Niger. J. Physiol. Sci. 30(2015) 005-009
www.njps.com.ng

Niger. J. Physiol. Sci.

Vitamin C Prevents Sleep Deprivation-induced Elevation in Cortisol and Lipid Peroxidation in the Rat Plasma

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Summary: Sleep deprivation (SD) is biological stressor that alters metabolic parameters, induced oxidative stress and lipid peroxidation. Previous studies have shown that antioxidants substances such as melatonin, tryptophan, vitamin E and vitamin C improved stress tolerance in laboratory animals. In this study, we examined the potential protective effects of administration of vitamin C on acute and chronic sleep deprivation-induced metabolic derangement. In addition, possible processes involved in vitamin C effects on acute and chronic sleep deprivation-induced metabolic derangement were determined. Thirty-five rats (120-250g) were used. The rats were divided into 7 groups of 5 rats each as Control (CTRL), Acute sleep deprived untreated with vitamin C (AC), Acute sleep deprived treated with vitamin C (AWC), Chronic sleep deprived untreated with vitamin C (CC), Chronic sleep deprived treated with vitamin C (CWC), Chronic sleep deprived + Recovery untreated with vitamin C (RC), and Chronic sleep deprived + Recovery treated with vitamin C (RWC). The SD was carried out for 20h for 1 day on the acute groups, and for 20h/day for 5 days on the chronic group, using the Multiple Modified Platforms (MMP) after oral administration of 300mg/kg of vitamin C to all vitamin C-treated groups. The recovery groups were further observed for five days after SD. The control group were treated with vitamin C and without stress in their home cages. At the end of the experiment, the animals were sacrificed and blood was collected for estimation of plasma glucose, insulin, cortisol and malondialdehyde (MDA). The results showed that acute and chronic SDs significantly ($p<0.05$) increased MDA and cortisol levels, while significantly ($p<0.05$) reduced the levels of insulin. Treatment with vitamin C reversed the changes in the MDA, cortisol and plasma insulin levels. Additionally, allowing the rats to recover for 5 days after sleep deprivation corrected the observed changes. Plasma glucose was significantly ($p<0.05$) reduced in all the sleep deprived groups compared to the control. In conclusion, sleep deprivation induced metabolic, hormonal and lipid peroxidation derangement, and treatment with vitamin C prevented these impairments. Thus, the effects of vitamin C could improve stress tolerance in rats.

Keywords*:* Sleep Deprivation, Lipid Peroxidation, Plasma Cortisol, Plasma Insulin, Plasma Glucose.

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Manuscript Accepted: October, 2015

INTRODUCTION

Sleep, defined as unconsciousness from which a person can be aroused by sensory or other stimuli (Hall, 2015), is an essential physiological need that must be satisfied to ensure normal physiological functions (Rechtschaffen and Bergmann, 2002). Sleep deprivation can be defined as the restriction of sleep below the level of basal sleep need (Lim and Dinges, 2007). This can be acute (a single period of extended wakefulness) or chronic (accumulation of sleep debt over multiple nights of sleep restriction) (Lim and Dinges, 2007).

Despite this importance of sleep, people sleep less in our contemporary environment. Sleep disruptions, which may be caused by several factors, have been said to affect daily life negatively (Chasens *et al.*, 2010). Therefore, sleep restriction in humans has been shown to alter multiple behavioural and metabolic pathways leading to increased prevalence of Insulin Resistance, Diabetes Mellitus, Obesity, Hypertension and Hyperlipidaemia (Lucassen *et al.*, 2012).

Since sleep deprivation is a biological stressor that simulates Corticotropin Releasing Hormone (CRH) and cortisol secretion (Leproult *et al.*, 1997); and negatively alters the body oxidant-antioxidant system through its effects on metabolism and endocrine functions (Spiegel *et al.*, 1999), this research studied effect of deprivation on some metabolic parameters (cortisol, insulin and glucose) and an oxidative parameter (malondialdehyde (MDA)). Cortisol was chosen as a stress marker because it has previously been used for the same purpose in rats (Prasad *et al.*, 2006; Jameel *et al.*, 2014; Joshi and Jameel, 2015). While many laboratory studies such as (Tochikubo *et al.*, 1996; Spiegel *et al.*, 1999; Kato *et al.*, 2000; Meier-Ewert *et al.*, 2004; Spiegel *et al.*, 2004) focused on short-term (acute) Sleep Deprivation, such studies for chronic sleep deprivation is limited, although the results from the short-term can give a potential mechanism through which chronic sleep deprivation affects health (Dinges and Banks, 2007). Also, most studies did not study recovery phenomena from the sleep restriction.

Therefore, this research studied the effect of both acute and chronic sleep deprivations on cortisol, insulin, glucose and malondialdehyde (MDA) as well as how vitamin C (an antioxidant) can affect the levels of these indices during sleep deprivation and recovery phenomena.

MATERIALS AND METHODS

Thirty-five rats of weight 120-250g were used in the research. The rats were acclimatised for 2 weeks under ambient temperature of 25^{o}C and standard photoperiod of 12hr light-dark cycle after which they were subjected to Paradoxical sleep deprivation for 20hrs (11:00am-7:00am next morning) for one day in the acute group or 20hrs for 5 days in chronic group with 4hr (7:00am-11:00am) rest each day using Modified Multiple Platform (MMP) method. The MMP has been previously described (Oh *et al.*, 2012; Medeiros *et al.*, 1998). The rats were grouped into 7 groups each containing 5 rats as follows:

A- Control group (CTRL)
B- Acute sleep deprived untreated with vitamin C (AC).
C- Acute sleep deprived treated with vitamin C (AWC).
D- Chronic sleep deprived untreated with vitamin C (CC).
E- Chronic sleep deprived treated with vitamin C (CWC).
F- Chronic sleep deprived + Recovery untreated with vitamin C (RC).
G- Chronic sleep deprived + Recovery treated with vitamin C (RWC)

Oral vitamin C was administered to the vitamin C-treated rats at a dose of 300 mg/kg body weight following previous researches in which higher dosages were used (Wadly and McConell, 2010; Derakhshanfar *et al.*, 2012). All the administrations were done in the morning before subjecting them to SD in the acute group and every morning before SD for 5 days in the chronic group. Recovery groups were kept for additional 5 days without SD for observation of their recovery processes. All the rats were sacrificed between 9:00hrs and noon in all groups at their respective days.

Before sacrifice, the body weights of the rats were taken. The rats were afterwards anaesthetised with intraperitoneal injection of 125mg/kg of ketamine (Youth *et al.*, 1973) and blood samples were collected from them into fluoride oxalate bottles through cardiac puncture. The blood samples were centrifuged at 3000rpm for 10 minutes and their respective plasma were removed and stored in plain bottles below -20^{o}C inside a freezer until the day of analyses.

Biochemical assays from all the groups were done the same day. Estimations of insulin and cortisol levels were done using commercially available kits (Agape Diagnostics, Switzerland Gmbh) according to manufacturers recommended protocols. Absorbance readings were obtained using an automated blood chemistry analyser (URIT-810 Chemistry Analyzer, URIT Medical Electronic Co., Ltd. Guangxi, PR China). Plasma MDA levels were analysed using GOD-PAP and thiobarbituric acid methods respectively. Glucose concentration was determined with AccuCheck (Roche Diagnostics, Indianapolis, IN, USA).

Statistical Analysis

All values are presented as mean ± SEM (Standard Error of Mean). Statistical analyses were done with Statistical Package for Social Sciences (SPSS) using one-way Analysis of Variance (ANOVA) and Least Significant Difference (LSD) multiple comparison analysis taking p-value <0.05 at any stage as significant.

RESULTS

Plasma Cortisol

The results, Fig. 1., showed a significant rise in plasma cortisol levels in both the acute sleep deprived untreated rats (AC) with a mean value of 7.78±0.34pg/dl ($p<0.01$) and the chronic sleep deprived (SD) rats (CC) with the mean value of 5.26±0.13pg/dl ($p<0.05$) compared with the control (3.98±0.14pg/dl). However, chronic sleep deprived rats treated with vitamin C (CWC) showed significant (2.78±0.09 pg/dl, $p<0.05$) reduction plasma cortisol concentration when compared with the control.

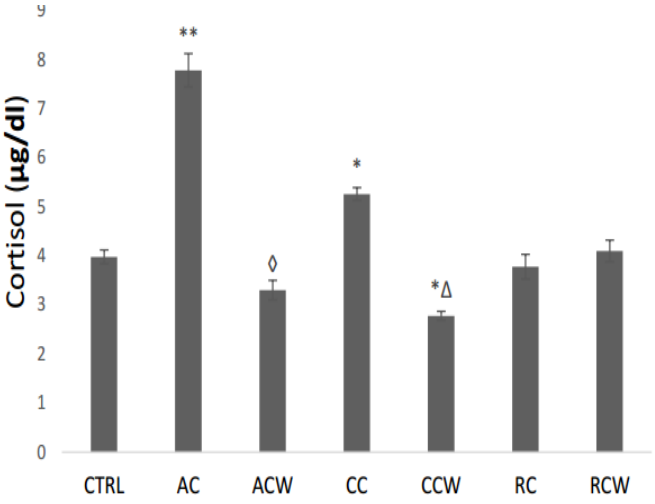

Figure 1. Effects of vitamin C on plasma cortisol level in sleep-deprived rats; *p<0.05 vs Control, **p<0.01 vs Control, ◊p<0.05 vs Acute, Δ p<0.05 vs Chronic. CTRL Control, AC Acute, AWC Acute treated with vitamin C, CC Chronic, CWC Chronic treated with vitamin C, RC Recovery, RWC Recovery treated with vitamin C.

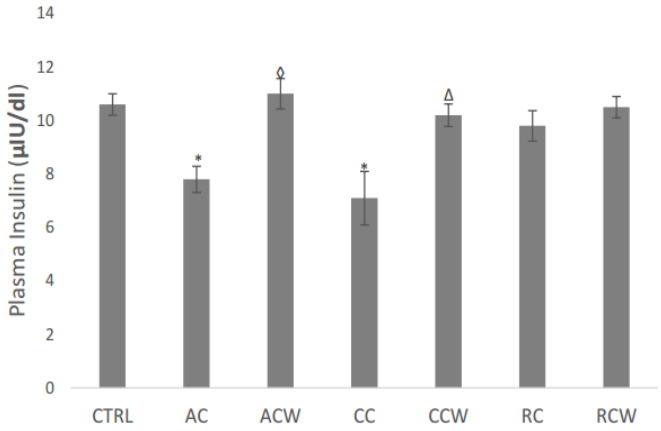

Figure 2. Effects of vitamin C on plasma insulin level in sleep-deprived rats; *p<0.05 vs Control, **p<0.01 vs Control, ◊p<0.05 vs Acute untreated, Δ p<0.05 vs Chronic untreated. CTRL Control, AC Acute, AWC Acute treated with vitamin C, CC Chronic, CWC Chronic treated with vitamin C, RC Recovery, RWC Recovery treated with vitamin C

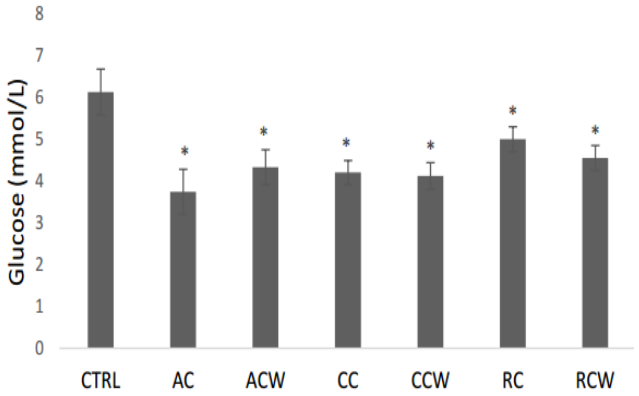

Figure 3. Effects of vitamin C on plasma glucose level in sleep-deprived rats; *p<0.05 vs Control. CTRL Control, AC Acute, AWC Acute treated with vitamin C, CC Chronic, CWC Chronic treated with vitamin C, RC Recovery, RWC Recovery treated with vitamin C.

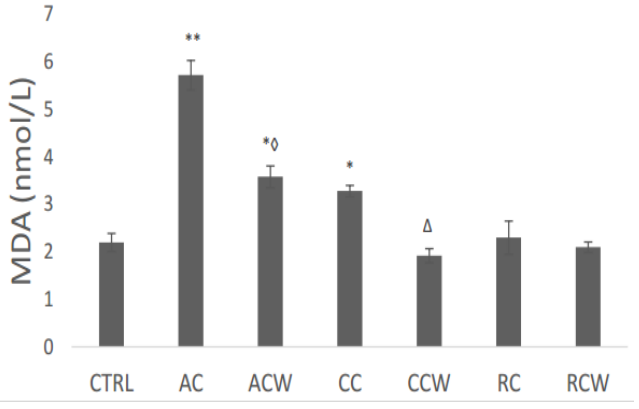

Figure 4. Effects of vitamin C on plasma MDA level in sleep-deprived rats; *p<0.05 vs Control, **p<0.01 vs Control, ◊p<0.05 vs Acute, Δp<0.05 vs Chronic. CTRL Control, AC Acute, AWC Acute treated with vitamin C, CC Chronic, CWC Chronic treated with vitamin C, RC Recovery, RWC Recovery treated with vitamin C.

Treatment with vitamin C in both acute and chronic sleep deprived groups significantly (p<0.05) reduced the levels of cortisol compared to the untreated groups.

Plasma Insulin

The results in Fig. 2, showed significant (p<0.05) decrease in insulin levels of acute (AC) (7.8±0.5pIU/dl, p<0.05), chronic (CC) (7.1±1.0pIU/dl, p<0.05) sleep deprivation compared to control (10.6±0.4pIU/dl). In addition, post-hoc multiple comparisons showed a significant (p<0.05) increase in plasma insulin concentration following vitamin C treatment in both acute and chronic sleep deprivation groups.

Plasma Glucose

The result (Fig. 3) showed a significant reduction (p<0.05) in glucose levels in all the treated groups compared to the control group.

Plasma MDA

The results, Fig. 4, showed significant increase in the levels of MDA of the acute sleep deprived groups (treated or untreated with vitamin C, 5.72±0.31nmol/L, p<0.01 and 3.58±0.23nmol/L, p<0.05 respectively) and chronic untreated with vitamin C (3.28 ± 0.12nmol/L, p<0.05) when compared to the control group (2.20 ± 0.19nmol/L). The results also showed significant reduction (p<0.05) in the levels of MDA in both acute and chronic sleep deprived groups following vitamin C treatment when compared with the untreated groups (5.72±0.31nmol/L vs 3.58±0.23nmol/L and 3.28±0.12nmol/L vs 1.92±0.15nmol/L). There were no significant differences in the levels of MDA of the recovery groups and the control group.

DISCUSSION

The reason for this study was to know the role of vitamin C, an antioxidant, in mitigating stress response in rats. Previous studies have shown that antioxidants such as tryptophan and vitamin E normalise oxidative stress markers due to sleep deprivation in laboratory animals (Alzoubi *et al*, 2012; Hosseini and Hoseini, 2013)

During periods of stress, cortisol levels are increased (Galliot *et al.*, 2007). Therefore, since sleep deprivation is a biological stressor, cortisol level generally increased in sleep deprivation process. In fact, sleep duration has been said to affect cortisol levels in the late afternoon and evening with a graded inverse relationship between sleep duration and cortisol (Spiegel *et al.*, 2004). Other studies such as (Gonzales-Oritz *et al.*, 2000; Schmid *et al.*, 2007 and Nedeltcheva *et al.*, 2009) found no significant changes in the cortisol levels in the morning following periods of sleep deprivation. However, in this study, increase

in cortisol levels was found in acute and chronic sleep deprived rats untreated with vitamin C.

The significant drop in cortisol levels observed in both the vitamin C treated acute and chronic sleep deprived rats in this study might be indirectly due to ability of vitamin C to mitigate stress response. Lower level of cortisol in recovery treated group than the control and untreated recovery group shows ability of vitamin C to hasten recovery from stressful situation of sleep deprivation. Reduction in cortisol levels of chronic vitamin C treated and untreated groups than their acute counterparts might be due to adaptation of the rats to the stress thereby reducing the effect of such stressful situation.

Increased insulin secretion in all vitamin C treated groups in both acute and chronic conditions compared to corresponding untreated groups might be accounted for by ability of vitamin C to stimulate insulin secretion as it has been previously shown (Gokkusu *et al.,* 2001; Shawer *et al.,* 2014). It has been said that the elevation of insulin hormone level with vitamins C and E is explained by effects of vitamins C and E in maintaining residual P-cell function though they act as a free oxygen radical scavenger hence, prevent P-cell cytotoxicity. Also vitamins C and E may improve the functions of P- cells, elevate plasma insulin and C-peptide levels possibly by increasing the antioxidant capacity. In addition, antioxidants may also block the ability of the immune system to recognise P-cells (Gokkusu *et al.,* 2001). The increase might also result from cortisol actions to increase free fatty acids and mobilise proteins or by cortisol actions to cause insulin resistance which can stimulate pancreas further to release more insulin.

Glucose levels in this study were reduced in all groups compare to the control and all the levels in the experimental groups were not significantly different. Generally reduced glucose level might be due to high utilisation of glucose by the body undergoing stress especially the brain as previously described (Galliot *et al.,* 2007). It might also be due to too much insulin level which may stimulate increased glucose uptake (Shashank, 2007) more than cortisol level that stimulates glucose release from tissues such as muscle (Hoehn and Marieb, 2010). Insignificant differences among glucose levels of experimental animals might be due to counteracting effect of both insulin and cortisol.

Wakefulness involves high neuronal metabolism to maintain neuronal electrical potentials, which requires a great amount of oxygen, resulting in a significant production of oxidants. Thus, sleep represent with an increased antioxidant activity which promotes a brain protection against free radicals via a diminution of in oxidant production (Reimund, 1994). Increased MDA (oxidative marker) levels which was significant in both acute and chronic SD groups denotes the effect of sleep deprivation as a stressor to increase tissue oxidation. This is in agreement with the result of a previous research (Thamaraiselvi *et al.,* 2012) which also showed increased MDA. Likewise, De Oliveira and colleagues (De Oliveira *et al.,* 2002) observed a decreased glutathione and an increase thiobarbituric acid reactive substance (an index of lipid peroxidation) levels in SD rats. However, in this study, MDA levels reduced in vitamin C treated groups compared to corresponding untreated groups. The recovery groups showed similar MDA levels as that of the control group.

Conclusion

It is concluded that vitamin C could mitigate stress response in rats, at least in the case of plasma cortisol, which could help to improve stress tolerance.

REFERENCES

Alzoubi, K.H., Khabour, O.F., Rashid, B.A., Damaj, I.M. and 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.

Chasens, E.R., Twerski, S.R., Yang, K. and Umlauf, M.G. (2010). Sleepiness and health in midlife women: results of the National Sleep Foundation's 2007 Sleep in America poll. Behav Sleep Med 8(3):157-171.

De Oliveira, A.C., D'Almeida, V., Hipolide, D.C., Nobrega, J.N. and Tufik, S. (2002). Sleep deprivation reduces total plasma homocysteine levels in rats. Canadian Journal of Physiology and Pharmacology 80(3):193-197.

Derakhshanfar A., Roshanzamir, M. and Bidadkosh, A. (2012). Dose-related protecting effects of vitamin C in gentamicin-induced rat nephrotoxicity: a histopathologic and biochemical study. Comp Clin Pathol 22:441-447.

Dinges, D. F. and Banks, S. (2007). Behavioural and Physiological Consequences of Sleep Restriction. Journal of Clinical Sleep Medicine 3(5):519-528.

Galliot, M.T., Baumeister, R F., DeWall, C.N., Plant E.A., Brewer, L.E., Schmeichel, B.J. *et al.* (2007). Self-control relies on glucose as a limited energy source: Willpower is more than a Metaphor. J Personal Soc Psychol 92(2):325-336.

Gokkusu, C., Palanduz, S., Ademoglu, E. and Tamer, S. (2001). Oxidant and antioxidant systems in NIDDM patients: influence of vitamin E supplementation. Endocr Res 27(3):377-386.

Gonzales-Ortiz, M., Martinez-Abundis, E., Baleazer-Muroz, B.R. and Pasroe-Gonzales, S. (2000). Effect of SD on Insulin sensitivity and control concentration in healthy subjects. Diabetes Nutr Metab 13:80-83.

Hall, J.E. (2015). Guyton and Hall textbook of medical physiology. 13th edition. Philadelphia: Elsevier Inc. Page 772.

Hoehn, K. and Marieb, E.N. (2010). Human Anatomy & Physiology. San Francisco: Benjamin Cummings. ISBN 0-321-60261-7.

Hosseini, S.A. and Hoseini, S.M. (2013). Effect of dietary tryptophan on stress response of wild common carp *Cyprinus carpio* L. World Journal of Fish and Marine Science 5(1):49-55.

Jameel, M.K., Joshi, A.R., Dawane J., Padwal, M., Joshi, A.R., Pandit, V.A. and MelinKeri, R.R. (2014). Effect of various physical stress models on serum Cortisol level in Wistar Rats. Journal of Clinical and Diagnostic Research 8(3):181-183.

Jameel, M.K. and Joshi, A.R. (2015). Effect of acute stress on serum cortisol level in female wistar rats. International J of Healthcare and Biomedical Research 3 (4): 109-113.

Kato, M., Phillips, B.G., Sigurdsson, G., Narkiewicz, K., Pesek, C.A. and Somers, V.K. (2000). Effects of sleep deprivation on neural circulatory control. Hypertension 35:1173-1175.

Leproult, R., Van Reeth, O., Byrne, M.M., Sturis, J. and Van Cauter, E. (1997). Sleepiness, performance, and neuroendocrine function during sleep deprivation: effects of exposure to bright light or exercise. J Biol Rhythms 12:245-258.

Lim, J. and Dinges, D. F. (2007). Sleep deprivation. Scholarpedia 2(8):2433.

Lucassen, E.A., Rother, K.I. and Cizza, G. (2012). Interacting epidemics? Sleep curtailment, insulin resistance, and obesity. Ann N.Y. Acad Sci 1264:110-134.

Medeiros, R., Lenneberg-Hoshino, C., Hoshino, K. and Tufik, S. (1998). Neuroethologic differences in sleep deprivation induced by the single and multiple-platform methods. Braz J Med Biol Res 31(5): 675-680.

Meier-Ewert, H.K., Ridker, P.M., Rifai, N., Regan, M.M., Price, N.J., Dinges, D.F. and Mullington, J.M. (2004). Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. J Am Coll Cardiol 43:678-683.

Nedeltcheva, A.V., Kessler, L., Imperial, J. and Penev, P.D. (2009). Exposure to recurrent sleep restriction in the setting of high caloric intake and physical inactivity results in increased insulin resistance and reduced glucose tolerance. J Clin Endocrinol Metab 94:3242-3250.

Oh, M.M., Kim, J.W., Jin, M.H., Kim, J.J. and Moon, D.G. (2012). Influence of paradoxical sleep deprivation and sleep recovery on testosterone level in rats of different ages. Asian Journal of Andrology 14:330-334.

Prasad, A., Naskar, R., Dubey, R., Raha, D. and Ahmed, M.F. (2006). Modulation of serum cortisol by Substance P in albino rats: evidence of a direct effect on adrenal gland. Indian Journal of Experimental Biology 44:163-164.

Rechtschaffen, A. and Bergmann, B.M. (2002). Sleep deprivation in the rat: an update of the 1989 paper. Sleep 25:18-24.

Reimund, E. (1994). The free radical flux theory of sleep. Med Hypotheses 43:231-233.

Schmid, S.M., Hallschmid, A., Jauch-Charaq, K., Bandorf, N., Born, J. and Schultes, B. (2007). "Sleep loss alters basal metabolic hormone secretion and modulates the dynamic counter-regulatory response to hyperglycaemia. J Clin Endocrinol Metab 92:3044-3051.

Shashank, R.J., Rakesh, M. and P- Das, A.K. (2007). Insulin History, Biochemistry, Physiology and Pharmacology. JAPI 55:19-26.

Shawer, G.A., Samaha, S.R. and Youssef, G.A. (2014). Effects of C-Peptide With and Without Antioxidant Supplementation on Diabetic Male Rats. Nat Sci 12(3):1-13.

Spiegel, K., Leproult, R. and Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. Lancet 354:1435-1439.

Spiegel, K., Leproult, R., L'hermite-Baleriaux, M., Copinschi, G., Penev, P. and Van Cauter, E. (2004). Leptin levels are dependent on sleep duration: relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. Journal of Clinical Endocrinology and Metabolism 89:5762-5771.

Thamaraiselvi, K., Mathangi, D.C. and Subhashini, A.S. (2012). Effect of increase in duration of REM sleep deprivation on lipid peroxidation. Int J Biol Med Res 3(2):1754-1759.

Tochikubo, O., Ikeda, A., Miyajima, E. and Ishii, M. (1996). Effects of insufficient sleep on blood pressure monitored by a new multibiomedical recorder. Hypertension 27:1318-1324.

Wadley, G.D., McConell, G.K. (2010). High-dose antioxidant vitamin C supplementation does not prevent acute exercise-induced increases in markers of skeletal muscle mitochondrial biogenesis in rats. J Appl Physiol 108:1719-1726.

Youth, R.A., Simmerman, S.J., Newell, R. and King, R.A. (1973). Ketamine anesthesia for rats. Physiology and Behavior 10(3): 633-636.