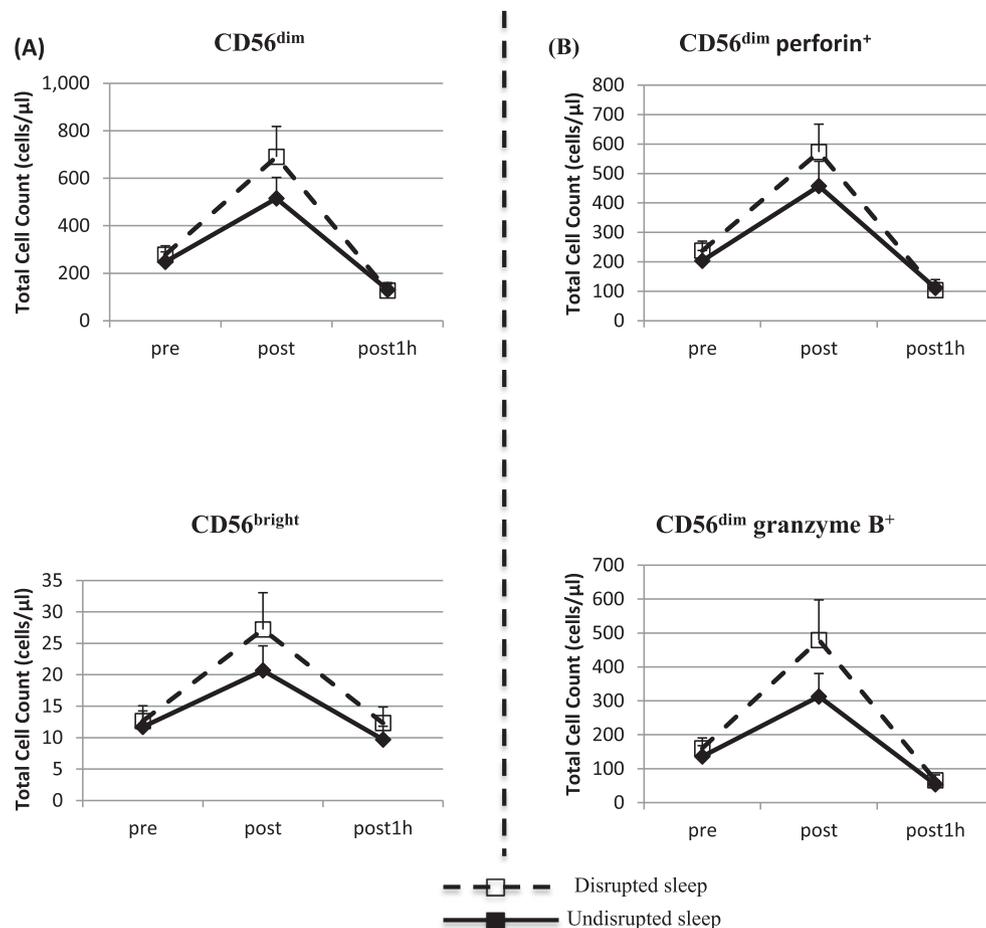


Fig. 2. The impact of disrupted sleep on the mobilisation of NK cell subsets. Panel A shows the total number of peripheral blood NK cells subsets (CD56^{dim} and CD56^{bright}) following an acute bout of aerobic exercise after a night of US or DS. Panel B shows the intracellular expression of Perforin and Granzyme B on the CD56^{dim} subset. Values are mean \pm SE.

($F_{(2/18)} = 3.14, p > 0.05$) ($p > 0.05$), although there was a trend for a larger nor-adrenaline response after exercise following DS.

4. Discussion

This is the first study to examine the impact of short-term sleep disruption on lymphocyte redeployment in response to an acute bout of strenuous exercise. The sleep disruption protocol used resulted in a heavy sleep debt the following day whilst also resulting in lower average heart rates and greater perceived exertion levels in response to 1 h of cycling exercise. This was coupled with an amplified redeployment of total lymphocytes and CD8⁺ T-cells in response to exercise. While the mobilisation of all major lymphocyte subsets tended to be larger in response to exercise following one night of disrupted sleep (DS) compared to undisrupted sleep (US), the larger egress of lymphocytes and lymphocyte subsets from the blood during the early stages of exercise recovery was more marked. Our findings indicate that a short-term period of sleep disruption amplifies lymphocyte redeployment in response to a single bout of exercise, suggesting that exercise-induced immunosurveillance may be enhanced due to short-term changes in sleep architecture.

In response to total sleep deprivation or prolonged periods of sleep disruption, T-cell counts have been reported to decrease (Dinges et al., 1994) or remain unaltered (van Leeuwen et al., 2009). Consistent with the findings of van Leeuwen et al. (2009),

we found that short-term alterations in sleep architecture did not alter baseline CD4⁺, CD8⁺ or $\gamma\delta$ T-cells numbers in blood. In contrast, a single night of DS markedly elevated circulating NK-cell numbers the following day. This is consistent with a previous study reporting increased NK-cell numbers after a night of sustained wakefulness (Born et al., 1997). Moreover, Matsumoto et al. (2001) reported increased levels of NK-cell activity following a single night of total sleep deprivation. However, during periods of chronically reduced sleep (<7 h for extended periods) or deprived sleep, NK cell activity has been reported to decrease (Fondell et al., 2011; Irwin et al., 1996), underscoring apparent dichotomous effects of short-term and long-term sleep disruption on the composition of lymphocyte subtypes in blood.

The rapid redeployment of lymphocytes between the blood and tissues is one of the most striking features of the acute stress response, and is known to occur immediately after dynamic exercise, psychological stress and beta-agonist infusion (Walsh et al., 2011; Benschop et al., 1994). In the present study, there was a trend for the mobilisation of all major lymphocyte subsets, particularly those with phenotypes associated with enhanced cytotoxic and tissue-migrating properties (NK cells, CD8⁺ T-cells $\gamma\delta$ T-cells), to be larger after exercise following a night of DS. This occurred concomitantly with a non-significant increase in plasma nor-adrenaline levels after the DS exercise trial compared to the US exercise trial. It is possible, therefore, that minor enhancements in exercise-induced lymphocyte mobilisation due to DS may be due to an augmented catecholamine response as such cells are

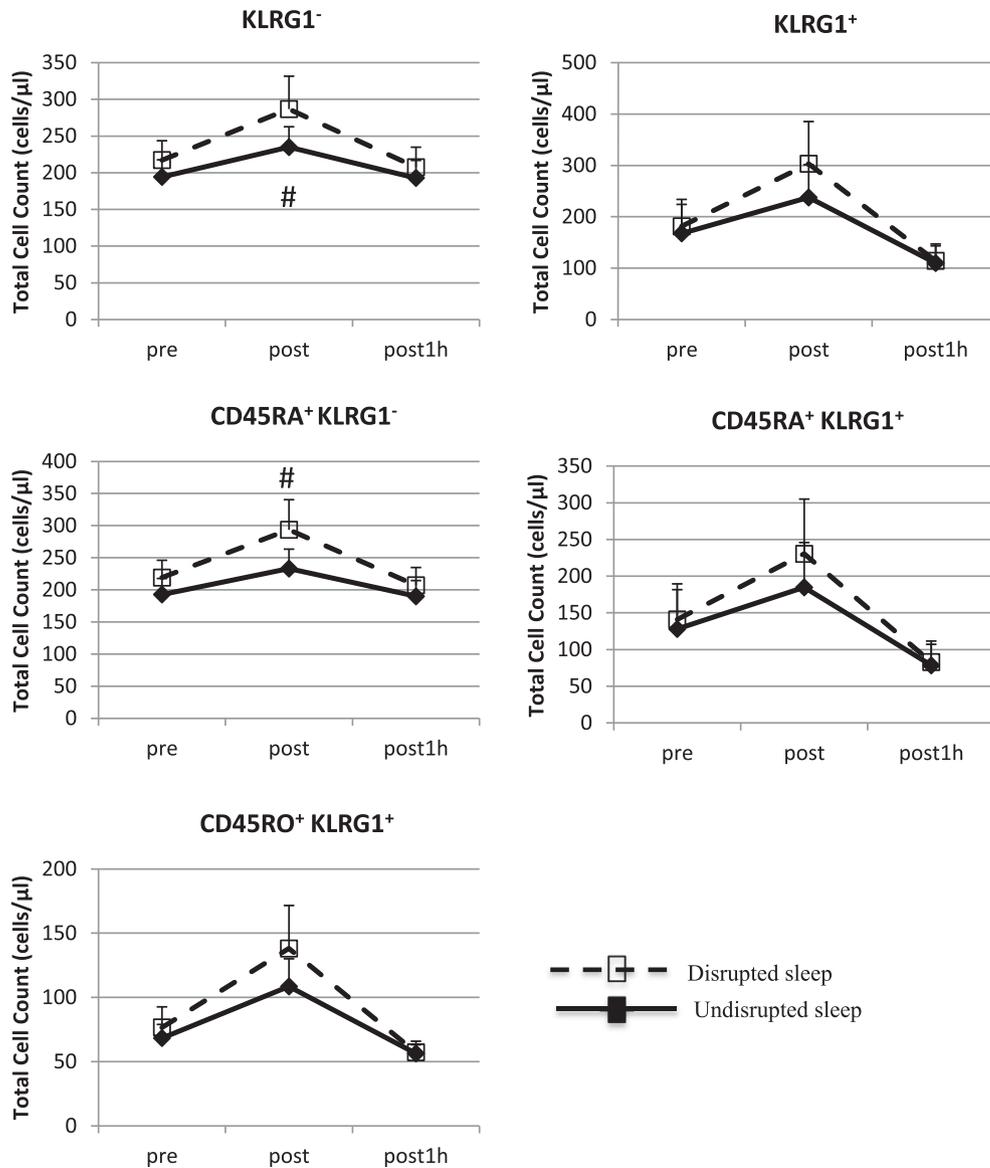


Fig. 3. The impact of disrupted sleep on the mobilisation CD8⁺ T-cell subsets. Values are means ± SE. Statistical significant differences indicated by #*p* < 0.05 for US vs. DS, results of post hoc *t*-tests. Lymphocyte populations are designated by their cell membrane markers. CD45RA⁺ KLRG1⁻, naïve cells; CD45RO⁺, memory cells; CD45RA⁺ KLRG1⁺, senescent cells.

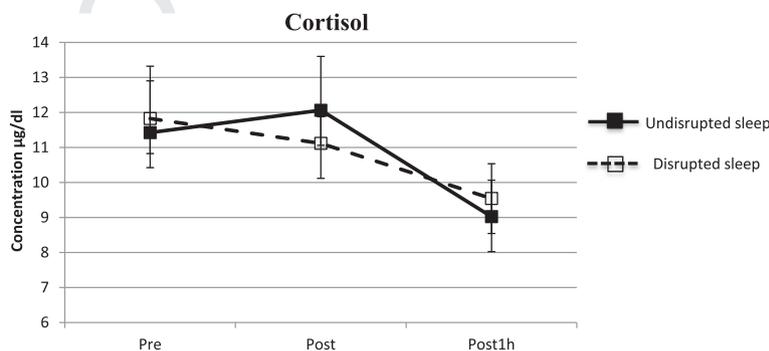


Fig. 4. The impact of sleep disruption on peripheral blood concentrations of cortisol. Values are mean ± SE.

419 known to express high levels of B₂ adrenergic receptors and are
420 mobilised in large numbers in response to catecholamine infusion
421 (Dimitrov et al., 2010). While acknowledging that the failure to

document statistically significant effects for this trend may be
422 due to low subject numbers, it nevertheless indicates that DS
423 amplifies exercise-induced lymphocyte redeployment by
424

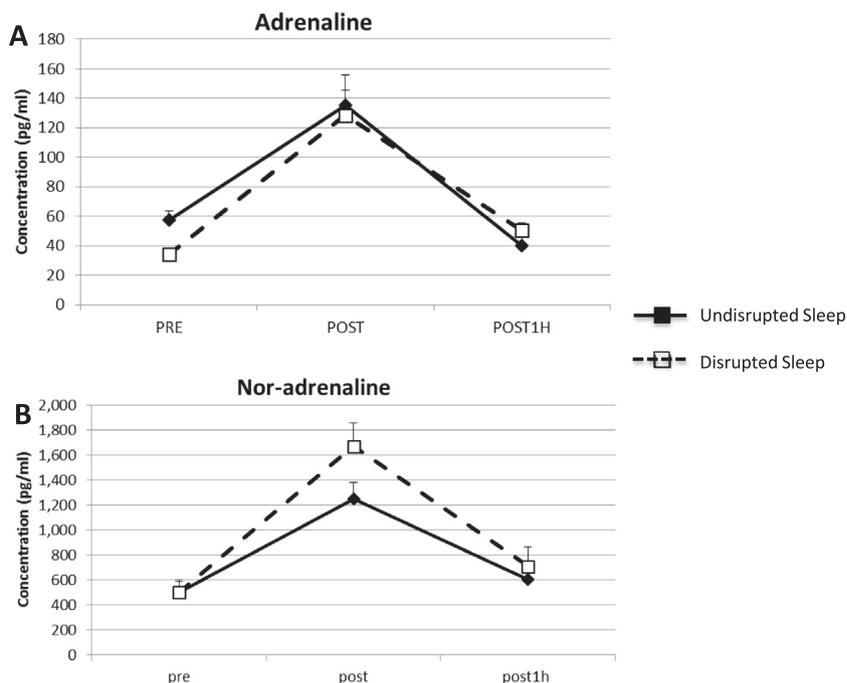


Fig. 5. The impact of sleep disruption on peripheral blood concentrations of adrenaline (A) and nor-adrenaline (B). Values are means \pm SE.

425 enhancing, for the most part, their egress from the blood during the
 426 early stages of exercise recovery. Indeed, compared to the US trial,
 427 DS caused a more profound egress of NK cells, CD8⁺ T-cells and $\gamma\delta$
 428 T-cells during the recovery phase of exercise. Although lymphocyte
 429 egress is more dependent on glucocorticoids than catecholamines
 430 (Dimitrov et al., 2009; Toft et al., 1992), plasma cortisol levels
 431 changed with exercise in a similar manner following the US and
 432 DS trials, indicating that factors other than glucocorticoid concentra-
 433 tion are responsible for the augmented egress of lymphocyte
 434 subsets. One possibility is that DS may alter the sensitivity of glu-
 435 cocorticoid and/or catecholamine receptors on blood lymphocytes
 436 and future studies exploring this would be illuminating.

437 In comparison to other lymphocyte subsets, DS appears to cause
 438 largest perturbations in the exercise response of CD8⁺ T-cells by
 439 markedly elevating their redeployment. The CD8⁺ cell subsets
 440 affected most were those with a naïve/early-differentiated pheno-
 441 type. This is somewhat surprising as these cell types are less-
 442 responsive to exercise in general (Simpson et al., 2007; Campbell
 443 et al., 2009) and, given that we observed an enhanced exercise-
 444 induced redeployment of broad lymphocyte subtypes with
 445 enhanced cytotoxic properties (e.g., NK-cells, $\gamma\delta$ T-cells) we would
 446 have expected the most cytotoxic CD8⁺ subsets (CD45RA⁺/KLRG1⁺)
 447 to also have been amplified after exercise with DS. Out of all major
 448 lymphocyte subsets, NK-cells show the most striking response to
 449 acute stress and exercise (Anane et al., 2009), however, their mobi-
 450 lisation to the exercise bout was largely impervious to short-term
 451 alterations in sleep architecture. There was, however, a trend for
 452 NK-cell mobilisation to be greater after the DS trial for both NK^{dim}
 453 (mature phenotype) and NK^{bright} (immature phenotype) subsets.
 454 Moreover, NK-cells redeployed with exercise appeared to retain
 455 their expression of perforin and Granzyme-B indicating that DS
 456 did not affect NK-cell ‘arming’.

457 Sleep disruption and deprivation typically have negative connota-
 458 tions regarding overall health and well-being (Mosendane et al.,
 459 2008; Coffey et al., 2006). The rapid redeployment of lymphocytes
 460 between the blood and tissues is an archetypal feature of the acute
 461 stress response, which is believed to enhance the speed, efficacy
 462 and regulation of an immune response by directing leukocyte

463 subpopulations to specific target organs (Dhabhar et al., 2012) thus
 464 facilitating pathogen clearance and wound healing (Biron et al.,
 465 1999; Lanier, 2001). Our finding that a single night of sleep disrup-
 466 tion enhanced lymphocyte trafficking in response to exercise per-
 467 formed the next day indicates that short-term changes in sleep
 468 architecture may actually augment this fundamental survival
 469 response. Nevertheless, it is important to differentiate between
 470 short-term periods of sleep disruption and long-term periods of
 471 sleep impairment. For instance, chronically shortened sleep
 472 (<7 h) is known to increase infection risk (Cohen et al., 2009),
 473 and deprived sleep has been shown to impair immune function
 474 by reducing NK cell activity, suppressing interleukin-2 production,
 475 and increasing circulating levels of pro-inflammatory cytokines
 476 (Irwin et al., 2006; Vgontzas et al., 2004). Moreover, when sleep
 477 is deprived, increases in circulating levels of pro-inflammatory
 478 cytokines are evident (van Leeuwen et al., 2009; Fondell et al.,
 479 2011), and, in an analysis of sleep duration (<8 h, 8–9 h and >9 h)
 480 in adolescents, those who slept 8–9 h/night demonstrated the low-
 481 est concentration of circulating inflammatory cytokines and mem-
 482 ory (CD45RO⁺) CD4⁺ T-cells (Pérez de Heredia et al., 2014). Thus,
 483 short-term periods of sleep disruption and long-term periods of
 484 sleep impairment may elicit dichotomous effects on immunity
 485 and the overall immune response to an acute stressor.

486 The current study aimed to address immune system responses
 487 associated with physical exertion and sleep disruption typically
 488 experienced by athletes and those working in professions that per-
 489 turb the circadian system (i.e. the military, fire service and police
 490 force). Although this is the first study, to our knowledge, to exam-
 491 ine the effects of DS in the context of the acute stress response, it is
 492 possible that certain subjects may have adapted to the hourly
 493 awakenings and further work may be required to optimise our
 494 DS protocol. Moreover, analysis of melatonin would have been use-
 495 ful in determining alterations made to the sleep-wake cycle when
 496 disruptions in sleep are evident. Another limitation of the present
 497 study is that that we did not determine if DS also causes changes in
 498 immune cell function. Indeed, short-term periods of sleep
 499 disruption may alter, for example, exercise-induced changes in
 500 plasma cytokine levels, stimulated cytokine production, T-cell

proliferation or NK-cell cytotoxic activity. We acknowledge that a more comprehensive assessment of immune function is required to bolster our intuition that short-term periods of sleep disruption may 'prime' the immune system and enhance immunosurveillance in response to an acute stress response.

In conclusion, we have shown for the first time that one night of DS amplifies lymphocyte redeployment to a single bout of strenuous exercise. This effect was largely due to an increased egress of lymphocyte subsets from blood during exercise recovery, with CD8⁺ T-cell redeployment being particularly sensitive to DS. In contrast to complete sleep deprivation or chronic sleep disruption where immune dysregulation is apparent and infection susceptibility increased (Cohen et al., 2009), our findings indicate that short-term changes in sleep architecture may 'prime' the immune system and cause minor enhancements in lymphocyte trafficking in response to an acute stressor. Future research should examine the impact of long-term DS on the immune response to acute stressor. Such studies are likely to have important implications for the health of athletes and those working in professions that require physical tasks to be performed during perturbations of the circadian system.

Conflict of interest

The authors declare that they have no conflict of interest.

Uncited reference

Appay et al. (2002).

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References

- Anane, L.H., Edwards, K.M., Burns, V.E., Drayson, M.T., Riddell, N.E., van Zanten, J.J.C.S.V., Wallace, G.R., Mills, P.J., Bosch, J.A., 2009. Mobilization of gamma delta T lymphocytes in response to psychological stress, exercise, and beta-agonist infusion. *Brain Behav. Immun.* 23, 823–829. <http://dx.doi.org/10.1016/j.bbi.2009.03.003>.
- Appay, V., Dunbar, P.R., Callan, M., Klenerman, P., Gillespie, G.M., Papagno, L., Ogg, G.S., King, A., Lechner, F., Spina, C.A., Little, S., Havlir, D.V., Richman, D.D., Gruener, N., Pape, G., Waters, A., Easterbrook, P., Salio, M., Cerundolo, V., McMichael, A.J., Rowland-Jones, S.L., 2002. Memory CD8⁺ T cells vary in differentiation phenotype in different persistent virus infections. *Nat. Med.* 8, 379–385. <http://dx.doi.org/10.1038/nm0402-379>.
- Atanackovic, D., Schnee, B., Schuch, G., Faltz, C., Schulze, J., Weber, C.S., Schafhausen, P., Bartels, K., Bokemeyer, C., Brunner-Weinzierl, M.C., Deter, H.C., 2006. Acute psychological stress alters the adaptive immune response: stress-induced mobilization of effector T cells. *J. Neuroimmunol.* 176, 141–152. <http://dx.doi.org/10.1016/j.jneuroim.2006.03.023>.
- Benschop, R.J., Nijkamp, F.P., Ballieux, R.E., Heijnen, C.J., 1994. The effects of beta-adrenoceptor stimulation on adhesion of human natural killer cells to cultured endothelium. *Br. J. Pharmacol.* 113 (4), 1311–1316.
- Biron, C.A., Nguyen, K.B., Pien, G.C., Cousens, L.P., Salazar-Mather, T.P., 1999. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu. Rev. Immunol.* 17, 189–220. <http://dx.doi.org/10.1146/annurev.immunol.17.1.189>.
- Borg, G., 1970. Perceived exertion as an indicator of somatic stress. *Scand. J. Rehabil. Med.* 2, 92–98.
- Born, J., Lange, T., Hansen, K., Molle, M., Fehm, H.L., 1997. Effects of sleep and circadian rhythm on human circulating immune cells. *J. Immunol.* 158, 4454–4464.
- Boudjeltia, K.Z., Faraut, B., Stenuit, P., Esposito, M.J., Dyzma, M., Brohee, D., Ducobu, J., Vanhaeverbeek, M., Kerkhofs, M., 2008. Sleep restriction increases white blood cells, mainly neutrophil count, in healthy young men: a pilot study. *Vasc. Health Risk Manag.* 6, 1447–1470.
- Bryant, P.A., Trinder, J., Curtis, N., 2004. Sick and tired: does sleep have a vital role in the immune system? *Nat. Rev. Immunol.* 4, 457–467. <http://dx.doi.org/10.1038/nri1369>.

- Campbell, J.P., Riddell, N.E., Burns, V.E., Turner, M., Veldhuijzen van Zanten, J.J.C.S., Drayson, M.T., Bosch, J.A., 2009. Acute exercise mobilises CD8⁺ T lymphocytes exhibiting an effector-memory phenotype. *Brain Behav. Immun.* 23, 767–775. <http://dx.doi.org/10.1016/j.bbi.2009.02.011>.
- Coffey, L.C., James, K., Skipper, J.K., Jung, F.D., 2006. Nurses and shift work: effects on job performance and job-related stress. *J. Adv. Nurs.* 13 (2), 245–254.
- Cohen, S., Doyle, W.J., Alper, C.M., Janicki-Deverts, D., Turner, R.B., 2009. Sleep habits and susceptibility to the common cold. *Arch. Intern. Med.* 169, 62–67. <http://dx.doi.org/10.1001/archinternmed.2008.505>.
- Dhabhar, F.S., Malarkey, W.B., Neri, E., McEwen, B.S., 2012. Stress-induced redistribution of immune cells – from barracks to boulevards to battlefields: A tale of three hormones – Curt Richter award winner. *Psychoneuroendocrinology* 39 (9), 1345–1368. <http://dx.doi.org/10.1016/j.psychneuen.2012.05.008>.
- Dimitrov, S., Lange, T., Born, J., 2010. Selective mobilization of cytotoxic leukocytes by epinephrine. *J. Immunol.* 184, 503–511. <http://dx.doi.org/10.4049/jimmunol.0902189>.
- Dimitrov, S., Benedict, C., Heutling, D., Westermann, J., Born, J., Lange, T., 2009. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. *Blood* 113, 5134–5143. <http://dx.doi.org/10.1182/blood-2008-11-190769>.
- Dinges, D.F., Douglas, S.D., Zaugg, L., Campbell, D.E., McMann, J.M., Whitehouse, W.G., Orne, E.C., Kapoor, S.C., Icaza, E., Orne, M.T., 1994. Leukocytosis and natural killer cell function parallel neurobehavioral fatigue induced by 64 hours of sleep deprivation. *J. Clin. Invest.* 93, 1930–1939. <http://dx.doi.org/10.1172/JCI117184>.
- Fields, A., 2009. Discovering statistics using SPSS, 3rd ed. SAGE publications.
- Fondell, E., Axelsson, J., Franck, K., Ploner, A., Lekander, M., Balter, K., Gainers, H., 2011. Short natural sleep is associated with higher T cell and lower NK cell activities. *Brain Behav. Immun.* 25, 1367–1375. <http://dx.doi.org/10.1016/j.bbi.2001.04.004>.
- Gleeson, M., 2007. Immune function in sport and exercise. *J. Appl. Physiol.* 103, 693–699. <http://dx.doi.org/10.1152/japophysiol.00008.2007>.
- Gottlieb, D.J., Punjabi, N.M., Newman, A.B., Resnick, H.E., Redline, S., Baldwin, C.M., Nieto, F.J., 2005. Association of sleep time with diabetes mellitus and impaired glucose tolerance. *Arch. Intern. Med.* 165 (8), 863–867. <http://dx.doi.org/10.1001/archinte.165.8.863>.
- Irwin, M., Minge Wang, M.S.N., Capella, O., Campomayor, M.S., Collado-Hidalgo, A., Cole, S., 2006. Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. *Arch. Intern. Med.* 166, 1756–1762. <http://dx.doi.org/10.1001/archinte.166.16.1756>.
- Irwin, M., McClintick, J., Costlow, C., Fortner, M., White, J., Gillin, J.C., 1996. Partial night sleep deprivation reduces natural killer and cellular immune response in humans. *FASEB J.* 10 (5), 643–653.
- Johns, M.W., 1991. A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. *Sleep* 14 (6), 540–545.
- Kerhofs, M., Boudjeltia, K.Z., Stenuit, P., Brohee, D., Cauchie, P., Vanhaeverbeek, M., 2007. Sleep restriction increase blood neutrophils, total cholesterol and low density lipoprotein cholesterol in postmenopausal women: a preliminary study. *Maturitas* 56, 212–215. <http://dx.doi.org/10.1016/j.maturitas.2006.07.007>.
- Kruger, M., Lechtermann, A., Fobker, M., Volker, K., Mooren, F.C., 2008. Exercise induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. *Brain Behav. Immun.* 22, 324–338. <http://dx.doi.org/10.1016/j.bbi.2007.08.008>.
- Krüger, K., Mooren, F.C., 2007. T cell homing and exercise. *Exerc. Immunol. Rev.* 13, 37–54.
- Lanier, L.L., 2001. On guard – activating NK cell receptors. *Nat. Immunol.* 2, 23–27. <http://dx.doi.org/10.1038/83130>.
- Matsumoto, Y., Mishima, K., Satoh, K., Tozawa, T., Mishima, Y., Shimizu, T., Hishikawa, T., 2001. Total sleep deprivation induces acute and transient increases in NK-cell activity in healthy young volunteers. *Sleep* 24, 804–809.
- Meier-Ewert, H.K., Ridker, P.M., Rifai, N., Regan, M.M., Price, N.J., Dinges, D.F., Mullington, J.M., 2004. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. *J. Am. Coll. Cardiol.* 43 (4), 678–683. <http://dx.doi.org/10.1016/j.jacc.2003.07.050>.
- Mosendane, T., Mosendane, T., Raal, F.J., 2008. Shift work and its effects on the cardiovascular system. *Cardiovasc. J. Afr.* 19 (4), 210–215.
- Pérez de Heredia, F., Garaulet, M., Gómez-Martínez, S., Díaz, L.E., Wörnberg, J., Androuetos, O., Michels, N., Breidenassel, C., Cuenca-García, M., Huybrechts, I., Gottrand, F., Ferrari, M., Santaliestra-Pasías, A.M., Kafatos, A., Molnár, D., Sjöström, M., Widhalm, K., Moreno, L.A., Marcos, A., HELENA Study Group, 2014. Self-reported sleep duration, white blood cell counts and cytokine profiles in European adolescents: the HELENA study. *Sleep Med.* 15, 1251–1258. <http://dx.doi.org/10.1016/j.sleep.2014.04.010>.
- Ricardo, J.S., Cartner, L., Oliver, S.J., Laing, S.J., Walters, R., Blizon, J.L., Walsh, N.P., 2009. No effect of a 30-h period of sleep deprivation on leukocyte trafficking, neutrophil degranulation and saliva IgA responses to exercise. *Eur. J. Appl. Physiol.* 105, 499–504. <http://dx.doi.org/10.1007/s00421-008-0931-3>.
- Simpson, R.J., Florida-James, G.D., Cosgrove, C., Whyte, G.P., Macrae, S., Pircher, H., Guy, K., 2007. High-intensity exercise elicits the mobilization of senescent T lymphocytes into the peripheral blood compartment in human subjects. *J. Appl. Physiol.* 103, 396–401. <http://dx.doi.org/10.1152/japophysiol.00007.2007>.
- Simpson, R.J., Florida-James, G.D., Whyte, G.P., Guy, K., 2006. The effects of intensive, moderate and downhill treadmill running on human blood lymphocytes expressing the adhesion/activation molecules CD54 (ICAM-1), CD18 (beta2 integrin) and CD53. *Eur. J. Appl. Physiol.* 97, 109–121.

- 653 Terry, P.C., Lane, A.M., Lane, H.J., Keohane, L., 1999. Development and validation of a
654 mood measure for adolescents. *J. Sports Sci.* 117, 861–872. 664
- 655 Toft, P., Tonnesen, E., Svendsen, P., Rasmussen, J.W., 1992. Redistribution of
656 lymphocytes after cortisol administration. *APMIS* 100, 154–158. 665
- 657 Turner, J.E., Aldred, S., Witard, O.C., Drayson, M.T., Moss, P.M., Bosch, J.A., 2010.
658 Latent cytomegalovirus infection amplifies CD8 T-lymphocyte mobilisation and
659 egress in response to exercise. *Brain Behav. Immun.* 24, 1362–1370. [http://](http://dx.doi.org/10.1016/j.bbi.2010.07.239)
660 dx.doi.org/10.1016/j.bbi.2010.07.239. 667
- 661 van Leeuwen, W.M.A., Lehto, M., Karisola, P., Lindholm, H., Luukkonen, R., Sallinen,
662 M., Harma, M., Porkka-Heiskanen, T., Alenius, H., 2009. Sleep restriction
663 increases the risk of developing cardiovascular diseases by augmenting
664 proinflammatory responses through IL-17 and CRP. *PLoS One* 4 (2), 4589. <http://dx.doi.org/10.1371/journal.pone.0004589>. 665
- 666 Vgnontzas, A.N., Zoumakis, E., Bixler, E.O., Lin, H.M., Follett, H., Kales, A., Chrousos,
667 G.P., 2004. Adverse effects of modest sleep restriction on sleepiness,
668 performance and inflammatory cytokines. *J. Endocrinol. Metab.* 85 (5), 2119–
669 2126. <http://dx.doi.org/10.1210/jc.2003-031562>. 670
- 671 Walsh, N.P., Gleeson, M., Shephard, R.J., Woods, J.A., Bishop, N.C., Fleshner, M.,
672 Green, C., Pedersen, B.K., Hoffman-Goetz, L., Rogers, C.J., Northoff, H., Abbasi, A.,
673 Simon, P., 2011. Position statement. Part one: immune function and exercise.
674 *Exerc. Immunol. Rev.* 17, 6–63. 675

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