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IMPACT OF REM SLEEP DEPRIVATION ON ENDOTHELIAL FUNCTION IN RAT MODEL: ROLE OF VITAMIN C SUPPLEMENTATION

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Abstract: Sleep deprivation increased risk for cardiovascular disease. This study aims to evaluate the effect of rapid eye movement (REM) sleep deprivation on the endothelium. The beneficial effect of vitamin C was also evaluated. Forty adult male Sprague–Dawley rats were equally divided into 5 groups**:** free-moving control (FMC), 72-h REM sleep-deprived rats (REMsd), tank control (TC), REMsd pre-treated with vitamin C (RVC) and FMC pre-treated with vitamin C (FVC). After 72 hours of adaptation, the experiments were carried out according to their respective paradigms for 72 hours. Rats were deprived of REM sleep using the inverted flowerpot technique. Endothelial function was assessed by myographic study, eNOS protein expression was determined by Western blotting while oxidative stress parameters were measured in the plasma using ELISA technique. Vitamin C was administered daily for 4 weeks (100 mg/kg/day) before the experiment. Impairment of endothelium-dependent relaxation and low levels of eNOS protein expression were observed in REMsd rats. Levels of glutathione reductase, superoxide dismutase (SOD) activities and total antioxidant capacity were significantly lower in REMsd rats. Vitamin C preserved the endothelial function, restored the eNOS protein expression and increased SOD activity. Thus, REM sleep deprivation is associated with endothelial dysfunction that improved with supplementation of vitamin C.

Keywords: REM sleep deprivation, endothelial dysfunction, oxidative stress.

Introduction

Good quality of sleep is crucial for human health and well-being**,** regardless of age and gender. Sleep is vital for conserving energy, cell functioning and increasing brain protein synthesis (Siran *et al*., 2014). Sleep deprivation has become an emerging public health issue globally (Chattu *et al*., 2019), thus sleep deprivation has attracted the interest of many researchers for many years. There is growing evidence that sleep deprivation is associated with cardiovascular related diseases such as hypertension (Vgontzas *et al*., 2009), atherosclerosis (May & Harrison, 2013) and diabetes (Xu *et al*., 2016). Oxidative stress plays a major role in the initiation and progression of cardiovascular diseases, such as hypertension, coronary artery disease, chronic heart failure and peripheral artery disease (Gopalakrishnan *et al*., 2004). It is noteworthy that sleep deprivation enhances generation of free radicals (Mahmoudi *et al*., 2017), and sleep removes free radicals or reactive oxygen species (ROS) that are produced during wakefulness (Reimund, 1994). A significant imbalance of oxidant-antioxidant levels has been demonstrated in the hippocampus (Alzoubi *et al*., 2012), hypothalamus and thalamus (D'Almeida *et al*., 1998), and brainstem of sleep-deprived rats (Rajendran *et al*., 2013). Thus, sleep has a protective role against oxidative stress.

Researchers have invested much effort over the years to determine the adverse effects of REM sleep deprivation on health, as this phase serves many vital physiological functions. The characteristics of REM sleep include vivid dreams, loss of muscle tone, increased brain metabolism and memory consolidation (Wiesner *et al*., 2015). Effects of REM sleep deprivation on memory (Wiesner *et al*., 2015), pain-related gene expression (Siran *et al*., 2014), behaviour (Hanlon *et al*., 2005) and

lipid peroxidation (Thamaraiselvi *et al*., 2012) have been reported previously. Extensive research has been done on the potential role of antioxidants in repairing and preventing damages due to oxidative stress. Vitamin C is an antioxidant that protects the blood vessels (May *et al*., 2005). It has also been reported to have anti-carcinogenic, immunomodulation and anti-atherogenic (Pham-Huy *et al*., 2008). Hence, many researchers have focused on the vitamin C research in the context of its importance to human health and disease prevention.

The endothelium produces numerous factors that regulate vascular tone, smooth muscle cell proliferation, cellular adhesion and vessel wall inflammation (Pham-Huy *et al*., 2008). Endothelial dysfunction is widely accepted as the early changes in the pathophysiology of cardiovascular disease (Jiang *et al*., 2017). Various mechanisms have been implicated in endothelial dysfunction, including oxidative stress (Abas *et al*., 2015; Di Meo *et al*., 2016), decreased nitric oxide bioavailability (Jiang *et al*., 2017), down-regulation of endothelial nitric oxide synthase (Suganya *et al*., 2016) and inflammation (Kearney *et al*., 2017). Although the link between sleep deprivation and endothelial dysfunction is being increasingly recognised, data on REM sleep deprivation-induced endothelial dysfunction is very limited. Therefore, this study was carried out to determine the protective influence of vitamin C against the adverse effects of REM sleep deprivation by evaluating the functional changes of the endothelium and its eNOS protein expression, as well as the plasma levels of oxidative stress markers, such as GR, SOD, TAC and MDA.

Materials and Methods

Animals

Forty Sprague-Dawley (SD) male rats weighing between 200–250 g and aged 8–10 weeks were used in this study. This study was approved by the Animal Ethics Committee Universiti Sains Malaysia (Ref USM / Animal Ethics Approval / 2013/ (89) (491)) and in accordance with the institutional guidelines. Rats were placed in polypropylene solid-floor cages and maintained in a room with 12:12-h light:dark cycle and controlled environment (25ºC temperature, 60–70% humidity). Food and water were provided *ad libitum*.

Chemicals

Vitamin C was purchased from HmbG® Chemicals, Malaysia.

Experimental Protocol

Rats were randomly divided into five equal groups. Group 1 is free-moving control rats (FMC) that were kept in polypropylene/normal dry lightproof cages. This group was used for evaluating the baseline values for the various parameters that were measured in this study. Group 2 is REM sleep-deprived rats for 72 hours (REMsd). Group 3 is tank control rats (TC). Group 4 is REM sleep-deprived rats for 72 hours that were pre-treated with vitamin C (RVC). Group 5 is free-moving control rats pre-treated with vitamin C (FVC). In the vitamin C groups, i.e. groups 4 and 5, rats were given daily vitamin C by oral gavage (100 mg/kg body weight/day) for 4 weeks before the experiment (Ergul *et al*., 2010).

Adaptation Period

To familiarise with the tank environment, rats from groups REMsd, TC and RVC were isolated and adapted individually in a dry tank model (without water) for 72 hours. During this period, the other groups were kept in polypropylene/normal dry lightproof cages for 72 hours.

REM Sleep Deprivation Model

The REM sleep deprivation model was carried out by modification of the inverted flowerpot technique (Siran *et al*., 2014). Two small platforms**,** of 6.5 cm in diameter each**,** were placed in the middle of a glass tank immersed in water. The height of the platform is 1 cm above a pool of water, and only one rat was placed in a tank at one time. The glass tank measured 30 cm in height, 30 cm in width and 60 cm in length, and the top was covered by wire mesh. This model is based on the concept that during

REM sleep, animals lose their muscle tone that causes the rats to fall off the platform into the water and wake up. Thus, this will prevent the development of the REM sleep component of the sleep cycle. Food and water were provided *ad libitum* by placing pellets and water bottles on a grid located on top of the chamber. It was shown to selectively deprive 90–99% of REM sleep in rats (May *et al*., 2005).

Tank Control

For TC group, a similar model to REM sleep deprivation was used except that the diameter of the platform**,** at 14 cm**,** was larger (Siran *et al*., 2014). The wide platform allowed the rats to sleep without falling into the water. The purpose of this control group was to expose them to the same aquatic environment as the REMsd but rats experienced both NREM and REM sleep. Food and water were placed similarly to the REM sleep deprivation model.

Free-moving Control Rats Pre-treated with Vitamin C

The free-moving control rats**,** pre-treated with vitamin C (FVC) group**,** served as a control to REM sleep-deprived rats for 72 hours that were pre-treated with vitamin C (RVC). In addition, the rats were also placed in a typical rat cage environment as FMC. Food and water are allowed *ad libitum* as FMC.

In Vitro Functional Study of Endothelium

Briefly, descending thoracic aortic rings were mounted horizontally between parallel hooks in tissue chambers filled with Krebs Hanseliet buffered solution (in mM: 118.4 NaCl, 4.7 KCl, 2.5 CaCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25.0 $NaHCO_4$ and 11.1 glucose). Tissue chamber solution was constantly aerated with carbogen (95% O_2, 5% CO_2) at 37°C. Rings were originally loaded with 1.50 g tension (basal tension) through incremental application for half an hour, followed by equilibration for another 30 minutes before the studies began (Zhang *et al*., 2012). Changes in isometric force were recorded by using LabChart programme (version 7.0) and a PowerLab data acquisition system (ADInstruments Ltd, Oxfordshire, UK). Rings were then exposed twice to KCl (40 mM) for viability assessment. Rings that gave contractile responses of more than 50% maximum contraction by KCl were considered for the following steps. Following viability assessment, rings were pre-contracted with phenylephrine (PE, 1 μM) (Lee *et al*., 2014). Once the tension plateau to PE developed, series of cumulative acetylcholine (ACh), 10^{-9}, 10^{-8} and 10^{-7} M, were added consecutively to evaluate endothelium-dependent versus endothelium-independent vasodilation (Furchgott, 1981). Vasorelaxation to cumulative dose of ACh was measured as: (tension PE – tension ACh) / tension PE × 100% (the percentage change in reactivity to dosage ACh after 1 μM PE). These arterial rings were exposed twice with cumulative concentration of ACh interspersed by washing, equilibration and re-exposure to PE.

Western Blot Analysis

Femoral arteries were homogenised in lysis buffer composed of Thermo Scientific RIPA buffer and Protease Inhibitor Cocktail (Promega Corporation, USA) using mortar pestle on ice at room temperature. Then, the homogenised sample was collected and centrifuged at 20,000 × *g* for 20 minutes at 4 °C. The resulting supernatant was then collected. Protein determination was done using Pierce™ BCA Protein Assay Kit (Thermo Scientific). For Western blot, equal amounts of protein lysate were separated using 10% sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gel electrophoresis. The gels were blotted onto a polyvinylidene difluoride (PVDF) membrane and incubated overnight with the primary antibody eNOS monoclonal antibody (Thermo Fisher Scientific) at 4 °C, followed by incubation with goat anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology). As for the loading control, the housekeeping protein, primary antibody to β-actin (Santa Cruz Biotechnology), was used with anti-mouse IgG kappa horseradish peroxidase-conjugated (Santa Cruz Biotechnology) secondary antibody. Bands were visualised by the enhanced chemiluminescence

method (Bio-Rad Laboratories, Inc., USA). Images of the bands were taken using an image analyser. Band intensities were analysed and compared using Image J software. Band for each eNOS protein was normalised as a ratio to the endogenous control (housekeeping protein, β-actin protein). The ratio between them was used to quantify eNOS protein level.

Measurement of Oxidative Stress Markers in Plasma

Oxidative stress markers in the plasma were measured by enzyme-linked immunosorbent assay (ELISA). Blood samples were collected into anticoagulant-treated tubes (ethylenediaminetetraacetic acid (EDTA)). Blood was left at room temperature for 20 minutes, then**,** it was centrifuged for 15 minutes at 3,000 rpm at 4 °C. The plasma obtained was used to measure oxidative stress markers. The activities of superoxide dismutase (SOD) and glutathione reductase (GR) were measured using EnzyChrom™ SOD Assay kit and EnzyChrom™ GR Kit (Bioassay Systems, California, USA), respectively. Levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured using MDA Assay Kit (North West Life Sciences, USA) and TAC assay kit (Bioassay Systems, California, USA), respectively.

Statistical Analysis

The data were analysed using one-way analysis of variance (ANOVA) with Bonferroni's post-hoc test. The level of significance was set at $p<0.05$, and the results were expressed as the mean ± SEM. Data analysis and graphs were plotted using GraphPad Prism version 6.01 software (GraphPad, San Diego, CA).

Results and Discussion

Effects of REM Sleep Deprivation on Food Consumption and Body Weight Gain

To evaluate the effects of selected REM sleep deprivation protocol on the physical changes, we measured food consumption (Fc) (Figure 1) and body weight gain (BWg) (Figure 2). The Fc was calculated as the difference in the food intake normalised as grams per day per kilogram of body weight taken to the power of 0.67 (g/day per $kg^{0.67}$). There was no significant difference in Fc between the groups during adaptation. Meanwhile, Fc was significantly higher in the REMsd group compared to FMC, TC and FVC during the experiment. In addition, during adaptation, there was no significant difference in BWg between the groups. In contrast, REMsd rats showed a significant reduction in BWg during the experiment compared to FMC, TC and FVC. The results of Fc and BWg in TC proposed that the source of stress on the rat was from REM sleep deprivation instead of the aquatic environment itself.

The occurrence of REM sleep deprivation using the inverted flowerpot technique is clearly demonstrated in the present study. Despite increased food consumption, the body weight gain was significantly reduced in the REMsd group compared to other groups. The findings were consistent with several previous studies (Koban & Swinson, 2005; Koban & Stewart, 2006; Koban *et al*., 2008; Siran *et al*., 2014). Different durations of REM sleep deprivation have been performed previously, including for 24 hours (Thamaraiselvi *et al*., 2012), 48 hours (Thamaraiselvi *et al*., 2012), 72 hours (Siran *et al*., 2014), 96 hours (Koban & Swinson, 2005), 5 days (Hanlon *et al*., 2005) and 10 days (Koban *et al*., 2008). In addition, different REM sleep paradigms have been used earlier, including single platform (Lima *et al*., 2014), multiple platform (Lima *et al*., 2014) and disk-over-water (Gulyani *et al*., 2000). Despite the differences in the duration and paradigms, the findings were similar, whereby they produced a characteristic feature consisting of an increase in food consumption but a decrease in body weight gain. Hyperphagia with body weight loss in the present study indicates a state of negative balance. Negative balance occurs when the expenditure is more than the calorie intake. It can be due to increases in resting metabolic rate in REM sleep-deprived rats (Koban & Swinson, 2005) or increases in energy expenditure (Koban & Swinson, 2005; Hipolide *et al*., 2006).

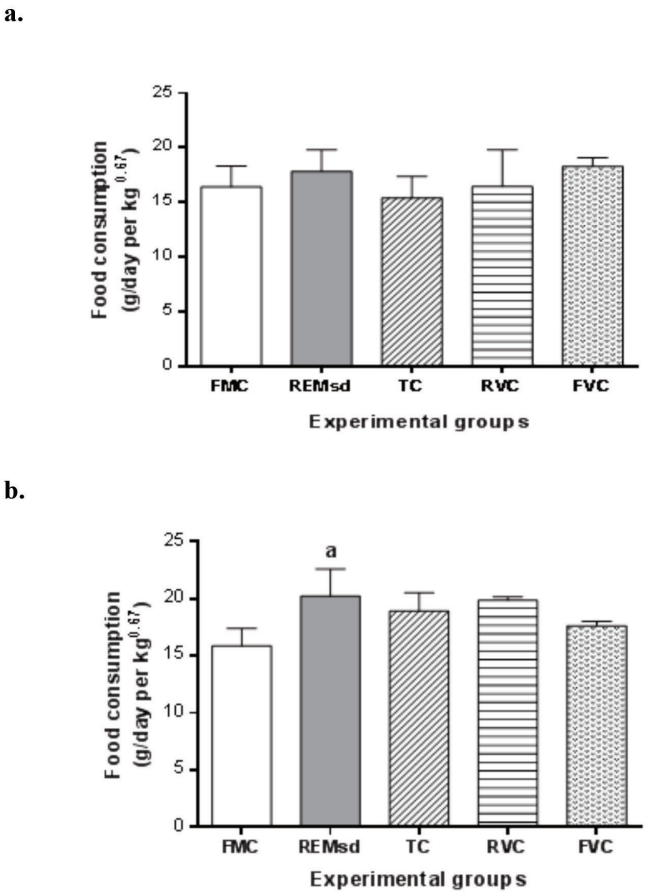

Figure 1: Effects of REM sleep deprivation on food consumption (g/day/kg$^{0.67}$) in all groups during adaptation (a) and experiment (b). FMC, free-moving control rats; REMsd, 72-h REM sleep-deprived rats; TC, tank control rats; RVC, REMsd pre-treated with vitamin C; FVC, FMC pre-treated with vitamin C. $^{a}p<0.05$ compared to FMC

Effects of 72-h REM Sleep Deprivation on Endothelial Function

We used *in vitro* functional vessel study (myographic study) to assess endothelium-dependent relaxation. The aortic ring was pre-contracted with phenylephrine (PE) and subsequently exposed to an endothelium-dependent relaxant, i.e. acetylcholine (ACh). The ability of the pre-contracted aortic ring to relax in response to ACh will determine whether its endothelial function is preserved. Contractile responses were measured as decreases in tension (g) after the aortic ring was pre-contracted with 1 µM of PE. This is expressed as percentage (%) reduction in contraction when exposed to serial concentrations of ACh. ACh at 10^{-7} M significantly reduced the percentage (%) reduction in contraction of REMsd rat compared to FMC, FVC and RVC (Figure 3). Thus, REMsd rats showed the lowest endothelium-dependent vasodilator responses to ACh.

In this study, REM sleep deprivation is associated functional changes in the endothelium. The endothelium is a crucial regulator of vascular physiology (Versari *et al*., 2009) that maintains vascular homeostasis (Wang *et al*., 2015; Incalza *et al.,* 2017). NO is the most significant endothelium derived- relaxing factor (EDRF) (Roberts & Porter, 2013). eNOS was measured in this study as it is essential for the biosynthesis

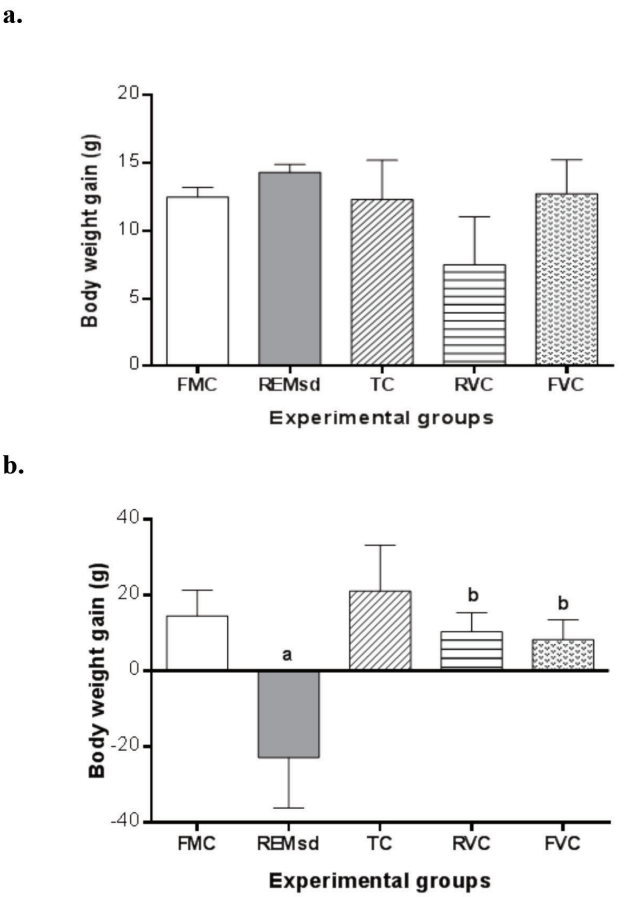

Figure 2: Effects of REM sleep deprivation on body weight gain in all groups during adaptation (a) and experiment (b). FMC, free-moving control rats; REMsd, 72-h REM sleep-deprived rats; TC, tank control rats; RVC, REMsd pre-treated with vitamin C; FVC, FMC pre-treated with vitamin C. [a]$p<0.001$ compared to FMC and TC; [b]$p<0.01$ compared to REMsd

of NO endogenously from L-arginine (Wang *et al*., 2015). In *in vitro* functional study, a pre-contracted aortic ring was subjected to an endothelium-dependent relaxant agent, i.e. acetylcholine (ACh). An aortic ring with a healthy endothelium is able to relax in response to ACh. In this study, the percentage (%) reduction in contraction of aortic ring from the REMsd group was significantly lower compared to FMC, FVC and RVC. The results suggest that REM sleep deprivation is associated with impaired function of the endothelium. A healthy endothelium produces NO at optimal levels. NO spreads through the cell membrane to the underlying muscle cells and causes the arteries to dilate (Srivastava & Singh, 2014). Regulation of vascular tone is affected by the availability of NO (Tanabe *et al*., 2003). This is because NO acts as a vasodilator via relaxation of vascular smooth muscle cells.

Effects of 72-h REM Sleep Deprivation on eNOS Protein Expression

We determined the expression of protein eNOS, a key regulator of nitric oxide (NO) production, in femoral arteries by Western blot analysis. Our results revealed a decreased expression of eNOS protein in REMsd compared to FMC, TC and FVC groups (Figure 4). Pre-treatment with vitamin C (RVC) increased the level of eNOS significantly compared to REMsd.

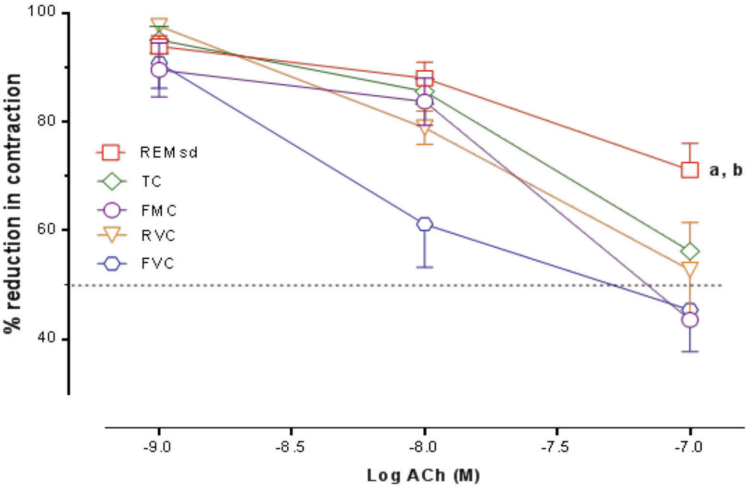

Figure 3: Effects of REM sleep deprivation on ACh-mediated relaxation of the descending thoracic aortic rings from all groups and the results are expressed as percentage (%) reduction in contraction when the aortic rings are exposed to serial concentrations of ACh. FMC, free-moving control rats; REMsd, 72-h REM sleep-deprived rats; TC, tank control rats; RVC, REMsd pre-treated with vitamin C; FVC, FMC pre-treated with vitamin C. [a]$p<0.05$ compared to FMC and FVC; [b]$p<0.01$ compared to RVC

A significant decrease in the eNOS protein expression by the endothelial cells of REMsd rats could be responsible for the impaired endothelium-dependent relaxation in the *in vitro* functional study. A reduction in NO bioavailability is the primary cause of endothelial dysfunction (Kolluru *et al*., 2012), and decreased eNOS expression is one of the factors (Coco & de Oliveira, 2015). Thus, there is an association between REM sleep deprivation and endothelial dysfunction, as evidenced by a reduction in the ability of the endothelium to vasodilate in the presence of ACh stimulation.

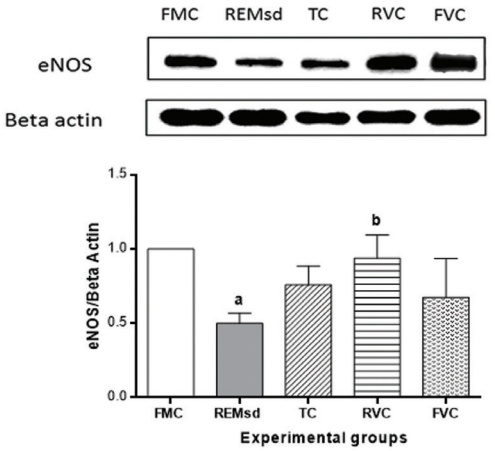

Figure 4: Representative Western blot and densitometric analysis of protein eNOS expression in the femoral arteries from all groups. FMC, free-moving control rats; REMsd, 72-h REM sleep-deprived rats; TC, tank control rats; RVC, REMsd pre-treated with vitamin C; FVC, FMC pre-treated with vitamin C. [a]$p<0.05$ compared to FMC; [b]$p<0.05$ compared to REMsd

Effects of 72-h REM Sleep Deprivation on Oxidative Stress Parameters

There was no significant difference in the MDA levels between the groups as shown in Figure 5. Levels of plasma GR and SOD activities were significantly lower in REMsd group compared to FMC, TC and FVC. The plasma TAC in REMsd group was also significantly decreased as compared to FMC and TC. Moreover, the level of SOD activity was significantly higher in RVC compared to REMsd.

Our results suggested that REM sleep deprivation induces oxidative stress. Levels of plasma GR, SOD activities and TAC were significantly lower in REMsd compared to FMC group. Oxidative stress occurs when there is an imbalance between ROS and antioxidants (Villafuerte *et al*., 2015) or due to inadequate removal of ROS by antioxidants (Valko *et al*., 2007). Hence, decreased levels of GR, SOD activities and TAC during REM sleep deprivation might induce oxidative stress in the present study. The results proposed that antioxidative capacity is reduced in REM sleep deprivation. Several lines of evidence indicate that there is a link between oxidative stress and endothelial dysfunction (Annuk *et al*., 2003; Hadi *et al*., 2005; Suganya *et al*., 2016). ROS plays a physiological role in controlling endothelial function, vascular tone and vascular integrity.

MDA levels were not significantly different between the groups. This indicates that lipid peroxidation did not increase during 72-hour REM sleep deprivation. Previous studies demonstrated no changes in the MDA levels in the brain and liver of sleep-deprived rats, short-term (8 hours) and long-term (3 to 14 days), compared to normal control rats (Gornik & Creager, 2004). Using the same protocol in the present study, others have demonstrated an increase in plasma levels of MDA compared to control (Thamaraiselvi *et al*., 2012). Among the antioxidant enzymes that were measured in this study, SOD is the most essential antioxidant defence in all cells exposed to O_2 (Villafuerte *et al*., 2015). SOD catalyses the dismutation of superoxide anion into oxygen and hydrogen peroxide. A degradation of SOD enzymes after prolonged activation during waking may contribute to the decrease in the levels of SOD (Reimund, 1994). Decreased SOD could increase the levels of superoxide anion, subsequently increasing ROS. Furthermore, among the various sources of ROS in the cells, superoxide anion is known to be the first to be generated, and it is essential for the formation of other ROS, including hydrogen peroxide and peroxynitrite (Incalza *et al*., 2017). Decreased NO bioavailability is a key adverse effect of increased ROS (Roberts & Porter, 2013) through degradation of eNOS or alterations of its functions (Coco& de Oliveira, 2015). In addition, effects of ROS on regulating factors like arginine (Gracia *et al*., 2017) and tetrahydrobiopterin (BH4) (Bevers *et al*., 2006) may alter the function of eNOS.

The regeneration of glutathione (GSH) from oxidised glutathione (GSSG) is catalysed by GR, and thus plays an essential role to combat oxidative stress by maintaining the intracellular glutathione (Bevers *et al*., 2006). GSH is an antioxidant that can scavenge ROS, such as hydrogen peroxide and superoxide anion. A reduction in TAC is attributed to increased oxidative stress (Ganjifrockwala *et al*., 2017), which can support REM sleep deprivation associated with oxidative stress in the present study. Moreover, excess utilisation of antioxidants against oxidative stress may decrease TAC (Ganjifrockwala *et al*., 2017).

Vitamin C Reduced the Adverse Effects of REM Sleep Deprivation

Vitamin C was able to reduce the adverse effects of REM sleep deprivation in the present study. The effects of this vitamin were demonstrated in the RVC group, i.e. REMsd rats pre-treated with vitamin C. Among the physical changes, vitamin C normalised the reductions in BWg, whereas no significant weight loss was observed in RVC compared to FMC group.

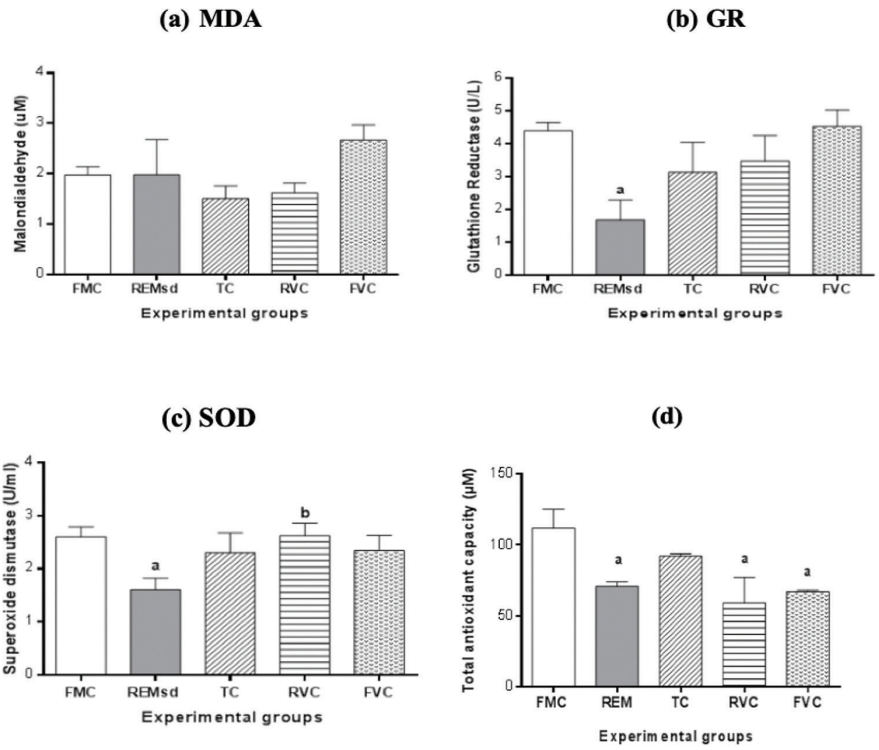

Figure 5: Plasma levels of (a) malondialdehyde, (b) glutathione reductase, (c) superoxide dismutase, and (d) total antioxidant capacity in all groups; FMC, free-moving control rats; REMsd, 72-h REM sleep-deprived rats; TC, tank control rats; RVC, REMsd pre-treated with vitamin C; FVC, FMC pre-treated with vitamin C. [a]$p<0.05$ compared to FMC; [b]$p<0.01$ compared to REMsd

In *in vitro* functional study, vitamin C preserved the endothelial function. Additionally, the eNOS protein expression was restored in the presence of vitamin C. Regarding the oxidative stress markers, vitamin C prevented the reduction of GR activity. It is interesting to note that SOD activity in RVC was significantly increased compared to REMsd group. The role of vitamin C in preventing oxidative stress has been widely investigated. Therein, vitamin C serves to scavenge free radicals (May *et al*., 2005). It reduces and neutralises ROS**,** thus, it is also known as a reducing agent (Padayatty *et al*., 2003).

In the blood vessels, vitamin C has been shown to stimulate endothelial proliferation, increase the synthesis and deposition of collagen in the basement membrane, scavenge radical species and inhibit apoptosis (May *et al*., 2005). Since vitamin C is a free radical scavenger, it is possible that the effects of oxidative stress during REM sleep deprivation are reduced by this antioxidant. Hence, supplementation of vitamin C was able to reduce the subsequent effects of REM sleep deprivation via its anti-oxidative property. In the FVC group (normal control rats pre-treated with vitamin C), administration of vitamin C did not affect the parameters measured. The results of the FVC group were comparable to the FMC group. Thus, vitamin C is not an enhancing agent.

Conclusion

We can conclude that oxidative stress induces endothelial dysfunction in REM sleep-deprived rat model. Endothelial dysfunction is shown in *in vitro* functional study that demonstrated an impairment of endothelium-dependent

vasorelaxation to acetylcholine. A significant decrease in eNOS protein expression may reduce NO bioavailability. In addition, vitamin C reduces the adverse effects of REM sleep deprivation. Further studies are needed to explore the molecular mechanism of endothelial dysfunction in REM sleep deprivation that may involve oxidative stress-related nitric oxide signalling pathways.

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References

Abas, R., Othman, F., & Thent, Z. C. (2015). Effect of Momordica charantia fruit extract on vascular complication in type 1 diabetic rats. *EXCLI Journal*, *14,* 179–189.

Alzoubi, K. H., Khabour, O. F., Rashid, B. A., Damaj, I. M., & Salah, H. A. (2012). The neuroprotective effect of vitamin E on chronic sleep deprivation-induced memory impairment: the role of oxidative stress. *Behavioural Brain Research*, *226*, 205–210.

Annuk, M., Zilmer, M., & Fellstrom, B. (2003). Endothelium-dependent vasodilation and oxidative stress in chronic renal failure: impact on cardiovascular disease. *Kidney International*, *63,* S50–S53.

Bevers, L. M., Braam, B., Post, J. A., van Zonneveld, A. J., Rabelink, T. J., …..Koomans, H. A. (2006). Tetrahydrobiopterin, but not L-arginine, decreases NO synthase uncoupling in cells expressing high levels of endothelial NO synthase. *Hypertension*, *47,* 87–94.

Chattu, V. K., Manzar, M. D., Kumary, S., Burman, D., Spence, D.W., Pandi-Perumal, S. R. (2019). The Global Problem of Insufficient Sleep and Its Serious Public Health Implications. *Healthcare (Basel)*, *7,* 1-16. doi:10.3390/healthcare7010001

Coco, H., & de Oliveira, A. M. (2015). Endothelial dysfunction induced by chronic psychological stress: a risk factor for atherosclerosis. *Cardiovascular Pharmacology: Open Access*, *4,* 168. doi:10.4172/2329-6607.1000168.

D'Almeida, V., Lobo, L.L., Hipolide, D.C., de Oliveira, A.C., Nobrega, J.N., & Tufik, S. (1998). Sleep deprivation induces brain region-specific decreases in glutathione levels. *Neuroreport*, *9,* 2853–2856.

Ergul, Y., Erkan, T., Uzun, H., Genc, H., Altug, T., & Erginoz, E. (2010). Effect of vitamin C on oxidative liver injury due to isoniazid in rats. *Pediatric International*, *52,* 69–74.

Furchgott, R. F. (1981). The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. *Trends in Pharmacological Sciences*, *2,* 173–176.

Ganjifrockwala, F. A., Joseph, J., & George, G. (2017). Decreased total antioxidant levels and increased oxidative stress in South African type 2 diabetes mellitus patients. *The Journal of endocrinology, metabolism and diabetes of South Africa, 22,* 21–25.

Gopalakrishnan, A., Ji, L. L., & Cirelli, C. (2004). Sleep deprivation and cellular responses to oxidative stress. *Sleep*, *27,* 27–35.

Gornik, H. L., & Creager, M. A. (2004). Arginine and endothelial and vascular health. *The Journal of Nutrition*, *134,* 2880S–2887S.

Gracia, K. C., Llanas-Cornejo, D., & Husi, H. (2017). CVD and oxidative stress. *Journal of Clinical Medicine, 6,* 22–44.

Gulyani, S., Majumdar, S., & Mallick, B. N. (2000). Rapid eye movement sleep and significance of its deprivation studies: a review. *Sleep and Hypnosis, 2,* 49–68.

Hadi, H. A., Carr, C. S., & Al Suwaidi, J. (2005). Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. *Vascular Health and Risk Management*, *1,* 183–198.

Hanlon, E. C., Andrzejewski, M. E., Harder, B. K., Kelley, A. E., & Benca, R. M. (2005). The effect of REM sleep deprivation on motivation for food reward. *Behavioural brain research*, *163,* 58–69.

Hipolide, D., Suchecki, D., Pinto, A. P., Chiconelli Faria, E., Tufik, S., & Luz, J. (2006). Paradoxical sleep deprivation and sleep recovery: effects on the hypothalamic–pituitary–adrenal axis activity, energy balance and body composition of rats. *Journal of Neuroendocrinology*, *18,* 231–238.

Incalza, M. A., D'Oria, R., Natalicchio, A., Perrini, S., Laviola, L., & Giorgino, F. (2017). Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Journal of Cardiovascular Pharmacology, 100,* 1–19.

Jiang, J., Gan, Z., Li, Y., Zhao, W., Li, H., …..Zheng, J. P. (2017). REM sleep deprivation induces endothelial dysfunction and hypertension in middle-aged rats: roles of the eNOS/NO/cGMP pathway and supplementation with L-arginine. *PLOS ONE*, e0182746.

Kearney, K., Tomlinson, D., Smith, K., & Ajjan, R. (2017). Hypofibrinolysis in diabetes: a therapeutic target for the reduction of cardiovascular risk. *Cardiovascular Diabetology*, *16,* 34–51.

Koban, M., Sita, L. V., Le, W. W., & Hoffman, G.E. (2008). Sleep deprivation of rats: the hyperphagic response is real. *Sleep*, 31: 927–933.

Koban, M., & Stewart, C. V. (2006). Effects of age on recovery of body weight following REM sleep deprivation of rats. *Physiology & Behavior*, *87,* 1–6.

Koban, M., & Swinson, K. L. (2005). Chronic REM-sleep deprivation of rats elevates metabolic rate and increases UCP1 gene expression in brown adipose tissue. *American Journal of Physiology-Endocrinology and Metabolism, 289,* E68–E74.

Kolluru, G. K., Bir, S. C., & Kevil, C. G. (2012). Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. *International Journal of Vascular Medicine,* 2012, 918267.

Lee, M. H. H., Chen, S. J., Tsao, C. M., & Wu, C. C. (2014). Perivascular adipose tissue inhibits endothelial function of rat aortas via caveolin-1. *PLOS ONE*, *9,* e99947.

Lima, A. M. A., de Bruin, V. M. S., Rios, E. R. V., & de Bruin, P. F. C. (2014). Differential effects of paradoxical sleep deprivation on memory and oxidative stress. *Naunyn-Schmiedeberg's Archives of Pharmacology, 387,* 399–406.

Mahmoudi, J., Ahmadian, N., Farajdokht, F., Majdi, A., & Erfani, M. (2017). A protocol for conventional sleep deprivation methods in rats. *Journal of Experimental and Clinical Neurosciences, 4,* 1–4.

May, J. M., & Harrison, F. E. (2013). Role of vitamin C in the function of the vascular endothelium. *Antioxidants & Redox Signaling*, *19,* 2068–2083.

May, M. E., Harvey, M. T., Valdovinos, M. G., Kline, R. H., Wiley, R. G., & Kennedy, C. H. (2005). Nociceptor and age specific effects of REM sleep deprivation induced hyperalgesia. *Behavioural Brain Research*, *159*, 89–94.

Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., ….Lee, J. H. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American College of Nutrition*, *22*, 18–35.

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*, *4*, 89–96.

Rajendran, P., Rengarajan, T., Thangavel, J., Nishigaki, Y., Sakthisekaran, D., ….Sethi, G. (2013). The vascular endothelium and

human diseases. *International Journal of Biological Sciences, 9*, 1057–1069.

Reimund, E. (1994). The free radical flux theory of sleep. *Medical Hypotheses*, *43*, 231–233.

Roberts, A. C., & Porter, K. E. (2013). Cellular and molecular mechanisms of endothelial dysfunction in diabetes. *Diabetes & Vascular Disease Research*, *10,* 472–482.

Siran, R., Ahmad, A. H., Abdul Aziz, C. B., & Ismail, Z. (2014). REM sleep deprivation induces changes of down regulatory antagonist modulator (DREAM) expression in the ventrobasal thalamic nuclei of Sprague–Dawley rats. *The Journal of Physiology and Biochemistry*, *70,* 877–889.

Srivastava, A., & Singh, A. (2014). Oxidative stress and nitric oxide: a significant marker in coronary artery disease. *International Journal of Technical Research,* Appl *2,* 19–25.

Suganya, N., Bhakkiyalakshmi, E., Sarada, D., & Ramkumar, K. (2016). Reversibility of endothelial dysfunction in diabetes: role of polyphenols. *British Journal of Nutrition*, *116*, 223–246.

Tanabe, T., Maeda, S., Miyauchi, T., Iemitsu, M., Takanashi, M., ….Irukayama Tomobe. Y. (2003). Exercise training improves ageing induced decrease in eNOS expression of the aorta. *Acta Physiologica*, *178*, 3–10.

Thamaraiselvi, K., Mathangi, D., & Subhashini, A. (2012). Effect of increase in duration of REM sleep deprivation on lipid peroxidation. *International Journal of Biological and Medical Research, 3,* 1754–1759.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, *39*, 44–84.

Versari, D., Daghini, E., Virdis, A., Ghiadoni, L., & Taddei, S. (2009). Endothelium dependent contractions and endothelial dysfunction in human hypertension. *British Journal of Pharmacology*, *157*, 527–536.

Vgontzas, A. N., Liao, D., Bixler ,E. O., Chrousos, G.P., & Vela-Bueno, A. (2009). Insomnia with objective short sleep duration is associated with a high risk for hypertension. *Sleep*, *32*, 491–497.

Villafuerte, G., Miguel-Puga, A., Murillo Rodríguez, E., Machado, S., Manjarrez, E., & Arias-Carrión, O. (2015). Sleep deprivation and oxidative stress in animal models: a systematic review. *Oxidative Medicine and Cellular Longevity*, 2015, 234952.

Wang, M., Chen, M., Ding, Y., Zhu, Z., Zhang, Y., …..Wei, P. (2015). Pretreatment with β-boswellic acid improves blood stasis induced endothelial dysfunction: role of eNOS activation. *Scientific Reports*, *5*, 15357.

Wiesner, C. D., Pulst, J., Krause, F., Elsner, M., Baving, L., ….Pedersen, A. (2015). The effect of selective REM-sleep deprivation on the consolidation and affective evaluation of emotional memories. *Neurobiology of Learning and Memory*, *122,* 131–141.

Xu, X., Wang, L., Zhang, Y., Su, T., Chen, L., …..Ma, W. (2016). Effects of chronic sleep deprivation on glucose homeostasis in rats. *Sleep and Biological Rhythms*, *14,* 321–328.

Zhang, R., Niu, H., Wang, N., Sun, L., Xu, Y., ….Zhao, R. (2012). Daming capsule restores endothelial dysfunction induced by high-fat diet. *BMC Complementary and Alternative Medicine*, *12,* 12–21.