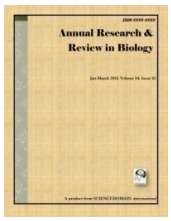

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Lipid Profile, Cortisol and Haematological Alterations in Simulated Microgravity Using the Bat Model

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Authors' contributions

This work was carried out in collaboration between the both authors. Author JOA designed the study, and interpreted the data. Author MSA anchored the field study, gathered the initial data, performed preliminary data analysis and produced the initial draft. Both authors read and approved the final manuscript

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ABSTRACT

The mode of blood lipid and cortisol expression in simulated microgravity has been poorly understood. This study determined the influence of simulated microgravity (prolonged inversion) on the level of expression of serum cortisol, lipid profile and haematological parameters in bats. Forty bats (*Eidolon helvum)*were used for this study; they were divided into groups A, B, C and D Groups A, B, C and D were exposed to zero, seven, fourteen and twenty-one days of prolonged inversion respectively. Group A served as the control group. Results of the study showed that prolonged inversion induced the elevation of cortisol and indicated that stress was involved. There was amelioration of atherogenic parameters such that total cholesterol, low density lipoprotein, trigycerides and very low density lipoprotein decreased as prolonged inversion progresses ($p< 0.05$). But high density lipoprotein increased with the progression of prolonged inversion ($p< 0.05$). Packed cell volume, haemoglobin concentration, red blood cell counts were significantly decreased with prolonged inversion ($p< 0.05$). But such parameters were partially ameliorated in the group (D)

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that underwent twenty-one days of prolonged inversion. This study concludes that simulated microgravity or prolonged inversion in bats is associated with stress, improvement of serum atherogenic indices and decline in haematological parameters, and that bats also showed adaptive responses of its haematological status in the prolonged inversion. This study could be useful in predicting health consequences of extreme environment such as space experienced by astronauts and possible countermeasures.

Keywords: Bat; inversion; head-down position; simulated microgravity; lipid profile; haematological parameters; cortisol.

1. INTRODUCTION

The microgravity condition initiates profound alteration in the biological systems, ranging from increases in intracranial pressure, fluid redistribution, fluid loss, electrolyte Imbalances, blood and immunologic compromises [1,2]. The exact role the microgravity environment plays on the stress level and serum lipid profile has been poorly understood. Although such extreme environment has been previously determined to cause red cell anaemia in humans, there are scarce literatures that have demonstrated such alterations in animal models and the probable countermeasures that could be derived. A study by Ashaolu and Ajao [3] has shown that cardiac myocytes undergo mechanical, structural and functional distortions in prolonged phase of inversion. Ashaolu et al. (in press) has also shown that the bat aortic tissue undergoes profound differential adaption in simulated microgravity. NASA has called scientists to develop more ground-based model for simulation of microgravity since there are tendencies for increased human frequency and prolonged stay in the space. Ground-based models will be useful in understanding the mechanism of adaptation of the biological systems to such conditions. This current study seeks to determine the changes in serum cortisol and lipid profile in bat ground-based model of simulated microgravity and to determine haematological responses that are accompanied.

2. MATERIALS AND METHODS

2.1 Acquisition of Animals

Forty (40) healthy bats (*Eidolon helvum*) were obtained by netting from the Bowen University Campus bat roosting colony, Iwo, Osun state, Nigeria. The bats were obtained between the periods of November to December, 2011 at which season the bats had maintained their routine daily flight and resting activities (i.e. they were not hibernating). Experimental procedures received approval of the Bowen University Wildlife Conservation Committees and Departmental Ethical Clearance Committee, which parallels those set down by the National Institute of Health for use of animal in scientific experiments. Bats breed in captivity are not suitable for this experiment. The bats were obtained irrespective of sex. They were conveyed immediately to the laboratory of the Department of Anatomy, Bowen University for commencement of experimentation. Only male bats were used for the study; this was intended to nullify mating or gestational influences on the study. Captured female bats were returned back into the bat roosting colony. The bats were kept in wooden cages at room temperature between 19ºC-26ºC and photo-periodicity of about 12 hours light and 12 hours darkness. The bats used were carefully examined to be healthy before the commencement of the experiment. The bats were fed liberally with fruits (water melon and banana) and water *ad libitum* throughout the period of the experiment.

2.2 Experimental Procedure

After capture, the bats were conveyed to the laboratory, and kept in specially designed cages, all the bats were hanging inverted on the wire gauze roof of the cage. The bats were grouped as A, B, C and D and were treated as described in the Table 1 below. The hook-like hind limbs of the bats was used in griping the wire gauze on the roof of the cage. Bats are known to rest continuously in inverted positions, and this is an inherited trait in bats.

Laboratory conditioning for prolonged inversion began at 12 noon of first day of capture. Throughout the period of the experiment, the roof of the cages to which all the bats hung their hind limbs was unopened. Movement of the bats hanging (to the roof of the cages) was also unrestricted.

Table 1. Showing experimental grouping of animals

Groups	Experimental conditioning	Sacrifice
A (n=10)	Animal underwent no laboratory inversion	Animal sacrificed at 12 noon on the first day of capture
B (n=10)	Animals underwent seven days of laboratory prolonged inversion (one week)	Animals sacrificed at 12 noon of eight day of capture
C (n= 10)	Animals underwent fourteen days of laboratory prolonged inversion (two weeks)	Animals sacrificed at 12 noon of fifteenth day of capture
D (n=10)	Animals underwent twenty-one days of laboratory prolonged inversion (three weeks)	Animal sacrificed at 12 noon of twenty-second day of capture

Foods and water were positioned on a platform closed to the roof of the cages to which the bats were hanging to limit downward creeping of the bats. The bats were intermittently observed to ensure they remained in the inverted position throughout the period of the experiment. The bats aligned their bodies parallel to the earth gravitational field except for when the bats were eating (when they flexed their trunks). Bats seldom flexed their neck against their thoracic region when resting [4]. The opening at the lower front part of the cage was the access point used in maintaining regular cleaning of the cages, whereas, another upper and frontward opening was the access point for the feeding of the animals.

2.3 Euthanasia of Animals, Blood Collection and Tissue Perfusion

The animals were euthanized with sodium pentobarbital (40 mg/kg), and were then placed on the dissecting board. A longitudinal incision was made through the mid- thoracic and mid-abdominal walls of the bats. The chondral cartilages of the thoracic cages were dissected bilaterally. Then, the thoracic and abdominal walls were reflected laterally. Blood samples were collected via cardiac puncture quickly after the animals were put to sleep. For each animal, the samples were dispensed partly into EDTA coated sample bottles and partly into Sodium heparin coated sample bottles. The EDTA preserved blood samples were used for the determination of red cell indices while the sodium heparin preserved blood samples were used for the biochemical assays.

The bats were perfused through a cannula inserted into left ventricle with the phosphate buffer solution containing 10 g/ml sodium nitroprusside and kept at 37ºC, under a pressure of 40 cm H_20 for 15min. Then the vasculature was perfused and *in situ* fixed with 250 ml of 10% formal saline. The ascending aorta was transected and removed. Tissues meant for histological studies were transferred to the specimen bottles that were prefilled with 10% formal saline.

2.4 Determination of Red Blood Cell Indices

Two milliliters of blood each were collected into heparinized sample bottles and were then analyzed for hematological parameters such as packed cell volume (PCV), hemoglobin concentration (Hb), total red blood cells (RBC) count, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), using an automatic hematological assay analyzer, Advia 60® Hematology System (Bayer Diagnostics Europe Ltd., Ireland).

2.5 Serum Cortisol Assay

The cortisol Express EIA kit (Cayman Chemical Company, USA) and appended manual instructions were used for the demonstration of serum cortisol level. Standard curve formulas provided with product manual was used to determine the actual concentration of cortisol. This assay is based on the competition between cortisol and cortisol-acetylcholinesterase conjugate (cortisol tracer) for a limited number of cortisol-specific mouse monoclonal antibody binding sites. Because the concentration of the cortisol tracer is held constant while the concentration of the cortisol varies, the amount of cortisol tracer that is able to bind to the mouse antibody will be inversely proportional to the concentration of the cortisol in the well. This mouse antibody-cortisol complex binds to the goat monoclonal anti-mouse IgG that has been previously attached to the well. The plate is washed to remove unbound reagents and then Ellman's reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm.

2.6 Determination of Serum Lipid Profile

Total Cholesterol Enzymatic Assay Kit (Xpress bio product, USA) and appended manual were used for the demonstration of total serum cholesterol concentration. High density Liporotein (HDL) and Low Density Lipoprotein concentration (LDL) were also determined by Assays kits produced by Xpress bio product, USA. Triglyceride and very low density lipoprotein levels were determined by methods described by Murherjee (1988). Very low density lipoprotein (VLDL) concentration was calculated by subtracting the addition of HDL and LDL from total cholesterol for each sample. Triglycerides concentration amount was calculated by multiplying the VLDL value by five (5).

3. RESULTS

3.1 Changes in Cortisol Level in the Experimental Groups

It is found that cortisol level increases from A (444.41±41.12), B (458±49.15), C (489±58.22) through D (846.92±42.64). But significant differences were only found between A and Dat P<0.05 (See Fig. 1).

3.2 Changes in Lipid Parameters in the Experimental Groups

As seen in Table 2 below, Groups B (87.71±7.31 mg/dL), C (43.20±3.12 mg/dL) and D (16.30±1.13 mg/dL) showed significantly reduced cholesterol level as compared with A (107.60±8.20 mg/dL) at p<0.05. Triglycerides level in B, C and D were also significantly reduced as compared with A (A; 393.90±9.07 mg/dL, B; 131.13±5.13 mg/dL, C; 63.26±4.63 mg/dL, D; 13.40±1.27 mg/dL) at p<0.05.Whereas HDL levels in B, C and D was significantly increased as compared with value in A (A; 6.02±1.82 mg/dL B; 23.18±2.73 mg/dL C; 15.94±1.12 mg/dLD; 8.51±0.42 mg/dL) at p<0.05. LDL values were reduced from group A (22.98±3.41 mg/dL), B (18.3±4.28 mg/dL),C (14.61±2.39 mg/dL)through D (5.12±0.03 mg/dL) and values in B, C, D were significantly reduced as compared to that in A at p<0.05. VLDL levels in B (26.23±1.01 mg/dL), C (12.65±2.21 mg/dL), D (2.68±0.05 mg/dL) was significantly reduced as compared with that in group A (78.61±4.90 mg/dL) at p<0.05.

3.3 Changes in Red Blood Cell Indices in the Experimental Groups

In Table 3. below, PCV values were significantly reduced in group B (27.60±1.04%)and C (23.00±2.53%) as compared to group A (33.40±2.10%) and D (35.00±2.05%) at p<0.05. PCV values were not statistically significantly different between A and D. Haemoglobin concentration was significantly reduced in group B (10.72±2.13 g/dL) and C (8.84±0.94 g/dL)as compared to group A (11.90±1.07g/dL) and D (12.64±3.66g/dL) at p<0.05. There were no statistically significant difference between A and D. RBC values in B ($1.03\pm0.62\times10^{12}$/L), C ($2.03\pm0.12\times10^{12}$/L), D ($3.59\pm0.52\times10^{12}$/L) are significantly reducedas compared with values in A ($5.59\pm1.13\times10^{12}$/L) at p<0.05. MCV values were significantly higher in groups B ($267.96\pm10.23\times10^{-15}$L) and C ($113.30\pm9.03\times10^{15}$L) as compared to groups A ($59.74\pm4.22\times10^{-15}$L) and D ($97.49\pm6.04\times10^{-15}$L at p<0.05). MCH values were significantly higher in groups B ($101.16\pm6.31\times10^{-12}$L) and C ($43.5\pm4.76\times10^{-12}$L) as compared to groups A ($21.29\pm2.33\times10^{-12}$L) at p<0.05. MCHC values in B (38.84±4.13 g/dL) and C (38.43±5.04 g/dL) were significantly higher as compared with A (35.63±3.07 g/dL) and D (36.11±1.04 g/dL) at p<0.05. There was no statistically significant difference between A and D at p<0.05.

4. DISCUSSION

This study showed that elevation of cortisol was associated with prolonged inversion in bats. Cortisol is a glucocorticoid produced by the adrenal cortex in response to adrenocorticotropic hormone [5] and its elevation typically marks stress. The finding of the current study corroborates the report by Rai and Kaur [6] which demonstrated that human 6º head-down tilt was associated with elevation of salivary cortisol. Development of physiological stress in microgravity environment can affect the health of astronauts and limit their performances in space. It is also importance for scientist working on bat laboratory conditioning to be aware of the stress that could be elicited in such state which could influence the results of their experiment. Cortisol is released in response to stress, sparing available glucose for the brain, generating new energy from stored reserves, and diverting energy from lower-priority activities in order to survive immediate threats or prepare for exertion [7]. However, prolonged cortisol secretion (which may be due to chronic stress) results in

significant physiological changes. In the hypothalamus-pituitary-adrenocortical (HPA) system, cortisol secretion is regulated by the adrenocorticotropic hormone secreted from the pituitary gland. It is probable that the HPA system was activated by the prolonged inversion [6]. The elevation of cortisol currently reported in simulated microgravity may partly explain the weakening of the immune system previously reported in microgravity, since cortisol prevents proliferation of T-cells by rendering the interleukin-2 unresponsive to interleukin-1 (IL-1), and unable to produce the T-cell growth factor [7].

Table 2. Changes in serum lipid parameters in the experimental groups

Experimental groups	A	B	C	D
Total cholesterol(mg/dL)	107.60±8.20	87.71±7.31[a d]	43.20±3.12[a d]	16.30± 1.13[a]
Triglycerides(mg/dL)	393.90±9.07	131.13±5.13[a d]	63.26±4.63[a d]	13.40±1.27[a]
HDL (mg/dL)	6.02±1.82	23.18±2.73[a d]	15.94±1.12[a d]	8.51±0.42[a]
LDL (mg/dL)	22.98±3.41	18.3±4.28[a d]	14.61±2.39[a d]	5.12±0.03[a]
VLDL(mg/dL)	78.61±4.90	26.23±1.01[a d]	12.65±2.21[a d]	2.68±0.05[a]

Values are mean ± SEM n=5 in each group, A, B, C and D indicate experimental groups exposed to zero, seven, fourteen and twenty-one days of prolonged inversion respectively, [a]statistically different from Group A (p<0.05), [d]Statistically different from Group D (p<0.05)

Table 3. Changes in red blood cell indices in the experimental groups

Experimental groups	A	B	C	D
PCV(%)	33.40±2.10	27.6±1.04[a d]	23.00±2.53[a d]	35.00±2.05
Hb(g/dL)	11.9±1.07	10.72±2.13[a d]	8.84±0.94[a d]	12.64±3.66
RBC Count ($x10^{12}$/L)	5.59±1.13	1.03±0.62[a d]	2.03±0.12[a d]	3.59±0.52[a]
MCV(10^{-15}L)	59.74±4.22	267.96 ±10.23[a d]	113.30±9.03[a d]	97.49±5.04[a]
MCH(10^{-12}L)	21.29±2.33	101.16±6.31[a d]	43.5±4.76[a d]	35.2±8.04[a]
MCHC(g/dL)	35.63±3.07	38.84±4.13[a d]	38.43±5.04[a d]	36.11±1.04[a]

Values are mean ± SEM n=5 in each group, A, B, C and D indicate experimental groups exposed to zero, seven, fourteen and twenty-one days of prolonged inversion respectively, [a] statistically different from Group A (p<0.05), [d]Statistically different from Group D (p<0.05)

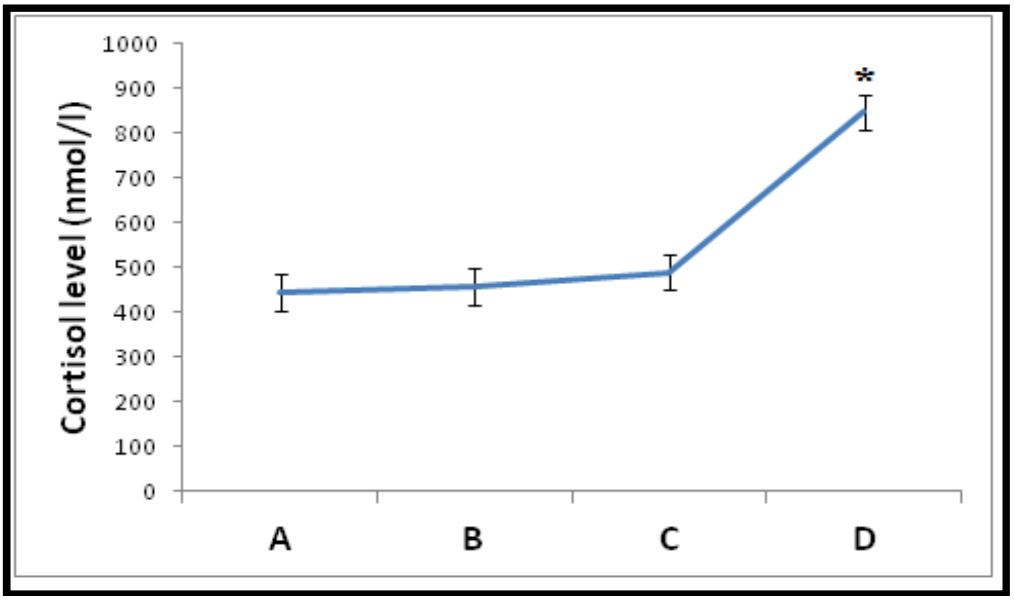

Fig. 1. Changes in cortisol levels in the experimental groups (A, B, C, D)

*Values are mean ± SEM n=5 in each group, * statistically different from Group A (p<0.05), A, B, C and D indicate experimental groups exposed to zero, seven, fourteen and twenty-one days of prolonged inversion respectively*

The reduced PCV, haemoglobin concentration and red cell count observed in the early and mid-phase of the prolonged inversion is indicative of anaemic condition in the bats. Meanwhile, previous human and animal experimental studies have established that red cell loss is associated with microgravity conditions [8]. However, the drastic amelioration of the condition in the latter phase of the experiment shows bat peculiarity in restoring its haematological status in such prolonged inversion conditioning. Splenic destruction of red blood cells has been implicated in microgravity [9], but since demineralization of bone has also been linked with microgravity condition, the tendency of reduced production of red blood cell at the bone marrow level becomes increased. Dai et al. [8] demonstrated that microgravity inhibits bone marrow stem cell proliferation and as a result may be responsible for some of the physiological changes, such as bone loss and anaemia that occur during spaceflight.

Since the very early manned missions in space, a state of anaemia associated with reduced erythropoietin levels and reduced plasma volume was disclosed [10]. The reduction in red blood cell mass is driven by a process of selective hemolysis, which has been named neocytolysis [10]. The origin of the signal leading to destruction of produced red blood cells probably is located in central circulation [10]. The reduction in red blood cell in response to prolonged inversion might be due to reduction in erythropoietin production that is regulated in the central circulation.

Increased Mean Cell Volume usually indicates spherocytic or macrocytic red blood cells. Therefore, the observed reduction is red blood cells may be due to splenic destruction, resulting from reduced maneuverability of red cell, as increased Mean Cell Volume was observed in the prolonged inverted bats. The non-reduction of Mean Cell Haemoglobin Concentration value signifies that the anaemia was not due to iron deficiency. Furthermore, the re-stabilization of red cell parameters seen in latter phase of the experiment may attenuate such anaemic condition observed in early and mid-phases of prolonged inversion.

In this study, the reduced level of LDL, VLDL, triglyceride and total cholesterol level indicate that bat prolonged inversion conditioning does not pose artherogenic cardiovascular risk. Although, paucity of information exist as regards the expression of lipid parameters in microgravity, studies by Hanpanich et al. [11] showed that under microgravity condition, cholesterol has more tendency to accumulate on the vessel wall than under normal gravity condition. LDL causes coronary heart damage by carrying 70 per cent of total cholesterol and transporting cholesterol to cardiac tissues and thus is the most potential atherogenic agent [5]. The raised HDL concentration would be beneficial and protective against coronary heart damage [5] since HDL is involved in reversed transport of cholesterol from peripheral tissues into the liver; which thus reduces the intracellular cholesterol content [5].

5. CONCLUSION

This study shows that anaemia, alleviated artherogenic cardiovascular risk and increased stress level is associated with bat prolonged inversion, and that it could serve as a suitable ground-based model for understudying the effects of microgravity condition and the countermeasures that could be developed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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