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Research Article

Comparative Effect of Daylight Restriction and Sleep Deprivation on the Immune and Oxidative Stress Response of Male Swiss Mice

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Abstract
Circadian rhythms modulate the body's immune system and subsequent response to infection. This study was designed to compare the effects of two circadian disruptors (daylight-restriction and sleep-deprivation) on the immune response in male Swiss mice. Mice were divided into group 1 (control; n=10), which were neither daylight-restricted nor sleep-deprived and groups 2, 3, 4 and 5 (n=20/group). 10-animals each from groups 2-5 were daylight-restricted for 12, 24, 48 and 72 hours respectively while the remaining animals per group were sleep-deprived for same time interval. Post-exposure, blood was collected into EDTA-coated bottles for haematology (white blood cell (WBC), neutrophils, lymphocyte, monocyte, eosinophils and platelet counts) and plain bottles for serum biochemistry (interferon-γ, superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA). Compared to control, daylight-restricted and sleep-deprived groups had reduced WBC (12-72 hours), neutrophils (12, 48 and 72 hours) and increased lymphocyte (12, 48 and 72 hours), eosinophil (72 hours) and MDA level (12-72 hours). Daylight-restricted groups exhibited reduced platelets and increased interferon-γ levels at 12-72 hours while sleep-deprived had reduced platelets and increased interferon-γ at 48 and 72 hours only. While SOD decreased in daylight-restricted at 12-72 hours, sleep-deprived had increased values at 12-48 hours, and lowered values at 72 hours. Daylight-restricted had increased GSH level at 12-24 hours and reduced at 48-72 hours while values in sleep-deprived groups were decreased from 12-72 hours respectively. This study suggests that the onset of anti-immune and oxidative stress effects of daylight-restriction is more sudden than that of sleep-deprivation in male Swiss mice.

Key Words: Daylight restriction, sleep deprivation, interferon-γ, immune system

INTRODUCTION

Circadian rhythms are daily oscillations in behavior and physiology that prepare organisms to better react to and anticipate changes in the environment that are consequences of the earth's rotation (Geiger *et al*., 2015). These rhythms are endogenously generated by a pacemaker-synchronizing center often referred to as the body's biological master clock located in the hypothalamic suprachiasmatic nuclei (SCN). They usually have a 24 hour periodicity and persist as long as environmental conditions such as light, temperature, posture, etc. are kept constant (Skene and Arendt, 2006).

Circadian rhythm is known to affect different aspects of body physiology but their roles as critical regulators of specific immune functions are just emerging (Scheiermann *et al*., 2013). Recently, Abele *et al*. (2019) noted that the activities of the circadian clock can greatly influence adaptive immune responses to invading pathogens and how efficiently they are processed them. Disruption in the activity of the biological clocks and resultant irregular circadian rhythms have been linked with various chronic health conditions such a sleep disorders (Zhu and Zee, 2012), obesity, diabetes (Sridhar and Sanjana, 2016), depression, bipolar disorder, seasonal affective disorder (Vadnie and McClung, 2017), heart attacks, atherosclerotic plaques (Zhang *et al*., 2019), susceptibility to infections and allergies (Pick *et al*., 2019). This suggests an intricate link between disruptions in circadian rhythms and impaired immune responses.

In humans, sleep/wake cycles and daylight represent the most important synchronizers of the bodies' circadian rhythms. Alteration in the exposure to daylight as seen following seasonal weather changes in duration of daytime and night-time, in miners and in underground train workers may alter circadian rhythms and result in impaired immune

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responses (Gonzalez *et al*., 2016). Sleep deprivation, a state of inadequate quantity and quality of sleep, has also been linked with alterations in the circadian rhythms resulting in weight loss or weight gain, impaired neural and cognitive functions and alterations in immune functions (Alhola and Polo-Kantola, 2007). Shift workers (Tamagawa *et al.*, 2007), students who study over-night (Ming *et al*., 2011) and air travellers (jet-lag syndrome resulting from changing time zones) often present with illness and sickness (Juda *et al*., 2013) suggesting impairment in homeostatic functions following sleep deprivation. While there is a preponderance of studies which show deleterious effects of sleep deprivation on the immune system, few studies have examined the effects of daylight restriction, i.e. limited exposure to daylight, on the immune system. This study therefore investigated and compared the effects of daylight restriction and sleep deprivation on immune response in male Swiss mice.

MATERIALS AND METHODS

Animals: Male Swiss mice (28 - 32g) were obtained from the Central Animal House, University of Ibadan, housed in well-aerated cages and exposed to natural alternating day and night cycles. They were acclimatized for 14 days in the animal house of the Department of Physiology prior to commencing experimental procedures. They were thereafter randomly assigned to control group (n=10), and groups 2, 3, 4 and 5 (n=20 respectively) and maintained on standard rat chow with free access to drinking water *ad libitum.* All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA).

Experimental Procedures: Animals in control group were neither daylight-restricted nor sleep-deprived. Ten animals each from groups 2 - 5 were taken from the animal house and kept in a dark room that was restricted from daylight for 12, 24, 48 and 72 hours respectively while the remaining 10 animals per group were sleep-deprived using the modified multiple platform method (Nunes and Tufik, 1994) for same time interval. The modified multiple sleep-deprivation platforms consisted of 20 cylindrical 14-cm high platforms each with a diameter of 3 cm, spaced 8 cm apart and fixed in a plastic cage 35.5 x 26.5 x 25 cm. The cage was filled with water up to 1 cm from the platform tops

Biochemical Analysis: At the end of each exposure period, blood samples were collected under light thiopentone anaesthesia from the retro orbital plexus into EDTA-lined and plain sample bottles. The EDTA-coated blood samples (n=5 per subgroup) were assayed for total and differential white blood cell (WBC), and platelet counts using standard laboratory procedures. Plain blood samples (n=5 per subgroup) were centrifuged at 3000 g for 10 min at 4◦C to obtain serum, which was subjected to interferon-γ (BioLegend ELISA Mouse IFN-γ assay kit), superoxide dismutase (SOD) (Misra and Fridovich, 1972), reduced glutathione (GSH) (Beutler *et al.*, 1963), and malondialdehyde (MDA) (Varshney and Kale, 1990) assays.

Statistical analysis

Results obtained are expressed as mean ± SEM. Statistical significance within and between experimental groups was taken at $P < 0.05$ using 2-Way ANOVA and Newman Keuls' post-hoc test.

RESULTS

Total and differential white blood cell counts in control experimental animals

The total white blood cell count in the daylight restricted group and sleep deprivation group were reduced ($P<0.05$) at 12, 24, 48 and 72 hours respectively compared to control (Figure 1). WBC increased ($P<0.05$) at 24 hours but reduced ($P<0.05$) at 48-hour in sleep-deprived group compared to daylight restricted (Figure 1).

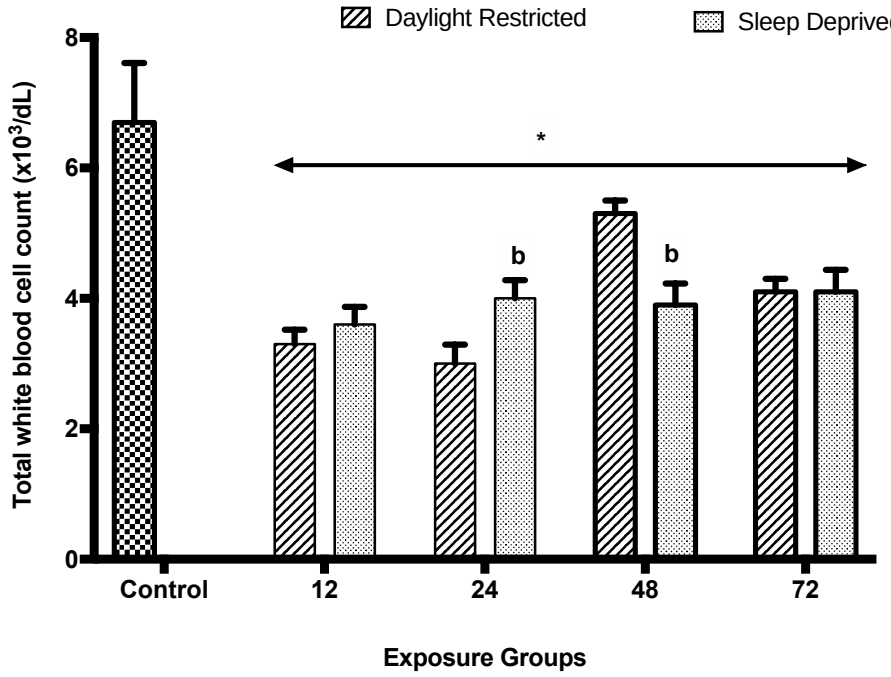

Figure 1.
Effect of Daylight restriction and Sleep Deprivation on Total White Blood Cell count in male Swiss mice. Values are Mean ± SEM, * indicates values that are significantly different from control. b indicates significant differences between sleep deprived and corresponding daylight restricted groups

Table 1.
Differential white blood cell counts in control and experimental groups

	Neutrophil (%)		Lymphocyte (%)		Eosinophil (%)		Monocyte (%)	
	DL	SD	DL	SD	DL	SD	DL	SD
Control	35.8 ± 2.78		61.2 ± 2.39		1.6 ± 0.46		1.4 ±0.22	
12 hours	26.8 ±0.77*	30.4 ±3.28	69.6 ±0.61*	66.2 ±3.04	2.2 ±0.52	1.4 ±0.36	1.4 ±0.22	2.0 ±0.28
24 hours	36.0 ±1.74	28.4 ±1.00*	60.6 0.61	68.4 ±1.04*	2.0 ±0.49	1.8 ±0.52	1.4 ±0.22	1.4 ±0.22
48 hours	24.4 ±1.46*	32.2 ±0.77	72.2 ±1.51*	64.4 ±1.08	1.8 ±0.52	1.6 ±0.46	1.6 ±0.36	1.8 ±0.33
72 hours	26.2 ±1.95*	26.0 ±0.22*	69.8 ±1.78*	70.0 ±1.36*	2.4 ±0.36*	2.4 ±0.22*	1.6 ±0.21	1.6 ±0.36

N=10, values are Mean ± SEM,
** indicate values that are significantly different from control.*
DL = Daylight-restricted;
SD = Sleep-deprived

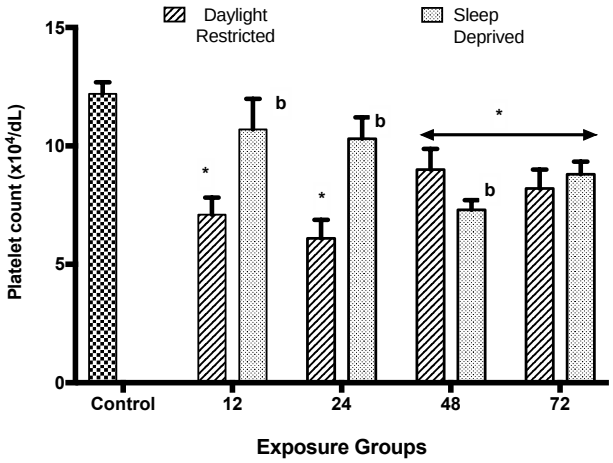

Figure 2.
Effect of Daylight restriction and Sleep Deprivation on Platelet count in male Swiss mice. N=10, values are Mean ± SEM, *indicate values that are significantly different from control. b indicates significant differences between sleep deprived and corresponding daylight restricted groups

Neutrophils reduced at 12, 48 and 72 hours respectively in daylight restricted group while only neutrophils reduced in sleep deprived group at 24 and 72 hours respectively compared to control. Compared to control (61.2 ± 2.39%), lymphocyte count in daylight restricted group were elevated at 12 (69.6±0.61%), 48 (72.2 ± 1.51%) and 72 (69.8 ± 1.78%) hours post exposure while sleep-deprived groups showed elevated values only at 24 (68.4 ± 1.04%) and 72 (70.0 ± 1.36%) hours respectively. Eosinophil count in the daylight restricted (2.4 ± 0.36%) and sleep deprived group (2.4 ± 0.22%) were both elevated only at 72 hours post exposure compared to control (1.6 ± 0.46%), while monocyte count did not differ significantly between control and all other experimental groups (Table 1).

Platelet counts in control and experimental animals
Platelet counts showed significant reductions in daylight restricted group at 12, 24, 48 and 72 hours while values obtained in the sleep deprived group reduced only at 48 and 72 hours post exposure respectively compared to control. When compared to sleep deprived, daylight restricted group exhibited a 50.7% and 68.9% increase at 12 and 24 hours while values obtained at 48 hours was 23.3% reduced (Figure 2).

Interferon-γ level in control and experimental animals
Interferon-γ level in daylight-restricted group was elevated at 12, 24, 48 and 72 hours (751.8 ± 31.8, 745.4 ± 33.8, 890 ± 31.0 & 773.4 ± 53.3 pg/mL), respectively compared to control (NR) (452.3 ± 43.6 pg/mL). In sleep-deprived group, Interferon-γ significantly reduced at 24 hours (310 ± 31.4 pg/mL) and elevated at 48 and 72 hours (1078 ± 133 & 909 ± 105.1 pg/mL) respectively compared to control (452.3 ± 43.6 pg/mL) (Figure 3). Compared to daylight-restricted, values obtained in the sleep deprivation group were only increased (P<0.05) at 12 and 24 hours respectively.

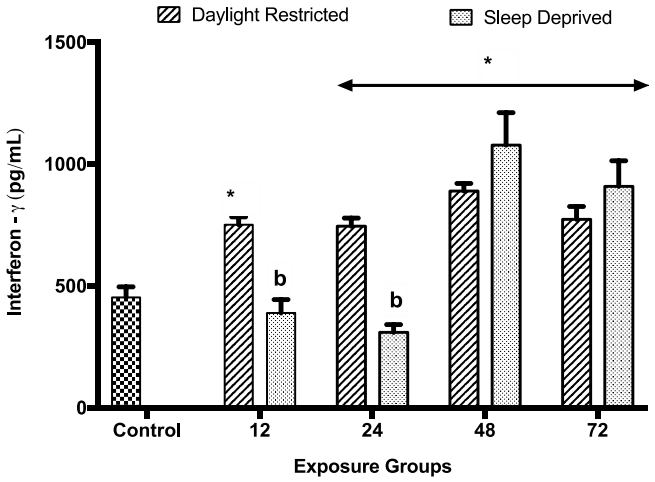

Figure 3.
Effect of Daylight restriction and Sleep Deprivation on Interferon-γ in male Swiss mice. N=10 Values are Mean ± SEM, * indicate values that are significantly different from control. b indicates significant differences between sleep deprived and corresponding daylight restricted groups

Oxidative stress status in control and experimental animals
Superoxide dismutase (SOD) levels in the daylight restricted group reduced significantly (P<0.05) at 12, 24, 48 and 72 hours (1.38±0.38, 0.82±0.16, 0.74±0.17 & 0.82±0.15 U/mg protein), respectively post exposure compared to control (2.12±0.31 pg/mL). In sleep-deprived group, SOD increased

(P<0.05) at 12, 24 and 48 hours (2.86±0.07, 2.74±0.08, 4.92±0.13 pg/mL), respectively but reduced at 72 (1.26±0.08 pg/mL) hour post-exposure compared to control. Compared to daylight-restricted groups, SOD values in the sleep deprivation groups increased (P<0.05) at 12-72 hours post-exposure, respectively (Figure 4).

Reduced glutathione level in daylight-restricted group increased at 12 and 24 hours but reduced at 48 and 72 hours compared to control. Values obtained in the sleep-deprived groups reduced (P<0.05) at 12 -72 hours post exposure compared to control (Figure 5). Compared to daylight restricted at 12 and 24 hours, values obtained in the corresponding sleep derivation groups were significantly (P<0.05) increased (Figure 5).

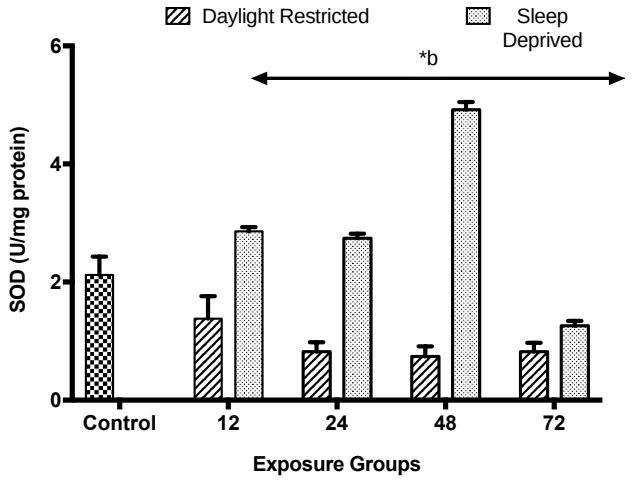

Figure 4.
Effect of Daylight restriction and Sleep Deprivation on Superoxide dismutase (SOD) activity in male Swiss mice**.** N=10, values are Mean ± SEM, * indicate values that are significantly different from control. b indicates significant differences between sleep deprived and corresponding daylight restricted groups

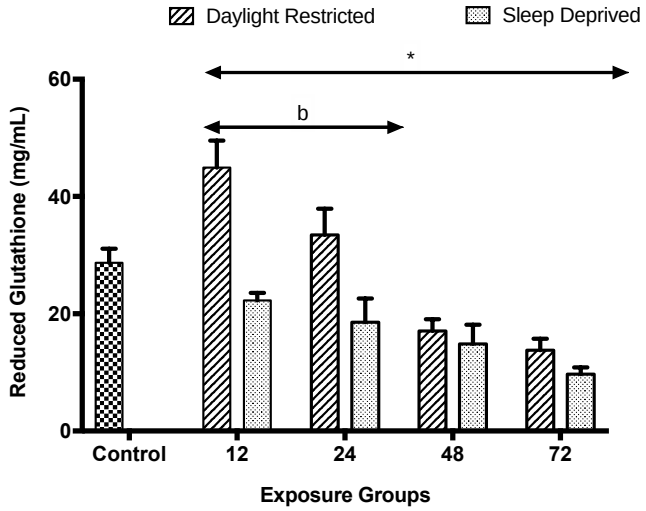

Figure 5.
Effect of Daylight restriction and Sleep Deprivation on Reduced Glutathione in male Swiss mice. N+??, values are Mean ± SEM, * indicate values that are significantly different from control (NR). b indicates significant differences between sleep deprived and corresponding daylight restricted groups

Lipid peroxidation, which was assessed via malondialdehyde (MDA) levels, was increased in both the daylight restricted (2.73±0.82, 3.62±0.99, 3.67±0.97, 3.14±0.78 mmol/pg protein) and sleep deprived (3.07±0.44, 3.65±1.31, 2.68±0.18, 2.76±0.34 mmol/pg protein) groups at 12 – 72 hours post-exposure compared to control (1.77±0.23 mmol/pg protein) (Figure 6).

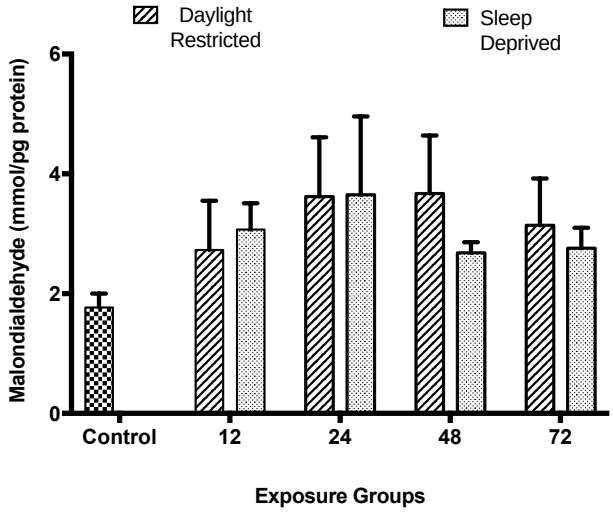

Figure 6.
Effect of Daylight restriction and Sleep Deprivation on Malondialdehyde level in male Swiss mice.
Values are Mean ± SEM

DISCUSSION

The impact of circadian rhythms on body physiology help living organisms adapt favourably to the changing environmental and respond effectively to the daily variations in light, temperature, or food availability (Yerushalmi and Green, 2009). The circadian control of immune functions allows organisms present the most appropriate response to the changing environment they live in. As a result of endogenous circadian (~24-h) rhythms in physiology and behaviour, organisms and cells are able to anticipate and respond effectively to the daily variations in light, temperature, or food availability experienced. These daily environmental variations may affect the risk of infection (Yerushalmi and Green, 2009). Hence, the circadian control of immune functions allows organisms present a most response when it is required. This study shows reductions in total white blood cell count following either daylight restriction or sleep deprivation for 12 – 72 hours (Fig. 1) suggesting that exposure to these stressors may lead to circadian rhythm disruption of the immune system that can result in a suppressed immune response and a likely impairment in the ability to fight invasion by foreign pathogens.

White blood cells (leukocytes) have been reported to possess a circadian rhythm with respect to their production and release into the blood stream. This circadian control has been suggested to be controlled by a synergy of impulses from the master clock, the supra chiasmic nucleus (SCN) and local clock molecules that reside within leukocytes (see Labrecque and Cermakian, 2015; Pritchett and Reddy, 2015 for reviews). These studies have shown that disruption of the circadian clock leads to dysregulation of immune responses, which underlie the pathophysiological basis of disease (Comas *et al.*, 2017). This may therefore account for the reduction in total WBC counts observed in the groups restricted from daylight. These light restricted groups may have had altered synchrony in SCN mediated and local clock gene control of WBC

activities that resulted in immune-suppression. Furthermore, reduced vitamin D levels have been observed in conditions of limited exposure to sunlight and or prolonged darkness (Palacios *et al*., 2016). Vitamin D has been reported to suppress B and T lymphocyte proliferation, decrease production of inflammatory cytokines and increases the production of anti-inflammatory cytokine (Aranow, 2011). Its reduction has been associated with immunosuppression and activation of proinflammatory processes (Aranow, 2011). Hence, the reduced total WBC count observed in the daylight-restricted groups may also be as a result of reductions in vitamin D level following exposure to prolonged darkness.

Reductions in neutrophil and an increase in lymphocyte count at 12, 48 and 72 hours of daylight restriction was also observed which suggests an immediate suppression of the phagocytic activity of neutrophils and likely stimulation of lymphocyte mediated inflammatory processes at 12 hours post exposure. A compensatory recovery in phagocytic and reduction in inflammation activity may also have occurred at 24 hours, as values obtained were not significantly different from controls. However, it is likely that following increased daylight restriction and hence increased darkness, further disruption of leukocyte circadian rhythm and reduction in serum vitamin D may have led to a sustained suppression of the neutrophil phagocytic and stimulation of inflammatory activity at 48 and 72 hours respectively. Platelet count was reduced while interferon gamma (IFN-γ) activity was increased after daylight-restriction of 12-72 hours, which further supports the likely presence of immunosuppression, infection, impaired blood-clotting ability, and activation of immune mediated inflammatory processes. Interferon gamma (IFN-γ) is a major proinflammatory cytokine that is important to both innate and adaptive immunity. It has been reported to facilitate leukocyte attraction and direct the growth, maturation and differentiation of many cell types, in addition to enhancing natural killer (NK) cell activity and regulating B cell function (Boehm *et al.*, 1997, Schroder *et al.*, 2003). An increase in the blood level of this cytokine is often associated with presence of infection and inflammation (Schroder *et al.*, 2003).

Sleep deprivation has been reported to be a risk factor that contributes to several diseases processes resulting in behavioral and hormonal alterations that can alter the immune response and compromise host defense mechanisms (Asif *et al.*, 2017). It has been reported to activate the innate immune system resulting in the increased serum levels of C-reactive peptide, cortisol as well as increased expressions of inflammatory cytokines and antimicrobial peptides in the periphery (Besedovsky *et al.*, 2019). This may likely account for the reduction on total white blood cell count observed in the sleep deprived animals and suggest an increased susceptibility to infection in this group. Neutrophil was reduced while lymphocytes were elevated only at 24 and 72 hours of sleep deprivation suggesting delayed immunosuppression, which began only after 24 hours of sleep deprivation. At 48 hours of sleep deprivation, there was a rebound in phagocytic and reduction lymphatic activity while at 72 hours post exposure, phagocytic ability became depressed and was accompanied by an increase in lymphatic and likely inflammatory processes. Sustained reductions in platelets and elevations in IFN-γ activity at 12-72 hours sleep-deprivation was also observed which further supports the likely presence of immunosuppression, infection and inflammation in this group.

In both daylight-restricted and sleep deprived groups eosinophil increased only at 72 hours post exposure, which further suggests the likely presence of increased parasitic host invasion, allergies and inflammation following exposure to these two stressors. However, the basophil counts were unchanged suggesting that the ability of the immune system to remove dead or damaged tissues, destroy cancer cells, may not compromised following either daylight-restriction or sleep deprivation for 12-72 hours respectively.

Altering environmental variables, such as light, temperature, pressure, and behaviours such as starvation and sleep deprivation, have being reported to induce stress, particularly oxidative stress, and increased inflammatory processes in the body (Aseervatham *et al.*, 2013). Vitamin D is one of the key controllers of systemic inflammation, oxidative stress and mitochondrial respiratory function, and thus, the aging process in humans (Wimalawansa, 2019). A deficiency of vitamin D, as would occur following daylight restriction (Palacios *et al.*, 2016), has been reported to result in oxidative stress, immune dysfunction, hyperparathyroidism, inflammation, thrombosis, vascular smooth muscle proliferation and hypertension (Mozos and Marginean, 2015). It is therefore not unlikely that in the daylight-restricted group, serum depletion of vitamin D may have led to increased oxidative stress, as evidenced by increased levels of malondialdehye, a marker of lipid peroxidation. Superoxide dismutase (SOD) is a first line defence antioxidant (Ighodaro and Akinloye, 2018). It is a detoxification enzyme and is said to be the most powerful antioxidant in the cell (Ighodaro and Akinloye, 2018). It is an important endogenous antioxidant enzyme that catalyzes the dismutation of superoxide anion to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Its steady depletion following daylight restriction at 12 hours - 72 hours as observed in this study suggests rapid overwhe lming of first line SOD-antioxidant mechanisms by oxidative stress. Reduced glutathione, a second line antioxidant defence mechanism that scavenges free radicals (Ighodaro and Akinloye, 2018), was however potentiated at 12 and 24 hours exposure to daylight restriction suggesting an initial potentiation of scavenging activity. However GSH values obtained following daylight restriction at 48 and 72 hours suggest a compromise in the ability of GSH to scavenge free radicals and hence lead to an eventual decline in the activity of both first and second line antioxidant defence mechanisms.

Sleep can be described as an active state characterized by reduced alertness and responsiveness that is rapidly reversible (Rasch and Born, 2013). It represents a state with an increased antioxidant activity, which promotes brain protection against free radicals via attenuation in oxidant production (Reimund, 1994; Villafuerte *et al.*, 2015). Sleep deprivation has been reported to represent an oxidative challenge for the brain, as it requires high neuronal metabolism to maintain neuronal electrical potentials. This requires a great amount of oxygen, resulting in a significant production of oxidants (Villafuerte *et al.*, 2015). Increased oxidant production may therefore be responsible for the increased serum malondialdehyde level observed in the sleep-deprived groups at 12 -72 hours post exposure. It is also likely that in the sleep-deprivation group, the production of superoxide radicals may not be as rapid as that for daylight restriction, hence the initial potentiation of

SOD from 12 - 48 hours which might be a response to slow but steady production of superoxide radicals. However, values obtained at 72 hours post exposure to sleep deprivation suggest increased oxidant production and oxidative stress leading to a suppression of SOD activity and hence first line antioxidant defence mechanisms. This observation is further supported by the steady decline in reduced glutathione in the sleep-deprived group from 12 – 72 hours, which suggests an overwhelming of the ability of reduced glutathione to scavenge the free radicals being produced.

In conclusion, this study suggests that daylight restriction and sleep deprivation for 12 - 72 hours affects the immune and the oxidant-antioxidant balance systems and thus might predisposes to infection and oxidative stress. The onset of anti-immune and oxidative stress effects of daylight restriction appears to be more sudden than that of sleep deprivation in male Swiss mice

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