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Research Article

Effect of vitamin C on glucose metabolism in partial and total paradoxical sleep-deprived male wistar rats

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ABSTRACT

Background: Chronic sleep deprivation is associated with increases in oxidative stress and impaired glucose homeostasis. The effect of vitamin C on glucose metabolism and oxidative stress in sleep deprived animals was investigated in this study. **Method:** Thirty male Wistar rats (150-200 g) divided into six groups as control; control + vitamin C (CC); partial sleep deprived (PSD); partial sleep deprived + vitamin C (PSDC); total sleep deprived (TSD); and total sleep deprived + vitamin C (TSDC) were used. Paradoxical sleep deprivation was by the modified multiple platform for 20 hours daily in PSD and PSDC or for 24 hours daily in TSD and TSDC for 7 days. Vitamin C (300mg/kg, daily) was administered to animals in CC, PSDC and TSDC groups. After the 7 days SD and/or vitamin C treatment, blood samples were obtained for fasting blood glucose, lactate, Malondialdehyde (MDA), Superoxide dismutase (SOD) and catalase. Muscle and liver samples were obtained for glycogen determination while hexokinase and glucose-6-phosphatase activities were determined in the liver sample. **Results:** Body weight decreased significantly in TSD rats; the percentage weight loss was however reduced in TSDC. Serum MDA increased in PSD and TSD compared with control; but lower in PSDC and TSDC compared with PSD and TSD, respectively. Catalase activity was decreased in TSD and TSDC. Fasting blood glucose level, plasma lactate and muscle glycogen were reduced in TSD and TSDC. Hepatic glycogen increased in TSD but decreased in TSDC. Glucose 6-phosphatase activity decreased in PSD but increased in TSD; also, the activity decreased in TSDC with no difference in PSDC compared with control. Hexokinase activity was decreased in TSD but increased in TSDC. **Conclusion**: Partial sleep deprivation had no effect on glucose metabolism but total sleep deprivation caused derailment in glucose metabolism which was not reversed by vitamin C despite reductions in oxidative stress.

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INTRODUCTION

The importance of sleep in the regulation of glucose metabolism, appetite, hormone secretion and immunologic activity is well documented (Levy *et al.*, 1987; Clore *et al.*, 1989; Van Cauter and Spiegel, 1997). A close relationship has been shown for sleep quality and glucose regulation in humans (Scheen *et al.*, 1996; Mathew *et al.*, 2012; Kim *et al.*, 2016; Nakajima *et al.*, 2017) and rats (Barf *et al.*, 2010; Xu *et al.*, 2016). Studies have also shown that circadian rhythmic activity involving the suprachiasmatic-paraventricular-autonomous nervous system (SCN-PVN-ANS) axis plays vital role in the daily rhythmic hepatic glucose output (Doi *et al.*, 2010; Kalsbeek *et al.*, 2014; Kim *et al.*, 2015). This rhythmic glucose output is entrained to melatonin (Mühlbauer *et al.*, 2009; Bähr *et al.*, 2011) which is secreted during sleep. Hence, long term sleep disturbance such as deprivation is associated with metabolic and endocrine alterations that have physiopathological consequences (Spiegel *et al.*, 1999). For instance, inadequate sleep increases insulin insensitivity (Gottlieb *et al.*, 2005; Spiegel *et al.*, 2005), risk of obesity (Buxton and Marcelli, 2010), metabolic syndrome (Wu *et al.*, 2012), and impairment of salivary secretion (Lasisi *et al.*, 2017).

Tissue oxidative damage has been reported by several workers (Silva *et al.*, 2004; Everson *et al.*, 2005; Chang

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et al., 2008; Chanana and Kumar 2017) as a causative agent in the physiological imbalances associated with sleep deprivation. The oxidative damage could be secondary to the elevation of glucocorticoid (Mirescu *et al.*, 2006) which causes increase generation of reactive oxygen species (You *et al.*, 2009; Feng and Tang, 2014); thus, use of antioxidants in sleep deprived animals were shown to be beneficial (Kumar and Singh, 2009; Zhang *et al.*, 2013). Vitamin C, a water-soluble antioxidant, prevented sleep deprivation-induced memory impairment (Mhaidat *et al.*, 2015) and lipid peroxidation (Olayaki *et al.*, 2015) in rats. It is however not known if vitamin C has any effect on impaired glucose metabolism during sleep deprivation. In type 2 diabetes, that is characterized by increased ROS, basal vitamin C level was significantly depleted (Chen *et al.*, 1991; Will and Byer, 1996) and vitamin C supplementation improved glycemic control in diabetic humans (Eriksson and Kohvakka, 1995; Afkhami-Ardekani and Shojaoddiny-Ardekani 2007). This study therefore investigated the effect of vitamin C on glucose metabolism in sleep deprived male rats.

MATERIALS AND METHODS

Animals

Thirty (30) male Wistar rats weighing between 150 and 200 g were used for this study. They were housed and acclimatized for two weeks in the Departmental Animal House, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria. They were maintained under standard laboratory conditions with natural photoperiod of 12 hours light: dark cycle and free access to rat chow (Ladokun Feeds) and water. All experimental protocols and handling were in compliance with the NIH publication No. 85-23 guidelines (NIH publication revised, 1985).

Sleep Deprivation Model

Paradoxical or Rapid Eye Movement (REM) sleep deprivation of the rats was achieved by using the modified multiple platform method of Coenen and van Luijtelaar, (1985) as described by Lasisi *et al* (2017). Briefly, a pedestal of 16 cylindrical platforms (6 cm in diameter and 10 cm high) covered with wire gauze was placed in a large acrylic tank measuring 69 X 69 X 31 cm filled with water up to about 1 cm below the platform surface. The tank was covered with a perforated lid with feeders and drinking bottles attached; thus, the rats were required to stand to reach the feed and water. In the chamber, REM sleep cannot be attained due to muscle atonia which makes the rat to either be in partial contact with the water or fall completely into the water (the wire gauze aid climbing back to the platform) with any attempt to sleep. Not more than 6 rats were placed in each chamber to allow free movement across the platforms.

Control animals were also placed in similar chamber with multiple platforms without water to allow sleep and access to feed and drinking water in comparable conditions. Observations were made in a room with a controlled temperature (24-25^{0}C). Commercial pellets were made available *ad libitum* in a feeder attached to the chamber lid and water was supplied through conventional drinking spouts.

Vitamin C Administration

Vitamin C was administered orally to the treated rats at a dose of 300 mg/kg body weight daily in accordance to the effective dose reported by Derakhshanfar *et al.* (2012).

Experimental Design

The rats were randomly divided into 6 groups (n=5) sleep deprived and/or administered vitamin C (300mg/kg) for 7 days. Studies have shown that rat can withstand 24 hours sleep deprivation up to 21 days, ranging from 11 days to 32 days (Everson *et al.*, 1989). Koban et al (2008) also reported no mortality following 10 days sleep deprivation in Sprague Dawley rats. The animals were grouped as follows:

1. Control: Administered distilled water daily and placed in the chamber without water to allow sleep
2. Control + vitamin C (CC): Administered vitamin C (300 mg/kg, daily) and placed in the chamber without water to allow sleep
3. Partial Sleep-Deprived (PSD): Administered distilled water and placed in the sleep deprivation chamber for 20 hours, allowed to sleep in their cages for 4 hours (8am-12pm) daily
4. Partial Sleep-Deprived + vitamin C (PSDC): Administered vitamin C (300 mg/kg, daily), placed in the sleep deprivation chamber for 20 hours, allowed to sleep in their cages for 4 hours (8am-12pm) daily
5. Total Sleep-Deprived (TSD): Administered distilled water daily and placed in the sleep deprivation chamber for the entire 7 days.
6. Total Sleep-Deprived + vitamin C (TSDC): Administered vitamin C (300 mg/kg, daily) and placed in the sleep deprivation chamber with water for 7 days

Body weight and fasting blood glucose (FBG) were monitored at the start and at the end of the 7 days of sleep deprivation. Blood sample for FBG was collected via the tail vein and determined using Accucheck® glucometer.

Sample collection

On the 8th day of the experiments (8-10am), under anaesthesia induced by ketamine (100mg/kg, *i.p*) and

xylazine (0.5mg/kg, *i.m*), blood samples were collected via cardiac puncture for determination of lactate, malondialdehyde (MDA), superoxide dismutase (SOD) and catalase. Samples for plasma lactate was obtained into Eppendorf bottles containing fluoride oxalate while the remaining blood was void into plain sample bottle for serum MDA, SOD and catalase determination. Liver was harvested for the determination of glycogen content and activities of glucose-6-phosphatase and hexokinase. The left soleus muscle was also harvested for the determination of its glycogen content. The total wet liver weight was noted and the ratio of liver to body weight (relative liver weight) was determined.

Determination of plasma lactate level

Plasma lactate was determined using a modification of Baker and Summerson (1941)'s method described by Pryce (1969). Briefly, 0.05ml of plasma was added to 3.95 ml of a precipitating reagent containing sodium tungstate, orthophosphoric acid and copper sulphate, it was centrifuged at 2000 rpm to obtain supernatant. One millilitre of the supernatant was dispensed into a test tube with wide aperture and 6ml concentrated sulphuric acid was rapidly added and cooled. Thereafter, 100µl of p-hydroxybiphenyl was added and allowed to stand at room temperature for 10 minutes prior to incubation at 100^0C for 90 seconds. The absorbance of the violet blue chromogen formed was compared with a standard of known lactate concentration at 565 nm.

Determination of serum MDA, SOD and Catalase

The assay method of Hunter *et al.* (1963) modified by Gutteridge and Wilkins (1982) was adopted for the determination of MDA. Briefly, 1 ml of 0.7% TBA and 0.5 ml of glacial acetic acid were added to 1 ml of serum. The mixture was thoroughly mixed and incubated in water bath at 80°C for 20 min. It was then allowed to cool and centrifuged at 400 rev/min for 10 min. Absorbance of the pink coloured supernatant was read at 532 nm against a blank wherein serum was substituted with distilled water. The results were expressed as nanomoles MDA/ml.

Superoxide dismutase (SOD) activity was determined using the method described by Misra and Fridovich (1972). Briefly, the serum was diluted to make a 1/10 dilution. 0.2 ml of the aliquot was supplemented with 2.5 ml of 0.05 M phosphate buffer (pH 10.2) and equilibrated at room temperature. 0.3 ml of 0.3 nM adrenaline solution was then added to the reference and the test solution, followed by mixing by inversion and absorbance at 480 nm was monitored every 30 s over 150 s. Increase in absorbance per minute was obtained and used to calculate percentage inhibition. The enzyme activity was expressed in arbitrary units considering inhibition of autoxidation as 1 unit of SOD specific activity which is the amount of SOD to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 min.

Catalase activity was determined using the method described by Cohen *et al.* (1970). Briefly, 0.25ml of sample/blank was added into 2.5 ml of 30mM H_20_2, mixed by inversion and allowed to stand for 3 minutes followed by the addition of 1 ml of 6M H_2SO_4 and mixed by inversion. A standard was made by adding 1 ml of 6M H_2SO_4 into 2.75 of phosphate buffer saline and mixed by inversion. Thereafter, 3.5 ml of 2mM $KMnO_4$ was added into the test, blank or standard and absorbance (Abs) at 480 nm was noted within 60 seconds. Catalase activity (Unit/ml) was calculated as:

$$\text{Catalase activity (Unit/ml)} = \frac{1}{log}\left(\frac{Abs.standard - Abs.blank}{Abs.standard - Abs.test}\right) X \frac{2.3}{t}$$

Determination of liver and muscle glycogen content

The glycogen content of the liver and left soleus muscle was determined using the method of Seifter *et al.* (1950) as modified by Jermyn (1975). Briefly, 1 g of liver or muscle was digested in 10ml of 30% KOH over heat. An aliquot of 4 ml of the digested tissue was washed by adding 5 ml of 95% ethanol, centrifuged for 5 minutes and drained. The precipitate was reconstituted with 0.5ml of distilled water and rewashed to obtain a white precipitate which was reconstituted with 2 ml of distilled water. The reconstituted precipitate or distilled water (0.5 ml) was pipetted into a test tube followed by stepwise addition of 0.5 ml concentrated HCl, 0.5 ml 88% formic acid and 4 ml of anthrone reagent (added slowly to minimize frothing). The solution was mixed thoroughly, incubated at 100^0C for 10 minutes and cooled. A standard curve was obtained by treating several double dilutions of 0.2 mg/ml of standard glycogen with HCl, formic acid and anthrone reagent as it was done for the test/blank. Absorbance of the blue colored solution formed was read at 630 nm against a reagent blank. Glycogen concentration (mg/ml) was obtained from the standard curve while glycogen content/100 g tissue was calculated as follows:

Glycogen content/100 g wet tissue =

$$glycogen\ concentration\ X \frac{10}{4} X \frac{2}{0.5} X \frac{100}{total\ tissue\ weight}$$

Determination of Hexokinase activity

Hepatic hexokinase activity was determined by the method described by Branstrup *et al.* (1957) wherein the rate of disappearance of glucose was determined at

38^0C in a buffer solution containing ATP, Magnessium, KCl and Fluoride. Briefly, 2 ml of Glucose buffer [0.0025 M glucose, 0.0025 M $MgCl_2$, 0.01 M K_2HP0_4, 0.077 M KCl, and 0.03 M Tris (Hydroxy-methyl) aminomethane, (Trizma base) pH 8] was pippetted into a test tube followed by 0.1 ml of 0.18 M ATP solution and 0.9 ml of distilled water. The mixture was preheated in water for 5 minutes at 38^0C, 1 ml of liver homogenate was added and 100µl of the homogenate-buffer substrate mixture was taken immediately for initial glucose analysis. The mixture was then incubated at 38^0C for 30 minutes and another 100 µl was taken for final glucose analysis. The difference in the level of glucose was calculated and hexokinase activity was expressed as glucose metabolised/mg. pr/30min. All assays were carried out in duplicates. In this assay, glucose was assayed using a commercially available Glucose GOD-PAP kit (Fortress Diagnostic®, United Kingdom).

Determination of Glucose-6-Phosphatase activity

Glucose-6-phosphatase activity was assayed according to the method of Koide and Oda (1959) based on the principle that the enzyme acts as phosphohydrolase and phosphotransferase. The reaction involves the formation of covalently bound enzyme-inorganic phosphate intermediate that can liberate inorganic phosphate in the presence of an acceptor. The liberated inorganic phosphate can then be quantified using a suitable method. Briefly, into a test tube, 0.3 ml of 0.1 M citrate buffer (pH 6.5), 0.5 ml of 150 mM glucose-6-phosphate solution and 0.2 ml of sucrose buffer extracted hepatic homogenate were mixed and incubated at 37^0C for 1 hour. At the end of the incubation period, 1.0 ml of 10% trichloroacetic acid (TCA) was added to stop the reaction and placed on ice. After 10 minutes on ice, the mixtures were centrifuged. Aliquot (1 ml) of the supernatant was then used for the determination of liberated phosphate by the method of Fiske and Subbarow (1925).

Statistical Analysis

Data were presented as Mean± SEM. Difference in mean values was determined using Analysis of Variance (ANOVA). Post hoc analysis was carried out using the Turkey post hoc test and Least Significant Difference (LSD) at $p \leq 0.05$. All analyses were done using the GraphPad prism®.

RESULTS

Effect of vitamin C on body weight changes and relative liver weight in sleep-deprived rats

The effect of vitamin C on body weight changes, liver weight and relative liver weight are shown in table 1.

Table 1. Effect of vitamin C on body weight and relative liver weight of partial and total sleep-deprived male Wistar rats

	Control	PSD	TSD	CC	PSDC	TSDC
Body weight before (g)	164.71± 5.83	175.00 ± 4.32	176.13 ± 6.86	165.33 ± 7.66	179.80 ± 9.99	185.00 ± 12.17
Body weight after (g)	163.86 ± 6.28	158.20 ± 4.93	137.38 ± 4.33†	166.00 ± 5.14	169.40 ± 7.47	164.50 ± 6.89#
body weight change (%)	-0.55 ± 1.17	-9.66 ± 1.21†	-21.71 ± 1.83†	0.82 ± 2.26	-5.49 ± 1.85*	-10.56 ± 2.90*#
Liver weight (g)	5.21 ± 0.22	4.82 ± 0.34	3.71 ± 0.23	6.44 ± 0.39	5.38 ± 0.41	3.56 ± 0.15
Relative Liver Weight (x 10^{-2} g/g)	3.19 ± 0.11	3.07 ± 0.22	2.84 ± 0.23	3.87 ± 0.14	3.20 ± 0.27	2.17 ± 0.08*#

*P<0.05 and †P<0.01 when compared with control; # p<0.05 when compared with corresponding group without vitamin C

There was no difference in the mean body weight of all the groups prior to sleep deprivation. After sleep deprivation, animals in the TSD group had significant reduction in body weight compared with the control and PSD animals. Although, loss in weight was evident in all the groups, the percentage changes in body weight was higher in PSD and TSD animals compared with the control. Treatment with vitamin C in the PSDC and TSDC group decreased the percentage changes in weight when compared with their corresponding untreated groups (PSD or TSD). However, the percentage weight losses in PSDC and TSDC were still significantly higher compared with the control. Liver weight was significantly reduced in TSD and TSDC compared with the control, PSD and PSDC while the relative liver weight was only significantly reduced in the TSDC animals.

Effect of vitamin C on serum MDA, SOD and catalase activity in sleep-deprived rats

Effect of sleep deprivation on serum MDA level is shown in table 2, MDA level was significantly elevated

Table 2. Effect of vitamin C on serum MDA, SOD and catalase activity of partial and total sleep-deprived male Wistar rats

	Control	PSD	TSD	CC	PSDC	TSDC
MDA (x 10^{-5} µmol/ml)	16.70± 1.25	64.57 ± 5.72†	73.84 ± 1.75†	5.33 ± 7.66#	48.28 ± 1.17†#	62.48 ± 4.70†#
SOD (Unit/ml)	1.15 ± 0.07	1.24 ± 0.05	1.18 ± 0.06	1.78 ± 0.03#	1.18 ± 0.08	1.32 ± 0.09
Catalase (Unit/ml)	2.52 ± 0.03	2.42 ± 0.03	2.26 ± 0.05†	2.60 ± 0.01#	2.47 ± 0.05	2.24 ± 0.05†

†P < 0.01 compared with the control, # p < 0.05 when compared with corresponding group without vitamin C

in the PSD and TSD groups compared with the control. Although, vitamin C treatment significantly decreased MDA levels in the three treated groups when compared with their respective untreated groups; the MDA levels in PSDC and TSDC were still significantly higher than the control (Table 2). The serum MDA levels in the PSD animals were significantly lowered than that of the TSD; similarly, in the sleep deprived groups treated with vitamin C, MDA level was lower in PSDC compared with TSDC (Table 2). Superoxide dismutase activity was significantly increased in the control animals treated with vitamin C while there was no difference in the levels among the sleep-deprived only and sleep-deprived animals that were concurrently administered vitamin C compared with the control (Table 2). On the other hand, as shown in table 2, catalase activity was significantly reduced in the TSD and TSDC compared with control.

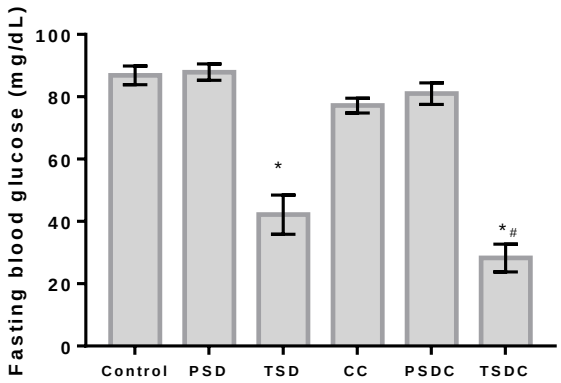

Fig. 1. Effect of vitamin C on fasting blood glucose levels of partial and total sleep deprived male Wistar rats. (n=5), *P<0.05 compared with control, #P<0.05 compared with TSD.

Effect of vitamin C on Fasting blood glucose and plasma lactate level in sleep-deprived rats

The effects of vitamin C on the fasting blood glucose of sleep deprived animals are shown in figure 1. Partial sleep deprivation had no significant effect on fasting blood glucose level in the PSD (87.89 ± 2.62 mg/dl) animals while total sleep deprivation significantly reduced fasting blood glucose of the TSD (42.20 ± 6.29 mg/dl) animals compared with the control (86.86 ± 3.00 mg/dl). Vitamin C treatment caused an apparent insignificant reduction in fasting blood glucose of animals in the CC (77.16 ± 2.3 mg/dl) and PSDC (81.00 ± 3.42 mg/dl) groups while it caused a further significant (P < 0.05) reduction in the fasting blood glucose of the TSDC (28.25 ± 5.43 mg/dl) animals compared with their respective untreated groups.

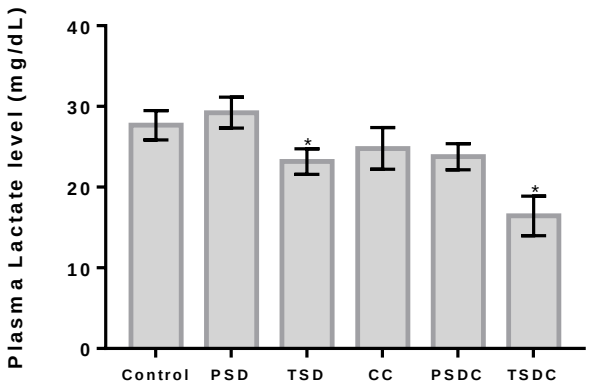

Fig. 2. Effect of vitamin C on plasma lactate levels of partial and total sleep deprived male Wistar rats. (n=5), *P<0.05 compared with control.

As shown in Figure 2, plasma lactate level was significantly reduced in the total sleep deprived untreated animals (23.2 ± 1.6 mg/dL) compared with the partial sleep deprived (29.2 ± 1.9 mg/dL) and control (27.6±1.8 mg/dL) animals. A generalized reduction was observed in the lactate levels of all the vitamin C treated groups, however, when compared with their corresponding untreated group, it was only the animals in TSDC (16.4 ± 2.4 mg/dL) that had further significant reduction in their plasma lactate level compared with the TSD (23.2 ± 1.6 mg/dL) animals.

Effect of vitamin C on hepatic and muscle glycogen content in sleep-deprived rats

Liver glycogen contents were significantly increased in the TSD animals compared with the control and PSD

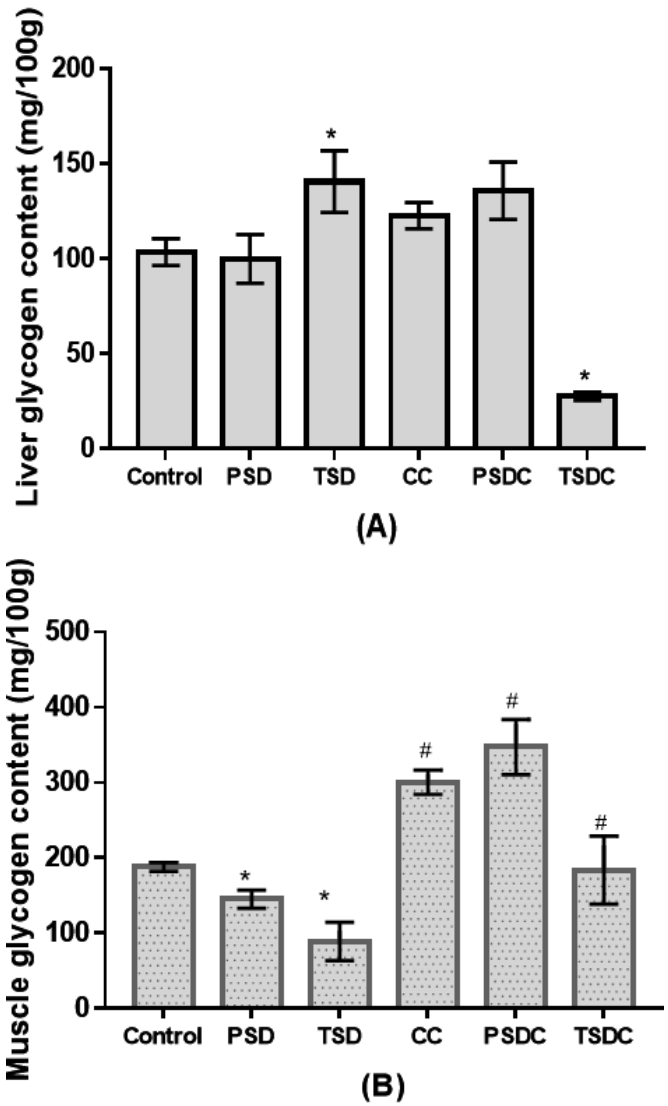

Fig. 3. Effect of vitamin C on (A) liver and (B) muscle glycogen contents of partial and total sleep deprived male Wistar rats. (n=5), *P<0.05 compared with control, #P<0.05 compared with the corresponding untreated group.

animals. Treatment with vitamin C increased hepatic glycogen content in both CC and PSDC compared to control while it decreased hepatic glycogen content in TSDC compared with control and TSD animals (Figure 3A). As shown in figure 3B, sleep deprivation caused a duration dependent decrease in the muscle glycogen content compared with the control. In other words, glycogen content of the TSD animals was significantly lower than that of the PSD animals. Vitamin C caused a generalised increase in muscle glycogen content of control and sleep deprived animals; while CC and PSDC animals had significantly higher muscle glycogen content compared with the untreated control, there was no difference in the muscle glycogen content of TSDC and the control animals (Figure 3B).

Effect of vitamin C on glucose-6-phosphatase and hexokinase activities in liver of sleep-deprived rats

Glucose-6-phosphatase activities decreased significantly in the PSD group while it increased in the TSD group compared with the control. Treatment with vitamin C had no effect on G6Pase activity in CC and PSDC while the activity which was hitherto elevated in the TSD was significantly decreased in the TSDC compared with the control (figure 4). Hexokinase activities was significantly decreased in TSD and increased in TSDC compared with the control while it was not significantly different in PSD, CC, and PSDC compared with the control (Figure 5).

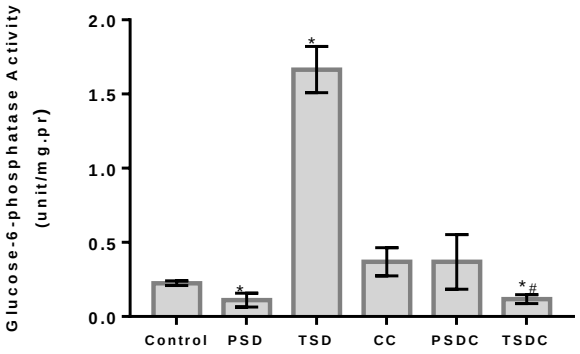

Fig. 4. Effect of vitamin C on hepatic glucose-6-phosphatase activities in partial and total sleep deprived male Wistar rats. (n=5), *P<0.05 compare with control, #P<0.05 compared with TSD.

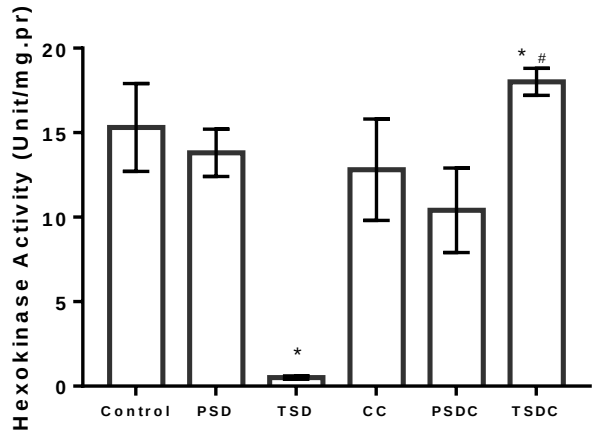

Fig. 5. Effect of vitamin C on hepatic glucose-6-phosphatase activities in partial and total sleep deprived male Wistar rats. (n=5), *P<0.05 compare with control, #P<0.05 compared with TSD.

DISCUSSION

The present study investigated the effect of vitamin C on 7 days partial and total sleep deprivation in male

Wistar rats. The loss in body weight following sleep deprivation in this study is in agreement with the earlier reports of weight loss following sleep deprivation in animals (Everson *et al.*, 1989; Everson *et al.*, 2005; Caron and Stephenson, 2010; Lasisi *et al.*, 2016). The loss of body weight or the inability to gain weight during sleep deprivation was associated with increased energy expenditure (Everson *et al.*, 1989; Kushida *et al.*, 1989; Everson and Wehr, 1993; Hipólide *et al.*, 2006) and increased metabolic rate (Koban and Swinson, 2005; Stephenson *et al.*, 2007) probably mediated by increase expression of uncoupling protein 1 gene in brown adipose mitochondria (Stephenson *et al.*, 2007). Although, food intake was not quantified in the present studies, the loss in body weight is documented to occur despite hyperphagia induced by sleep deprivation (Bhanot *et al.*, 1989; Brock *et al.*, 1994; Everson *et al.*, 2005; Koban and Stewart, 2006; Thamaraiselvi *et al.*, 2012). The hyperphagic response was challenged by Martins et al (2006) as an artefact occasioned by decrease food availability resulting from sloppiness in handling chow by the rats leading to food spillage; however, Koban and colleagues (2008) after corrections for food spillage using liquid diet, showed that while hyperphagia may not be evident in the first 5 days of sleep deprivation, it occurs when sleep deprivation is beyond 6 days via a mechanism involving hypothalamic Neuropeptide Y and peripheral leptin secretions (Koban *et al.*, 2008). The reduction in the percentage weight loss in the sleep deprived animals treated with vitamin C is consistent with the reported reversal in rapid weight loss associated with conditions characterized by increased oxidative stress (Adeneye and Olagunju, 2008;2009) following vitamin C administration.

Elevated MDA level is a hallmark during prolonged sleep deprivation (Silva *et al.*, 2004; Everson *et al.*, 2005; Chang *et al.*, 2008; Abd El-Aziz and Moustafa, 2012; Thamaraiselvi *et al.*, 2012; Arjadi *et al.*, 2015; Olayaki *et al.*, 2015; Mourad and Fahmy, 2017), it is a product of lipid peroxidation involving an autocatalytic mechanism that leads to the oxidative damage of polyunsaturated fatty acids and cellular membranes thereby reducing membrane fluidity (Cheese-man, 1993; Halliwell, 1996). Lipid peroxidation could be evident as early as 48 hours of paradoxical sleep deprivation (Mourad and Fahmy, 2017). Accordingly, partial and total sleep deprivation increased serum MDA levels in this study.

The reduction in MDA level of the vitamin C treated sleep deprived animals in the present study is in line with the well-known free radical scavenging activities of vitamin C (Niki, 1991; Retsky et al, 1993; Vojdani *et al.*, 2000). The incomparability of the reduced MDA level in the vitamin C treated animals to that of the control in this study was earlier observed by Olayaki *et al.* (2015). A plausible explanation for this might be the poor liposolubility of vitamin C which limits its permeation of cell membrane (Oh *et al.*, 2010); hence, vitamin C cannot scavenge lipophilic radicals within the lipid compartment by itself but could acts as a synergist with vitamin E for the reduction of lipid peroxyl radicals within the lipid compartment by reacting with tocopheroxyl radical and regenerating active tocopherol (Niki *et al.*, 1991). Studies using vitamin E (Alzoubi *et al.*, 2012; Akindele *et al.*, 2014) in sleep deprived animal however, reported reduction in MDA level/ lipid peroxidation that was comparable with the control.

In the present study, SOD activities were not significantly different in the sleep deprived animals despite elevated MDA levels. This is contrary to the decreased (Ramanathan *et al.*, 2002) or increased (Lungato *et al.*, 2013) SOD activity reported in rat hippocampus or mice splenocyte respectively, following sleep deprivation. The decreased catalase activity of the total sleep deprived animals in this study is in line with the reported decreased catalase activity following 5 days total sleep deprivation reported by Everson *et al.* (2005). However, the decreased catalase activity was not reversed by vitamin C treatment in the sleep deprived animals. In the general context of oxidative stress, SOD activity can increase, decrease or unchanged despite consistent increased lipid peroxidation (Szaleczky *et al.*, 1999). For instance, Lungato *et al.* (2013) reported reduced catalase activity but increased total SOD activity after sleep deprivation in rats, Ramanathan *et al.* (2002) reported decreased SOD activity with no change in glutathione peroxidase activity following prolonged sleep deprivation while Gopalakrishnan *et al.* (2004) did not find any change in all measured endogenous antioxidants after 14 days of sleep deprivation.

Majority of the epidemiological and experimental studies on sleep deprivation and glucose metabolism focused on postprandial glucose/ glucose load handling with pointers to insulin resistance and impaired glucose tolerance (Scheen *et al.*, 1996; Barf *et al.*, 2010; Mathew *et al.*, 2012; Kim *et al.*, 2016; Xu *et al.*, 2016; Nakajima *et al.*, 2017). Reports on fasting blood glucose levels of sleep deprived animals are scanty and conflicting; available literature ranged from unaltered fasting glucose level (Rechtschaffen *et al.*, 1989; Nedeltcheva *et al.*, 2009; Donga *et al.*, 2010; Wehrens *et al.*, 2010) to reduced fasting blood glucose level (Brianza-Padilla *et al.*, 2016). Our finding in the

total sleep deprived rats agrees with the latter report who finds significant hypoglycemia starting from 96 hours of sleep deprivation (Brianza-Padilla *et al.*, 2016). The observation could be due to impaired nutrient absorption (Wehrens *et al.*, 2009) despite increased caloric intake given that studies documenting no change in fasting blood glucose level also did not find any difference in food intake (Brianza-Padilla *et al.*, 2016). The decreased fasting blood glucose in the vitamin C treated groups in this study is consistent with the blood glucose lowering effect of vitamin C (Afkhami-Ardekani and Shojaoddiny-Ardekani, 2007; Dakhale *et al.*, 2011; Christie-David *et al.*, 2015; Khodaeian *et al.*, 2015; Aluwong *et al.*, 2016; Zhou *et al.*, 2016; Molz *et al.*, 2017). The precise mechanism by which vitamin C reduces blood glucose is yet to be discerned. However, Zhou *et al.* (2016) suggested that it may be mediated by inhibition of oxidative stress and insulin resistance. Oxidative stress induces insulin resistance by activation of NFκB pathway which causes impairments in IRS-1 phosphorylation and PI 3-kinase activation (Ogihara *et al.*, 2004). The inhibition of insulin resistance by vitamin C was however recently challenged by Ali et al (2018) who observed that it may predisposes insulin resistance by downregulation of the hepatic components of insulin signaling pathway including IR, p-IR, IRS-1, p-Akt, p-GSK-3β and GLUT2 following an eight months administration in normal rats. The enhancement of the hypoglycaemic effect of total sleep deprivation by vitamin C in the TSDC animals of this study was earlier observed by Adeneye and Olagunju (2008, 2009) following acetaminophen-induced hypoglycaemia wherein vitamin C treatment did not reversed hypoglycemia rather, it became more pronounced. Thus, vitamin C may not be beneficial in reversing or preventing hypoglycemia during total sleep deprivation.

Reduced plasma lactate observed in the total sleep deprived animals in this study is contrary to the reported no difference in plasma lactate level (Schmid *et al.*, 2007) and elevated cortical lactate level (Naylor *et al.*, 2102) during sleep deprivation. In the present study, there is a relationship between fasting blood glucose level and plasma lactate in the total sleep deprived animals. Hyperglycaemia is reported to actively increase tissue lactate release (Hagström *et al.*, 1990; Henry *et al.*, 1996), it therefore indicates that the reduced plasma lactate in the total sleep deprived animals in the present study is probably a reflection of their glycemic level.

Activation of glycogen synthase activity and consequence increased glycogen synthesis is a well-documented phenomenon in liver of normal, diabetic and adrenalectomized rats following glucocorticoid administration (Steiner *et al.*, 1961; Hornbrook *et al.*, 1966; De Wulf and Hers, 1967; Mersmann and Segal, 1969). Therefore, conditions characterized by increased glucocorticoid could increase hepatic glycogen content. Sleep deprivation induces elevations in corticosterone and ACTH (Mirescu *et al.*, 2006; Machando *et al.*, 2010; 2013) via activation of hypothalamic-pituitary-adrenal axis (Tobler *et al.*, 1983; Coenen and van Luijtelaar, 1985; Galvao *et al.*, 2009). Although, corticosterone level was not assessed in this study, its involvement in the observed increase hepatic glycogen level in the total sleep deprived animals of this study cannot be completely ruled out. Also, the decreased hepatic glycogen content in total sleep deprived animals treated with vitamin C could be linked to the antagonistic effect of vitamin C on stress-induced glucocorticoid release. Studies have suggested that vitamin C supplementation reduces stress-induced cortisol (Brody *et al.*, 2002) and corticosterone (McKee and Hurrison, 1995; Choi *et al.*, 2011) release via a direct adrenal 'braking effect' (Brody *et al.*, 2002). Depletion in muscle glycogen following sleep deprivation in this study is consistent with the report of Skein *et al.* (2011) on muscle glycogen depletion in athletes after 30 hours of sleep deprivation. The depletion was however not reversed by vitamin C treatment.

Furthermore, the increased hepatic glucose-6-phosphatase activity in the total sleep deprived animals observed in the present study may support our glucocorticoid hypothesis in the hepatic glycogen sparing effect of total sleep deprivation. Increased activity of glucose-6-phosphatase has been reported in the liver of normal and adrenalectomized rats (Ashmore *et al.*, 1956; Nordlie *et al.*, 1965; Garland, 1986) as well as increase in its mRNA in cultured hepatoma cells (Lange *et al.*, 1994; Schmoll *et al.*, 1996) following glucocorticoid/dexamethasone administration. The G6Pase gene promoter has potential glucocorticoid response elements which when binds at its cognate site plays an essential role in the effect of glucocorticoids via the involvement of Hepatocyte nuclear factor 1alpha (Lin *et al.*, 1998; Van Schaftingen and Gerin, 2002).

Findings from this study showed that partial sleep deprivation had no effect on glucose metabolism in the rats while total sleep deprivation caused derailment in glucose metabolism characterized by hypoglycaemia

which was not reversed by vitamin C treatment despite decline in oxidative stress.

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