Original Research

Effect of Sleep Restriction on Cardiometabolic and Haemoinflammatory Parameters in Adult Male Wistar Rats

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Abstract

Background and Objective: While insufficient sleep remains an under-recognized public health issue across the globe, there is paucity and heterogeneity of data regarding its cardiometabolic and haemoinflammatory implications. We, therefore, aimed to evaluate the impact of chronic sleep restriction on cardiometabolic and haemoinflammatory parameters in rats.

Materials and Methods: 16 male Wistar rats (aged 8-10 weeks) were randomly assigned into equal control or sleep restriction groups. Gentle handling was used to induce sleep restriction for six weeks. Fasting weight and blood sugar were obtained and lipids were analyzed using their respective Randox kits. Malondialdehyde (MDA) levels and catalase (CAT) and superoxide dismutase (SOD) activities were assayed. Full blood count and $CD4^+$ T cell count were determined using automated analyzer. Data were analyzed using Student's t-test, with level of significance set at $P \leq 0.05$, via SPSS software.

Results: Chronic sleep restriction caused significant initial weight loss, increase in feed consumption, and percentage increase in fasting blood sugar (FBS) (32% vs. 15%). We also noted the triglyceride-glucose (TyG) index of sleep-restricted rats to be significantly higher (6.22) than that of controls (5.62). In addition, a significant reduction in monocyte count, monocyte-lymphocyte ratio (MLR), and absolute $CD4^+$ cell count among the sleep-restricted rats was observed.

Conclusion: Our findings have provided objective evidence that, over the course of 6 weeks, 5 hours of sleep restriction had caused body weight gain, hyperglycaemia, insulin resistance, and impairment in immunoinflammatory status; hence, it could be a risk factor for developing cardiometabolic syndrome and immune-related disorders.

Keywords: Sleep deprivation; Metabolic syndrome; Blood cell count; $CD4^+$ cells

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Introduction

Humans sleep for approximately one-third of their lifetime, which upon appropriate timing, allows for molecular replenishment, body detoxification, neuronal downscaling, and memory consolidation (1). For optimal health, the National Sleep Foundation (NSF) consensus report recommends 7-9 hours of sleep for those aged 18 to 64 years (2). Compared to a few decades ago, a worldwide changing trend of electric lightening, round-the-clock lifestyles, and sustained stress has resulted into a significant reduction in our total sleep quantity, quality, and timing (3), resulting in a 10-fold increase in the risk of premature death when the average sleep is less than six hours per night (4).

While reduced sleep duration is acknowledged to be a public health epidemic and has been linked to immunoinflammatory, metabolic, and oxidative stress-associated disorders (5, 6), it has received

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non-commensurate public health attention across the globe (6). Equally, relevant literature has been sparse and findings have been quite heterogeneous. In addition, while most studies have adopted acute protocols, the chronic studies have mostly failed to typify the real world scenarios of delayed timing of sleep and its accumulated debt. The present study, therefore, was designed to simulate sleep restriction during the first 5 hours of biological night of rats with the primary aim of evaluating its impact on cardiometabolic and haemoinflammatory parameters.

Materials and Methods

Experimental animals and grouping: A total of 16 male Wistar rats aged between 8-10 weeks, weighing 100 ± 12 g were purchased from the animal house of Department of Human Physiology, Bayero University, Kano, Nigeria. The animals were randomly divided into control (n = 8) and sleep restriction (n = 8) groups and were housed in metallic cages measuring 38 × 46 × 24 cm, with sawdust beddings, under a prevailing natural room temperature of 22-25 ^{o}C.

Research protocol: In order to properly realign the animals' circadian rhythm and allow for acclimatization with group members and the research environment, two-week acclimation period was allowed. During this period, the animals were maintained on the prevailing light/dark cycles (12L:12D) and were allowed free access to feed and water during their activity period (12D). Sleep restriction was employed by means of gentle handling during the first five hours of photophase (i.e., from 6:30 am to 11:30 am) to simulate 5 hours of sleep delay in the early biological night of humans as reported earlier (**7**). Briefly, whenever a behavioral sign of sleep was observed, the rats were aroused by light touching and/or gently knocking their cage. The research protocol was reviewed and approved by Animal Use and Care Committee of Ahmadu Bello University, Zaria, Nigeria (ABUCAUC/2020/64), while the National Institute of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) were strictly adhered to.

Determination of body weight changes and feed intake: Fasting weight of the rats was determined using an American triple beam scale (Model: TB-2610, Georgia, USA). The measurements were obtained on days 0, 15, 22, 29, 36, 43, 50, and 56 of the experiment. Measurements were done between 6:00 pm and 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., w_2 - w_1, w_3 - w_2, etc.). On the other hand, feeds were pelletized and oven dried for maximum particular cohesion and the weight of feed intake per day was obtained by subtracting the weight of leftover pellets from the weight of provided feeds. Thereafter, cumulative total weekly feed intake was obtained by adding daily intakes.

Animal sacrifice, sample collection, and biochemical analysis: The research protocol lasted for 6 weeks after which, the two groups were allowed to resume acclimation protocols for 24 hours. They were later anaesthetized using an intraperitoneal injection of a cocktail of diazepam (2 mg/kg) and ketamine (20 mg/kg) as previously described (7). Blood samples were then taken via cardiac puncture and were put in an ethylenediamine tetra-acetic acid (EDTA) and plain sample bottles. Blood samples in EDTA bottles were used for blood cell count and $CD4^+$ T cells, while blood samples in the plain containers were allowed to stand at room temperature for 30 minutes before being centrifuged at 2000 g for 15 minutes at room temperature using a benchtop centrifuge. Using a Pasteur pipette, the serum layers were aspirated and transferred into smaller, sterile, labeled, and blank tubes and stored in a refrigerator at 0 ℃ for subsequent analysis of biochemical parameters.

Biochemical analysis: Biochemical analysis of samples was done at the laboratory units of Department of Haematology, Aminu Kano Teaching Hospital, Kano and Department of Human Physiology of Yusuf Maitama Sule University, Kano.

Determination of fasting blood sugar (FBS): This was done using a digital glucometer and strips (Accu-Check Active® Roche Diagnostics, GmbH D-68298, Germany) on days 0, 14, and 56 between 5:30 pm and 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. The blood was then drawn into reaction cell automatically. The blood glucose level was then read on the meter screen as a unit of milligram per deciliter (mg/dl).

Determination of lipid profile and ratios: Total cholesterol (TC), serum triglyceride (TG), and serum high-density lipoprotein (HDL) were quantified using their various Randox kits and chemistry

analyzer (Mindray BA-88A). Serum low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were computed as LDL = TC - (HDL + TG/5) and VLDL = TG/5, whereas total lipid (TL) was calculated as the sum total of all the components of the lipid profile. Triglyceride-glucose (TyG) index was computed as ln [TG (mg/dl) × fasting plasma glucose (FPG) (mg/dl)/2] (8). Cardiac risk ratio (CRR) and atherogenic index of plasma (AIP) were calculated as TC/HDL and log(TG/HDL), respectively (9). Atherogenic coefficient (AC) and Castelli's Risk Index-II (CRI-II) were determined as (TC–HDL)/HDL and LDL/HDL, respectively (9, 10).

Determination of oxidative stress biomarkers: Lipid peroxidation was estimated calorimetrically by measuring malondialdehyde (MDA) (11, 12), whereas catalase (CAT) activity was measured spectrophotometrically using Abebi's method (13) and superoxide dismutase (SOD) was determined by the method described by Fridovich (14).

Determination of haematological parameters and ratios: Full blood count was done using an Automated Hematology Analyzer (Mindray BC-10), while $CD4^+$ T cells were estimated by impedance-based flow cytometry using an automated Cyflow counter 1 (Partec, Germany, 2017). Monocyte to HDL ratio (MHR) and monocyte-lymphocyte ratio (MLR) were obtained by dividing monocyte with HDL and lymphocyte count, respectively, whereas platelet-lymphocyte ratio (PLR) was obtained by dividing platelets by lymphocyte count.

Statistical analysis: Data were analyzed using the SPSS software (version 20, IBM Corporation, Armonk, NY, USA). Student's t-test was used to investigate difference between groups and data were summarized as mean ± standard error of mean (SEM). In all cases, $P \leq 0.05$ was considered as statistically significant.

Results

Five hours of sleep restriction among the animals was observed to cause significant weight loss with an associated insignificant increase in feed consumption in the first intervention week. Subsequently, body weight of the sleep-restricted rats continued to increase until the end of the intervention. Although the higher total body weight gain of the sleep-restricted rats was not statistically significant (Figure 1), their total feed consumption at the end of the intervention period was significantly higher than that of controls (Table 1).

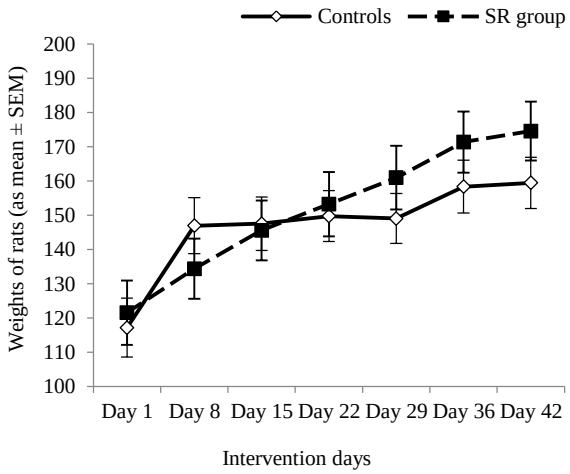

Figure 1. Comparison of body weight and body weight changes across the intervention period

While the initial FBS of the two groups was statistically similar following the 6-week intervention, sleep-restricted rats displayed a 32% increase in their FBS compared to 15% increase among the controls (Table 2). In addition, the TyG index of the sleep-restricted rats was significantly higher than that of the controls (Table 2). Interestingly, except for TG and VLDL, all other lipid parameters and ratios were statistically similar between the groups. However, the raised TG and VLDL as well as the insignificant increase in AIP and decreased HDL (Table 2) were pointers towards increasing risks of cardiovascular morbidity.

Table 1. Weekly and total weight of feeds consumed during the intervention period

Variables	Controls	Sleep-restricted group	t-value	P-value
Week 1	181.8 ± 4.7	192.0 ± 8.8	-1.022	0.324
Week 2	175.8 ± 4.1	181.5 ± 6.2	-0.778	0.449
Week 3	168.4 ± 4.1	211.0 ± 8.5	-4.503	0.001*
Week 4	168.8 ± 7.7	205.3 ± 7.0	-3.503	0.004*
Week 5	166.0 ± 10.6	178.3 ± 11.6	-0.782	0.447
Week 6	127.1 ± 9.1	146.9 ± 11.2	-1.365	0.194
Total	1409.0 ± 51.4	1519.0 ± 45.1	-4.555	0.001*

*: Statistically significant

Table 2. Comparing fasting blood sugar (FBS), lipid profile, and lipid ratios between the groups

Variables	Controls	Sleep-restricted group	t-value	P-value
Initial FBS (mg/dl)	96.00 ± 4.70	96.30 ± 2.30	-0.047	0.963
Final FBS (mg/dl)	110.10 ± 4.00	126.80 ± 4.10	-2.877	0.012*
FBS changes (mg/dl)	14.10 ± 5.50	30.50 ± 5.10	-2.181	0.047*
TyG index	5.62 ± 0.11	6.22 ± 0.17	-2.909	0.011*
TG (mg/dl)	6.04 ± 0.61	8.66 ± 1.08	-2.123	0.050*
HDL (mg/dl)	3.42 ± 0.66	2.81 ± 0.17	0.909	0.379
LDL (mg/dl)	18.74 ± 1.71	17.25 ± 2.18	0.540	0.597
VLDL (mg/dl)	1.21 ± 0.12	1.73 ± 0.22	-2.123	0.050*
TC (mg/dl)	23.37 ± 1.80	21.79 ± 2.26	0.549	0.592
TL (mg/dl)	52.79 ± 3.59	52.23 ± 4.57	0.095	0.926
CRR	7.72 ± 0.72	7.73 ± 0.61	-0.006	0.996
CRI	6.28 ± 0.66	6.11 ± 0.64	0.184	0.856
AC	6.72 ± 0.72	6.73 ± 0.61	-0.006	0.996
AIP	0.27 ± 0.11	0.46 ± 0.07	-1.488	0.159

All values are presented as mean ± standard error of mean (SEM); *: Statistically significant
FBS: Fasting blood sugar; TyG: Triglyceride-glucose; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very-low-density lipoprotein; TC: Total cholesterol; TL: Total lipid; CRR: Cardiac risk ratio; CRI: Castelli's Risk Index; AC: Atherogenic coefficient; AIP: Atherogenic index of plasma

When markers of oxidative stress and systemic inflammation were compared between the two groups, we noted the sleep-restricted rats to have a slight increase in serum MDA and decrease in both serum CAT and SOD (Table 3). This was observed to be associated with significant decreases in MLR and a slight reduction in MHR (Table 3), suggesting a reduced inflammatory status. In addition, we observed a mild reduction in granulocyte cell count and a significant reduction in percentage and absolute monocyte count as well as reduced $CD4^+$ T cell count (Table 4). These demonstrate an impaired numerical and functional immunological competence among the sleep-restricted rats.

We also noted that erythrocyte and thrombocyte cells and indices were essentially similar between the sleep-restricted rats and controls (Table 5).

Discussion

The purpose of the present study was to evaluate the impacts of chronic sleep restriction on cardiometabolic and haemoinflammatory parameters in adult male Wistar rats. Overall, this study indicated that 5 hours of sleep restriction led to an initially slow but subsequently accelerated body weight gain. This was noted to be associated with a significant increase in feed consumption. In addition, FBS, VLDL, TG, and TyG index were found to be significantly higher, while on the other hand, MLR, $CD4^+$ T cells, and monocyte count and percentage were observed to be significantly lower in the sleep-restricted group as compared to controls.

Our reported significant weight loss with an associated non-significant increase in feed consumption in the first week of our study is similar to a previous acute sleep restriction study (15), perhaps due to the reported marked increase in energy expenditure associated with acute sleep restriction (16). On the other hand, even though the difference in final body weight was not statistically significant, we observed subsequent increase in body weight gain in the sleep-restricted group across the intervention weeks. This weight increase could be explained by the observed consistent weekly increase in feed consumption among the sleep-restricted rats. Since feeding during the dark period was provided ad libitum, the animals would have likely fed in excess of their energy requirements for basal metabolism and compensation for their extended wakefulness.

Table 3. Biomarkers of oxidative stress and systemic inflammation of the groups

Variables	Controls	Sleep-restricted group	t-value	P-value
MDA (µmol/l)	5.15 ± 0.98	6.54 ± 2.60	-0.501	0.624
CAT (U/l)	0.19 ± 0.04	0.13 ± 0.02	1.432	0.174
SODa (U/minute)	1.98 ± 0.01	1.89 ± 0.09	1.063	0.306
SODi (%)	99.20 ± 0.34	94.40 ± 4.51	1.063	0.306
MHR	0.48 ± 0.06	0.36 ± 0.05	1.643	0.123
MLR	0.14 ± 0.01	0.10 ± 0.01	2.463	0.027*
PLR	44.80 ± 6.50	43.90 ± 4.00	0.117	0.909

All values are presented as mean ± standard error of mean (SEM); *: Statistically significant
MDA: Malondialdehyde; CAT: Catalase; SOD: Superoxide dismutase; a = Activity level; i = Percentage inhibition; MHR: Monocyte to high-density lipoprotein ratio; MLR: Monocyte-lymphocyte ratio; PLR: Platelet-lymphocyte ratio

Table 4. Leukocyte parameters of the groups

Variables	Controls	Sleep-restricted group	t-value	P-value
WBC ($x10^3/\mu l$)	13.91 ± 0.91	12.33 ± 1.13	1.098	0.291
Lymphocyte ($x10^3/\mu l$)	10.85 ± 0.68	10.04 ± 0.99	0.676	0.510
Monocyte ($x10^3/\mu l$)	1.45 ± 0.15	0.96 ± 0.11	2.593	0.021*
Granulocyte ($x10^3/\mu l$)	1.58 ± 0.19	1.31 ± 0.06	1.323	0.222
Lymphocyte (%)	78.50 ± 1.50	81.10 ± 0.83	-1.515	0.152
Monocyte (%)	10.40 ± 0.87	7.90 ± 0.56	2.444	0.028*
Granulocyte (%)	11.10 ± 0.95	11.10 ± 0.67	-0.043	0.966
CD4 (cells/μl)	72.00 ± 8.00	37.00 ± 6.00	3.687	0.002*

All values are presented as mean ± standard error of mean (SEM); *: Statistically significant
WBC: White blood cell

In addition, the observed weight gain may be due to the reported ability of chronic sleep restriction to promote a compensatory neuroendocrine, metabolic, and behavioral response that provides a conducive platform for weight gain over an extended period of time (17).

The use of TyG index as a putative discriminatory risk factor for insulin resistance, even among normoglycemic subjects, has recently gained popularity. Our observed increase in FBS and TyG index among the sleep-restricted rats is, therefore, a pointer towards a metabolic state of hyperglycemia and insulin resistance. This may be due to the reported tendencies of sleep restriction to slow glucose metabolism, reduce insulin secretion and sensitivity (18-20), and ultimately result to insulin resistance, hyperglycaemia, and diabetes mellitus (DM).

Higher values of atherogenic indices have been associated with adverse metabolic and cardiovascular events. Our findings of raised AIP, increased TG and VLDL as well as decreased HDL among the sleep-restricted rats, although did not show significance, have raised concern towards the increasing risks of adverse cardiovascular events associated with sleep restriction. Contrary to our findings, significantly lower HDL, high TG, LDL, TC as well as impaired lipoprotein (a) level (21, 22) have been observed following sleep restriction protocols. On the other hand, a recent systematic review and meta-analysis of 13 articles involving 83037 participants concluded that sleep duration was not associated with lower HDL, higher TG, or higher LDL (23).

In Wistar rats, maximal paradoxical sleep is attained during the second portion of the light period (24) during which, a significant antioxidative potential is achieved; hence, sleep restriction during this period would be more likely to cause oxidative stress (25). In this study, the sleep restriction of our animals in the first portion of the light period could explain the non-significant oxidative stress findings.

Although sleep deprivation has been associated with systemic inflammation (20) via reduced HDL (26), the observed reduced inflammatory status in this study could have been as a result of our finding of decreased granulocyte cell count and significantly reduced absolute and percentage monocyte cell count among the sleep-restricted rats.

Table 5. Haemato-thrombotic cell counts and indices of the groups

Variables	Controls	Sleep-restricted group	t-value	P-value
RBC ($x10^6/\mu$)	7.00 ± 0.20	7.10 ± 0.20	-0.374	0.714
HGB ((g/d)	13.80 ± 0.30	13.80 ± 0.30	0.000	> 0.999
HCT (%)	35.20 ± 0.60	35.50 ± 0.60	-0.397	0.697
MCV (μm^3)	50.40 ± 0.40	50.40 ± 0.70	0.079	0.938
MCH (pg)	19.70 ± 0.20	19.40 ± 0.20	0.883	0.392
MCHC (g/d)	39.10 ± 0.20	38.70 ± 0.20	1.497	0.157
RDW-CV (%)	17.90 ± 0.20	17.40 ± 0.40	1.105	0.288
RDW-SD (μm^3)	29.40 ± 1.90	28.70 ± 0.70	0.342	0.737
PLTC ($x10^3/\mu$)	465.00 ± 47.00	417.00 ± 17.00	0.954	0.356
MPV (μm^3)	7.60 ± 0.10	7.40 ± 0.10	0.705	0.492
PCT (%)	0.35 ± 0.04	0.31 ± 0.02	0.995	0.337
PDW (%)	19.10 ± 0.60	18.80 ± 0.80	0.256	0.802
PLCR (%)	9.80 ± 0.70	9.80 ± 0.70	0.051	0.960

All values are presented as mean ± standard error of mean (SEM)
RBC: Red blood cell; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW-CV: Red cell distribution width coefficient of variation; RDW-SD: Red cell distribution width standard deviation; PLTC: Platelet count; MPV: Mean platelet volume; PCT: Plateletcrit; PDW: Platelet distribution width; PLCR: Platelet large cell ratio

However, our observed low HDL is not statistically significant; hence, it could not have significantly affected systemic inflammatory status of the sleep-restricted rats. In essence, increased monocyte count is associated with inflammation (26); therefore, the significant reduction of both absolute and percentage monocyte counts among our sleep-restricted rats could have explained the reduced inflammatory status.

Our observation of impaired numerical and functional immunological competence among the sleep-restricted rats may not be unexpected, because chronic sleep deprivation is considered as a stress-causing factor that leads to sustained increase in glucocorticoid secretion, subsequent immunosuppression (27), and negative impacts on population diversity and function of circulating neutrophils (28). Our finding corroborates the immunosuppressive status of reduced peripheral natural killer, $CD4^+$ and $CD8^+$ T cells, reported in sleep-restricted mice (27) and agrees with the decreased levels of $CD4^+$ T cells and reduced phagocytic and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in neutrophils reported by Said et al. (29). Similarly, since $CD4^+$ T cells are required for effective memory B cell and humoral responses and have a direct role in recognizing capsular polysaccharides of encapsulated bacteria that cause pneumonia, our present finding of lower $CD4^+$ T cells supports previous reports that sleep restriction was associated with increased likelihood of developing colds, pneumonia, and poor post-vaccination antibody production and immune response (30-32).

Although poor sleep disrupts the number of bone marrow progenitor cells and their ability to form colonies (33), reduces platelet count (PLTC) (34), haematocrit (HCT), hemoglobin (Hb), and mean corpuscular hemoglobin (MCH) (35), and increases mean platelet volume (MPV), mean corpuscular volume (MCV) (34, 35), and platelets hypersensitivity (34), our findings have shown the contrary. Even though our finding has corroborated similar results (33, 36, 37), the underlying mechanism through which sleep disruption could bring about this finding is subject to further research.

In general, although the molecular mechanisms underlying the adverse health outcomes of modern society's imposed sleep disruption are poorly understood, it has been suggested that lower than 1:2 sleep to wake ratio could disrupt physiological and behavioral functions via circadian desynchronization (38). Since it is difficult to perturb sleep without affecting the circadian system (39), our overall findings could have been compounded by circadian disruption, because we have earlier reported a reduced nocturnality and a 4.5 hour phase advancement among the sleep-restricted rats (7).

Even though we did not establish a significant causal relationship for dyslipidaemia, our findings of increased weight gain, hyperglycaemia, and raised TyG index in the sleep-restricted rats provide objective evidence that delaying sleep long into the night for five hours could confer a risk for poor immune response and cardiometabolic syndrome. This is buttressed by the consistent association of metabolic syndrome with short sleep duration (6, 40, 41) and the reported reversal of some pathophysiological mechanisms of metabolic syndrome by weekend catch-up sleep (42).

Conclusion

Provided that animal to human extrapolation is allowed, our findings implicate the common attitude of delaying night sleep in developing cardiometabolic and immunocompromised related disorders.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

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