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Alterations in Lipid Profile and Oxidative Stress Markers Following Heat Stress on Wistar Rats: Ameliorating Role of Vitamin C

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Abstract: Global warming and the increased frequency of heat waves have generated more interest in the effect of heat stress on human physiology. The present study evaluated the effect of 30 days of controlled heat exposure (38.1±1°C) on the lipid profile and oxidative stress markers of male Wistar rats. Twenty-five (25) male Wistar rats (3 - 4 months old, 200-250g) were used for the study and grouped into five (5) groups of five (5) rats per group. Group 1 served as the control, while groups 2 – 4 were allowed exposed to controlled heat for two (2), four (4) and eight (8) hours respectively while group 5 were allowed an 8-hour heat exposure plus oral administration of vitamin C. The result of the study indicates that heat stress caused a significant increase in serum low-density lipoprotein (LDL), total cholesterol (TC), malondialdehyde (MDA) and catalase (CAT) among the heat-exposed group compared to the control ($p<0.05$) while the serum levels of superoxide dismutase (SOD), glutathione (GPH) and glutathione peroxidase (GPx) significantly reduced compared to the non-exposed control ($P<0.05$). The result also indicates that supplementation with vitamin C positively modulated the effects of heat stress on lipid profile and oxidative stress markers. The current evidence suggests that heat stress can cause an increase in oxidative stress leading to increased lipid peroxidation and cellular damage with a possible ameliorative role by vitamin C supplementation.

Keywords: Heat Stress, Lipid Peroxidative, Lipid Profile, Oxidative Stress, Vitamin C

1. Introduction

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 [1, 2]. A hot environment imposes on the body the need to maintain physiological stability as the thermal and water pressure gradients between the body and the environment decreases, hence impairing heat exchange. Over the years, human and animal populations have acclimatized to prolonged exposure to their local high temperature in behavioural and cultural terms often taking several weeks to adapt. For example, heat stress enhances the capacity of sweat glands to secrete sweat, increase plasma volume and there is the activation of the renin-angiotensin-aldosterone

system [3]. Though there are no clear and absolute limits to heat exposure, heat stress remains a natural hazard with attendant physiological alterations leading to heat exhaustion and heat stroke [4, 5]. Heat exhaustion is a milder form of heat-related illness and occurs when the body is unable to cool itself properly in hot temperatures. They are associated with symptoms like heavy sweating, dizziness and headache while heat stroke refers to a more serious condition that occurs when the body is overwhelmed by high temperatures. A core body temperature above 39.4°C with consequences such as rapid pulse, headache, dizziness, nausea, confusion, and unconsciousness at 49° to 50° [4, 6, 7].

Oxidative stress is a condition in which the body's antioxidant defences are overwhelmed by the amount of harmful reactive oxygen species (ROS) present [3, 8-10]. Excessive levels of reactive oxygen species (ROS) can be stimulated by heat stress

resulting in a disturbance between oxidation and antioxidant defence, causing lipid peroxidation (LPO) and eventual oxidative damage to proteins and DNA [11-14]. Vitamin C (ascorbic acid) is a water-soluble vitamin with diverse functions including an essential role in hydroxylation reactions for collagen formation and carnitine synthesis as well as iron absorption due to its chemical property as a reducing and chelating agent [15, 16]. It is a potent scavenger of free radicals and destructive oxygen-derived species like singlet oxygen, hydroxyl radicals and hydrogen peroxide (H_2O_2), hence its ability to prevent or ameliorate the effects of disease states caused by oxidative stress [17-19].

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 [20, 21]. This may cause an increase in the breakdown of fats in the body, releasing fatty acids into the bloodstream. These fatty acids can then be used as an energy source by the body's cells, but in excess, they can contribute to oxidative stress and inflammation. Additionally, heat stress can also alter the expression of genes involved in lipid metabolism, leading to changes in lipid levels in the body [22, 23]. There is a paucity of data regarding the effect of heat stress on the lipid profile and oxidative stress markers of homeotherms as available data have shown varying results. The present study, therefore, aims to evaluate the effect of heat stress on lipid profile and oxidative stress markers and the possible ameliorative role of vitamin C using Wistar rat models.

2. Materials and Methods

2.1. Research Animals

Twenty-five (25), healthy, male Wistar rats, about 3-4 months old weighing 200-250g were sourced from the animal house of the Department of Human Physiology, University of Port Harcourt and used for the study. The animals were housed in well-ventilated, clean wooded cages with optimal conditions: 12 hr day/night cycle, temperature 28 - 31°C, humidity 45 – 50%. They were allowed free access to standard rat pellets and tap water. The floor of the cages was lined with sawdust and cleaned daily. The animals were allowed two (2) weeks of acclimatization before the commencement of the study.

2.2. Heat Box

A heated wooden box measuring 30cm x 50cm x 25cm with a ventilation perforated hole of 5cm x 5cm was used for the study. The box was heated by a 100W Heat Emitter ceramic bulb (Simple Deluxe, China) and fitted with a digital thermometer (Shenzhen Brav Electronic Technologies Co., Ltd, China). The heat was regulated by adjusting a heat switch (Popu Electric, China) and maintained at 38 – 39°C.

2.3. Research Design

Twenty (25) male Wistar rats were weighed and randomly divided into five (5) groups of five (5) animals each and treated as shown in table 1.

Table 1. Research design and grouping of experimental animals.

Groups	Treatment
Group 1 (control)	Housed inside a heat box with the heating light off
Group 2 (Test 1)	Two (2) hours of heat exposure, regulated at 38-39°C
Group 3 (Test 2)	Four (4) hours of heat exposure, regulated at 38-39°C
Group 4 (Test 3)	Eight (8) hours of heat exposure, regulated at 38-39°C
Group 5 (Test 4)	Eight (8) hours of heat exposure + Vit. C, regulated at 38-39°C

Controlled heat exposure lasted for thirty (30) days. Animals were returned to their standard cage and allowed food and water *ad libitum* after daily heat exposure.

2.4. Laboratory Analysis

Animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture and transferred into a dry sample bottle. The supernatant serum was carefully collected using a micropipette and stored at 2-5°C pending laboratory analysis. Total Cholesterol (TC), high-density lipoproteins (HDL) and triglycerides (TG), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase were determined by standard laboratory methods [24, 25].

2.5. Ethical Considerations

The animals were treated according to the highest ethical considerations governing animal experimentation. The research protocol and design were approved by the University of Port Harcourt Research Ethics committee (Ref: UPH/CEREMAD/REC/MM78/004).

2.6. Statistical Analysis

Data were analyzed using IBM Statistical Product and Service Solutions (SPSS) version 25. The mean and standard error of the mean were determined and the ANOVA was used to determine the difference in mean for all the parameters among the research groups followed by Fisher's Least Significant Difference (LSD). Each test group (groups 2 – 4) was compared against the control group (group 1) and (group 5). A p-value of less than 0.05 was considered statistically significant.

3. Results

Table 2 shows the effect of different durations of 30 days-controlled heat exposure on the lipid profile of male Wistar

rats. The data indicate that the mean values of total cholesterol (TC) and low-density lipoproteins were significantly higher among the animals exposed to heat for 2 hours compared to the control group (P<0.05). The animals exposed for 4 and 8 hours did not show any difference in the mean values of their lipid profile. There was no significant difference among animals exposed to 8 hours of heat plus an oral administration of vitamin C when compared to the control for serum TC, TG, HDL, LDL and VLDL.

Table2. *The effect of heat stress on the lipid profile of Wistar rats.*

Groups	TC (mg/ml)	TG (mg/ml)	HDL (mg/ml)	LDL (mg/ml)	VLDL (mg/ml)
Control	116.38±9.45	96.28±4.42	25.75±1.94	71.37±8.04	19.26±0.89
2Hrs heat exposure	172.20*±7.29	88.85±3.78	37.21±5.12	117.22*±7.25	17.77±0.75
4Hrs heat exposure	130.00±12.90	91.50±5.30	33.15±3.17	78.54±12.97	18.30±1.06
8Hrs heat exposure	119.86±9.53	97.87±11.76	27.45±2.10	72.83±12.00	19.57±2.35
8Hrs heat exposure + Vit. C	110.73±10.89	92.21±3.60	33.97±8.40	58.32±8.11	18.44±0.72

Data expressed as mean±standard error of mean. *significantly different compared to the control (p<0.05)

Table 3. *The effect of heat stress on oxidative stress markers.*

Groups	MDA (µmol/L))	CAT (U/ml)	SOD (U/ml)	GSH (mmol/L)	GPx (U/ml)
Control	5.80±0.73	4.72±0.68	16.36±8.04	36.12±1.43	0.83±0.52
2Hrs of heat exposure	14.25*±0.53	10.56*±1.59	7.04*±7.25	34.00*±1.47	0.58±0.84
4Hrs of heat exposure	12.80*±1.07	6.54±0.77	7.57*±12.97	29.45* ±2.09	0.49*±0.04
8Hrs of heat exposure	9.93*±0.77	5.88.45±1.23	7.63*±12.00	28.66* ±0.82	0.53±0.06
8Hrs of heat exposure + Vit. C	7.35±0.30	3.56±0.97	28.74*±5.11	26.02*±1.88	1.09±0.21

Data expressed as mean±standard error of mean. *significantly different compared to the control (p<0.05)

Table 3 shows the effect of different durations of 30 days-controlled heat exposure on the oxidative markers of male Wistar rats. The data indicates that there was a significant increase in mean values for malonaldehyde (MDA) among the animals exposed to 2, 4 and 8 hours of heat compared to the non-exposed control group (p<0.05). The mean values for catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) were significantly lower among the animals exposed to 2, 4 and 8 hours while the mean values for glutathione peroxidase were found to be significantly lower only among the animals exposed for 4 hours when compared to the non-exposed control P<0.05.

4. Discussion

Global warming and the increased frequency of heat waves have generated more interest in heat-related illnesses [3]. The present study evaluated the effect of 30 days of exposure to heat on the lipid profile and oxidative stress markers of male Wistar rats.

4.1. Effect on Lipid Profile

Data from the present study (Table 2) show that the animals exposed to heat for 2 hours had significantly elevated serum TC and LDL when compared to the non-exposed control group (p<0.05). No significant changes were observed in the serum TG, HDL and VLDL. The observed increased serum TC and LDL is attributable to the effect of heat stress on lipid metabolism. One of the first responses to heat stress is the activation of stress hormones such as cortisol and adrenaline with a concomitant decrease in thyroid hormones [26, 27]. Cortisol can decrease the sensitivity of insulin in the muscle and hence increase the levels of TC and LDL as observed in this study [28, 29]. Adrenaline on the hand can enhance lipolysis, causing an increase in the breakdown of fat leading to an increase in the release of free fatty acids into the bloodstream and contributing to the development of dyslipidemia. Additionally, heat stress-induced depressed thyroid function can lead to an increase in the production of lipoproteins, further contributing to the increase in lipid levels. Furthermore, heat stress has been shown to enhance hepatic *de novo* lipogenesis and promotes preadipocytes differentiation while inhibiting lipolysis, causing a release of free fatty acids into the blood [22, 23, 30]. Hence the present study hypothesizes that heat stress up-regulated the deposition of cholesterol (TC) and lipoproteins (LDL) in the blood.

4.2. Effect on Oxidative Stress

Heat stress is heavily linked with oxidative stress at the cellular level, requiring a system of oxidative enzymes to maintain healthy ROS levels to sustain cellular metabolism. The present study show (Table 3) that serum MDA and CAT significantly increased following heat exposure compared to the non-exposed control group while SOD, GSH and GPx significantly reduced among the exposed groups compared to the non-exposed controls (p<0.05). Malondialdehyde (MDA) remains the principal product of polyunsaturated fatty acid peroxidation and is a marker of cell or tissue injury level [31, 32]. Hence oxidized lipids produce MDA as a decomposition product. The observed higher MDA levels among the heat-exposed animals could be linked to the effect of heat on promoting lipid peroxidation [21, 22, 32] and tissue injury

[33, 34]. The values of serum MDA, though significantly higher among the exposed groups (Table 2), gradually reduced with the duration of heat exposure showing possible signs of adaptation of the animals to oxidative stress. MDA can impair several physiological mechanisms of the human body through its ability to react with molecules such as DNA and proteins [35]. Altan et al. [36] observed significantly elevated serum MDA among broilers exposed to 3 hours of heat at $38\pm1°C$ while Malyar et al., [37] observed higher MDA levels among heat-stressed Wistar rats. Catalase (CAT) remains the most important antioxidant enzymes present in almost all aerobic cells with the primary two hydrogen peroxide molecules into one molecule of oxygen [8]. Hence, its primary role is in the regulation of the cellular level of hydrogen peroxide, thereby protecting the cells from oxidative assault [38, 39]. The present study shows that the animals exposed to heat for 2 hours had significantly increased CAT levels compared to the non-exposed controls ($p<0.05$). This could be due to the effect of heat which possibly triggered the activity of CAT. An increase in CAT levels is often seen as a response to increased levels of ROS generated during heat stress. This increase in CAT activity helps to reduce the levels of ROS and prevent oxidative damage to cellular components. Serum catalase levels were found to be highest among the 2-hour exposed group and gradually reduced with the increase in the duration of exposure. Similar to the findings from the present study, Lin et al., [40] and Shamuni et al., [41] observed higher CAT levels for heat-stressed broilers and Wistar rats respectively. Superoxide dismutase (SOD) is the first detoxification enzyme and the most powerful antioxidant in the cell against ROS. It scavenges harmful superoxide radicals, converting them to hydrogen peroxide and molecular oxygen [42]. A depression of this important antioxidant enzyme as observed among the heat-exposed animals compared to the non-exposed control group ($p<0.05$) further shows heat stress can alter the expression and activity of SOD, leading to a decrease in its levels. This reduction in SOD levels can increase the risk of oxidative damage in cells and tissues, and potentially contribute to heat stress-related health problems. However, Lin et al. [12] and Altan et al. [36] observed higher SOD levels among heat-stressed broilers while Shamuni et al. [41] observed no changes in SOD among heat-stressed rats. Glutathione (GSH) and glutathione peroxidase (GPx) are important antioxidant enzymes protecting cells from ROS and reactive nitrogen species (RNS). While GSH is involved in diverse reactions in response to oxidative stress, GPx uses GSH to neutralize hydrogen peroxide to water [43, 44]. The reduced levels of GSH and GPx among the heat-stressed animals suggest that heat exposure increased the demand for antioxidant protection leading to a decrease in the levels of GSH and GPx. Altan et al. [36] observed higher GPx levels among heat-stressed broilers while Shamuni et al. [45] observed no changes in GPx among heat-stressed rats while Dehghan et al. [46] noticed a reduction in GSH levels among heat-stressed rams.

4.3. Ameliorative Role of Vitamin C

Vitamin C (or ascorbic acid) is a naturally occurring organic antioxidant found in both plants and animals. It serves as a redox buffer, reducing and neutralizing ROS. It is also a co-factor in the regeneration of other antioxidants [15, 18]. The present study shows that animals exposed to heat for 8hr with a concomitant administration of vitamin C did not show any significant difference in the mean values of TC, TG, HDL, LDL and VLDL when compared with the non-exposed group (Table 2). This is suggestive of the ability of vitamin C to maintain normal lipid metabolism despite the presence of heat stress [14, 47]. This could be attributed to the antioxidant role of vitamin C which has been shown to prevent oxidative changes to LDL [48, 49]. When LDL is oxidized, it becomes a target for scavenger receptors, which incorporate it into plaques [50, 51]. Hence, the inhibition of LDL oxidation by vitamin C may prevent atherosclerosis. Vitamin C is also known to improve liver function which leads to a reduction in the production of cholesterol [52, 53]. Also, it is known to improve endothelial function which may aid in lipid metabolism [54]. The study also observed that vitamin C supplementation improved serum levels of oxidative stress marker, MDA as there were no significant changes between the control and the animals that there were exposed to heat stress and vitamin C. Also, Vitamin C appeared to improve the oxidative stress enzymes (CAT, SOD and GPx), significantly increasing the mean serum SOD levels among the heat exposed/vitamin C-supplemented rats compared to the non-exposed control ($p<0.05$) while maintaining the levels of serum CAT and GPx in the presence of heat stress (Table 3).

5. Conclusion

The Increasing heat waves due to global warming have necessitated several studies on the effect of heat stress on the physiology of homeotherms. The current evidence suggests that heat stress can cause an increase in oxidative stress leading to increased lipid peroxidation and cellular damage. The present study indicates that vitamin C supplementation was able to ameliorate the effect of heat stress on Wistar rat heat-exposed rat models.

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