NIGHT SHIFTWORK-INDUCED OBESITY, ADVERSE GLUCOSE METABOLISM AND OXIDATIVE STRESS IN MALE WISTAR RATS

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ABSTRACT

Background: Due to social, economic and work demands for a round-the-clock availability in health care and other services, night shiftwork (NSW) has become so common in developing societies. Observational studies have documented adverse health outcomes following NSW, however, interventional researches mimicking NSW are dearth.

Aim: The present study therefore aimed to evaluate the obesogenic, oxidative stress and dysglycaemic potentials of NSW in adult male Wistar rats.

Methodology: Sixteen rats (aged 8-10 weeks) were randomly assigned into control group (n=8) or NSW group (n=8) whom were sleep restricted and exposed to light at night (LAN) for six weeks. Fasting body weight and fasting blood sugar (FBS) were obtained using a digital weighing scale and Glucometer respectively. Malondialdehyde was determined using the method of Albro *et al*. (1986) and Das *et al*. (1990) whereas catalase and superoxide dismutase activities were assayed using Abebi's (1974) and Fridovich (1989) methods respectively. Data were analyzed using SPSS $V_{20.0}$ and summarized using Mean±SEM. Student's t-test was used to investigate differences between the groups and $p \leq 0.05$ was considered as statistically significant.

Results: The study findings have demonstrated that NSW increases body weight by 62% as compared to 40% in controls. Similarly, fasting blood sugar (FBS) (136 mg/dl vs. 110 mg/dl; p=0.001) and TriG index (6.1 vs. 5.6; p=0.020) were higher, while serum catalase activity is lower (p=0.003) in the NSW group than in controls.

Conclusion: The present study has demonstrated the obesogenic, diabetogenic and oxidative stress potentials of NSW in male Wistar rats.

Keywords: Night shiftwork, body weight, fasting blood sugar, TriG index, oxidative stress

INTRODUCTION

The invention of electric light bulb in 1879 by Thomas Edison has, undoubtedly, contributed to, and supported the progress of the recent world towards civilization (Potter *et al*., 2016). It has also made humans to shift their way of life away from the natural 12 hours of light and 12 hours of darkness towards a 24 hours constant light society and round-the-clock lifestyles (Fleury *et al*., 2020). Importantly, these opportunities and lifestyles coupled with societal, economic and work demands for a round-the-clock availability in health care, security, transportation, communications and other services have consequentially made work schedules irregular, often resulting to shift and/or night work (Vetter *et al*., 2016 and ouitou *et al*., 2017).

Compared to the conventional daytime-work schedule, shiftwork, particularly night shift, involves working irregularly mostly during hours when the body is assuming a physiological state of rest. Working during these irregular or unusual hours, necessitates exposure to artificial light at night (LAN), sleep disruption and disorganized circadian-time structure and physiological rhythms.

In the long run, these desynchronizations increase the risks of adverse health conditions, as consistently reported (Brown *et al*., 2009; Wang *et al*., 2014; Lin *et al*., 2015; Demir *et al*., 2016; Vetter *et al*., 2016; Wegrzyn *et al*., 2017; Vetter *et al*., 2018; Cakan & Yildiz 2020 and Hong *et al.,* 2020).

While shiftwork involving circadian disruption has, in 2007, been categorized as group 2A carcinogen by the International Agency for Research on Cancer (Touitou *et al.,* 2017 and Wegrzyn *et al.,* 2017), it appeared highly common in developed societies (Demir *et al.,* 2016), accounting for almost 75% of their workforce (Touitou *et al.,* 2017; Hong *et al.,* 2020). Data on shift work, although grossly lacking in this part of the world, but its burden is likely high, hence, with regards to its universal burden, carcinogenic and adverse health potentials, it could be regarded as an evolving pandemic requiring research interests and public health attention. Although several attempts have been made to investigate functional perturbations of shift work (Brown *et al*., 2009; Wang *et al*., 2014; Lin *et al*., 2015; Demir *et al*., 2016; Vetter *et al*., 2016; Wegrzyn *et al*., 2017; Vetter *et al*., 2018 and Cakan & Yildiz 2020) and LAN exposure (Gale *et al.,* 2011; Qian *et al.,* 2013; Potter *et al.,* 2016; Touitou *et al.,* 2017; Aisling 2018; Fleury *et al.,* 2020 and Hong *et al.,* 2020), most of the available studies were observational (Brown *et al.,* 2009; Demir *et al.,* 2016; Vetter *et al.,* 2016; Wegrzyn *et al.,* 2017; Vetter *et al.,* 2018 and Cakan & Yildiz 2020), and the interventional ones have not considered the contributions of sleep loss that is associated with night shifts in their protocols (Potter *et al.,* 2016; Touitou *et al.,* 2017; Aisling 2018; Fleury *et al.,* 2020 and Hong *et al.,* 2020).

Since nightshift work involves sleep restriction (SR) (Touitou *et al.,* 2017 and Cakan & Yildiz 2020) and LAN exposure (Vetter *et al.,* 2018 and Cakan & Yildiz 2020), and because both LAN and SR could disrupt the circadian system (Cakan & Yildiz 2020), dysregulate physiology and cause adverse health conditions (Touitou *et al.,* 2017); this study conceived designing an animal model to simulate the SR and LAN exposures encountered during NSW and to investigate its obesogenic, oxidative stress and dysglycaemic potentials in male Wistar rats.

MATERIALS AND METHODS

Experimental animals, animal groupings and research protocol: A total of 16 male Wistar rats aged between 8-10 weeks, weighing 100g±12g were purchased from the animal house of the Department of Human Physiology, Bayero University, Kano, and were housed in metallic cages measuring 38cm×46cm×24cm with saw dust beddings and kept under a room temperature of 22^0C-25^oC. The animals were randomly divided into controls (n=8) and nightshift work (n=8) groups and were allowed an acclimation period of two weeks during which they were maintained under the prevailing natural light:dark (12L:12D) conditions. For the six weeks of the intervention period, feeds and tap water were made accessible throughout the dark portion of the day. Night shiftwork was simulated by SR and exposure to LAN during the first five hours of photophase and scotophase respectively. Sleep restriction was employed using gentle handling protocol while LAN exposure was done using ≈750 lx of white light, all, as previously described (Dissi *et al.,* 2020). The research protocol was reviewed and approved by the Animal Use and Care Committee of Ahmadu Bello University, Zaria, Nigeria (ABUCAUC/2020/64).

Determination of body weight changes and feeds intake: A weighing scale (American weigh triple beam scale, model: TB-2610; Readable load of 610 g with readability and sensitivity of 0.1g) was used to obtain the fasting weight of the experimental animals on days 0, 14, 21, 28, 35, 42, 49 and 56 of the experiment. Measurements were done between 6:00 pm to 6:30 pm of the respective days, accordingly.

Weekly weight changes were deduced by subtracting weight of a particular week from the weight of a previous week, i.e, week two weight minus week one weight, week three weight minus week two weight and so on. The animals were deemed obese when they gain a total body weight of more than 40 percent (Zou *et al.,* 2017). On the other hand, Feeds were pelletized and oven dried for maximum particular cohesion and the weight of feeds intake per day was obtained by subtracting the weight of leftover pellets from the weight of provided feeds (Goh *et al.,* 2016).

Animal sacrifice, samples collection and biochemical analysis: The two groups were allowed to resume their acclimation protocols for 24 hours after which the rats were anaesthetized using an intraperitoneal injection of a cocktail of diazepam (2 mg/kg) and ketamine (20 mg/kg). Blood samples were then taken via cardiac puncture and were put in plain containers, allowed to stand at room temperature for 30 minutes before being centrifuged at 2000G for 15 minutes at room temperature using a bench top centrifuge. Using a Pasteur's pipette, the serum layers were aspirated and transferred into smaller, sterile, labeled, blank tubes and stored in a refrigerator at 0°*C* for subsequent analysis of triglycerides and oxidative stress parameters.

Biochemical analysis of samples was done at the laboratory unit of Human Physiology Department, Yusuf Maitama Sule University, Kano while fasting blood sugar (FBS) was determined using a drop of blood from the rats' tail, using a digital Glucometer and strips (Accu-Check Active® Roche Diagnostics, GMBH 68298; Germany). Fasting blood sugar levels were obtained on days 0, 14 and 56 between 5:30 pm to 6:00 pm accordingly. The test strips were inserted into the strip box of the meter which then turned on automatically. A small drop of blood was put on the top white edge of the test strip which then automatically draws the blood into the reaction cell where the reaction takes place. The blood glucose level was then read on the meter screen as a unit of milligram per deciliter (mg/dl).

Serum triglyceride (Trigs) was assessed using the protocols of Tietz (Tietz, 1990), with commercially available Randox kits and chemistry Autoanalyser (mindry Ba-88a) while TriG index was computed as Ln [TG(mg/dl) × FPG (mg/dl)/2] (Du *et al.,* 2014). Lipid peroxidation was estimated calorimetrically by measuring malondialdehyde (MDA) (Albro *et al.,* 1986; Das *et al.,* 1990) whereas serum catalase (CAT) activity was measured spectrophotometrically (Abebi, 1974) and superoxide dismutase (SOD) was determined by the method described by Fridovich (Fridovich, 1989).

Statistical Analysis

Data was analyzed using the Statistical Package for Social Sciences (IBM SPSS version 20.0). Student's t-test was used to investigate the difference between groups and data were summarized as Mean±Standard error of means (SEM). In all cases, $p \leq 0.05$ was considered as statistically significant.

Results

From the results, it was observed that night shift work model (NSW) rats had an initial slight decrease in body weight, but, by the second experimental week, they assumed a steady significant increase in body weight (62%) compared to controls (40%) as shown in table 1. This is in consistent with a significant increase in weekly feed consumption (table 2). These observations demonstrate night shift work conditions' tendency to increase feed consumption and induce obesity.

Table 1: Mean (±SEM) Body Weight Changes (in grams) of Animals over the Research Period

Variables	Controls	NSW Group	t-value	P-value
Post acclimation weight	117.38±8.56	133.75±10.68	-1.196	0.251
week 1	29.63±5.54	18.38±2.79	1.815	0.091
week 2	0.63±6.36	14.88±1.41	-2.187	0.046*
week 3	2.13±2.21	10.25±1.58	-2.994	0.010**
week 4	0.63±3.25	12.63±1.93	-3.511	0.003**
week 5	9.25±1.44	14.63±1.57	-2.527	0.024*
week 6	1.13±1.47	4.50±2.80	-1.068	0.303
Final weight	159.50±7.46	209.00±6.79	-4.907	0.001**
Total weight changes	42.13±6.02	75.25±8.56	-3.167	0.007**
Percentage weight gain (%)	39.67±9.08	61.88±11.24	-1.537	0.147

NSW= night shift work; *=statistically significant at ≤ 0.05; **=statistically significant at ≤ 0.01

Table 2: Mean (±SEM) Weekly and Total Weight (in grams) of Feeds Consumed by Animals During the Intervention Period

Variables	Controls	NSW Group	t-value	P-value
week 1	181.80±4.68	194.55±15.39	-0.793	0.441
week 2	175.75±4.05	216.95±6.49	-5.387	0.001**
week 3	168.38±4.11	216.75±8.71	-5.023	0.001**
week 4	168.75±7.68	226.38±8.74	-4.953	0.001**
week 5	166.0±10.58	203.25±5.96	-3.068	0.008**
week 6	127.13±9.12	158.75±17.34	-1.586	0.135
Total	1409.24±18.19	1626.26±26.83	-6.695	0.001**

NSW= night shift work; **=statistically significant at ≤ 0.01

Table 3 shows that although the initial FBS of the two groups appeared similar, their FBS was statistically different at the end of the intervention period. In essence, NSW rats were found to have higher FBS changes and TriG index than controls. It can also be observed that serum MDA and SOD are statistically similar between the groups. In contrast, serum catalase activity is significantly lower in the NSW group than in controls (table 3).

Table 3: Mean (±SEM) Fasting Blood Glucose, TriG index and Markers of Oxidative Stress among the Groups

Variables	Controls	NSW Group	t-value	p-value
Initial FBS (mg/dl)	96.00±4.74	103.75±3.03	-1.376	0.190
Final FBS (mg/dl)	110.13±4.03	136.25±4.46	-4.345	0.001**
FBS Changes (mg/dl)	14.13±5.47	32.50±4.21	-2.664	0.019*
TriG index	5.62±0.11	6.12±0.15	-2.617	0.020*
MDA (μmol/L)	5.15±0.98	4.64±1.34	0.305	0.765
CAT (U/L)	0.19±0.04	0.05±0.01	3.600	0.003**
SOD (U/min)	1.984±0.007	1.981±0.006	0.272	0.790

NSW= night shift work; FBS=fasting blood sugar; TriG index=triglyceride-glucose index; MDA= malondialdehyde; CAT= catalase; SOD= superoxide dismutase; *=statistically significant at ≤ 0.05; **=statistically significant at ≤ 0.01

DISCUSSION

The NSW group was concomitantly exposed to sleep restriction and LAN exposure; these experimental conditions have been found to independently alter energy balance and disturb metabolism. For example, sleep restriction has been reported to increase ghrelin production which could increase feed intake (Taheri *et al.,* 2004), and also reduce resting metabolic rate (Leonard, 2012; Spaeth *et al.,* 2015), diet-induced thermogenesis and the level of physical activity (Leonard, 2012). In a similar vein, LAN exposure is known to decrease metabolic rate (Coomans *et al.,* 2013), decrease energy expenditure (Coomans *et al.,* 2013), increase energy intake during the resting phase (Fonken *et al.,* 2010; Coomans *et al.,* 2013 and Hong *et al.,* 2020) and decrease locomotor activity (Shi *et al.,* 2013; Chalfant *et al.,* 2020 and Rumanova *et al.,* 2020). Notwithstanding these observations, this study found an initial decrease in body weight gain with an associated non-significant increase in feed consumption during the first intervention week. This could be a consequence of the acute effect of sleep restriction which is known to increase sympathetic activities (Spiegel *et al.,* 2005) energy expenditure (Everson *et al.,* 1993)' neuronal metabolism (Villafuerte *et al.,* 2015), reduce body weight gain (Hart *et al.,* 2013) and cause significant weight loss (Ho *et al.,* 2018). On the other hand, the consequential effects of increased energy intake during the resting phase (Fonken *et al.,* 2010; Coomans *et al.,* 2013 and Hong *et al.,* 2020), reduced locomotor activity (Leonard, 2012; Shi *et al.,* 2013; Chalfant *et al.,* 2020; Rumanova *et al.,* 2020), decreased metabolic rate (Leonard, 2012; Spaeth *et al.,* 2015) and energy expenditure (Coomans *et al.,* 2013) inducible by the NSW protocols, could provide a compensatory neuroendocrine, metabolic and behavioral response that increase the intake and conservation of energy, hence providing a conducive platform for weight gain over an extended time.

The present study's finding of higher total body weight gain and increased feed consumption among the NSW rats is in contrast with the findings of Dauchy and colleagues, Qian *et al*. and Chalfant *et al*., following LAN exposure (Dauchy *et al.,* 2010; Qian *et al.* 2015 and Chalfant *et al.,* 2020) as well as Robertson and colleagues following sleep restriction (Robertson *et al.,* 2013). The finding is however, in agreement with the findings of increased feed intake and increased body weight induced by LAN exposure (Coomans *et al.,* 2013 and Hong *et al.,* 2020) and sleep restriction in mice (Ho *et al.,* 2018) and humans (Spaeth *et al.,* 2015).

Glucotoxicity and lipotoxicities are critical modulators for insulin resistance, hence, the popularity of Triglycerides-Glucose (TriG) index's utility as a superior marker for insulin sensitivity (Du *et al.,* 2014). Higher values of TriG index have been widely accepted as a putative marker for insulin resistance even among normoglycemic subjects (Du *et al.,* 2014). More so, fasting hyperglycaemia is a metabolic state of impaired glucose tolerance, pre-diabetes or diabetes mellitus. Therefore, the demonstration of higher fasting blood sugar and TriG index, by this study, among the NSW models is indicative that night shift work is associated with reduced insulin sensitivity, significant fasting hyperglycaemia and thus, a diabetogenic potential. While circadian expressions of pancreatic islets is essential for proper beta-cell survival and function (Qian *et al.,* 2013), both light-at-night exposure (Dauchy *et al.,* 2010; Robertson *et al.,* 2013; Qian *et al.,* 2013; Qian *et al.,* 2015 and Rumanova *et al.,* 2020) and Sleep restriction (Reynolds *et al.,* 2012) have been reported to disturb these rhythms. The present study findings could therefore be a consequence of this, especially because phase advancement of glucose rhythm has been described as an early sign of a pre-diabetic metabolic state (Gubin *et al.,* 2017) while blood glucose dysrhythmia is a classical finding in diabetes mellitus (Gale *et al.,* 2011 and Gubin *et al.,* 2017).

In support of the present findings, sleep restriction has been shown to slow glucose metabolism (Mullington *et al.,* 2009), cause impaired glucose tolerance (Spiegel *et al.,* 2005), reduce insulin sensitivity (Reynolds *et al.,* 2012 and Robertson *et al.,* 2013) as well as cause insulin resistance (Mullington *et al.,* 2009; Zou *et al.,* 2017) and diabetes mellitus (Zizi *et al.,* 2010), whereas, exposure to LAN has been found to abolish the circadian rhythm of β-cell function and insulin sensitivity (Qian *et al.,* 2013; Qian *et al*., 2015; Opperhuizen *et al.,* 2017 and Rumanova *et al.,* 2020), accelerates β-cell apoptosis (Gale *et al.,* 2011; Qian *et al.,* 2013 and Qian *et al.,* 2015) and decrease insulin secretion (Hong *et al.,* 2020), the concomitant application of these protocols among the NSW model rats could therefore alter glucose metabolism and cause hyperglycaemia with subsequent development of diabetes mellitus over an extended time. This deduction and the present findings have equally been demonstrated by previous shift work protocols (Qian *et al.,* 2013 and Vetter *et al.,* 2018).

The connection between exposure to LAN, sleep restriction, circadian rhythm disruption and oxidative stress are overlapping and the molecular mechanisms linking them are complex and remain a subject of further research. The present study's finding of significantly lower serum catalase activity in the NSW group demonstrates a reduced oxidative stress handling capacity and increased oxidative stress potential. It has been reported that both LAN (Opperhuizen *et al.,* 2017 and Rumanova *et al.,* 2020) and Sleep restriction (Sargent *et al.,* 2016) could cause circadian desynchronization. It is also worth noting that we have previously reported significant circadian desynchronization and a substantial 7 hours phase delay among NSW model rats (Dissi *et al.,* 2020). Therefore, since circadian rhythm disruption has been noted to decrease the expression of nuclear factor erythroid-2-related factor-2 (Nrf2) and its targets ultimately resulting to increased reactive oxygen species accumulation (Lee *et al.,* 2013) and subsequent oxidative stress, the present oxidative stress finding could partly be explained by this circadian rhythm disruption previously observed (Dissi *et al.,* 2020). In addition, sleep restriction has been known to cause significant production of oxidants (Villafuerte *et al.,* 2015) and hyperglycaemia is reported to induce oxidative stress (Thanan *et al.,* 2015), therefore the observed hyperglycaemia among the NSW rats could have compounded the oxidative stress findings of this study. Notwithstanding the possible contributions of hyperglycaemia, LAN exposure has been observed to lower energy expenditure (Raap *et al.,* 2016), reduce metabolic rate (Coomans *et al.,* 2013) and subsequently reduce the production of oxidants (Villafuerte *et al.,* 2015 and Raap *et al.,* 2016). Similarly, in Wistar rats, maximal paradoxical sleep is attained during the second portion of the light period (Bodosi *et al.,* 2004), however, the animals were sleep-restricted during the first portion of the light period. Consequently, since paradoxical sleep is known to have significant antioxidative potential (Villafuerte *et al.,* 2015), its prevention is more likely to translate into more oxidative stress. This intricate relationship, between sleep restriction, LAN exposure and circadian disruption could have therefore, resulted to the current observation of reduced antioxidants but with a slight decrease in serum MDA among the NSW models. On the other hand, the present study used young (10 weeks old) experimental animals, and because, LAN induces oxidative stress in a tissue (McLay *et al.,* 2018) and age (Verma *et al.,* 2020) specific manner, it is likely that the NSW protocol could have caused more oxidative stress in other tissues than blood or if the experimental animals were older, since aged rats are known to be more susceptible to LAN induced redox imbalance (Verma *et al.,* 2020).

Nevertheless, the findings of the present study agrees with the reduced brainstem and hippocampal SOD (Ramanathan *et al.,* 2002) as well as the similar concentrations of lipid peroxidation products reported in sleep deprived Wistar rats (Gopalakrishnan *et al.,* 2004). It is also in tandem with the significant oxidative stress observed in brain homogenates (Voiculescu *et al.,* 2016), retinal tissue (Yang *et al.,* 2020) and blood tissue of rats (Verma *et al.,* 2020), as well as humans (Demir *et al.,* 2016) exposed to night shifts. In contrast to the present study findings, nonsignificant findings of oxidative stress have been reported following acute (Raap *et al.,* 2016) and chronic (Casasole *et al.,* 2017) LAN exposure among free-living nestling songbirds and female Drosophila melanogaster flies (McLay *et al.,* 2018) as well as sleep deprived Wistar rats (Gopalakrishnan *et al.,* 2004).

In conclusion, night shift work model rats exhibited an overall state of oxidative stress, hyperglycaemia, obesity and raised insulin resistance index.

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