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Profile of Biochemical and Oxidative Stress Markers in Male Athletes Following Strenuous Exercise Session

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Authors' contributions

This work was carried out in collaboration among all authors. Author KUN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ANC managed the analyses of the study and author FSAT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

With the increasing number of people who participate in one form of exercise or the other, believing that it improves health and enhances professional sporting career, the question of its health benefit can only be answered with the knowledge of the metabolic changes that occur during exercise. This study therefore, was aimed at investigating the implications of physical exercise on some metabolic biomarkers, and to identify the changes that occur in subjects who undergo strenuous regular physical exercise in the tropics. Blood samples were collected from 86 male athletes and 100 male non-athletes aged between 22 and 30 years following established protocols. The athletes were at the peak of their training in preparation for a major sporting event and the blood samples were collected at rest and immediately after strenuous exercise sessions; while blood samples from the non-athletes were collected only at rest and served as control. The samples were prepared and used for the evaluation of some metabolic and oxidative stress markers. All experimental evaluations were done following well established methods. Result showed that serum

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concentrations of creatinine, urea, bilirubin, testosterone and cortisol all significantly increased in athletes after strenuous exercise. However, there was no statistical significant change in these biomarkers between athletes at rest and non-athletes. Also except glutathione reductase which significantly decreased in athletes after exercise, other oxidative stress markers assayed significantly increased in athletes after exercise; there was also no significant change between athletes at rest and non-athletes. From the result, this study concludes that strenuous exercise may increase oxidative stress in the cells, cause hemolysis or muscle damage and subsequent myoglobin degradation.

Keywords: Oxidative stress; cortisol; bilirubin; exercise.

1. INTRODUCTION

How the body responds to intense demands placed on it by both chronic and acute physical activity has been the interest of exercise physiologist in recent times. In dealing with this, the knowledge of biochemical control mechanisms provides an explanation of much of the well-established physiology involved. Even though the concentration of several biochemical biomarkers in the body are used in assessing a number of human disorders [1]; their values in physically active subjects could also be used to study adaptations to training or changes that have occurred during and following strenuous exercise, rather than the presence of a given disease.

Exercise is known to increase basal energy demands and, hence, basal metabolic rate [1,2,3]; which may in turn cause fluctuations in the serum concentrations of several biochemical components of the body. There are variations in biological biomarkers after exercise and this has been linked to the type, duration and intensity of exercise performed. Studies have shown that middle and long-term strenuous exercises trigger transient elevations of muscular and cardiac biomarkers like cardiac troponins (Tn), natriuretic peptides, neutrophil gelatinase associated lipocalin (NGAL) alanine aminotransferase (ALT), creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LD) [4-12]. In a small study on adolescents, more than half of the participants developed Tn increase after basketball training [9]. The same has been reported after a treadmill test [10]. These elevated values could precipitate into acute myocardial infarction, and they are neither related to athlete's age, nor to the presence of cardiovascular risk factors. A study has shown that samples from trained marathoners obtained before a race had CK-MB (11%) and ALT (5%) exceeding the reference range [11]. An acute bulk of aerobic physical exercise also produced significant changes in the activity of biomarkers of liver injury such as gamma-glutamyl transpeptidase (GGT), AST, LD, CK [12]. In another study involving a rugby match lasting 10 minutes, only LD increased after the match; while CK, AST, and ALT remained basically unchanged [13]. On the other hand, other studies showed that maximal physical exercise induces transient elevation of muscle and hepatic enzymes [14]. When exercise loading exceeds the limit of muscle ability, CK and LD leak into the interstitial fluid and are released into circulation [5].

Generally, there is increasing number of people worldwide who participate in one form of exercise or the other, either with the belief that it improves health or for professional sporting reasons. However, the question of its health benefit can only be answered with the knowledge of the metabolic changes that occur during exercise. These metabolic changes can be reflected on the serum concentration of biomarkers. Hence, the variability of these biomarkers in non-Caucasian athletes before and after exercise were investigated in this study. Therefore, this study is aimed at investigating the implications of physical exercise on variability of some biomarkers, and to identify the changes that occur in subjects who undergo regular physical activity following strenuous exercise in the tropics.

2. METHODOLOGY

2.1 Experimental Design

With ethical approval from the University of Port Harcourt ethical committee and with signed informed consent, 86 male athletes and 100 male non-athletes aged between 22 and 30 years were recruited in this study. The athletes were drawn from University of Port Harcourt sports contingents who were at the peak of their training, in preparation for the West African University games (WAUG) held at the University of Port Harcourt between October and November, 2018. The athletes have been in the

profession for not less than two years; and were at the peak of their training, in preparation for the West African University games (WAUG) held at the University of Port Harcourt between October and November, 2018. Exercise sessions were not less than 3 days per week for a minimum of 60 minutes per session as recommended by the Physical Activity Guidelines for Americans [15]. Subjects also completed a health history, drug usage, and physical activity questionnaires to determine eligibility and were encouraged to avoid smoking, drinking alcohol or use of tobacco products during this period of training. We employed anonymity in which case, the subject's names were codified in the study. All subject recruitment and data collection were completed over a 1-month period of similar environmental conditions.

2.2 Collection of Blood Sample

Following established protocols [16], 5 mL of venous blood sample was taken through the antecubital veins of athletes using sterile syringes before exercise; between the hours of 7 am-8 am and immediately after exercise between 10-11am. Also, following the same protocols, 5 mL of venous blood was taken from non-athletes. The blood samples taken were dispensed in Ethylenediaminetetraacetic Acid (EDTA) and stored at -4°C pending analysis of desired variables.

2.3 Measurement of Experimental Parameters

Urea and creatinine were measured by the colorimetric method with reagents from RANDOX laboratories limited UK. and on RX Monza analyzer following instructions of the manufacturers. Cortisol and testosterone assays were done using the microplate enzyme-linked immunosorbent assay (ELISA) method with reagents from monoband Inc. USA and also following the manufacturer's instructions and the absorbance was measured on a microtiter plate reader (SM-300A, SURGIFIELD MEDICAL, ENGLAND). Bilirubin was determined by the spectrophotometry method.

The method used in the measurement of the serum concentration of malondialdehydedy (MDA) was based on the principle of the ability of MDA produced from peroxidation of fatty acid membranes to react with 2-thiobarbaturic acid (TBA) to give a pink coloured complex with maximum absorbance at 532 nm. [17]. Glutathione (GSH) in serum was estimated based on the principle of the development of a stable yellow colour when 5,5'-dithiobis-2-nitrobenzoic acid is added to sulfhydryl compounds. The product of this reaction (2-nitro-5-thiobenzoic acid) has an absorption of 412 nm [18].

A modified method of Clairborne in which catalase (CATA) in a sample splits hydrogen peroxide, which can be measured using by spectrophotometry at 240 nm and was used for the estimation of catalase activity [19]. The principle is based on the fact that one unit of catalase activity equals the amount of protein that converts 1 umol H_2O_2/min.

The principle of superoxide dismutase (SOD) estimation was based on the ability of SOD to inhibit the autoxidation of epinephrine at pH of 10.2, taking note of the fact that superoxide radicals are generated by xanthine oxidase reaction as a result of the oxidation of epinephrine to adrenochrome [20].

The estimation of Glutathion-s-tranferase (GST) followed the fact that GST has high activity with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. When this substrate is conjugated with reduced glutathione, its maximum absorption moves to a longer wavelength of 340 nm in the spectrometer. This can therefore provide a direct measurement of the enzymatic reaction [21].

The assay method for glutathione peroxidase (GPx) is based on the measurement of the residual remaining glutathione after the action of glutathione peroxidase. The absorbance is determining at 412 nm in the spectrometer [22].

2.4 Statistical Analysis

At the end of the study, data collated on the selected variables were statistically analysed using statistical package and service solution (SPSS, version 20.0). The mean of descriptive statistics was recorded as mean± standard deviation**.** Comparison of parameters between athletes and non-athletes were done using paired t-test with the level of statistical significance accepted at $p<0.05$**.** Multiple comparison between non-athletes, athletes before exercise and athletes after was done using post-hoc multiple comparison test (LSD). Percentage change was calculated by subtracting values before exercise from values after exercise, divide by values after exercise multiply by 100. This calculation was done using Microsoft excel 2016 version.

3. RESULTS

Presented in the tables and figures below are the results of this study. Table 1 showed the effect of exercise serum concentrations of cortisol, testosterone, bilirubin, urea and creatinine in athletes at rest and after exercise; and in non-athletes at rest while Table 2 showed the effect of exercise on serum concentrations oxidative stress markers in athletes at rest and after exercise; and in non-athletes at rest. Fig. 1 showed the percentage changes in biochemical parameters in male athletes after exercise while Fig. 2 showed the Percentage changes in oxidative stress markers in male athletes after exercise.

4. DISCUSSION

4.1 Exercise and Some Metabolic Markers

The metabolic biomarkers assayed in this study were cortisol, testosterone, bilirubin, urea and creatinine.

Table 1. Effect of exercise on some biochemical parameters

Variables	non-athletes (n=100)	athletes before exercise (n=86)	athletes after exercise (n=86)
cortisol(µg/dL)	16.92±0.46	18.01±0.82	26.16±0.83*
Testosterone (µg/dL)	4.03±0.40	3.52±0.38	8.64±1.15*
bilirubin(µmol/L)	12.86±0.39	12.81±0.94	21.31±1.68*
Urea(mmol/L)	3.29±0.36	3.10±0.36	4.88±0.58*
Creatinine(µmol/L)	115.15±7.03	123.30±5.24	158.66±4.22*

*Results presented as mean±standard deviation (M±SD); * shows that there was a statistically significant change at p<0.05 when compared with non-athletes, n= sample size*

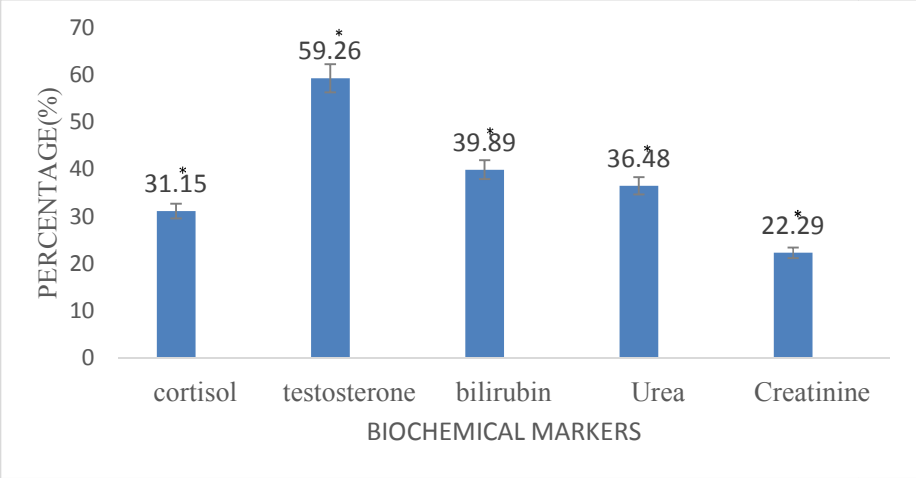

Fig. 1. Percentage changes in biochemical parameters in male athletes after exercise
** shows that there was a statistically significant increase at p≤0.05*

Table 2. Effect of exercise on oxidative stress markers

Variables	NON-athletes (n=100)	athletes before exercise (n=86)	athletes after exercise(n=86)
SOD (U/ML)	0.17±0.04	0.19±0.04	0.60±0.03*
MDA(µmol/L)	0.28±0.03	0.36±0.04	0.77±0.06*
CATA (U/g)	2.63±0.36	2.93±0.35	4.38±0.32*
GSH(ug/ml)	1.95±0.27	2.57±0.25*	1.89±0.32*
GPX (ug/ml)	0.13±0.02	0.13±0.01	0.77±0.07*
GST (ug/ml)	0.23±0.02	0.24±0.03	0.60±0.04*

*Results presented as mean±standard deviation (M±SD); significant value (p-value) set at <0.05. * shows that there was a statistically significant change at p=0.05 when compared with non-athletes, n= sample size*

Cortisol is an important regulatory glucocorticoid secreted by the adrenal cortex, it increases blood glucose concentration when released [23]. In this study, serum concentration of cortisol significantly increased in athletes from 18.01ug/dl at rest to 26.16 ug/dl after exercise (Table 1) representing about 31% (Fig. 1) increase and agreeing with the result of a study that has reported that physical or mental stress can lead within minutes to greatly enhanced secretion of ACTH and consequently cortisol as well, often increasing cortisol secretion as much as 20-fold [24]. It has also been reported that plasma cortisol increased after acute exercise which exceeded 60% of the VO2max, and also after intense resistance exercise [25]. The use of cortisol measurement as an indicator of physical stress has been demonstrated by the rapid and strong adrenocortical secretory responses after trauma. Physical exercise induces oxidative stress to the body as seen in the elevated oxidative stress markers in this study (Table 1). Physical training exercise challenges the body and the body's responses to challenging stimulus triggers a series of physiological reactions that has been shown to be mediated by the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) system [24]. In particular, the HPA axis responds to a stimulus by releasing hormones the corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) [26]. These hormones are directly associated with the mobilization of energy reserves to face an imminent or expected challenges [24]. This may therefore explain the statistically significant increase in the serum concentration of cortisol seen in male athletes after exercise

Testosterone (T) is an anabolic hormone, promoting protein synthesis and therefore is used as an indicator of anabolic processes in the body. However, it has been reported that during exhaustive exercise, the concentration of this hormone decreases [27]. Contrary to this report, this study observed a significant increase in the serum concentration of T in male athletes after exercise; increasing from 3.52 ug/dl before exercise to 8.64 ug/dl after exercise (Table 1); this represents 58% (Fig. 1) increase after exercise; agreeing with a study that showed that transient elevations in T may be important for hypertrophy and strength adaptations [28], as well as the psychological preparedness of an athlete for competition [29]. As an anabolic hormone, testosterone (T) can trigger the activation of anabolic processes including increases in transcription, translation, signaling-enzymes, and structural proteins [28]. Also, T has been reported to stimulate transient increases in intracellular calcium [30], which may temporarily increase force production [31] and enable the utilization of greater training intensity and volume-loads. Additionally, elevations in T have been positively associated with competitive anxiety/anticipation, motivation, and aggression prior to and during a competitive event, all of which may also affect the post-exercise response [29]. The significant increase in serum concentration of T seen in this study may be as a result of the adrenal cortex and gonads responding similarly to the physical and psychological elements of competition via the hypothalamic-pituitary-adrenal axis.

For serum concentration of bilirubin, it significantly increased from 12. 81uml/L to 21.31umol/L, representing about 40% increase in male athletes after exercise; agreeing with the report of a similar study [32]. Bilirubin is the end-product of hemoglobin catabolism; therefore, its significant increase in serum as seen in this study may indicates a certain degree of hemolysis, owing to mechanical trauma, but also oxidative injuries of the erythrocytes, as reported by Szygula, [33,34]. On the other hand, it may be as a result of increased myoglobin levels, due to muscle damage, and subsequent myoglobin degradation. Increased levels of bilirubin detected in the present study could suggest hemolysis and release of free iron in the blood. When red blood cells are hemolysed and their haemoglobin released, the hemoglobin is phagocytized by macrophages especially by the Kupffer cells of the liver. Within a few hours, the macrophages release iron from the hemoglobin and pass it back into the blood, to be carried by transferrin either to the bone marrow for the production of new red blood cells or to the liver and other tissues for storage in the form of ferritin [35]. Iron is essential for normal cell growth and proliferation. However, excess iron occasioned by increased hemolysis might be potentially harmful, as it can catalyze the formation of toxic reactive oxygen species [32].

For urea, the concentration in serum significantly increased in male athletes after exercise, from 3.1 mmol/l to 4.88 mmol/l (40% increase) after exercise. However, there was no statically change between athletes at rest and non-athletes. The significant increase in the serum concentration of urea seen this study suggests that during exercise there is massive breakdown of protein for energy generation.

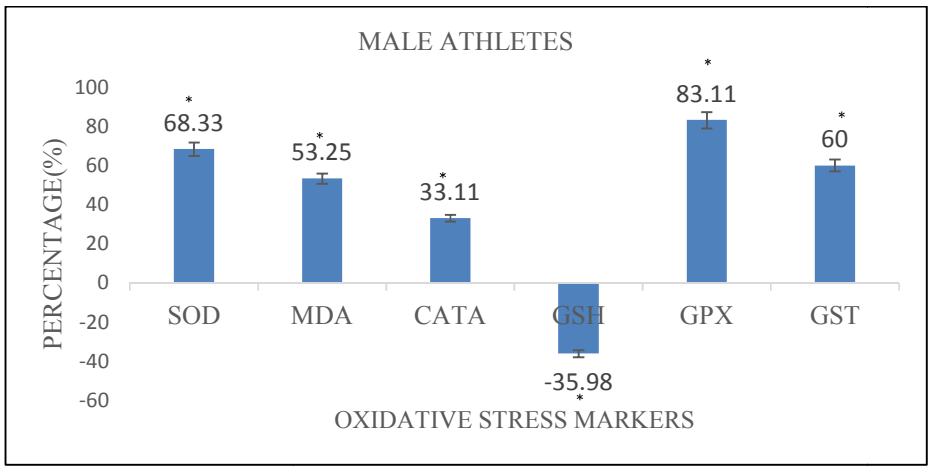

Fig. 2. Percentage changes in oxidative stress markers in male athletes after exercise
** shows that there was a statistically significant change at p≤0.05*

Creatinine (Cre) is produced from the decomposition of creatine, a nitrogen compound used by muscle cells to store energy. The serum concentration of creatinine varies according to creatine synthesis and the amount of muscle tissue [36]. In this study, creatinine followed a similar trend with the bilirubin; increasing to about 26% in athletes after exercise. However, there were no significant changes in its serum concentration when compared between athletes

at rest and non-athletes. Previous studies reported lower serum Cre in cyclists, Nordic skiers and swimmers [37,38] and higher serum Cre levels in soccer and rugby players than those observed in controls [32]. The elevated serum concentration of creatinine seen in this study may be linked to the generally higher muscle mass in athletes, because total muscle mass is the most important determinant of the creatine pool size and creatinine production [39]. Previous studies have demonstrated a significant correlation between the Cre concentration and BMI [31,37].

4.2 Exercise and Oxidative Stress Markers

In normal physiological condition, the generation of reactive oxygen species (ROS) are is tightly regulated by different antioxidants, but overproduction of ROS results in oxidative stress, which is important mediator of damage to cell structures and DNA [40]. The human system is endowed with a cascade of ROS scavengers that ultimately neutralize the effects of the free radicals to the cells. The free radical scavengers include superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CATA), glutathione (GSH), glutathione peroxidase (GPX) and glutathione-s-transferase (GST); and their serum concentration are usually used in determining the level of stress in the cells.

GSH is the most important antioxidant in the human body and its quantification is a popular technique to assess oxidative stress [41]. The result of this study showed that GSH is statistically significantly higher in male athletes at rest than male non-athletes. After exercise, GSH significantly decreased in male athletes compared to their values at rest (Table 2). The reduction in GSH may be explained by its consumption either to regenerate ascorbic acid and alpha tocopherol or to scavenge certain ROS; this decrease in GSH conforms with a similar study which reported a decrease in GSH immediately following both cycling and squatting [42]. Also, serum concentration of catalase (CAT) significantly increased in athletes after exercise; meanwhile, there was no statistically significant change between athletes at rest and non-athletes; disagreeing with a study which reported a 20% decrease in erythrocyte CAT activities at the end of submaximal exercise in trained cyclists [43]; but agrees with a study which reported a 33% increase in catalase activities after an exercise session in athletes [44]. The significant increase in CAT activity after exercise as seen in this study may be attributed to an increase in anti-oxidative activity

owing to increase in the generation of ROS as a result of exercise induced oxidative stress to the cells. Furthermore, glutathione peroxidase (GPx) significantly increased in male athletes after exercise. However, there was no significant change between athletes at rest and non-athletes. There are reports that GPx activity increases significantly with increasing of peroxides [45,46,47]. The increased GPx activity after exercise seen in this study may be as a result of increased production of free radicals and may be interpreted as a marker of oxidative stress also in response to exercise.

In this study also, SOD was seen to significantly increased by 68.3% in male athletes after exercise. At rest, serum concentration of SOD in male athletes was statistically unchanged compared to male non-athletes. Glutathione-S-transferase (GST) which catalyzes the reaction between the –SH group and potential alkylating agents to render them suitable for transport out of the cell statistically increased in athletes after exercise; and MDA which is a major peroxidation product derived from polyunsaturated fatty acids also statistically increased in athletes after exercise. The measurement of these markers is used as index of structural oxidative injury of the cell membrane. Result also showed that there was no statically significant change the serum concentration of these enzymes in athletes at rest when compare to non-athletes (Table 2). The post exercise elevation of MDA in athletes is in tandem with the studies that reported increases in MDA and thiobarbituric acid reactive substances (FBARS) immediately post-aerobic exercise [48,49]. This therefore suggests that exercise may increase lipid peroxidation and damage cell membranes. Damaged cell membranes may disrupt fluidity and permeability which may cause massive leakage of SOD and hence, the increased in serum concentration of SOD.

5. CONCLUSION

From the results of this study it could be inferred that while regular exercise training is associated with numerous health benefits, it can be viewed as an intense physical stressor leading to increased oxidative cellular damage, most likely due to enhanced production of ROS. We conclude that the observed significant increase in T and C immediately after exercise may be as a result of the adrenal cortex responding to physical and psychological elements of competition via the H-P-A axis. The study also concludes that exercise may cause hemolysis or muscle damage and subsequent myoglobin degradation; this is evidence in the significant increase in serum bilirubin concentration. This study further concludes that strenuous exercise increases oxidative stress and may disrupt endogenous free radical scavenging capability of the body and enhance production of ROS, leading to increased oxidative cellular damage, which will in turn further excite the H-P-A axis and increase the secretion of cortisol.

Finally, from the result of this study, strenuous exercise may cause mobilization of muscle proteins and creatine for energy, hence, the observed significant increase in the serum concentration of urea and creatinine.

CONSENT AND ETHICAL APPROVAL

With ethical approval from the University of Port Harcourt ethical committee and with signed informed consent, 86 male athletes and 100 male non-athletes aged between 22 and 30 years were recruited in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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