Original Research Article

Haematological Alterations in Heat-Stressed Male Wistar rats

ABSTRACT

Background
Heat stress (HS) occurs due to the global rising temperatures and the exposure of certain industrial workers to hot ambient temperatures. Physiological adaptability to heat stress involves long-term hemorheological modifications.

Objectives
The present study evaluated the effect of heat stress on haematological profile using heat-stressed Wistar rat models.

Materials and Methods
The study involved using twenty (20) male apparently healthy Wistar rats, with a weight range of 200-250g and an age range of 12-16 weeks. Before the study, the rats were acclimated for two weeks under standard animal husbandry conditions. To simulate heat stress (HS), a heating chamber was utilized and maintained at a temperature of 38±1˚C. The animals were randomly grouped into five (5), comprising five (5) animals per group. Group 1 served as the control and was not exposed to HS, while Groups 2, 3 and 4 were exposed to HS inside the heating chamber, regulated at 38±1˚C for 2, 4 and 8 hours respectively for thirty 30 days. Animals were anaesthetized by cervical dislocation and blood was collected by cardiac puncture for haematological analysis: packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC), white blood cell (WBC), MID cells percentage (MID), lymphocytes, neutrophil and platelet counts were determined using a haematology auto-analyzer. Other haematological indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), total lymphocyte count (TLC), plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW) and platelet large ratio (PCLR) were determined in line with standard formulae.

Results
Data from the study indicated a significantly raised PCV, RBC, Hb, NLR and PLR among the heat-exposed groups compared to the control ($p<0.05$). Also, mean values of WBC, TLC, lymphocytes and MPV decreased compared to the control ($p<0.05$).

Conclusion
Long-term hemorheological changes are involved in the physiological adaptation to heat stress. According to the information from the current study, it appears that HS caused a rise in the PVC, Hb RBC, NLR, and PLR levels and a decrease in WBC, TLC, lymphocytes, and MPV. The data

points to HS as a potential cause of increased blood viscosity, inflammation, and tissue damage, as well as immune system suppression and disruption of platelet synthesis and activation.

Keywords: heat stress, haematology, red blood cell, white blood cell, mean platelet volume

INTRODUCTION

In the past century, the earth's temperature has been gradually rising with strong evidence linking it to human activity, particularly the burning of fossil fuels such as coal, oil, and gas [1, 2]. The impact of global warming and rising temperature on human physiology has continued to gain traction as the world continues to experience rising temperatures due to climate change [3, 4]. Heat is a natural as well as an occupational hazard as it affects workers in various industries, particularly those who work outdoors or in hot environments [5, 6]. With the human capacity to adapt to varied climates and environmental conditions in physiological and behavioural terms, there are clear and absolute limits to the amount of heat exposure an individual can tolerate [7, 8].

Heat stress results when a change in body temperature exceeds the upper critical limits, producing a strain in the biological systems and overwhelming heat conservation/dissipation mechanisms, and affecting productivity and metabolic rate [9]. Homeothermic creatures regulate their body temperature within a narrow range and hence when heat is generated in the course of metabolic activity, a steady state is maintained by activating a heat loss mechanism to dissipate the excess heat [10, 11]. Physiological adaptability to heat stress involves behavioural or metabolic modifications in response to heat to increase the dissipation of excess body heat to the environment to negate heat load in the body [12, 13]. Thermoregulation involves a neural process that integrates external and internal information from the thermal environment to an appropriate efferent response, allowing the organism to maintain a stable internal environment relative to a variable external environment [9, 14, 15]. The general homeostatic responses to HS stress include increased core body temperature, respiration rate, water consumption and peripheral vasoconstriction [16, 17]. These responses are divided into two phases (acute and chronic) which correspond to the two stages of adaptation to heat stress. The acute response involves the activation of thermal receptors of the skin and hypothalamus which activates the autonomic nervous system leading to the release of glucocorticoids and catecholamines which modify metabolism. On the other hand, the chronic response is driven by continuous exposure to the HS causing changes in the homeorhetic and endocrine systems.

The adverse impact of heat has been fairly documented. As many intracellular molecular structures function on relatively weak interactions for stabilization, heat stress can cause significant damage cellular alterations and damage, impairing protein, DNA and RNA synthesis [18, 19]. Also, heat stress increases oxidative stress by damaging the mitochondria and increasing intracellular reactive oxygen species (ROS) leading to protein and DNA damage as well increased lipid peroxidation [20-23]. Long-term exposure to heat stress can lead to

endocrine responses involved in the release of stress hormones which can depress the thyroid hormones and hence affect energy utilization and lipid metabolism [24, 25]. Furthermore, heat increases the level of plasma cortisol which down-regulates the activity of neutrophils, depressing immunity [26]. Despite the various studies on the impact of heat stress, there is a paucity of data regarding the effect of heat stress on haematological parameters. Available data show varying and conflicting results. The present study, therefore, aims to evaluate the effect of heat stress on haematological profile using heat-stressed Wistar rat models.

MATERIALS AND METHODS

Research Animals

Twenty (20) apparently healthy male Wistar rats (200-250g, 12- 16 weeks) were sourced from the animal house of the Department of Human Physiology, University of Port Harcourt and used for the study. The animals were allowed to acclimatize for two (2) weeks before the start of the study. The animals were housed under standard animal husbandry conditions in well-ventilated, clean wooden cages with optimal conditions: 12 hr day/night cycle, temperature 28 - 30ºC, humidity 45 – 50%. The animals had access to standard rat chow and water *ad libitum*.

Heat Chamber

Heat stress (HS) was simulated using a perforated heated wooden chamber (30cm x 50cm x 25cm). The chamber was heated using a non-light heat emitter ceramic bulb (Simple Duluth xe, China), fitted with a digital thermometer (Shenzhen Brav Electronic Technologies Co., Ltd, China) and regulated using a heat switch (Popu Electric, China). The chamber was maintained at 38±1˚C.

Research Design and Heating Protocol

Twenty (20) male Wistar rats were weighed and randomly divided into four (4) groups of five (5) animals each. The animals in Group 1 served as the control and were not exposed to heat but were housed inside a heating chamber without food or water for the duration of the experiment. Groups 2, 3 and 4 served as the study groups and were exposed to HS inside the heating chamber with the temperature regulated at 38±1˚C for 2, 4 and 8 hours respectively. After each heating session, the animals were removed from the heating chamber and allowed free access to rat chow and water while they cooled passively at an ambient temperature of 26 –30°C. Daily controlled heat exposure lasted for thirty (30) days.

Laboratory Analysis

Animals were anaesthetized by cervical dislocation and blood was collected by cardiac puncture and transferred into an EDTA sample bottle for haematological assay. The packed cell volume (PCV, haemoglobin concentration (Hb), red blood cell (RBC), white blood cell (WBC), MID cells percentage (MID), lymphocytes, neutrophil and platelet counts were determined using a using a haematology auto-analyzer (Automatic Haematology Analyzer, Mindray, China). Other haematological indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), total lymphocyte count (TLC), plateletcrit (PCT), mean

platelet volume (MPV), platelet distribution width (PDW) and platelet large ratio (PCLR) were calculated in line with standard formulae [27-31].

Animals used for the study were housed and handled in compliance with standard guidelines and care of the experimental use of laboratory animals [30, 31].

Statistical Analysis

Data obtained from laboratory investigations were analyzed using IBM Statistical Product and Service Solutions (SPSS version 25). The mean and standard error of the mean of each parameter was calculated for each research group. The mean values obtained for study groups (2,3 & 4) were compared to the control (Group 1) using the analysis of variance (ANOVA) followed by a least significant difference (LSD) post hoc analysis (ANOVA). A p-value less than 0.05 ($p<0.05$) was considered statistically significant.

RESULTS

Table 1: Effect of Heat Stress on packed cell volume, haemoglobin concentration, red blood cell count and red cell indices of male Wistar rats

Parameters	Control (n=5)	2 hr HS (n=5)	4 hr HS (n=5)	8 hr HS (n=5)
Packed cell volume (%)	44.30±0.63	49.66*±1.68	48.32*±1.26	51.08*±1.11
Haemoglobin concentration (g/dl)	12.94±0.21	13.68±0.16	13.92*±0.29	14.46*±0.33
Red blood cell ($x10^{12}$/L)	7.05±0.09	8.38*±0.77	7.67±0.18	7.86±0.10
Mean corpuscular volume (fL)	62.81±0.80	60.35±3.27	62.10±0.64	64.93±0.64
Mean corpuscular haemoglobin (pg)	18.34±0.23	16.80±1.32	18.16±0.26	18.38±0.19
Mean corpuscular haemoglobin concentration (g/dL)	29.22±0.36	27.69±1.05	28.83±0.22	28.31±0.17

Result is given as mean±standard error of mean;*significantly different compared to control ($p<0.05$)

The effects of heat stress on the PVC, Hb, RBC, MCV and MCHC of male Wistar rats are shown in table 1 above. There was a significant increase in the PCV, Hb and RBC among the HS animals compared to the control ($p<0.05$).

Table 2: Effect of Heat Stress on total white blood cell count, white cell differential count and some haematological indices of male Wistar rats

Parameters	Control (n=5)	2 hr HS (n=5)	4 hr HS (n=5)	8 hr HS (n=5)
While blood cells ($x10^9$/L)	4.00±0.64	1.62*±0.27	4.12±0.80	4.04±0.98
Lymphocytes ($x10^9$/L)	3.20±0.50	1.56*±0.42	2.58±0.45	3.22±0.73
Neutrophil ($x10^9$/L)	0.36±0.10	0.22±0.02	0.28±0.07	0.52±0.12
MID cells percentage ($x10^9$/L)	0.24±0.07	0.10±0.03	0.20±0.04	0.30±0.10
Neutrophil-Lymphocyte ratio	2.7±0.58	12.15*±4.26	4.16±0.86	4.88±1.51
Platelet-Lymphocyte ratio	232.53±36.95	573.02*±173.04	277.33±49.60	245.93±44.01
Total Lymphocyte Count	339.59±51.36	134.28*±25.29	351.68±76.57	322.77±72.77

Result is given as mean±standard error of mean;*significantly different compared to control ($p<0.05$)

Table 2 shows the effect of HS on the WBC, lymphocyte, neutrophil, MID cell percentage, NLR, PLR and TLC of male Wistar rats. There was a significant reduction in WBC, lymphocytes, and TLC among the HS rats compared to the control ($p<0.05$). Conversely, the HS rats had a significantly increased NLR and PLR compared to the control ($p<0.05$).

Table 3: Effect of Heat Stress on platelet count and other platelet parameters of male Wistar rats

Parameters	Control (n=5)	2 hr HS (n=5)	4 hr HS (n=5)	8 hr HS (n=5)
Platelets ($x10^9$/L)	676.80±35.17	668.80±55.49	636.80±47.26	667.20±48.57
Plateletcrit (%)	0.58±0.24	0.74±0.24	0.51±0.04	0.54±0.04
Mean platelet volume (fL)	8.66±0.20	8.34±0.25	8.06*±0.21	8.14±0.06
Platelet distribution width (%)	12.32±0.75	11.78±0.98	10.54±0.66	11.04±0.26
Platelet large ratio	19.86±1.91	17.60±2.18	15.48±1.59	15.680.53

Result is given as mean±standard error of mean;*significantly different compared to control ($p<0.05$)

The effect of HS on PLT and other platelet indices is shown in table 3 above. Although the PLT, PCT, PDW and PCLR all showed a marginal decrease among the HS animals when compared to the control ($p>0.05$), only the MPV showed a significant reduction for 4 hr HS animals compared to the control ($p<0.05$).

DISCUSSION

Heat stress occurs due to rising global temperatures and the exposure of certain industrial workers to hot ambient temperatures [9, 14]. Physiological adaptability to heat stress involves long-term metabolic and hemorheological modifications [9, 12, 15]. The present study evaluated the effect of a 30 days-controlled HS on the haematological profile of Wistar rats. Haematological parameters serve as vital indicators of the overall health status and the functional state of the body. They also serve as physiological gauges to changes in environmental temperature, stress and diseases [32, 33]. The plasma fluid serves as a thermoregulatory medium to reduce hyperthermia with alterations in erythrocytic, leucocytic and immunologic variables [26, 34].

Data from the present study showed that animals exposed to HS had significantly raised PCV, Hb and RBC compared to the non-heat exposed control ($p<0.05$) (Table 1). The packed cell volume (PCV) is a measure of the proportion of blood cells (majorly RBCs) in whole blood relative to the plasma. Heat stress can cause dehydration which shrinks the size of the plasma, thereby elevating the PCV [35]. Furthermore, when exposed to high temperatures, the body may experience heat-induced polycythemia, a condition where the number of red blood cells (RBCs) increases as a response to heat stress [36]. Since RBCs are responsible for transporting oxygen to

the body's tissues, the body may produce more RBCs to meet the elevated demand caused by heat. Also, exposure to HS leads to the activation of the sympathetic nervous system and the subsequent release of catecholamines such as epinephrine and norepinephrine. These hormones trigger the contraction of the spleen, resulting in the release of stored red blood cells into the bloodstream. As a result, there is an increase in packed cell volume (PVC), red blood cell count, and haemoglobin concentration [35, 37]. It has also been hypothesized that the increase in PVC and Hb in response to elevated high temperature could be due to an increase in the availability of nutrients required for haemoglobin synthesis as the animals may have consumed more food due to HS [35, 38]. This increase in PCV, RBC and Hb is similar to earlier findings [33, 39, 40]. However, Srikandakumar *et al.* and Sivakumar *et al.* [41, 42] observed a decrease in PCV RBC and Hb concentration in heat-stressed goats and sheep respectively.

The study observed a significantly reduced WBC, TLC and lymphocytes among the 2 hours HS animals compared to the non-exposed control group ($p<0.05$) (Table 2). WBCs are very significant components of the immune system which help the body to fight against infections and other foreign bodies [32, 43]. Exposure to HS may have suppressed either the production of white blood cells (WBCs) in the bone marrow or their survival in circulation. This can lead to a reduction in the number of available WBCs to combat infections [40]. Furthermore, HS can lead to the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which leads to the release of cortisol. Cortisol is a stress hormone that can suppress the immune system, leading to a decrease in the number of lymphocytes [26, 33, 34, 44]. Data from the study also indicate a significantly increased neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) among HS animals compared to the control ($p<0.05$). NLR is employed as an easy and reliable marker of immune response to various infectious and non-infectious stimuli as it incorporates the innate immune response mainly due to neutrophils and adaptive immunity supported by lymphocytes [45, 46]. Elevated NLR suggests systemic inflammatory response (SIRS) due to bacterial infection, cancer, stroke, severe trauma and tissue damage [47]. HS has been shown to cause tissue damage characterized by elevated serum malondialdehyde (MDA) [13, 48]. The platelet-lymphocyte ratio (PLR) is a novel inflammatory biomarker used in the prediction of inflammation and mortality with higher values suggesting systemic inflammation, atherosclerosis and platelet activation [30, 49]. The present study hypothesizes that HS may have depressed immunity and increased systemic inflammation.

According to the study, the platelet counts and other platelet indices exhibited a slight decrease, but the reduction was not statistically significant. There was a significant reduction in the mean platelet volume (MPV) in animals subjected to 4 hours of HS compared to the control group ($p<0.05$). Mean platelet volume (MPV) is a measure of the average size of platelets in the blood. Platelets play a crucial role in blood clotting and inflammation. Dehydration and changes in blood viscosity can affect platelet size and function [50]. The MPV have been investigated in connection with inflammation and platelet activation [51, 52]. Also, HS can trigger SIRS with inflammatory mediators such as interleukin-6 (IL-6) and C-reactive protein (CRP) changing the size and shape of platelets, promoting platelet destruction or even interfering with platelet

production [53, 54]. This suggests that HS may be associated with increased SIRS and platelet dysfunction.

CONCLUSION

Physiological adaptability to heat stress involves long-term hemorheological modifications. Based on the available data from the present study, it appears that HS led to elevated levels of PVC, RBC, NLR, and PLR and a reduction in WBC, TLC, lymphocytes and MPV. The evidence suggests that HS could be responsible for increasing blood viscosity, inflammation and tissue damage, depressing immune function and disrupting the production and activation of platelets.

Ethical Approval

The research protocol and design were approved by the University of Port Harcourt Research Ethics committee.

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