AN ASSESSMENT OF CARDIOVASCULAR AND BIOCHEMICAL PARAMETERS IN AIRCREW WITH PROLONGED EXPOSURE TO HIGH ALTITUDE: NIGERIA AS A CASE STUDY

A THESIS SUBMITTED TO THE FACULTY OF HEALTH AND WELLNESS SCIENCES, CAPE PENINSULA UNIVERSITY OF TECHNOLOGY, BELLEVILLE, SOUTH AFRICA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE: DOCTOR OF PHILOSOPHY IN BIOMEDICAL SCIENCES

IN THE

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BY

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DECLARATION

I, Abiola Mahroof Adekilekun, declare that the content of this thesis represents my own work, and that the thesis has not been previously submitted for any academic qualification or examination. Furthermore, it represents my own opinion and not necessarily those of the Cape Peninsula University of Technology.

…….. 5th July, 2024

Signed Date

ABSTRACT

Many factors, including tourism, migration, and business, are causing an exponential increase in the number of individuals exposed to high altitudes worldwide. About 100 million lowlanders travel to mountainous areas above 2500 m yearly, while roughly 400 million people are residents in areas above 1500 m. While highlanders suffer from the harmful impacts of high altitude, lowlanders who visit high altitudes also encounter similar disadvantages. There is evidence linking high altitude to hypoxia, hypobaria, and hypothermia. Despite conflicting information regarding the effects of high-altitude exposure on renal and hepatic damage, dyslipidemia, and insulin resistance, studies illustrating the impact of high altitude on physiological systems are available. Thus, this study aims to investigate how exposure to high altitude and associated pathways affect insulin sensitivity, gluco-lipid regulation, and renal and hepatic function in aircrew members of local airlines (PHA-L) and international airlines (PHA-I) who have been traveling for at least five years.

The cross-sectional study consists of a total of 300 aviation workers assigned to 3 groups (n=100) subjects per group with a sample selection carried out using the random sampling method. An open-ended structured questionnaire was administered to the participants prior to sample collection to obtain socio demographic data. Anthropometric data including weight, height, blood pressure, waist and hip circumference, as well as biochemical parameters such as lipid profile, kidney function, and inflammatory and oxidative stress markers were determined. Participants were fasted overnight for 12 hours and then blood samples were obtained from them before analysis.

The first stage of this research investigated if lowlanders who have traveled to high altitudes were more likely to develop obesity and atherogenic indices because of their high altitudes exposure. Exposure to high altitude markedly increased the incidence of obesity independent of

the dietary patterns of the subjects when comparison with the control. More specifically, when compared to the control, exposure to high altitude increased the levels of serum total cholesterol, triglycerides, and low-density lipoprotein but decreased the amount of high-density lipoprotein. TNF-α and IL-1β levels were also elevated, along with higher Castelli risk indices I and II. In comparison to PHA-L, the detected disturbances in PHA-I were much higher. This study's second section looked into how exposure to high altitude affected renal function and lipid profiles. The high-altitude group showed increased triglycerides, low-density lipoprotein (LDL),

very low-density lipoprotein, and atherogenic indices levels but decreased high-density lipoprotein (HDL) levels when compared to the control group. In participants exposed to high altitude, these observations were accompanied by decreased Ca2+-ATPase and Na+/K+-ATPase activities and increased serum urea, blood urea nitrogen (BUN), creatinine, C-reactive protein, TNF-α, IL-1β, and malondialdehyde.

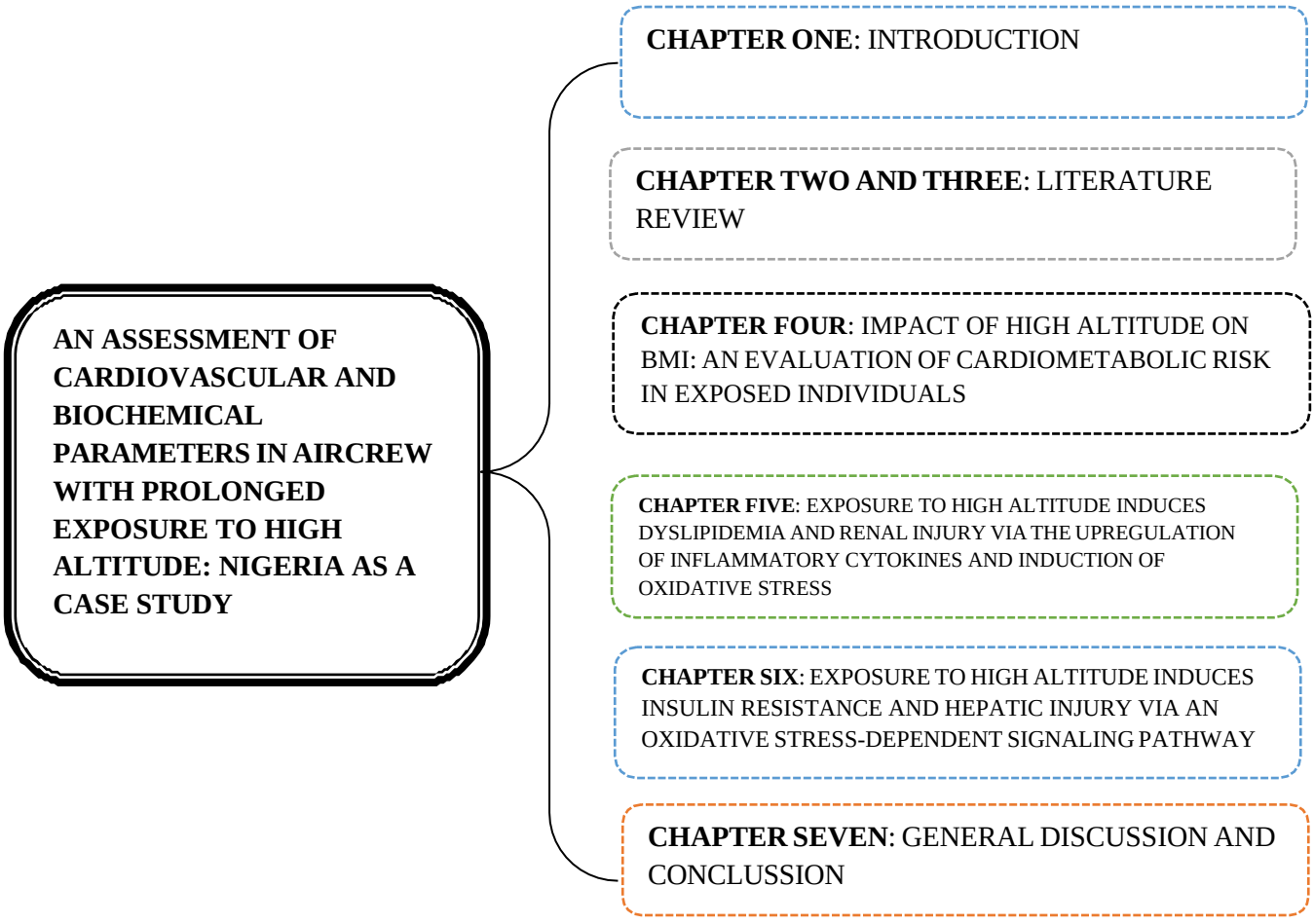
This study's third section assessed how exposure to high altitude affected liver function and insulin sensitivity. Additionally, the possible roles of inflammation, specifically, C-reactive protein (CRP), tumor necrosis factor-α (TNF- α), interleukin-1β (IL-1 β) signaling pathway and oxidative stress were evaluated in lowlanders who were high-altitude travelers. It was demonstrated that the individuals who had been exposed to high altitude had considerably lower levels of total protein and higher levels of AST, ALT, and ALP, indicating altered liver function, as compared to the control group. Furthermore, exposure to high altitudes raised insulin resistance and glucose plasma levels.

In addition, when compared to the control, high altitude exposure resulted in a substantial increase in triglycerides, low-density lipoprotein (LDL), TC/high-density lipoprotein (HDL), and LDL/HDL, but a decrease in HDL levels. These events were accompanied by significant reductions in GSH level, GPx, GST, SOD, catalase, Ca2+-ATPase and Na+/K+-ATPase activities, and an increase in malondialdehyde, TNF-α, IL-1β, and C-reactive protein levels.

Overall, high altitude exposure led to impaired renal and hepatic functions, insulin resistance, and dyslipidemia. These harmful effects, which were mediated by the activation of oxidative stress, and the elevation of pro-inflammatory cytokines, were much greater in PHA-I than in PHA-L.

PREFACE

This thesis is organized in an article-based structure, with each article discussing relevant components of the study objectives and presented in seven different chapters. The chapters are written according to the journals where they were published or submitted for review.



Chapter one is a brief introduction which contains general background information about the subject matter, justification, and the aim and objectives of the study. Chapter two contains a comprehensive review of data available in the literature including a review article on the ' high altitude and human reproduction function-a review‟, which has been published in the African Journal of Biomedical Research (Scopus-indexed). Chapter three is also a comprehensive review

entitled “high altitude exposure and cardiovascular circulation: friends or foe”, which has been published in the African Journal of Biomedical Research (Scopus-indexed). The subsequent three chapters are separate original articles from this study. Chapter four, titled „Impact of High Altitude on BMI: An Evaluation of Cardiometabolic Risk in Exposed Individuals“ submitted to African Journal of Biomedical Research, undergoing review. Chapter five, tittled „Exposure to high altitude induces dyslipidemia and renal injury via the upregulation of inflammatory cytokines and induction of oxidative stress“ demonstrates that high altitude exposure induces dyslipidaemia and renal injury through the activation of oxidative stress and upregulation of inflammatory cytokines. The full original article is at the moment undergoing peer-review for possible publication in the Current Research in Physiology (Pubmed-indexed). Chapter six titled

„Exposure to high altitude induces insulin resistance and hepatic injury via an oxidative stress-dependent signaling pathway“ reveals that high altitude exposure impaired glucose and lipid metabolism by triggering insulin resistance through the activation of oxidative stress and inflammation This is also undergoing peer-review for possible publication in Frontiers in Physiology (Scopus- and Pubmed-indexed). Chapter seven is the last chapter of the thesis and contains a general discussion of all the main findings of the study, conclusion and a brief analysis of key areas identified for further studies as a result of questions generated from the findings reported in this present study.

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To every other person I cannot mention because of space and time, I say Jazakumullah khairan (Aameen).

DEDICATION

This thesis is dedicated to almighty Allah and my parents, Sheikh Abdullateef Ahmad Tijani Adekilekun and my beloved late mum Hajia Raihanat Bolarinwa Adekilekun.

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LIST OF ABBREVIATIONS

AC: Atherogenic coefficient

AIP: Atherogenic index of plasma

ALP: Alkaline phosphatase

ALT: Alanine transaminase

AMS: Acute mountain sickness

AST: Aspartate transaminase

BH_4: Tetrahydrobiopterin

BMI: Body mass index

BP: Blood pressure

BUN: Blood urea nitrogen

Ca^{2+}-ATPase: Calcium ATPase (pump)

CBF: Cerebral blood flow

cGMP: Cyclic guanosine 3,5-monophosphate

CO: Cardiac output

COX: Cyclo-oxygenase

CRI: Castelli risk index

CRP: C-reactive protein

ED: Erectile dyfunction

EGFR: Epidermal growth factor receptor

eNOS: endothelial nitric oxide synthetase

ER: Estrogen receptor

ERK: Extracellular signal-regulated kinase

ET-1: Endothelin-1

FAAN: Federal Airports Authority of Nigeria

FSH: Follicle-stimulating hormone

GnRH: Gonadotropin-releasing hormone

GPx: Glutathione peroxidase

GSDMD-N: Gasdermin D N-terminal

GSH: Reduced glutathione

GST: Glutathione-S-transferase

HA: Higher altitude

HAH: High altitude hypoxia

HDL: High-density lipoprotein

HMGB1: High-mobility group protein B1

HR: Heart rate

IL-1β: Interleukin- 1beta

iNOS: inducible nitric oxide synthetase

LDL: Low-density lipoprotein

LH: Luteinizing hormone

LOX: Lipooxygenase

MAP: Mitogen-activated protein

mASL: Meter above sea level

MDA: Malondialdehyde

Na^+/K^+-ATPase: Sodium/potassium ATPase (pump)

NAFLD: Non-alcoholic fatty liver disease

NAMA: Nigerian Airspace Management Agency

NCAA: Nigerian Civil Aviation Authority

NF-kB: Nuclear factor-kappa B

NO: Nitric oxide

NOS: Nitric oxide synthetase

NOX: NADPH oxidase

OxLDL: Oxidized LDL

PA: Pulmonary arteries

PAF: Platelet-activating factor

PaO_2: Arterial partial pressure of oxygen

PASMC: Pulmonary arterial smooth muscle cells

PGE: Prostaglandin E

PHA-I: People (aircrew members) exposed to high altitude internationally

PHA-L: People (aircrew members) exposed to high altitude locally

PKC: Protein kinase c

PO_2: Partial pressure of oxygen

RAAS: Renin-angiotensin-aldosterone system

REM: Rapid eye movement

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

RV: Right ventricle

SACM: Simulated aerial combat maneuvers

SBP: Systolic blood pressure

SMC: Smooth muscle cells

SOD: Superoxide dismutase

SRE: Sleep-related erections

SV: Stroke volume

TBARS: Thiobarbituric acid reactive substances

TC: Total cholesterol

TG: Triglycerides

TNF-α: Tumour necrotic factor-alpha

VEGF: Vascular endothelial growth factor

VLDL: Very low-density lipoprotein

VO_2 max: Maximal oxygen uptake

WHO: World Health Organization

XO: Xanthine oxidase

Chapter I

Introduction

1.1 Statement of Research Problem

There is an increase in the number of individuals visiting high-altitude locations worldwide. Approximately, 100 million lowlanders travel every year to regions above 2500 m and another 400 million people are resident in places above 1500 m (Mallet *et al.,* 2021). When lowlanders visit high-altitude regions, they are subject to the same negative consequences of high altitude as Highlanders. (Burtscher *et al.,* 2018). This exposure alters some physiological processes and increases the individual‟s susceptibility to pathologies such as cardiometabolic derangement. These adverse events are mediated by the associated extreme conditions like hypoxia, hypobaria, and low temperature (Hackett and Roach, 2001; Moore, 2001; Basnyat and Murdoch, 2003).

1.2 Background

There is an increasing number of people exposed to high altitudes over time. This may be due to the rate of migration and tourism globally. As of 2021, approximately 400 million people live in places above 1500 m, while another 100 million lowlanders travel annually to mountainous areas that are over 2500 m (Mallet *et al.*, 2021). Both lowlanders and highlanders who are high- altitude travelers are exposed to the deleterious impact of high altitude, although the effects on lowlanders who are high-altitude travelers may be milder (Burtscher *et al.,* 2018). Studies have shown that adverse effects from exposure to high elevations include acute mountain sickness, high-altitude cerebral edema, high-altitude pulmonary edema, and cardiometabolic changes (Karakucuk and Mirza, 2000; Paven *et al.*, 2004). It has also been demonstrated to negatively impact male fertility. (Kim *et al.*, 1991, Kim *et al.,* 1993) and induce organ injury (Laustsen *et*

al., 2014; Yang *et al.,* 2023). These deleterious effects are mediated by hypoxia, hypobaria, and hypothermia (Hackett and Roach, 2001; Moore, 2001; Basnyat and Murdoch, 2003).

The reduced partial oxygen pressure associated with exposure to high altitude causes germinal epithelium degeneration and triggers testicular apoptosis, leading to impaired spermatogenesis (Oyedokun *et al.,* 2023). Although hypoxia may initially stimulate testosterone production, over time, it induces Leydig cell dysfunction, resulting in reduced testosterone secretion which consequentially impairs libido and penile erection (Saxena, 1995; Oyedokun *et al.,* 2023). Hypoxia may also cause a marked reduction in nitric oxide (NO)-dependent erection (Kim *et al.,* 1993; Oyedokun *et al.,* 2023).

More so, high altitude causes insulin resistance (Woolcott *et al.,* 2014), dyslipidemia (Deng *et al.,* 2012; Lopez-Pascual *et al.,* 2018), and altered systemic arterial pressure (Mingji *et al.,* 2015; Song *et al.,* 2020). Mingji *et al.* (2015) documented a 2% rise in the prevalence of hypertension, whereas Song *et al.* (2020) found that high altitude decreased the prevalence of hypertension from 40.6% to 20.4%. The effects of divergent adaptation on the cardio-vascular system, including stimulation of gene transcription in response to hypoxia by hypoxia-inducible substances (Beall, 2000), could explain the variation in the effects of high altitude on blood pressure that has been described. (Mallet *et al.,* 2021). Conversely, high altitude raises triglycerides and cholesterol and lowers high-density lipoproteins (HDL-C). (Sherpa and Deji, 2011; Deng *et al.,* 2012).

Exposure to high altitude also induces renal injury (Swenson, 2001; Laustsen *et al.,* 2014) through the modulation of renal metabolism (Laustsen *et al.,* 2014) and nitric oxide (NO) generation (Swenson, 2001). However, research examining how exposure to high altitude affects liver functions yields inconsistent results. While Kametas *et al.* (2003) reported that high-altitude

exposure did not alter liver marker enzymes, Song *et al.* (2020) found that chronic hypoxia following high-altitude exposure improved liver functions and ameliorated obesity-induced non-alcoholic fatty liver diseases. However, some studies (Ou, 1974; Yeh *et al.*, 2022; Yang *et al.* 2023) documented impaired liver functions in high altitude exposure via the induction of caspase 1 activity, high- mobility group protein B1 (HMGB1), gastrin D N- terminal (GSDMD- N), and with nuclear factor-kappa B (NF-kB). Pro-inflammatory cytokines that are dependent on oxidative damage (Yang *et al.,* 2023).

The evidence that is now available is contradictory, despite strong research and plenty of documentation demonstrating the impact of high-altitude exposure on physiological processes. Therefore, this study aimed to investigate the impact of high altitude exposure on insulin sensitivity, glucose regulation, and hepatic and renal function, as well as the mechanisms associated with these effects.

1.3 Rationale for this Study

Increasing evidence shows that the prevalence of cardiometabolic disorders is rising globally. Not just high-resource countries but even poor nations with emerging economies are seeing an increase in the incidence of cardiometabolic diseases. (Akhigbe and Ajayi, 2021). Several studies have demonstrated that reduced cardio-tolerance accompanied by an inflammatory state and oxidative stress plays a central role in the pathogenesis of cardiometabolic disorders (Abete *et al.,* 1999; Woolcott *et al.,* 2014; Lopez-Pascual *et al.,* 2018; Akhigbe and Ajayi, 2021). Although there are numerous modifiable and non-modifiable risk factors for cardiometabolic disorder, emerging predisposing factors have been reported. A prevalent emerging risk factor is exposure to high altitude.

High altitude modulates systemic arterial pressure (Mingji *et al.,* 2015; Song *et al.,* 2020), and induces insulin resistance (Woolcott *et al.,* 2014) and dyslipidemia (Deng *et al.,* 2012; Lopez-Pascual *et al.,* 2018). Although Song *et al.* (2020) showed that high altitude exposure reduced hypertension prevalence from 40.6% to 20.4%, Mingji *et al.* (2015) reported a 2% rise in hypertension prevalence following high altitude exposure. The observed variation might be due to the cardiovascular consequences of divergent adaptation (Beall, 2000), which includes hypoxia-inducible factors-activated hypoxia-responsive gene transcription (Mallet *et al.,* 2021). Additionally, exposure to high altitude raises cholesterol, triglyceride, and low levels of HDL-C, or high-density lipoproteins. (Deng *et al.,* 2012). Additionally, high altitude exposure distorts renal metabolism (Laustsen *et al.,* 2014) and generation of nitric oxide (NO) (Swenson, 2001), which may contribute to adverse cardiometabolic events via the uncoupling of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), and depletion of tetrahydrobiopterin (BH_4), leading to endothelial dysfunction (Otani, 2011; Akhigbe and Ajayi, 2021). Despite the aforementioned information, reports on high altitude-induced cardiometabolic derangement are scarce and the available are conflicting.

1.4 Aim

Therefore, the thesis study evaluated the cardiometabolic status indicators in aircrew members who have been exposed to high altitudes regularly.

1.5 Objectives

The objectives of the study were to:

1. explore the impact of exposure to high altitude on lipid profile and renal function

2. evaluate the impact of exposure to high altitude on hepatic function, glucose regulation, and insulin sensitivity

3. determine the influence of oxidative stress and inflammation in high-altitude exposure-induced modulation of hepatorenal function

1.6 Research Questions

- Is high altitude exposure associated with increased body mass index (BMI)?
- Does high altitude exposure induce dyslipidemia, increased atherogenic indices, and impaired renal metabolism?
- Does high altitude exposure induce insulin resistance and hepatic injury?
- Is high altitude exposure associated with the induction of oxidative stress and inflammation?

1.7 Hypothesis

H_0: High altitude exposure will not induce dyslipidemia and insulin resistance and is not associated with increased BMI and the induction of oxidative stress and inflammation.

H_1: High altitude exposure will induce insulin resistance and dyslipidemia and is associated with increased BMI and the induction of oxidative stress and inflammation.

1.8 Significance of the Study

Data regarding the effect of high altitude exposure on the likelihood of acquiring cardiometabolic illnesses are presented in the study. It also looks at how oxidative stress and inflammation affect how high altitude exposure affects the regulation of glucolipids. The data

from this study could contribute to understanding the pathophysiology underlying the impact of high altitude exposure on cardiometabolic derangement and open new therapeutic strategies in the management of high altitude-induced cardiometabolic disorders.

The thesis is structured as a series of articles, each appearing in a separate chapter and covering pertinent aspects of the research goals. The first chapter, which serves as the introduction, includes a basic overview of the topic, rationale, and the goals and purposes of the research.

The second chapter includes a comprehensive review of data available in the literature including a review article on the ' high altitude and human reproduction function-a review‟, which has been published in the African Journal of Biomedical Research (Scopus-indexed). Chapter three is a comprehensive review entitled “High altitude exposure and Cardiovascular Circulation: friends or Foe”, published in the African Journal of Biomedical Research (Scopus-indexed). The successive two chapters are distinct original articles from this study. Chapter four, titled „Exposure to high altitude induces dyslipidemia and renal damage via the upregulation of inflammatory cytokines and induction of oxidative stress‟ demonstrates that high altitude exposure induces dyslipidemia and renal injury through oxidative stress activation and inflammatory cytokines upregulation. The manuscript is undergoing peer review for possible publication in the Current Research in Physiology (Pubmed-indexed). Chapter five titled „Exposure to high altitude induces insulin resistance and hepatic injury via an oxidative stress-dependent signaling pathway‟ reveals that high altitude exposure impaired lipid and glucose metabolism by triggering insulin resistance via the activation of oxidative stress and inflammation This is also undergoing peer-review for possible publication in Frontiers in Physiology (Scopus- and Pubmed-indexed). The final chapter of the thesis, chapter six, includes a summary of all the major findings of the investigation, a conclusion, and a brief examination of

important areas that will require additional research because of problems raised by the findings of this study.

1.9 Ethical Consideration

The Nigerian Civil Aviation Authority (NCAA), the KUPA Medical Center in Lagos, an aviation medical laboratory and clinic, and the Faculty Research Committee of the Faculty of Clinical Sciences College of Health Sciences, Osun State University, Osogbo, Nigeria, as well as the Ethical Review Committees of the aviation regulatory body in Nigeria, approved the study. The Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa's Research Ethics Committee (REC) (Approval number: NHREC: REC-230408-014) provided additional ethical approval.

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Chapter 2

2.1 Literature Review

Title: High altitude and human reproduction function: A review

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2.1.1 Abstract

Altitude has also been known to have significant changes on the metabolic, cardiovascular and ophthalmological parameters due to prolonged exposure. However, the human body can adjust to acute and chronic reductions in its oxygen supply by increasing respiratory rate, chemical changes in the blood, and by increasing the production of red blood cells. In males, reproduction could be affected by hypoxia at different levels. It could be at hormonal level, spermatogenesis while epidydimal, seminal vesicular and testicular weight are reduced greatly. Chronic state of hypoxia can alter erectile physiology, thus triggering the onset of erection dysfunction (ED).

Reproduction in species that have adapted to conditions at high altitude was possible, making adaption to high altitude important for survival and for successful reproduction. Acute hypoxia increases the afferent sympathetic activation which increases vasoconstriction activity.

Keywords: High altitude, erectile dysfunction, sperm, ovary

2.1.2 Introduction

By definition, altitude or height is based on the contest in which it is used. Generally, altitude is a measure of distance, usually in the up or vertical direction, between a reference datum and a point.

Movement by humans in hostile environment such as humid coastal area, scorching heat of the desert and frigid high altitude leads to detrimental physiological effects. Of the aforementioned harsh climate adversities, high altitude is one of the most severe environment, posing challenges to human survival and performance. Intense low ambient temperature, low humid, high power solar radiation and low atmospheric pressure are different stresses being faced during travel or living in high altitude.

High altitude (beyond 9000 feet) has low atmospheric pressure which decreases the oxygen partial pressure in the ambient air implying that the number of molecules of oxygen present per breath decreases, even with unaltered relative percentage of oxygen. Hypobaric hypoxia is known as the state of the sub-optimal oxygen availability due to decreased ambient barometric pressure. Oxygen being a primary requirement for life processes, its inadequate bio-availability to tissues or organs leads to inadequate biological processes such as energy production, biosynthesis and breakdown of cellular component, reproduction, mental coherence, etc.

Interestingly, adaptation to high altitude in human is an instance of evolutionary modification in certain human populations, including those of Tibet in Asia, the Andes of the Americas, and Ethiopia in Africa, who have acquired the ability to survive at extremely high altitudes. This adaptation means irreversible, long term physiological responses to high altitude environments, associated with heritable behavioural and genetic changes.

Although other human populations would suffer serious health consequences, the indigenous inhabitant of these regions thrive well in the highest part of the world. Especially in the regulatory system of oxygen respiration and blood circulation, these people have undergone extensive physiological and genetic changes when compared to the general lowland population.

Different population and societies have a long antiquity at high altitudes, yet much of the early knowledge of whether or not male and females had normal reproductive fitness comes from chronicles written by Europeans colonizing the Andes. Likewise studies of the genetic components associated with high altitude living are novel avenues of investigation among researchers working with population in high altitude.

Given the difference of fertility rates between newcomers to high altitude and indigenous peoples, local biologists, biological anthropologists, and genetics began to investigate the manner in which high altitude environment impact reproduction. Yet, studies tend to produce contradicting results. The first fertility studies investigate the effect of altitude on fertility among sheep, cattle, cats and rabbits and demonstrated that short term exposure of high altitude resulted in temporary infertility (Monge, 1942, Monge and Mori Chavez, 1942). Studies in the late 20th century have shown that fertility is lower in high altitude than in low altitude (Collins 1983). Studies that show the fecundity rate among Peruvian highlanders have found to be from one to

two births less than of lowland Peruvians of the same ethnic background. In fact, highland native who move to low altitudes show markedly higher rates of fertility than their counterparts who remain in the highlands (Abelson, 1976). On the contrary, some studies have also noted that population fertility appears to be unaffected among natives to high altitude environments (Hoff, 1984).

The oxygen transport system must offset ambient hypoxia in order to maintain tissue oxygen levels to support maintenance, growth and development, and reproduction at high altitude. Altitude-initiated stress, hypoxia in particular, may act to affect the process of reproduction at several stages: formation of gametes and gametogenesis, the ovarian cycle and menstruation, birth weights, still birth rates, infant mortality, postpartum behaviours, and age of menopause. These reproductive categories will be explored.

High attitude has generally been defined as an elevation above 3000m (approximately 10,000ft) (International Society for Mountain medicine, 2011*; Cymerman and Rock, 1994).* In healthy persons, clinically significant changes are difficult to demonstrate elevations lower than this (Poothirikovil, 2014). Similarly, the development of modern transportation facilities has increased the number of individuals visiting high– altitude locations (>2500mm) and exposure to this condition triggers a series of physiologic responses intended to maintain adequate tissue oxygenation (Rimoldi *et al.,* 2010). There are enormous inter individual variability in these responses that may be further amplified by environmental factors such as cold temperature, low humidity, exercise and stress. These adaptive mechanisms, although generally tolerated by most healthy subjects, may induce major effects on biological system with prolonged exposure.

Previous research works have shown that altitude has several effects on humans. This include acute mountain sickness, high –altitude pulmonary edema and high altitude cerebral edema (Karakucuk and Mirza, 2000). Altitude has also been known to have significant changes on the metabolic, cardiovascular and ophthalmological parameters due to prolonged exposure (Paven *et al.,* 2004).

Previous studies have shown various physiological responses to prolonged exposure to high altitude in aircrews. The rate of hypertension in 1,000 pilots was estimated to be 4.2% and there were obvious differences among the different age teams, though no obvious difference was found among those who fled different kind of planes (Li-Huirounge*t al.,* 2008).

Solorera *et al.,* 2013 also reported maximum blood pressure and heart rate values were higher in hypertensive pilots as compared with the control group. (Tang and Wil, 2011) concluded that there was high prevalence of controllable cardiovascular risk factors in Chinese pilots. It was reported that significance difference existed on the rates of hypertension, total cholesterol, triglyceride, low high density lipoprotein – cholesterol smoking and abnormal BMI among pilots from different regions (all $p<0.01$). Results from the annual aeromedical examination on the analysis of ECG of pilots exposed to prolonged high altitude also showed decadal changes in aircraft pilots (Orhui *et al.,* 2011).

Karacucuk and Mirza, 2000 also reported the various effects of prolonged high altitude on human beings as altitude related illness are a frequent cause of morbidity and occasional mortalily in travelers and aircrew throughout the world.

Abdias *et al.,* 2012 reported that exposure to prolonged high altitude can be associated with both beneficial and detrimental effects on health. This includes reduced frequencies of obesity,

diabetes and coronary heart disease and the dark side is the increased frequency of systemic and pulmonary hypertension and the potential consequences of high altitude renal syndrome.

Vangelova and Zlatev, 1994 in a study of gravitational induced hormonal changes and blood concentrations of serotonin and histamine also revealed significant increase in biochemical parameters in pilots using simulated aerial combat maneuvers (SACM). Malcolm *et al.,* 1976 in a study of some aircrew exposed to prolonged altitude also revealed some changes in the biochemical and physiological parameters indicating that climatic conditions can prestress an aircrew before a flight, and lead to impaired adaptation to the additional strains of exacting work in rapidly changing surroundings of temperature, humidity and time.

2.1.3 High altitude and male reproduction

At high altitude, atmospheric pressure and oxygen partial pressure are lower than at sea level which results in hypoxia due to low availability of oxygen which inhibits diffusion of oxygen from the air into the lungs. Various physiological conditions could be severely affected at high altitude in which human reproduction is among. In males reproduction could be affected by hypoxia at different levels. It could be at hormonal level i.e release of LH (luteinizing hormone), FSH (follicle stimulating hormone) and testosterone which are the important reproductive hormones in males. Spermatogenesis could also be another physiological process which could be affected by hypoxia, studies have shown that hypoxia could cause oligospermia and promote increased apoptosis (Gasco *et al.,* 2003). Epididymal, seminal vesicular and testicular weight reduced greatly. Human male reproductive function depends on a complex interaction between a physiological erection and sperm production. Verratti *et al* (2012) that a chronic state of hypoxia can alter erectile physiology, thus triggering the onset of erectile dysfunction (ED). Oxygen availability and delivery could play an important role in the regulation of local penile erection-

related mechanisms and low oxygen supply levels may be considered an aetiological factor in ED (Verratti *et al.,* 2007, Verratti *et al.,* 2011). In a study by Gustavo *et al* 2007, it was observed that reproduction in species that have adapted to conditions at high altitude was possible which makes adaption to high altitude important for survival and for successful reproduction.

2.1.4 High altitude and Sperm function

The human sperm cell is haploid so that its 23 chromosomes can join that of 23 chromosomes of the female egg to form a diploid cell during fertilization. Sperm develops in the seminiferous tubules of the testicles and stored in the epididymis. Spermatogenesis is a physiological process in which mature sperm cells are formed. This process requires an array of proteins, steroids and glycans, which are needed for nutrition, growth/development, orientation/polarization and protection of maturing germ cells. The main function of sperm is to reach the ovum and fuse with it to deliver two sub-cellular structures;

This process (spermatogenesis) represents a delicate balance between cell proliferation, differentiation, and apoptosis. In most mammals, the testicles are kept in the scrotum 2 to 7°C below body core temperature, and the spermatogenic process proceeds with blood and oxygen supply that is fairly independent of changes in other vascular beds in the body. Despite this apparently well-controlled local environment, pathologies such as varicocele or testicular torsion and environmental exposure to low oxygen (hypoxia) can result in changes in blood flow, nutrients, and oxygen supply along with an increased local temperature that may induce adverse effects on Leydig cell function and spermatogenesis (Juan *et al.*, 2012). Germ cell apoptosis and DNA damage are common features in hypoxia which may lead to infertility.

Rat seminiferous tubules are thought to be under an oxygen tension lower than the interstitial oxygen tension in normal conditions (Giaccia *et al.,* 2004). Despite various controversies about

the correct values of oxygen tension in the seminiferous tubules, it has been proven that the testicular interstitial oxygen tension is approximately 20% of the testicular artery blood oxygen pressure (i.e., 12 to 15 mmHg) (Free *et al.,* 1976). It is expected that the oxygen tension decreases under low atmospheric oxygen pressure (which is the condition at high altitude) or under reduced blood flow to the testis (testicular torsion). The distribution of oxygen in the testis is determined by the testicular microvasculature, thereby making the access of spermatogenic cells to oxygen to be determined by the diffusion of oxygen in the interstitium and seminiferous tubules (Juan *et al.,* 2012).

According to Juan *et al.,* (2012) chronic hypoxia induces a state of reversible oligozoospermia (low sperm concentration in semen) in healthy men, sperm motility and count was also reduced which can be related to increased germ cell apoptosis in hypoxic state. Degeneration of germinal epithelium, folding of the basement membrane, degeneration and detachment of germ cells changes in lipid droplets in Sertoli cells, and an increase in lipoperoxidation has been associated with hypoxia (Farias *et al.,* 2005, Liao *et al.,* 2010).

The degeneration of testicular and increased apoptosis of the germ cells experienced at high altitude is mediated by an increase in intratesticular or seminal reactive oxygen species (ROS). In several studies, intracellular ROS can increase in hypoxic conditions which induces oxidative stress (Farias, *et al.,* 2010, Vargas *et al.,* 2011) and this was as a result of vascular changes that are associated with increase in testicular temperature on an average of 1.5°C (Farias *et al.,* 2005). In the testis, the generation of ROS seems to be of paramount importance in germ cell apoptosis and DNA damage (Makker *et al.,* 2009). At physiological levels, ROS are essential for normal reproductive functioning, acting as metabolic intermediates and regulating vascular tone, gene expression, and sperm capacitation (Sikka *et al.,* 2001; Makker *et al.,* 2009). Heat stress induces

oxidative stress, triggering cell survival or apoptosis depending on the cell type and the extent of the insult. This heat stress appears related to ROS-generating enzymes that produce ROS as by-products of their enzymatic activity. Xanthine oxidase (XO) catalyzes the conversion of hypoxanthine and xanthine to uric acid, producing hydrogen peroxide as a by-product, and XO inhibitors suppress testicular germ cell apoptosis induced by experimental cryptorchidism (testis subjected to the core body temperature) (Kumagai *et al.,* 2002). There is a lack of information, however, about whether or not other ROS-generating enzymes such as cyclooxygenase (COX), lipoxygenase (LOX), NADPH oxidase (NOX), and the mitochondrial NADH-CoQ oxido-reductase are activated after testicular heat stress. In other oxidative processes, nitric oxide (NO) is synthesized intracellularly through the action of a family of nitric oxide synthetase (NOS) enzymes. These NOS enzymes catalyze the NADPH- and -dependent oxidation of L-arginine to L-citrulline, producing NO (Hill *et al.,* 2010). This molecule is a free radical and is chemically more stable and less reactive than other ROS such as the superoxide anion or hydrogen peroxide (Pacher *et al.,* 2007). Furthermore, NO in the presence of ROS can form the highly reactive oxidant peroxynitrite(Calcerrada *et al.,* 2011). In monkey testes, endothelial nitric oxide synthetase (eNOS) and inducible NOS (iNOS) were found to be expressed in Sertoli and germ cells. No obvious alterations in eNOS levels were detected after heat stress, but the levels of iNOS increased three days after heat treatment compared with the controls showing a robust increase in iNOS expression in germ cells (Guo *et al.,* 2009). Thus, heat stress seems to induce NO production and it might contribute to oxidative damage in germ cells. The molecular targets that are modified by NO production and the consequences of this RNS in testis physiology and pathophysiology are still unknown, however.

2.1.5 High altitude and Erection/ejaculation/ erectile dysfunction

An erection or penile erection/tumescence is a physiological phenomenon in which the penis becomes firmer, engorged and enlarged. Penile erection is the result of a complex interaction of psychological, neural, vascular and endocrine factors, and is often associated with sexual arousal or sexual attraction, although erections can also be spontaneous. ED is the inability to achieve or sustain an erection suitable for sexual intercourse. Causes include medications, chronic illnesses, poor blood flow to the penis, drinking too much alcohol, or being too tired. Higher altitudes (HA) bring about various changes in physiology of the body due to decreased atmospheric pressure and less oxygen tension. As the human body goes beyond 7,000 feet above sea level, the saturation of oxy hemoglobin begins to fall (Young, *et al.,* 2002). In fact, recent trials have shown that nitric oxide (NO) synthesis and functional integrity of penis smooth muscles depend on an adequate oxygen supply (Moreland *et al.,* 1998, Sa´enz de Tejada *et al.,* 2004).

In a study by Verratti *et al.,* (2007) the relationship between sleep related erections (SRE), erectile dysfunctions and hypoxia was carried out. It was seen that SREs (sleep-related erections) are involuntary physiological phenomenon that occur in healthy men 4– 5 times a night during rapid eye movement (REM) sleep phase, each of 30–45 min for a total of 80– 180 min (Karacan *et al.,* 1989). The physiological role of SREs is not completely known, probably they play a role in metabolic processes at the base of erectile function and in corpus cavernosum perfusion and oxygenation (Kim *et al.,* 1998). Nitric oxide is thought to be a very important agent in erection as it is a vasodialator. Nitric oxide is a gas that diffuses into target tissues where it activates guanylate cyclase and catalyzes the formation of cyclic guanosine-30 ,50 -monophosphate (cGMP) from guanosine-50 -triphosphate. cGMP initiates a cascade of intracellular events and reduces intracellular calcium which leads to relaxation of penis smooth muscles (Arnold *et al.,*

1977). Nitric oxide synthesis is mediated by NO synthetase, which requires both L-arginine and oxygen as substrates. Oxygen is involved in penis erection mechanism through regulation of NO synthesis in the corpus cavernosum tissue and through the regulation of other vasoactive substances (Palmer *et al.,* 1989, Kwon *et al.,* 1990). Vasoconstrictor substances prevail when there are low O_2 tensions, while there is a prevalence of NO and prostaglandin E1 (PGE) when there are high O_2 tensions (Moreland *et al.,* 1998, Hirshkowitz *et al.,* 2005). Low O_2 tensions can interfere with NO synthesis and secretion or alter its availability upon release. Moreover, it is possible that the target cell (the smooth muscle), under hypoxic conditions, is less responsive to NO. Thus low O_2 tensions can inhibit the relaxation of trabecular smooth muscle. A direct demonstration of regulation role of oxygen is provided by the measurement of NO synthase activity in rabbit corpus cavernosum cytosol preparations. Hypoxia causes a significant reduction of NO synthase activity. This suggests that oxygen can be a rate-limiting factor for NO production in the penile corpus cavernosum (Kim *et al.,* 1991, Kim *et al.,* 1993). Acute hypoxia increases the afferent sympathetic activation (Hansen *et al.,* 2000, Xie *et al.,* 2001) which increases vasoconstriction activity (Rowell *et al.,* 1989). If erection is affected ejaculation may also be affected since erection preceeds ejaculation.

2.1.6 High altitude and Hormone; Testosterone, LH, FSH

At high altitude the testicular tissue is greatly affected and affects physiological processes as a result. Dysfunction of leydig cell a condition that decreases testosterone production (Saxena, 1995) seen at high altitude/hypoxic conditions. Testosterone: A "male hormone" -- a sex hormone produced by the testes that encourages the development of male sexual characteristics, stimulates the activity of the male secondary sex characteristics, and prevents changes in them following castration. Chemically, testosterone is 17-beta-hydroxy-4-androstene-3-one. Both

steroidogenesis and spermatogenesis are stimulated by the the release of gonadotropins LH and FSH. Gonadotropins are glycoprotein polypeptide hormones secreted by gonadotrope cells of the anterior pituitary of vertebrates (Parhar *et al.,* 2002). These are the follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Gonadotropins are released under the control of gonadotropin-releasing hormone (GnRH) from the arcuate nucleus and preoptic area of the hypothalamus. The target of gonadotropins in males is the testis.

Gonadotropins released by the hypophysis enter the blood stream to reach the testicle, where Luteinizing hormone (LH) stimulates Leydig cell steroidogenesis in the interstitium, whereas FSH, by stimulation of Sertoli cells, helps to maintain spermatogenesis in the seminiferous tubule. Control mechanisms for FSH secretion seem to be influenced not only by testosterone and its metabolic derivative, estradiol, but also by activins and inhibins produced by Sertoli cells (Clermont *et al.,* 1972). Little is known about the relationship between hypoxia and steroidogenesis, and the scarce studies performed in humans have been carried out with reduced sample sizes. It has been observed that in a small group of men exposed to an altitude of 4,300 m above sea level, their plasma testosterone level rose by 30% after the third day of exposition (Barnholt *et al.,* 2006). In another study in which 10 mountaineers stayed in the Himalayas for a period of 60 days at 5000 m above sea level, hormonal measures indicated reduced testosterone levels at the end of the period (Benso *et al.,* 2007). In experimental mice exposed to normobaric hypoxia, testosterone levels (plasma and intratesticular) were highest at 24 hours for plasma testosterone and 48 hours for intratesticular testosterone. The early increment of both intratesticular and plasma testosterone might be mediated by VEGF (vascular endothelial growth factor,), as postulated by Hwang *et al* (Hwang *et al.,* 2007) and consistent with a raise in VEGF in mice after 24 hrs of hypoxia (Madrid, 2011, Madrid *et al.,* 2011). Plasma and testicular

testosterone return to normal levels after 48 and 72 hours, respectively (Madrid, 2011, Madrid *et al.,* 2011). These results are in agreement with published data on early testosterone increments in mountaineers exposed to high altitude or in newborns exposed to neonatal hypoxia (Barnholt *et al.,* 2006, Boksa *et al.,* 2008, Gonzales *et al.,* 2011). Testosterone has a well-known relaxing effect on smooth muscle which can induce a vasodilator effect in minutes (Costarella *et al.,* 1996, Honda *et al.,* 1999, Webb *et al.,* 1999), an effect that, hypothetically, could be part of the hypoxia response mechanisms in the testicles (Madrid, 2011). Testosterone seems to have a relevant role in high altitude adaptation owing to its identity as an erythropoietic hormone which acts directly on bone marrow at the level of polychromatophilic erythroblasts (Gonzales *et al.,* 2011). Thus, testosterone administration has been shown to stimulate the production of red blood cells in males, especially elderly males, and it is associated with the increment of hemoglobin that occurs during puberty in young men (Bassil *et al.,* 2009, Coviello *et al.,* 2008). Thus, an early rise in testosterone in hypoxia and its role as a vasodilation agent is consistent with its possible role in early vascular changes in the hypoxic testis, as well as its being a likely coactivator of the erythropoietic response in hypoxia, acting both as a local paracrine hormone and as an endocrine signal toward bone marrow cells.

2.1.7 High altitude and male reproductive organs

Proper functioning of all the male reproductive organs is required to accomplish coitus and reproduction. Sexual stimulation leads to filling of erectile tissue which leads to erection of the penis in normal males. At climax, when organs is experienced various organs contribute to production of semen (seminal fluid containing sperm cells).

During the process of ejaculation, sperm passes through the ejaculatory ducts and mixes with fluids from the seminal vesicles, the prostate, and the bulbourethral glands to form the semen.

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The seminal vesicles produce a yellowish viscous fluid rich in fructose and other substances that makes up about 70% of human semen (Mann 1954). The prostatic secretion, influenced by dihydrotestosterone, is a whitish (sometimes clear), thin fluid containing proteolytic enzymes, citric acid, acid phosphatase and lipids (Mann 1954). The bulbourethral glands secrete a clear secretion into the lumen of the urethra to lubricate it (Guyton, 1991).

Sertoli cells, which nurture and support developing spermatocytes, secrete a fluid into seminiferous tubules that helps transport sperm to the genital ducts. The ductuli efferentes possess cuboidal cells with microvilli and lysosomal granules that modify the ductal fluid by reabsorbing some fluid. Once the semen enters the ductus epididymis the principal cells, which contain pinocytotic vessels indicating fluid reabsorption, secrete glycerophosphocholine which most likely inhibits premature capacitation. The accessory genital ducts, the seminal vesicle, prostate glands, and the bulbourethral glands, produce most of the seminal fluid (Guyton, 1991).

Seminal plasma of humans contains a complex range of organic and inorganic constituents.

The seminal plasma provides a nutritive and protective medium for the spermatozoa during their journey through the female reproductive tract. The normal environment of the vagina is a hostile one for sperm cells, as it is very acidic (from the native microflora producing lactic acid), viscous, and patrolled by immune cells. The components in the seminal plasma attempt to compensate for this hostile environment. Basic amines such as putrescine, spermine, spermidine and cadaverine are responsible for the smell and flavor of semen. These alkaline bases counteract and buffer the acidic environment of the vaginal canal, and protect DNA inside the sperm from acidic denaturation. According to Fahim *et al* (2009) it is thought that the gonads of people living at high altitudes gradually lose their potency, a surprisingly small amount of published

material is available concerning the effect of low atmospheric pressures on the andrology of male organs (Monge *et al.,* 1937; Monge *et al.,* 1942). Several low-pressure experiments in animals exposed to high altitude have reported decreased testis weight and destruction of germinal epithelium which may account for the impairment of spermatogenesis, and destruction of germinal epithelium; however, the duration of these changes is not precisely known nor is it known whether adaptation takes place (Fahim *et al.,* 2009). The reduction in testicular weight which is experienced at high altitude may be as a result of testicular degeneration, tends to affect the normal physiology of male reproduction and coitus where the organs have shown reduced potency (Fahim *et al.,* 2009).

2.1.8 High altitude and female reproduction

As much as high altitude affects male reproduction, same goes for females. Various physiological functions are also affected in females just as it was seen in males. In this review, we will take a look at female reproduction at high altitude which is a state of hypoxia. Successful reproduction requires efficient functioning of the female reproductive organs and the proper control of reproductive hormones to avoid infertility. From various studies it was evident that the condition at high altitude (hypoxic state where there is low partial and atmospheric pressure of oxygen) tends to induce stress (Crognier *et al.,* 2002). Victor *et al* 2013 demonstrated that the deleterious effects of hypoxia-induced oxidative stress at a high altitude may be prevented by daily administration of antioxidant vitamins, which increase the plasma progesterone concentrations throughout pregnancy (Parraguez *et al.,* 2010) and improve placental structure and fetal growth (Parraguez *et al.,* 2010). Menstrual cycle is affected and the hormones (progesterone and estrogen) regulating different phases are also affected. A number of different

factors may affect female fertility by affecting the functionality of the hypothalamus-hypophysis-

ovarian axis, cyclic ovulatory activity, the quality of preovulatory follicles/oocytes/embryos and/or subsequent embryo/fetal viability. All of these critical roles require the presence of a fully functional corpus luteum.

2.1.9 High altitude and menstrual cycle

Monthly ovum is released into the uterus waiting to be fertilized by sperm. The absence of sperm leads to menstruation. Menstrual cycle is the regular natural change that occurs in the female reproductive system (specifically the uterus and ovaries) that makes pregnancy possible (Silverthorn *et al.,* 2013, Sherwood *et al.,* 2013). The cycle is required for the production of ovocytes, and for the preparation of the uterus for pregnancy (Silverthorn *et al.,* 2013). The first period usually begins between twelve and fifteen years of age, a point in time known as menarche. They may occasionally start as early as eight, and this onset may still be normal. The average age of the first period is generally later in the developing world and earlier in developed world. The typical length of time between the first day of one period and the first day of the next is 21 to 45 days in young women and 21 to 35 days in adults (an average of 28 days) (Laufer *et al.,* 2006). Menstruation stops occurring after menopause which usually occurs between 45 and 55 years of age. Bleeding usually lasts around 2 to 7 days.

The menstrual cycle is governed by hormonal changes. Each cycle can be divided into three phases based on events in the ovary (ovarian cycle) or in the uterus (uterine cycle) (Silverthorn *et al.,* 2013). The ovarian cycle consists of the follicular phase, ovulation, and luteal phase whereas the uterine cycle is divided into menstruation, proliferative phase, and secretory phase.

Stimulated by gradually increasing amounts of estrogen in the follicular phase the latter part of this phase overlaps with the proliferative phase of the uterine cycle., discharges of blood

(menses) flow stop, and the lining of the uterus thickens. Follicles in the ovary begin developing under the influence of a complex interplay of hormones through the influence of a rise in follicle stimulating hormone (FSH) during the first days of the cycle, a few ovarian follicles are stimulated., and after several days one or occasionally two become dominant (non-dominant follicles shrink and die). Approximately mid-cycle, 24–36 hours after the luteinizing hormone (LH) surges, the dominant follicle releases an ovocyte, in an event called ovulation. After ovulation, the ovocyte only lives for 24 hours or less without fertilization while the remains of the dominant follicle in the ovary become a corpus luteum; this body has a primary function of producing large amounts of progesterone. Under the influence of progesterone, the uterine lining changes to prepare for potential implantation of an embryo to establish a pregnancy. If implantation does not occur within approximately two weeks, the corpus luteum will involute, causing a sharp drop in levels of both progesterone and estrogen. The hormone drop causes the uterus to shed its lining in a process termed menstruation.

Follicular phase ranges vary among women, a luteal phases lasting for less than 2 weeks is considered a “luteal defect” due to low levels of the hormone progesterone and an insufficient production of uterine lining, which inhibits a female‟s reproductive abilities. Fecundability may be correlated to cycle length, which determines the number of opportunities for conception in a given time span (Wood and Weinstein, 1988). Because ovarian follicle growth is characterized by cell growth and rapid cell divisions, hypothetically, hypoxia may slow this process and thereby disrupt phase lengths (Wood and Weinstein, 1988).

2.1.10 High altitude and female fertility

Infertility is a condition which has been associated with women at high altitude. Assessing how hypoxic stress impacts fertility alone is problematic because fertility is also affected by many cultural, social, and behavioral factors. Populations residing at high altitudes may have less developed health, social, and communication infrastructures than those residing at sea level.

In a review by Crognier *et al.,* 2002 it was concluded that, the elements of Aymara fertility presented composed of a reproductive pattern shaped by a late onset of fertility and a late beginning of childbearing, associated with a rather short reproductive span and large birth intervals. These characteristics could fit well with a scenario of physiological stress, in which poor conditions of health and nutrition, exacerbated by hypoxia, induce impairment of either fecundity or fertility processes. In particular, this could be the reason for the late age at menarche recorded among peasant girls. The huge discrepancy between the current observations and those of various other authors on adolescents living in the city of La Paz emphasizes the deep contrast in living conditions between rural and urban settlements, and the variety of environmental effects that are known to affect sexual maturation, James (James, 1966) argued that the physiological effects of altitude were responsible for a seemingly lower fertility among indigenous populations. In research carried out by Parraguez (Parraguez *et al.,* 2013) it was observed that the corpora lutea of sheep that were native and naïve to a high altitude were, overall, smaller in size than the corpora lutea of ewes at a low altitude. The occurrence of growth deficiencies in the corpora lutea has been classically linked to ovarian causes (inadequate development and maturation of preovulatory follicles (Keisler *et al.,* 1989) and/or systemic causes (inadequate LH secretion, which is necessary for the final maturation of the preovulatory follicles and,

subsequently, adequate development of the corpora lutea (Campbell *et al.*, 1999, Adams *et al.*, 1999).

The existence of augmented plasma progesterone concentrations during the late luteal phase may compromise female fertility by affecting the final development and maturation of the preovulatory follicle of the subsequent cycle. Furthermore, alterations in follicular development diminish a follicle‟s ability to ovulate an oocyte that can be fertilized and develop into a viable embryo (Gonzalez-Bulnes *et al.,* 2005). Progesterone exerts an inhibitory action on gonadotropin-releasing hormone release from the hypothalamus (Nett *et al.,* 1987), resulting in inadequate stimulation of gonadotrophs for LH synthesis. The hormone LH is pivotal for final maturation of preovulatory follicles (Campbell *et al.,* 1999), progesterone levels that are too high may interfere with this process. In sheep, as in other species, it is well known that large follicles have lower functionality in the mid-luteal phase (Contreras-Solis *et al.,* 2007) when progesterone concentrations are at the maximum level. Ovulation in defective follicles results in lower fertility (Viñoles *et al.,* 1999), which can contribute to the lower fertility observed at a high altitude.

2.1.11 High Altitude and Reproductive Hormone; Estrogen, Progesterone, LH, FSH etc.

In normal physiology in females, certain hormones are responsible for successful reproduction. At high altitude certain changes occur which lead to changes in the level of these hormones thereby affecting reproduction and menstrual cycle as illustrated above.

Progesterone has a characteristic profile during the menstrual cycle, levels being relatively low and flat during the follicular (pre-ovulatory) phase, then rising and peaking approximately half-way through the luteal (post-ovulatory) phase before returning to basal levels, signaled by the onset of menstrual bleeding. Because the absence or reduction of a rise and peak in progesterone

is considered indicative of subfecundity, progesterone levels are an excellent proxy for measuring fecundity. Vitzthum (Vitzthum *et al.,* 2001) reviewed works on hormonal level in females at high altitude and deduced that two higher altitude samples (Bolivia and winter Nepal) are nearly identical (251 pmol l−1 and 253 pmol l−1 respectively) and substantially higher than the low-altitude Zaire sample (201 pmol l−1). Interestingly, while lower than that of Polish women (299 pmol l−1) during the post-harvest season (a period roughly comparable with the seasons during which the winter Nepal and Bolivia data were collected), these samples from higher altitudes display progesterone levels greater than that of the low-altitude (700 m) sample of Polish women during the peak of harvesting (237 pmol l−1). The lowest value (124 pmol l−1) is seen in the Nepal sample during the monsoon season, a period of far greater energetic stress than characterizes the seasons when data for the other samples were collected (Vitzthum *et al.,* 2001).

In a study, the differential hormone profiles, specifically time between the gonadotropin peak/release of luteinizing hormone (LH) and ovulation, between high and low altitude populations may indicate an ovulatory disorder. Escudero *et al.* (1996) compared samples from Lima (sea level) (150 m) and Cerro de Pasco (high altitude region) (4340), Peru. Females in Cerro de Pasco had smaller pre-ovulatory follicle diameters and lower estrogen production during the late follicle phase. Estradiol levels only increased 80% between Cerro de Pasco females compared to 137.3% among females in Lima. Additionally, the luteinizing hormone peaked earlier among women in Cerro de Pasco compared to women in Lima. Yet, both groups of females exhibited the same duration of the luteal phase and the same endometrium measurements between high and low altitudes (Escudero *et al.,* 1996). Escudero and colleagues

(1996) conclude that the differences in hormone profiles during menstrual cycle between high altitude and sea level samples are a result of low barometric pressure.

Conclusion

The cumulation of the effects of high altitude (which is a state of hypoxia i.e reduced partial and atmospheric oxygen pressure) affects male fertility. From above illustration it could be seen that reduced partial oxygen pressure affects spermatogenesis by germinal epithelium degeneration and increased apoptosis which affects male fertility. The association of hypoxia to leydig cells dysfunction which affects testosterone production may affect male sex drive and erection (Saxena, 1995). Although, it has been seen that hypoxia tends to initially increase testosterone levels as a compensation for low oxygen and blood flow since it acts as a stimulator of erythropoesis. The relationship between hypoxia and erectile dysfuction shows that hypoxia causes a significant reduction of NO synthase activity which NO is a signal for erection being a vasodialator and aids filling of erectile tissue. This suggests that oxygen can be a rate-limiting factor for NO production in the penile corpus cavernosum (Kim *et al.,* 1991, Kim *et al.,* 1993). Acute hypoxia also, increases the afferent sympathetic activation (Hansen *et al.,* 2000, Xie *et al.,* 2001), which increases vasoconstriction activity (Rowell *et al.,* 1989). These breaches of physiological processes are thought to affect male fertility. This can cause infertility or sub- fertility.

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Chapter 3

3.1 Literature Review

Title: High Altitude Exposure and Cardiovascular Circulation: Friends or Foe?

Running title: High altitude and cardiovascular changes.

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3.2 Abstract

Cardiovascular function encounter changes on exposure to altitude. High altitude hypoxia (HAH), is the fall in arterial blood oxygen saturation due to low amount of breathable oxygen caused by low atmospheric pressure at high altitude. The human body is capable of adapting to HAH via short- and long-term mechanisms. HAH affects the vascular tone of pulmonary and systemic vessels thereby increasing ventilation, which is the first notable change at high altitude (HA).

Acute exposure to altitude increases cerebral and coronary blood flow and causes pulmonary hypertension. Pulmonary hypertension, is characterized by an increase in pulmonary vascular resistance secondary to hypoxia-induced vasoconstriction and vascular remodeling. Initially exposure to HA decreases systolic blood pressure which increases activation of sympathetic nervous system. This also is responsible for the increase in heart rate and cardiac output which returns to normal few days after acclimatization, but heart rate remains increased while stroke volume remains decreased.

The adaptation of the cardiovascular system to chronic exposure to hypoxia involves both structural and functional changes. These include right ventricle (RV) hypertrophy, persistent pulmonary hypertension, lower CBF and reduced uteroplacental and fetal volumetric blood flows. This review focuses on cardiovascular adaptation to high altitude exposure.

Keyword: High Altitude, hypoxia, circulation, cardiovascular adaptation, hypertension.

3.3 Introduction

Humans are faced with unique challenges at high-altitude environments including cardiac stress (Naeije, 2010). High altitude (HA) is generally defined as greater than 2500 meters above sea level (mASL) approximately 8200ft (Leon-Velarde and Reeves, 1999) and characterized by lower partial pressure of O_2 (PO_2) relative to sea level. A recent estimation revealed that ≈83million people live at >2500 mASL, which includes populations mainly from South America, Central Asia, and Eastern Africa (Beall, 2014). These highlanders are chronically exposed to relative hypoxia, which has important consequences on the cardiovascular system and blood pressure (BP) regulation (Beall, 2014).

High-altitude hypoxia (HAH) is conventionally defined by the fall in arterial blood O_2 saturation

(SaO_2) in the body at altitudes >2500 m (Moore *et al.,* 2011). The hypoxemic type, which is due to a decrease in the amount of breathable oxygen caused by the low atmospheric pressure of high altitudes, and in turn low maximal oxygen uptake (VO_2 max), and the arterial partial pressure of O_2 (PaO_2) in the body (Naeije, 2010). Decreased oxygen availability at high altitude is associated with significant changes in cardiovascular function and increased risk of cardiovascular diseases. Lowered amount of oxygen in the atmosphere can be partially compensated for, by both long- term and short-term adaptations to altitude by the human body (Wagner, 2000). The first notable physiologic change at HA is hyperventilation. Several endocrine changes are induced by HA such as activation or inhibition of hormonal systems (Jean *et al.,* 2010). Red blood cells are important players in the transport of oxygen in the body and are regulated by erythropoietin. Prolonged exposure to HA leads to increased erythropoietin production resulting in increase in hemoglobin concentration increase (Naeije, 2010).

Adults at high altitude experience cardiovascular death as a leading cause of non-traumatic deaths (Burtscher and Ponchia, 2010). Exposure to high altitude leads to increase in resting heart rate, compared with that at sea level, also paradoxically, maximal heart rate is decreased (Bärtsch and Gibbs, 2007). The rise in stroke volume observed with exercise at sea level is decreased at HA (Bärtsch and Gibbs, 2007, Boushel *et al.*, 2001, Boos *et al.,* 2014). Consequently, while resting cardiac output is higher at HA, versus sea level, at peak exercise it is comparatively lower (Bärtsch and Gibbs, 2007, Boos *et al.,* 2014, Naeije, 2010). These factors along with the notable decrease in arterial oxygen content act to limit peak exercise capacity and oxygen consumption (Bärtsch and Gibbs, 2007, Naeije, 2010). Other cardiovascular responses include an increase in resting brachial artery systolic blood pressure (SBP) and 24hours arterial blood pressure (BP),

which in conjunction with the elevation in resting heart rate could be potential implicating factors in cardiovascular risk (Bilo *et al.*, 2015).

Systemic hypertension is influenced by environmental factors such as high-altitude, genetics, race and geographic location. Studies have focused on the acute and subacute hemodynamic changes in lowlanders who are suddenly exposed to HA. Although, little is known about the definite pathophysiologic mechanisms of chronic hypertension at HA, differences in the characteristics of chronic hypertension between various HA settings, or the efficacy of anti- hypertensive agents in chronic highlanders. People living in low-income countries, such as the majority of highlanders, demonstrate higher prevalence of non-communicable diseases, including hypertension (Islam *et al.,* 2014). Although, the current extent of hypertension is considered as a global public health issue by the World Health Organization (WHO, 2017), the real burden of hypertension and its complications in HA locations worldwide is not well defined. Given the large number of highlanders around the world, and the well-established role of hypertension in cardiovascular risk, hypertension at HA is a highly relevant clinical and public health problem that requires increased awareness and study. This review presents the physiologic and pathologic adaptation of cardiovascular system to short-term and long-term HA and its underlying mechanisms.

3.4 High Altitude Exposure and the Cardiovascular Function

i. Heart rate

Acute hypoxia leads to increase in cardiac output (which falls at rest and on exercise with acclimatization), heart rate (at rest and on exercise), and myocardial contractility for the first few days on exposure (Riley and Gavin, 2017).

The electrocardiogram at HA shows variably increased amplitude of P wave, right QRS axis deviation, and signs of RV overload and hypertrophy(Riley and Gavin, 2017).Sometimes, an electrocardiogram may remain unchanged up to extreme altitudes (Naeije, 2010).Altitude and exercise may be related to supraventricular and ventricular premature beats, there is no evidence of an increased case of life threatening arrhythmias in either normal subjects or patients with heart disease (Bartsch and Gibbs, 2007).

One of the earliest responses to high altitude is increase in heart rate (HR). The increase is related to increased sympathetic activity and vagal withdrawal (Riley and Gavin, 2017). In a study of 139 healthy men, HR increased by 20.44 beats per min at an altitude of 3700m compared with 500m (Rao *et al.,* 2015). Although, the underlying mechanism for this increase, particularly during exercise, is not understood; undoubtedly, increased sympathetic activity and circulating catecholamines play essential roles in increasing HR at rest but few studies reported that preliminary administration of beta blockers can overcome this increase (Mirrakhimov and Winslow, 2011). In other studies, only an obstruction of both sympathetic and vagal activities simultaneously can totally overcome the rise in HR induced by hypoxia at rest (Siebenmann *et al.,* 2015). Therefore, it is possible that both vagal withdrawal and an increase in sympathetic activity are responsible for the rise in resting HR with altitude. Chronic exposure to altitude leads to enhancement in parasympathetic activity, causing reduction in maximum HR. During exercise HR is also decreased from sea-level values (Boushel *et al.,* 2001). However, at rest, there are discordant data on whether HR is normalized or remain slightly elevated with acclimatization (Siebenmann and Lundby, 2015).Vagal withdrawal (which is important at rest) appears to play a more central role during exercise-induced tachycardia in hypoxia. In a study by Siebenmann *et al.* (2015), observations showed that propranolol had no effect on exercise-induced tachycardia

at altitude. Also, a combination of beta-adrenergic and muscarinic blockade only caused partial reversal of HR which suggests that other factors are involved (Siebenmann *et al.,* 2015). They also observed that cardiac alpha adrenoceptors may respond to elevated levels of circulating catecholamines to control HR at HA. Animal studies also showed that pulmonary stretch receptors may play a role in regulating HR at high altitude, although the mechanisms of this is not fully understood and may simply be through an increase in sympathetic activity (Mirrakhimov and Winslow, 2011). Further studies are required on these possibilities, particularly the role cardiac alpha receptors play in the regulation of HR in hypoxia. There is possibility also that endothelin-1 (ET-1) plays a role in the control of HR HA. Receptors for ET- 1 (a potent vasoconstrictor peptide) are present incardiomyocytes (Riley and Gavin, 2017). Plasma ET-1 is elevated with ascent to altitude (Riley and Gavin, 2017). Activation of the ET-1 b-type receptors in cardiomyocytes triggers a leak of calcium ions from the sarcoplasmic reticulum, delivering positive inotropic and chronotropic effects (Kohan *et al.,* 2011; Karppinen *et al.,* 2014). Parasympathetic activity is enhanced with chronic exposure to HA, leading to a decrease in maximum HR. The change in HR during exercise is also decreased from sea-level values (Boushel *et al.,* 2001). However, there are conflicting data at rest if HR is normalized or maintains slightly elevated with acclimatization (Siebenmann and Lundby, 2015). Heart rate variability (HRV) is another area of interest of late for some studies. Reduction in HRV is a common finding at HA as a result of increased sympathetic activity (Karinen *et al.,* 2012). In a study by Karinen *et al.,* (2012) observations revealed that HRV parameters could be used as a predictor of acute mountain sickness (AMS). A study by Mairer *et al.* (2013) showed a contradicting observation that HRV indices were not associated with the prediction of AMS (Mairer *et al.,* 2013). Older subjects showed less adaptation and a decreased cardiovascular

response to HA, suggesting that age may affect the increase in HR (Richalet and Lhuissier, 2013). Altitude simulation has recently allowed researchers to look at how different environmental components of altitude, such as hypoxia and hypobaria, affect HR. One such study found that post-exercise resting HR was 50% higher in hypobaric hypoxia compared with normobaric hypoxia (Di Pasquale *et al.,* 2015). This example shows that both the hypobaric and hypoxic components of the high altitude environment are responsible for our physiological response and not hypoxia alone.

ii. Cardiac output and Stroke volume

Acute exposure to HA increase cardiac output (CO) to preserve O_2 delivery to tissues. Although, maximal cardiac output remains the same (Ke *et al.,* 2017) and maximum oxygen consumption (VO_2 max) falls by 1% per 100 m above 1500m. The increase in cardiac output is explained by the increased heart rate which may be offset by reduced stroke volume, which is detectable on the first day. The role of stroke volume (SV) in CO increase with altitude remains controversial Siebenmann and Lundby (2015) which suggestthat SV remains the same in acute hypoxia, while the results from studies on the subject are varied (ranging from a decrease (Sime *et al.,* 1974) to increase in SV (Rao *et al.,* 2015). A study by Rao *et al.,* (2015) revealed a significant increase in SV with acute exposure to HA from 64.65mL at 500m to 68.09mL at 3700m in 139 healthy males. They hypothesize that the reason for this increase in SV is likely due to increased venous return due to enhanced sympathetic activity on vasculature. Following this acute increase in SV, evidence shows that chronic reduction in SV following acclimatization, returns CO to normal physiological levels. While ejection fraction is increased after time, ruling it out as the cause of SV reduction during (Siebenmann and Lundby, 2015), SV reduction during acclimatization may be as a result of altered filling of the ventricles (Stembridge *et al.,* 2016) which is likely due to

decreased preload, as a result of pulmonary vasoconstriction (Siebenmann and Lundby, 2015). Blood volume rises with acclimatization due to polycythemia, which would alter venous return and CO, this is counteracted by the vascularization of tissues involved in venous return to normal levels (Mirrakhimov and Windslow, 2011). Maximum achievable CO decreases with chronic HA exposure (Wagner, 2000; Calbet, 2003). The leading theory for this is that muscle function is decreased at altitude, limiting its need for increased blood flow. Another theory is reduced myocardial function due to hypoxemia with the support of in vitro experiments. In vivo experiments have shown that myocardial function is maintained up to 5000m (Stembridge *et al.,* 2016).

iii. Blood pressure

Blood pressure (BP) changes during both acute and chronic altitude exposure are properly defined. Initially, there is a decrease in systolic BP due to hypoxic vasodilation, which is quickly (within a few hours) counteracted by the sympathetic nervous system activation, which raises systolic blood pressure (SBP) above sea-level values. Systemic hypertension is well documented (even in HA natives) in chronic HA exposure (Mirrakhimov and Windslow, 2011). A study by Lang *et al.* (2016) revealed that diastolic BP did remained unchanged between sea level and altitude in mildly hypertensive patients (Lang *et al.,* 2016). Chronic intermittent exposure to HA causes no long-term effect on BP (Vinnikov *et al.,* 2016). The increased SBP is evident even after moderate HA exposure. A study of 46 healthy people showed an increase in 24-hour ambulatory BP after an exposure to just 2035m (Torlasco *et al.,* 2015). There is enhancement in BP response of hypertensives to exercise at HA (Lang *et al.,* 2016). This systemic vasoconstriction is crucial in the maintenance of arterial O2 concentrations (Bender *et al.,* 1988), although, the increase in SBP is linear with plasma noradrenaline concentration (Mirrakhimov

and Windslow, 2011) which shows the importance of sympathetic activity in this response. Similar to HR, simultaneous alpha and beta blockade does not reverse it, which implies that other mechanisms must partially be responsible (Bartsch and Gibb, 2007). Compensatory polycythemia is a possibility, which elevates blood volume and the role of the renin-angiotensin system. Despite the crucial changes that have been observed, the effects of HA on the renin- angiotensin aldosterone system (RAAS) and the resulting effect on BP are widely ignored by some studies. Studies on RAAS at HA revealed acute decline in plasma levels of renin, aldosterone, and angiotensin II, but increase to normal levels with acclimatization (Maher *et al.,* 1975; Keynes *et al.,* 1982; Parati *et al.,* 2014; Lang *et al.,* 2016). The activity of renin and aldosterone also reduces (Lang *et al.,* 2016; Riley and Gavin, 2017). The mechanism for the reduction in these components of RAAS is not known since the increase in sympathetic activity seen at HA in conjunction with renal artery vasoconstriction would be expected to increase plasma renin levels. Also, there are peculiarities in the levels of angiotensin-converting enzyme (ACE) at HA; a study revealed that peripheral ACE increases with acute altitude exposure (Kamikomaki and Nishioka, 2004) despite several studies showing that trans-pulmonary ACE activity decreases (Li *et al.,* 2016). It is known that RAAS inhibition is a way to regulate the increase in BP at HA, although it may not be as effective as it is at sea level (Parati *et al.,* 2014; Lang *et al.,* 2016). Sympathetic regulation may be more effective (Bilo *et al.,* 2011).

3.5 High Altitude Exposure and the Cardiovascular Circulation

The vascular tone of the systemic and pulmonary vessels is affected by hypoxia, which increases ventilation and sympathetic activities via stimulation of the peripheral chemoreceptors (Bärtsch and Gibbs, 2007). Interactions occur between the direct effects of hypoxia on blood vessels and the chemoreceptor-mediated responses in the systemic and pulmonary circulation. Regulation of

local oxygen delivery according to the needs of the tissues is achieved by several mechanisms (Singel and Stamler, 2005; Lundberg and Weitzberg, 2005). One of such mechanisms is the release of ATP from red blood cells and the generation of nitric oxide (NO) by various ways to regulate local oxygen delivery according to the needs of the tissue. Prolonged stay at high altitude decrease these mechanisms when oxygen content of the blood increases because of ventilatory acclimatization, an increase in hematocrit associated with plasma volume reduction, and an increase in red blood cell mass due to erythropoiesis. Peripheral chemoreceptor afferent activity rises hyperbolically as hypoxia increases (Marshall, 1994). Exposure ranging from days to weeks increases the sensitivity of the peripheral chemoreceptors to hypoxia, leading to further enhancement of ventilation (ventilatory acclimatization). Presumably, this accounts for the further increase of sympathetic activity documented by micro-neurography after 3 weeks at 5200m (Hansen and Sander, 2003) and elevated catecholamines in urine and plasma (Mazzeo *et al.*, 1991). Blood pressure is reduced during the first few hours of exposure to HA which results from the nullification of sympathetic vasoconstriction in the systemic circulation by hypoxic vasodilatation. Blood pressure and systemic vascular resistance rises for about 3 to 4 weeks because of increasing sympathetic activities and decreased tissue hypoxia associated with acclimatization. Oxygen administration does not fully reverse the increased blood pressure (Bärtsch and Gibbs, 2007) which suggests the involvement of additional mechanism.

i. Coronary Circulation

On acute exposure to hypoxia, the epicardial coronary arteries dilate. An increase in resting myocardial blood flow compensates for the reduced oxygen content of the blood and contributes to the maintenance of cardiac function (Koepfli *et al.*, 2004), whereas exercise-induced coronary flow reserve is maintained at least to 4500 m (Wyss *et al.*, 2003). In a study, healthy young men

showed no myocardial ischemia on exercise during a simulated ascent to the summit of Mount Everest (8840 m) over 40 days (Malconian *et al.,* 1990). After 10 days at 3100 m (Bärtsch and Gibbs, 2007), coronary blood flow is decreased compared with at sea level and in proportion to the fall in left ventricular work because of the increased oxygen content of arterial blood with acclimatization. Hence, myocardial oxygen extraction per volume of blood increases to maintain myocardial oxygenation.

ii. Pulmonary Circulation

Pulmonary hypertension is the most common effect of long term exposure to HAH. Elements of the vascular walls and endothelial dysfunction, extension of smooth muscle into previously non-muscular vessels and adventitial thickening are involved in pulmonary vascular adaptations.

Oxygen breathing does not completely reverse HA induced pulmonary hypertension from chronic exposure to HA, this suggests that pulmonary arterial structural remodeling is crucial in pulmonary hypertension during chronic exposure to HA (Ke *et al.,* 2017). Several studies have reported the prevalence of HA pulmonary hypertension between 5% and 18% of the population living at high altitude (Xu and Jing, 2009). High-altitude pulmonary hypertension is characterized by a rise in pulmonary vascular resistance secondary to hypoxia-induced pulmonary vasoconstriction and vascular remodeling. Pulmonary arteries (PAs) remodeling, this involves cellular hypertrophy and hyperplasia in all three structural layers of PAs, namely adventitia, media, and intima. Also, chronic exposure to HA causes other structural modifications such as; migration of medial smooth muscle cells (SMCs) into the intima, fibroblast proliferation and increased collagen deposition in the adventitia, increased extracellular matrix proteins secretion by endothelial cells, and the appearance of SM-like cells in previously non-muscular vessels of the alveolar wall. These changes lead to a decrease in

vascular lumen diameter and a rise in pulmonary vascular resistance (Stenmark *et al.,* 2006). The molecular mechanisms underlying the pathogenesis of high altitude-induced pulmonary hypertension are not fully understood, but several hypoxia-mediated signaling pathways are considered as players. Membrane-bound receptors in pulmonary vessels and signaling proteins are sensitive to hypoxia and are crucial in vascular medial proliferation. Recently, a study using sheep model of in utero exposure to chronic HAH shows that pulmonary vascular remodeling was similar to that seen in other animal models of pulmonary hypertension (Sheng *et al.,* 2009). This results indicates that pulmonary arteries of fetuses exposed to chronic HAH displayed medial wall thickening and distal muscularization attributed to a rise in epidermal growth factor receptor (EGFR) protein expression in the pulmonary arteries. In a study, it was revealed that that EGFR was crucial in fetal ovine pulmonary vascular remodeling following exposure to chronic HAH and EGFR signaling inhibition may reverse high altitude-induced pulmonary vascular remodeling. EGFR, Platelet-activating factor (PAF) and PAF receptor have also been implicated in the pathogenesis of chronic exposure to HAH-induced pulmonary remodeling and hypertension in different animal models (Ono *et al.,* 1992, Bixby *et al.,* 2007). High PAF and PAF receptor expression levels in pulmonary arteries have been reported in exposure to chronic hypoxia in animal models (Ono *et al.,* 1992; Bixby *et al.,* 2007). Changes in ionic homeostasis of pulmonary arterial smooth muscle cells (PASMCs) caused by chronic exposure HAH have pronounced effects on PA remodeling. Membrane depolarization of PASMCs following of O_2 sensitive K^+ Channels inhibition activates Ca^{2+} influx and thereby elevating cytoplasmic ionized Ca^{2+} through voltage-gated Ca^{2+} channels. These changes in potassium and calcium ion transport regulates these processes by altering membrane potential, apoptosis, gene transcription, cell volume, and cell-cycle progression. These adaptations are involved in pulmonary arterial

remodeling. PASMCs, despite being a major component of arteries actively involved in the mediation of sustained vasoconstriction and enhanced medial hypertrophy, endothelial cells on the other hand can detect humoral and hemodynamic changes resulting from exposure to chronic HAH, thereby triggering the production of vasoactive and mitogenic factors that then affect the growth and function of PASMCs (Emery, 1994, Gerasimovskaya *et al.*, 2005, Stenmark *et al.*, 2006). Exposure to chronic hypoxia increases the gene transcription and peptide synthesis of Endothelin (ET)-1, a key player in hypoxia-induced pulmonary vasoconstriction and vascular remodeling in cultured endothelial cells (Chen and Oparil, 2000). Patients with primary pulmonary hypertension have selectively upregulated ET-1 and its receptors and when exposed to HA (Chen and Oparil, 2000). Administration of ET receptor antagonist can prevent and reverse hypoxic pulmonary vascular remodeling which suggests the importance of ET-1 and its receptor-mediated signaling in chronic hypoxia-induced pulmonary hypertension and vascular remodeling (Chen and Oparil, 2000).

iii. Cerebral circulation

With exposure to high altitude, there is a rise in cerebral blood flow (CBF). However, increased exposure to altitude leads to a fall to near sea level of the increased CBF within 1-3 weeks. This shows that time is an important factor during acclimatization. Generally, natives of HA have lower CBF values compared to sea level natives. The underlying mechanism in the reduction in CBF of high-altitude residents is as a result of the elevation in hematocrit and consequently increased arterial oxygen content (CaO2), this suggests an inverse relationship between CBF and CaO2. Some of the mechanisms responsible for the regulation of CBF are hypoxic ventilator response, hypercapnicventilatory response, hypoxic cerebral vasodilation and hypocapnic cerebral vasoconstriction (Ainslie and Subudhi, 2014).

Initial exposure to HA causes hypobaric hypoxia changes in mediators of CBF, because of a reduction in arterial oxygen tension, which is an independent mediator of cerebral arteriolar dilatation. Also, hypoxemia can excite hyperventilation related decrease in arterial carbon dioxide tension, this causes cerebral arterial constriction due to an associated increase in periarteriolarpH. Therefore, over a few days of constant exposure to HA, the effect of the arterial oxygen tension-induced threshold for cerebral vasodilation is attenuated and the degree of hypocapnia is promoted. Thus, prolonged exposure to HA increases the hematocrit, which results in a rise in the arterial oxygen composition at an unchanged oxygen tension. This change will tend to decrease CBF. Therefore, cerebral hemodynamics during acclimatization to altitude is the result of these homeostatic mechanisms. In addition to these reflex responses, CBF is also controlled by some other hypoxia-induced changes for instance, high-altitude hypoxia-induced changes of cerebral capillary density, hypoxia induced factor (HIF), nitric oxide, endothelin-1, reactive oxygen species (ROS), and neurotransmitters may be responsible for the falling CBF during long-term HAH (Ainslie and Ogoh, 2010).

iv. Uteroplacental circulation

Pregnancy, a condition related to significant increase in uterine blood flow which raises the oxygen delivery and nutrients to the developing fetus. Uteroplacental vascular resistance falls greatly which causes preferential direction of blood flow to this vascular bed, thus raising the uterine blood flow from 20–50 ml/min in the non-pregnant state to 450–800 ml/min in the pregnant state (Palmer *et al.,* 1992). Uterine circulation adaptations to pregnancy are complex and are mainly achieved through the refurbishment of the uterine vasculature, reduced vasoconstrictor response, enhanced vasodilator response and reduced pressure-dependent myogenic reactivity. At sea level, the uterine diameter during pregnancy doubles due to

alterations in vasoreactivity, changes in the active and passive properties of the uterine artery and vascular growth and refurbishment. Although, the underlying molecular mechanism for uterine vascular growth and enlargement of the vascular diameter are not properly understood. However, hormonal stimuli may be one of the major mechanisms responsible for pregnancy-mediated decreased uterine vascular resistance. Estradiol has been reported to be a key player because of its angiogenic properties and stimulatory effects on nitric oxide-mediated vasodilation (Burton *et al.,* 2004). Estrogen receptors (ERs) have been identified in uterine artery vascular smooth muscle and their expressions are significantly increased in pregnant uterine arteries as compared with non-pregnant uterine arteries (Chang *et al.,* 2010). Pregnancy-associated increased ER expression may directly upregulate vascular endothelial growth factor (VEGF), mitogen- activated protein (MAP) kinase, and endothelial nitric oxide synthase (eNOS) expression and their activities, leading to enhance uterine vascular growth and vasodilation (Chang *et al.,* 2010). Reduced uterine vascular resistance can also be controlled by contractile agonists or related proteins. Also, myogenic tone and distensibility are additional factors that can alter uterine arterial intraluminal diameter and uterine vascular resistance. It has been reported that pregnancy significantly downregulates pressure-dependent myogenic tone and increases the pressure- dependent passive uterine arterial diameter. The decreased myogenic tone is mediated by an increase in the inhibitory effect of extracellular-signal regulated kinase (ERK) and a decrease in the protein kinase C (PKC) signal pathway (Xiao *et al.,* 2006).

Exposure to high-altitude has profound effects on uteroplacental circulation which includes alterations in uteroplacental and fetal volumetric blood flows, which results in fetal intrauterine growth restriction. It was reported Julian *et al.* 2008, HAH decreases pregnancy-associated uterine blood flow increase (Julian *et al.,* 2008). Decreased uterine blood flow and insufficient

perfusion of the placenta has been attributed to the rise in the cases of preeclampsia and fetal intrauterine growth restriction (Moore *et al.,* 2011). One of the mechanisms that contributes to the decreased uterine blood flow may be a significant inhibition of pregnancy-associated increase in uterine vascular growth. The proliferative response to serum stimulation in cultured uterine arterial smooth muscle cells is also attenuated by hypoxia exposure (Rockwell *et al.*, 2006). Also, HAH can alter pregnancy-associated responses to contractile proteins and vasodilator- mediated signaling pathways. Report has shown that, long- term exposure of sheep to high- altitude during pregnancy showed significant increase in the pressure-dependent myogenic tone of resistance-sized uterine arteries by suppressing the ERK1/2 activity and increasing the PKC signaling pathway (Chang *et al.,* 2010). Furthermore, HAH exposure selectively downregulate estrogen-α receptor expression in the uterine arteries of pregnant animals and inhibited the steroid hormone-mediated adaptation of ERK1/2 and PKC signaling pathways to raise the myogenic tone of the uterine arteries in pregnancy (Chang *et al.*, 2010). Large-conductance Ca^{2+-} activated K^{+} (BKca) is abundantly expressed in vascular smooth muscle cells. The BKca channel, a major effector in response to hypoxia in vascular smooth muscle has been suggested to be involved in the regulation of uterine circulation and the increase in uterine blood flow during pregnancy (Rosenfold *et al.,* 2005). Evidence from pregnant sheep model exposed to long-term high-altitude (3801 m) provide evidence that, long-term high-altitude hypoxia during pregnancy adversely affects the uterine circulation by down regulating BKca channel function in uterine vasculatures (Hu *et al.,* 2012). During gestation, HAH significantly inhibits pregnancy- associated upregulation of BKca channel activity and diminishes BKca channel current density in pregnant uterine arteries (Hu *et al.,* 2012). This was moderated by a selective downregulation of BKca channel β1 subunit expression in the uterine arteries. In accordance, high-altitude

hypoxia impaired the role of the BKca channel in regulating pressure-induced myogenic tone of uterine arteries that was enhanced in pregnant animals acclimatized to high altitude. These results suggest that selectively targeting BKca channel may be another key mechanism in the maladaptation of uteroplacental circulation caused by HAH, which may contribute to the decreased uterine blood flow and fetal intrauterine growth restriction associated with maternal hypoxia. The molecular mechanisms underlying high-altitude hypoxia-mediated alteration of targeting gene expression in pregnant uterine arteries are not completely understood. Although, studies suggest that epigenetic mechanism plays a crucial role in the control of gene expression in adaptation to HA (Chen *et al.,* 2015). Chronic hypoxia increases estrogen receptor α subunit (ER-α) promoter DNA methylation at both specific protein-1 and upstream stimulatory factor binding sites, decreased specificity protein-1 and upstream stimulatory factor binding to the promoter, and suppressed ER-α expression in uterine arteries of pregnant animals (Chen *et al.,* 2015). Also, evidence shows that hypoxia-mediated DNA methylation plays an intermittent role in ER-α gene repression and deletion of estrogen-mediated adaptation of uterine arterial BKca channel activity, resulting in increased uterine arterial myogenic tone in pregnancy (Chen *et al.,* 2015). Significant differences can be observed in the uterine arterial adaptation to pregnancy based on the duration of exposure to HA. Birth weight of babies born to Tibetan residents at high altitude is higher than that of those born to Han women living at the same altitude, which is associated with a higher uterine flow velocity and larger uterine arterial diameters (Moore *et al.,* 2011). Andean pregnant women have almost doubled increase in their uterine arterial diameters at high altitude whereas, European pregnant women have about half as much increase (Niermeyer *et al.,* 2007). As a result, Andean pregnant women have higher uterine blood flow and birth weight than Europeans at high altitude. However, at sea level, the values are the same

in both Andean and European women, this suggests a higher protective effect of Andean ancestry at high altitude. Reports suggest that genetics are crucial in altitude-related changes in birth weight and uterine blood flow (Moore *et al.,* 2011).

Conclusion

Cardiovascular adaptation to altitude varies, depending on individual predisposition, rate of ascent and duration of exposure. The initial response to HAH exposure is hyperventilation and increase in sympathetic activity (which results partly from chemoreceptor reflexes and baroreceptor function) leading to increase in systemic vascular resistance, blood pressure, heart rate, cardiac output and pulmonary hypertension caused by pulmonary vasoconstriction. Much of the individual variability is dependent upon the severity of associated pulmonary hypertension, which is most often mild but may be severe in a proportion of cases. In response to HAH, CBF increases to ensure adequate supply of oxygen to the brain tissues.

Cardiovascular adaptation includes changes in vascular structures, remodeling and functional proteins through different molecular mechanisms such as epigenetic regulatory and/or genetic factor mediated mechanisms. Adaptations to HAH involve both compensatory and pathologic mechanisms. Maladaptation such as; pulmonary hypertension, pulmonary edema, heart failure, cerebral edema, chronic mountain sickness, and fetal intrauterine growth restriction develops from pathologic adaptations.

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Chapter 4

Title: Impact of high altitude on BMI: an evaluation of cardiometabolic risk in exposed individuals

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4.1 Abstract

Background: Cardiometabolic disorder (CMD) is a constellation of metabolic dysfunctions, which prone an individual to developing dyslipidemia, hypertension, diabetes mellitus, and central obesity. It remains a global challenge and poses a threat to human life with a decrease in longevity. Although some studies reported exposure to high height as a predisposing factor to the development of CMD, others reported a negative association. **Aim:** Thus, this study investigated whether or not high-altitude exposure increases the risk of obesity development and atherogenic indices in lowlanders who were high-altitude travelers. **Materials and methods:** A total of 300 aviation workers were allotted to 3 groups (n= 100 subjects per group); the control who were

non-crew members and did not travel by air, the PHA-L group who were crew members and traveled by air locally for at least 5 years, and the PHA-I who were crew members and traveled by air internationally for at least 5 years. **Results:** High altitude exposure significantly increased incident obesity independent of the dietary patterns of the subjects when compared with the control. Also, exposure to high altitude led to increased levels of serum total cholesterol, low-density lipoprotein, and triglyceride, but decreased levels of high-density lipoprotein when compared with the control. These findings were associated with raised Castelli risk indices I and II and elevated TNF-α and IL-1β. The observed perturbations were markedly increased in PHA-I than in PHA-L. **Conclusion:** High altitude exposure is a predisposing risk factor to cardiometabolic disorder evidenced by increased obesity development, lipid deregulation, and elevated Castelli risk indices. This may be mediated by the upregulation of pro-inflammatory cytokines.

Keywords: Atherogenic indices; cardiometabolic disorder; high altitude; inflammation; lipids; lipoproteins

4.2 Introduction

Cardiometabolic disorders (CMD) are collections of metabolic dysfunctions, that prone an individual to developing certain cardiovascular diseases like dyslipidemia, hypertension, diabetes mellitus, and central obesity (Grundy *et al.,* 2005; Akhigbe *et al.,* 2021). The increase in CMD has become a global challenge and poses a threat to human life with a decrease in longevity (WHO, 2021). CMD is prevalent in developed, underdeveloped, and developing nations (Chen *et al.,* 2007; Cevenini *et al.,* 2010; Taheri *et al.,* 2023) and poses a financial burden on individuals and a challenge to the economic growth of nations (Akhigbe and Ajayi, 2021). In addition to being inflammatory, CMD also induces oxidative damage (Abete *et al.,* 1999) and risk factors

like hypothyroidism (Ajayi *et al.*, 2017), insulin resistance, hypertension, dyslipidemia, abnormal lipolysis, and abnormally increased BMI (Ferreira *et al.*, 2019).

Obesity is characterized by an increase in BMI above 30 kg/m2 (Giroud *et al.*, 2022). In another study, the WHO described obesity as the excessive clustering of fat that increases the health risk and leads to a life-threatening condition (Chung, 2018). Excess fat accumulation or increased BMI has been demonstrated to raise the prevalence of CMD (Fangjian *et al.*, 2014; Hruby and Hu, 2015). It is now understood that adipose tissue functions as an active endocrine organ that produces adipokines, which in turn cause oxidative stress, reduced insulin sensitivity, and a role in decreased metabolic activity that results in cardiometabolic diseases (Jung and Jung, 2021). Over-nutrition and physical inexertion cause adipose tissue buildup and adipocyte impairment via downregulation of fat breakdown, leading to the buildup of fat in the liver, heart, muscles, and blood vessels (Britton and Fox, 2011). Increased BMI is caused by non-modifiable factors like developmental factors, genetic factors, gender, and age, and modifiable factors such as physical inactivity, diet, environmental factors, and exposure to high altitude (Touvier *et al.*, 2015).

High altitude according to Woolcott *et al.* (2023) is an elevation of about 2,400 meters or 8000 feet. Individuals at high altitudes are prone to hypoxia from birth, leading to acclimatized changes that include hormonal and metabolic changes such as an increase in insulin sensitivity, glucose uptake increase in peripheral tissues, and blood glucose decrease (Expert Panel on Detection, 2001). It was revealed that individuals who lived at a high altitude in comparison to those who lived at sea level have hypercholesterolemia, hypertriglyceridemia, abdominal obesity, low HDL, abnormal waist circumference, and increased incidence of obesity (Mohanna

et al., 2006). Also, Sherpa *et al.* (2011) reported an imbalance in lipid distribution among the Tibean population who lived at high altitudes, which was associated with hypercholesterolemia, hypertriglyceridemia, and low HDL-C. Conversely, some studies suggest a decrease in BMI among populations living at high altitudes. At high altitudes, reduction in food, calorie intake, and weight loss were observed according to Tschöp and Morrison. (2001). In a study that was carried out among women from low-middle-income nations, it was demonstrated that high altitude causes a decrease in BMI and lower chances of obesity development (Maxfield *et al.*, 2024). An increase in leptin level due to a higher altitude consequently increases basal metabolic rate (BMR), leading to less calorie intake as a result of anorexia, therefore causing a disparity between calorie intake and energy expenditure that can lead to loss of weight (Quintero *et al.*, 2010; Kayser and Verges, 2013). Lippl *et al.* (2010) revealed that about a week spent at a high altitude of about 8,700 feet with a regular food intake and without exercise was associated with weight loss, possibly due to a lower intake of calories after about four weeks out of a high altitude.

Hence, there are conflicting reports on the effect of high-altitude exposure on BMI and the risk for metabolic disorders. The effect of high altitude on BMI as a precursor for cardiometabolic risk was evaluated. Also, the effect of high-altitude exposure on lipid profile, atherogenic indices, and the levels of pro-inflammatory mediators (specifically, TNF-α and IL-1β) were explored.

4.3 Materials and Methods

4.3.1 Study design

The present study is a cohort study that investigated the impact of high altitude exposure on BMI, blood pressure and hemodynamic indices, lipid profile, atherogenic indices, and inflammatory markers in pilots and other aircrew members.

4.3.2 Study population and setting

Workers in the aviation sector were chosen for the study from among aircrew members (pilots and cabin crew) who had received the necessary certification from the Federal Airports Authority of Nigeria (FAAN), ground handlers (SAHCOL and NAHCO), Nigerian Airspace Management Agency (NAMA), and Nigerian Civil Aviation Authority (NCAA). The study concentrated on male and female aviation professionals in particular.

4.3.3 Research Setting

The study was conducted at Kupa Aviation Medical Laboratory and Clinic, an approved and certified Aeromedical facility by the Civil Aviation of Nigeria, the Aeromedical Standard Department of NCAA, and some designated local and international airports in Nigeria such as Lagos, Abuja, Kano, and Ilorin Airports. These facilities are Aviation environments in which workers and aircrew members operate primarily.

4.3.4 Sampling Method

Using the random sampling approach, the study's designated statistician assisted in the selection of the sample. To get consent and accurately choose individuals who fit the study's inclusion criteria, participants were contacted with the help of a certified nurse and an aeromedical assessor from the Nigerian Civil Aviation Authority.

4.3.5 Sample Size

A total number of 300 participants were randomly selected into 3 groups. Determination of the sample size was done using the Guilford and Flrucher formula.

$$\frac{N}{1 + Q^2N}$$

Where N= population size 1200

Q= 0.05

$$\frac{1200}{1 + (0.05)^2 x1200}$$

$$sample\ size = \frac{1200}{1+3}\ 300$$

Sample Size = 300

Participants were randomized into three, namely:

Group A (Control)**:** 100 aviation workers who are non-aircrew and have no flight experience for at least two (2) years.

Group B: 100 local aircrew members from local airlines and have been traveling for not less than five years; PHA-L

Group C: 100 aircrew members who have traveled for not less than five years internationally; PHA-I.

Inclusive criteria: Adults between the ages of 30 and 45 who gave their consent, worked in the aviation sector for at least five years, were not pregnant, had no history of cardiovascular or lipid

disorders, were free from known infections or cancers, and were not dependent on cigarettes or alcohol (optional).

Exclusive criteria: participants who are less than 30 years and older than 45 years of age, non-consenting participants, non-aviation workers, participants with infections, disorders, known to have cardiovascular or lipid disorders were all excluded from the study.

4.3.6 Data collection tools

Before the sample was collected, the participants were given an open-ended structured questionnaire to provide sociodemographic information. The open-ended structured questionnaire contains two sections of twenty-two (22) questions and lasted for about 30 minutes to complete. Section one contained bio data and industry experience of the participants while the other section focused on their demographics and lifestyles.

4.3.7 Participant Recruitment

Participants were informed about the research study before commencement after which they all gave written informed consent for participation in the study. After giving their consent, participants were given information regarding the nature and goals of the study and given a time slot for participation.

4.3.8 Ethical consideration

The Faculty Research Committee of the Faculty of Clinical Sciences College of Health Sciences, Osun State University, Osogbo, Nigeria, duly approved the study. Following permission for the study on site from the Aviation Medical Laboratory and Clinic at KUPA Medical Center in Lagos, ethical approval was also requested and received from the Nigerian Civil Aviation Authority (NCAA), the country's aviation regulatory agency. An additional consultation was

conducted to obtain ethical approval with the research ethics committee (REC) of the Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa (HW-REC), registration number NHREC: REC-230408-014 of Cape Peninsula University of Technology. The goal of the study was explained to the participants before the distribution of any questionnaires.

4.3.9 Data and sample collection

Participants' sociodemographic information was gathered using a standardized, pilot-tested questionnaire. The BMI was determined by dividing the individuals' height (in meters) by their weight (in kilograms). Using an ACCUSON sphygmomanometer, the systolic and diastolic blood pressures (SBP and DBP) were measured, and the pulse pressure (PP) was calculated as SBP-DBP. DBP + 1/3 (PP) was used to calculate the mean arterial pressure (MAP), and a stethoscope was used to auscultate the heart rate (Littman). The baseline values of the blood pressure and the hemodynamic indices were referred to as the initial values, while the values during the study were referred to as the final values. The change was determined and reported based on the variations between the original and final numbers. The fasting plasma level of lipids and inflammatory markers (TNF-α and IL-1β) were also determined. Atherogenic indices were calculated from the lipid profile. After a 12-hour fast, blood samples were taken from the participants using the medial cubital vein and placed into the proper sample vials. The obtained blood samples were spun at 3000 rpm for 10 minutes to obtain the serum/ plasma that was kept frozen at -80^0C at the KUPA Aviation Medical Clinic, Lagos, Nigeria until they were needed for analysis.

4.3.10 Sample Analysis

Using conventional laboratory reagents, the colorimetric approach was used to determine the lipids in fasting plasma (Randox Laboratory Ltd., UK). Castelli risk index-I (CR-I), likewise identified as cardiac risk ratio and a pointer to the formation of coronary plaques was determined as the ratio of total cholesterol (TC) to high-density lipoprotein (HDL), while Castelli risk index- II (CR-II) was determined as the ratio of low-density lipoprotein (LDL) to HDL (Akhigbe *et al.,* 2021; Akhigbe and Hamed, 2021).

Plasma concentrations of tumor necrotic factor-alpha (TNF-α) and interleukin-1beta (IL-1β) were assayed as inflammatory markers using an ELISA kit (Elabscience, Biotechnology Co., Ltd, USA) following the manufacturers‟ guidelines.

4.3.11 Statistical Analysis

Data was analyzed and bar charts were obtained using GraphPad Prism (version 9). Chi-square analysis was used for discrete variables, and one-way analysis of variance (ANOVA) and Tukey's posthoc test were used for continuous variables for group comparisons and pairwise comparisons, respectively. Results are presented as frequencies or percentiles and median. Values of *P* less than 0.05 were considered statistically significant.

4.4 Results

Socio-demographic data

The baseline characteristics of the participants are presented in Table 1. Most of the participants in the control group (42%) were between 20 and 29 years old, while most of the participants in the PJHA-L (45%) and PHA-I groups (51%) were between 30 and 39 years old. More so, the majority of the participants in the control (51%), PHA-L (79%), and PHA-I (83%) were male.

The distribution of the participants was not significantly different in terms of religion (*P*= 0.6206) and ethnicity (*P*= 0.5009). Most of the participants were Christians in the control (53%), PHA-L (59%), and PHA-I (58%). Also, the majority of the participants in the control (52%), PHA-L (55%), and PHA-I (68%) were Yorubas, while the least (3%, 5%, and 2% for the control, PHA-L, and PHA-I respectively) were Ebiras. In addition, most of the participants were Nigerians (98%, 83%, and 85% for the control, PHA-L, and PHA-I respectively).

In the control group, 37% had worked as aircrew members or pilots for 5-9.9 years, while 34% had worked for 10-15 years and 29% for 0-4.9%. On the other hand, all (100%) of the subjects in the PHA-L group had worked for just 0-4.9 years, while 78% of those in the PHA-I group had worked for 5-9.9 years and 22% for 10-15 years. Furthermore, all (100%) the participants in the control group fly for about 1-199 hours, while just 47% of those in the PHA-L fly for 1-199 hours, and the remaining 39% and 14% fly for 200-399 and 400-599 hours respectively. About 15%, 26%, and 59% of those in the PHA-I group fly for 1-199, 200-399, and 400-599 hours respectively.

The marital status, eye color, and hair color of the subjects were not significantly different across the groups. About 76%, 81%, and 85% of the subjects were married in the control, PHA-L, and PHA-I groups respectively. Also, 97%, 98%, and 96% of the subjects had brown-colored eyes in the control, PHA-L, and PHA-I groups respectively; while 90%, 86%, and 81% of the subjects had black-colored hair in the control, PHA-L, and PHA-I groups respectively.

The dietary pattern of the subjects was similar across the groups (*P*= 0.2432). About 89%, 82%, and 81% of the subjects were carnivores in the control, PHA-L, and PHA-I groups respectively; while 11%, 18%, and 19% of the subjects were vegetarians in the control, PHA-L, and PHA-I groups respectively (Table 2).

Blood pressure and hemodynamic indices

The changes in SBP and MAP were significantly increased in subjects in the PHA-I group when compared with those in the PHA-L group. In addition, the change in HR was markedly increased in PHA-I subjects when compared with PHA-L subjects. However, the changes in SBP and PP were comparable in subjects in the PHA-I group when compared with those in the PHA-L group (Table 3).

Anthropometric indices

The height of the subjects in the PHA-L group was significantly increased when compared to the control and PHA-I groups. Also, the height of the subjects in the PHA-I group was significantly lower when compared to the control. In addition, the weight of subjects exposed to high altitude was significantly higher when compared to the control. The increased weight in high altitude exposure was higher in the PHA-I when compared with the PHA-L subjects. Furthermore, exposure to high altitude significantly increased the BMI when compared with the control. The increase in BMI was significantly increased in the PHA-I when compared with the PHA-L subjects (Figure 1).

Lipid profile and atherogenic indices

Exposure to high altitude led to a significant increase in serum levels of total cholesterol, triglyceride, and LDL-C when compared with the control. The observed rise was significantly higher in PHA-I subjects when compared with the PHA-L subjects. More so, exposure to high altitude significantly reduced HDL-C when compared to the control. This perturbation was more significant in PHA-I subjects when compared with the PHA-L subjects. Additionally, exposure to high altitude significantly increases CR-I and CR-II when compared with the control. The observed rise was significantly higher in PHA-I subjects when compared with the PHA-L subjects (Table 4).

Inflammatory markers

Exposure to high altitude led to a significant increase in serum levels of TNF-α when compared with the control. The observed rise in TNF-α was significantly higher in PHA-I subjects when compared with the PHA-L subjects. Furthermore, high altitude exposure caused a significant increase in serum levels of IL-1β when compared with the control. The observed increase in IL-1β was significantly higher in PHA-I subjects when compared with the PHA-L subjects (Table 5).

4.5 Discussion

In the present cohort study, we observed that participants who were exposed to high altitude had significantly increased body weight and BMI, which were associated with dyslipidemia, raised SBP, MAP, and HR, and increased atherogenic indices viz. CR-I and CR-II but independent of the dietary pattern. These findings were accompanied by an upregulation of TNF-α and IL-1β.

The present finding that high altitude exposure was associated with obesity development irrespective of the dietary pattern of the individual aligns with previous reports (Mohanna *et al.*,

2006; Baracco *et al.*, 2007) that documented the positive association between exposure to high altitude and obesity development. However, this does not agree with the findings of Díaz-Gutiérrez *et al.* (2016) and Merrill (2020) that demonstrated a negative association between high altitude exposure and incident obesity. The studies that reported a negative association between high altitude exposure and obesity development attributed the link to low-calorie intake and high energy expenditure, while our present data revealed that obesity development following exposure to high altitude was independent of dietary pattern; hence, likely not associated with calorie intake. Our finding that high altitude exposure increases the risk of obesity development may be ascribed to the observed lipid deregulation seen following exposure to high altitude evidenced by hypercholesterolemia, hypertriglyceridemia, increased LDL-C, and reduced HDL-C, which may result in abnormal lipid distribution and fatty deposition in body tissues. These observations corroborate the data documented by Sherpa *et al.* (2013) and Vats *et al.* (2013) that revealed dyslipidemia following exposure to high altitude. High altitude-induced dyslipidemia may result in abnormal fat deposition and increased waist-hip ratio (Sherpa *et al.*, 2011), abnormal waist and abdominal circumferences, and increased incidence of obesity (Mohanna *et al.*, 2006,).

In addition, the observed lipid deregulation in individuals exposed to high altitude was accompanied by a rise in CR-I and CR-II, which are established atherogenic indices and pointers to atherosclerosis. These observations were also accompanied by a rise in SBP, MAP, and HR, indicating that high altitude exposure led to a rise in atherogenic indices and systemic blood pressure. This is in agreement with previous studies that revealed that high altitude exposure induces adverse cardiovascular events that are associated with elevated arterial blood pressure (Mohanna *et al.*, 2006; Baracco *et al.*, 2007; Sherpa *et al.*, 2011; Sherpa *et al.*, 2013). Increased levels of triglycerides and LDL-C are accompanied by oxidation of this lipoprotein by

endothelial nitric oxide synthase (eNOS)-dependent ROS into oxidized LDL (OxLDL), which the scavenger receptor CD36 on macrophages picks up to produce foam cells (Collot-Teixeira *et al.*, 2007; Akhigbe and Ajayi, 2021) that undergo apoptosis and formation of atherosclerotic plaques, resulting in the narrowing of the arterial lumen and elevated intraluminal pressure (Akhigbe and Ajayi, 2021).

It was also observed that high altitude led to upregulation of IL-1β and TNF-α. Although the present study did not report any data on oxidative stress and adiponectin, it is known that a rise in these pro-inflammatory cytokines (IL-1β and TNF-α) in obesity is usually triggered by oxidative stress and accompanied by suppression of circulating adiponectin (Akhigbe and Ajayi, 2021). Adiponectin enhances insulin sensitivity and exerts anti-atherogenic activity (Yamauchi *et al.,* 2001; Maeda *et al.,* 2002); hence, a significant decline in adiponectin causes adipocyte dysregulation and NF-kB-dependent systemic inflammation that results in insulin resistance and atherosclerosis by promoting endothelial dysfunction (Akhigbe and Ajayi, 2021). Therefore, exposure to high altitude increases cardiometabolic risk via multiple pathways, which may include lipid deregulation and oxidation of LDL-C that concludes in the generation of foam cells, resulting in the formation of atherosclerotic plaques (Pham et al., 2022). In addition, high altitude exposure may upregulate TNF-α and IL-1β which is associated with the induction of systemic inflammation, endothelial dysfunction, and atherosclerosis.

4.6 Conclusion

Summing up, the data presented demonstrate that high altitude exposure causes lipid deregulation and obesity development, which is accompanied by increased cardiovascular risk as depicted by elevated SBP, MAP, HR, CR-I, and CR-II, and upregulated pro-inflammatory factors that may promote atherosclerosis. These findings revealed that high altitude exposure

predisposes to the development of cardiometabolic disorder. It is nonetheless recommended that more well-designed epidemiological studies be carried out among other populations to validate the present findings and demonstrate other possible pathophysiological pathways.

Declarations

Ethical Approval

Approval for the study was obtained from the Faculty Research Committee of the Faculty of Clinical Sciences College of Health Sciences, Osun State University, Osogbo Nigeria and Ethical Review Committees of the Aviation regulatory body in Nigeria, The Nigerian Civil Aviation Authority (NCAA), KUPA Medical Center in Lagos, an Aviation Medical Laboratory and Clinic. Ethnical clearance was also obtained from the Research Ethnics Committee (REC) of the Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa (HW-REC) (Approval number: NHREC: REC-230408-014).

Competing Interests

The authors have no conflicts of interest.

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This study did not receive external funding.

Data Availability

The data used to support the findings of the present study are available from the corresponding author upon request.

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Figures

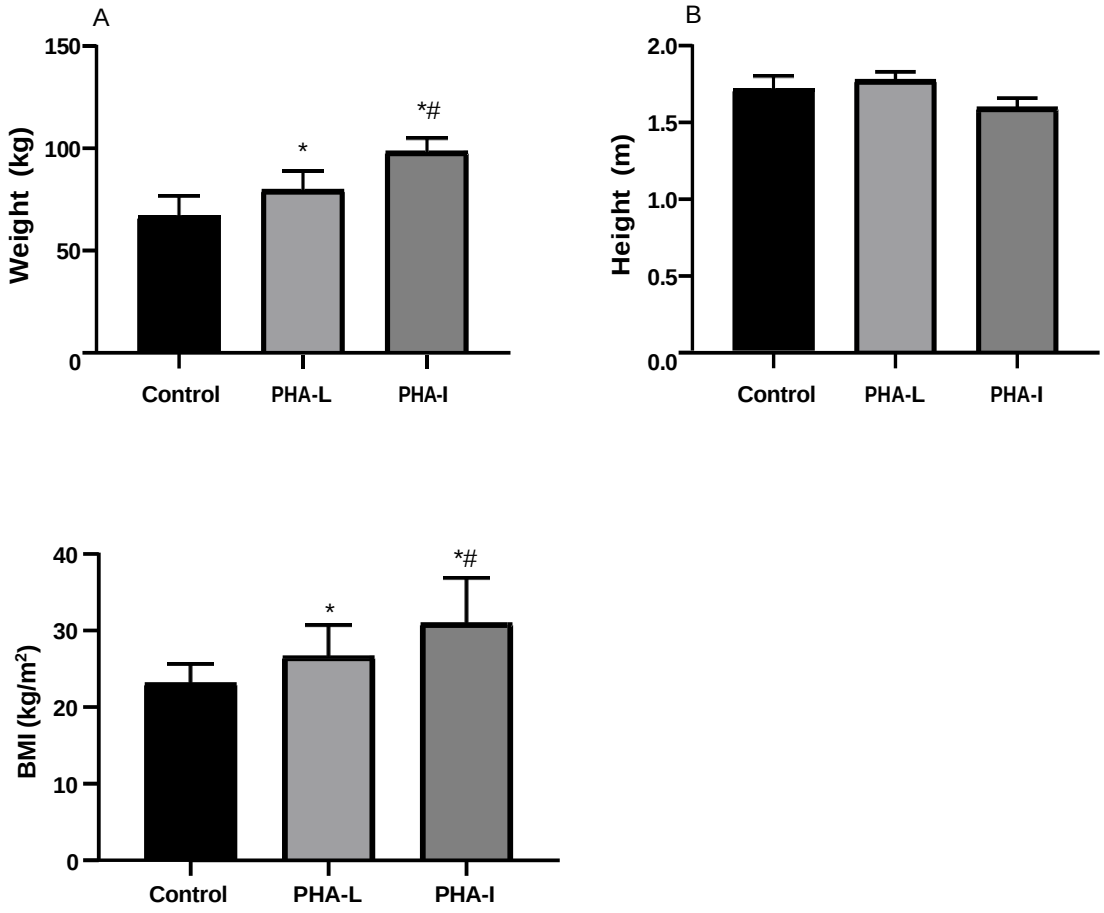

Figure 1: Effect of high altitude exposure on body weight (A), body mass index, BMI (C) and height (B) distribution of participant.

*$p<0.05$ compared to control, #$p<0.05$ compared to PHA-L.

Table 1: Sociodemographic pattern

VARIABLES (BIODATA)	GROUP PERCENTAGE			*P*-values
	Control	**PHA-L**	**PHA-I**	
AGE				
20-29 years	42	17	12	<0.0001
30-39 years	35	45	51	
40-49 years	23	38	37	
GENDER				
Male	51	79	83	<0.0001
Female	49	21	17	
RELIGION				
Christianity	53	59	58	0.6206
Islam	40	31	36	
Others	7	10	6	
ETHNICITY				
Yoruba	52	55	68	0.5009
Igbo	20	16	12	
Hausa/ Fulani	12	10	8	
Ijaw	10	8	5	
Ebira	3	5	2	
Others	3	6	5	
NATIONALITY				
Nigerian	98	83	85	0.0009

Non-Nigerian	2	17	17	
WORKING EXPERIENCE				
0-4.9 years	29	100	0	<0.0001
5-9.9 years	37	0	78	
10-15 years	34	0	22	
FLYING HOURS				
1-199	100	47	15	<0.0001
200-399	0	39	26	
400-599	0	14	59	
MARITAL STATUS				
Married	76	81	85	0.2715
Not married	24	19	15	
EYE COLOUR				
Brown	97	98	96	0.7092
Others	3	2	4	
HAIR COLOUR				
Black	90	86	81	0.3638
Blonde	10	13	17	
Red	0	1	2	

Data are presented as frequencies

Table 2: Dietary pattern of the subjects

DIET	GROUP PERCENTAGE			*P*-values
	Control	PHA-L	PHA-I	
Carnivore	89	82	81	0.2432
Vegetarian	11	18	19	

Data are presented as frequencies

Table 3: Effect of high-altitude exposure on lipid profile and atherogenic indices

Parameter	Control	PHA-L			PHA-I		
		Before	After	Change	Before	After	Change
SBP (mmHg)	128.4 ± 7.4	135.8 ± 6.4	139.6 ± 5.4	3.8 ± 1.8	138.5 ±7.2	148.4 ±6.2	9.9 ±2.1*
DBP (mmHg)	80.5 ± 8.4	83.2 ±5.8	84.6 ± 4.8	1.4 ± 1.5	82.8 ±5.7	85.9 ± 5.2	3.1 ±1.2
MAP (mmHg)	89.7 ± 6.3	87.5 ±7.6	85.1 ± 6.5	-2.4 ± 1.4	86.4 ± 4.9	89.8 ± 5.0	3.4 ±1.6*
PP (mmHg)	47.5 ±7.2	48.1 ± 6.7	50.1 ±5.9	2.0 ±0.8	48.7 ±5.3	51.6 ± 4.3	2.9± 1.4
HR (mmHg)	73.3 ± 8.1	75.2 ±7.3	79.4 ± 8.3	4.2 ±2.1	77.4 ±5.6	82.9 ±6.7	5.5 ± 1.3*

Values are expressed as means ±SEM. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP) and heart rate (HR). *$p<0.05$ compared to control, #$p<0.05$ compared to PHA-L.

Table 4: Effect of high-altitude exposure on lipid profile and atherogenic indices

	Minimum	25th percentile	Median	75th percentile	Maximum	*P* values		
						Control vs. PHA-L	Control vs. PHA-I	PHA-L vs. PHA-I
Total cholesterol (mg/dL)								
Control	112.00	123.00	124.00	126.00	138.00	<0.0001	<0.0001	<0.0001
PHA-L	123.30	134.30	136.30	145.30	156.40			
PHA-I	123.30	145.40	162.80	176.00	226.40			
Triglyceride (mg/dL)								
Control	83.37	92.00	94.23	99.00	110.00	<0.0001	<0.0001	<0.0001
PHA-L	92.48	110.30	113.30	119.00	130.00			
PHA-I	97.58	122.90	126.60	132.20	155.70			
HDL-C (mg/dL)								
Control	43.00	47.00	50.00	53.00	58.69	<0.0001	<0.0001	<0.0001
PHA-L	29.56	33.00	34.05	36.00	40.12			
PHA-I	20.49	25.49	29.39	34.49	40.02			
LDL-C (mg/dL)								
Control	41.23	56.46	63.48	66.00	87.49	<0.0001	<0.0001	<0.0001

PHA-L	58.18	67.11	74.28	81.00	97.99			
PHA-I	47.49	76.78	85.00	87.59	99.98			
CR-I								
Control	2.90	3.69	4.41	5.51	6.66	<0.0001	<0.0001	<0.0001
PHA-L	5.06	6.23	7.59	8.30	9.96			
PHA-I	7.16	9.43	11.00	11.00	16.29			
CR-II								
Control	1.31	1.79	2.17	2.57	3.88	<0.0001	<0.0001	<0.0001
PHA-L	2.00	2.57	2.96	3.31	4.07			
PHA-I	3.10	4.16	4.70	4.97	7.30			

HDL-C: High-density lipoproteins, LDL-C: low-density lipoproteins, CR-I: Castelli risk index-I, CR-II: Castelli risk index-II

Table 5: Effect of high altitude exposure on inflammatory markers

	Minimum	**25th percentile**	**Median**	**75th percentile**	**Maximum**	***P* values**		
						Control vs. PHA-L	**Control vs. PHA-I**	**PHA-L vs. PHA-I**
TNF-α (ng/mL)								
Control	3.00	8.59	10.61	13.97	20.67	<0.0001	<0.0001	<0.0001
PHA-L	13.00	20.25	23.00	26.95	36.10			
PHA-I	17.76	25.97	29.83	36.33	56.33			
IL-1β (ng/mL)								
Control	1.86	6.22	8.00	9.00	20.25	<0.0001	<0.0001	<0.0001
PHA-L	3.22	8.37	13.73	18.63	46.05			
PHA-I	12.30	28.93	37.47	44.50	54.30			

TNF-α: tumour necrosis factor-alpha, IL-1β: interleukin-1beta

Chapter 5

Title: Exposure to high altitude induces dyslipidemia and renal injury via the upregulation of inflammatory cytokines and induction of oxidative stress

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Running title: High altitude alters antioxidant and inflammatory biomarkers in humans.

This manuscript has been submitted and undergoing peer-review for possible publication in Current Research in Physiology (Pubmed-indexed)

5.1 Abstract

Background: Cardiovascular events, dyslipidemia, and renal damage are just a few of the harmful outcomes that have been linked to exposure to high altitude. There is a lack of data regarding the involvement of oxidative stress and inflammation in high altitude-induced dyslipidemia and renal injury, even though these conditions have been linked to the pathogenesis of both conditions. **Aim:** This study assessed the potential involvement of inflammation and

oxidative stress in lowlanders who traveled to high altitudes. **Materials and methods:** A total of 300 aviation workers were assigned to 3 groups (n= 100 subjects per group); the control who were non-crew members and did not travel by air, the PHA-L group who were crew members and traveled by air locally for at least 5 years, and the PHA-I who were crew members and traveled by air internationally for at least 5 years. **Results:** Subjects exposed to high altitude had higher levels of total cholesterol, triglyceride, very low-density lipoprotein, low-density lipoprotein, and atherogenic indices but lower levels of high-density lipoprotein when compared with the control. These findings were associated with increased serum urea, creatinine, BUN, C- reactive protein, TNF-α, IL-1β, and malondialdehyde, and reduction in Ca2+-ATPase and Na+/K+-ATPase activities in subjects exposed to high altitude. The alterations were significantly higher in PHA-I than in PHA-L. **Conclusion:** Exposure to high altitude induces dyslipidemia and renal injury via the activation of cytokine-driven inflammation and oxidative stress.

Keywords: High altitude; hypoxia; inflammation; lipids; oxidative stress; renal injury

5.2 Introduction

Worldwide, 100 million lowlanders travel annually to mountainous regions over 2500 m, while approximately 400 million are resident in places higher than 1500 m (Mallet *et al.*, 2021). While highlanders are exposed to the adverse effects of high altitude, lowlanders who are high-altitude travelers are also exposed to similar effects although possibly to a lesser degree (Burtscher *et al.,* 2018). The high-altitude adverse effects are mediated by the associated extreme conditions like hypoxia, hypobaria, and low temperature (Hackett and Roach, 2001; Moore, 2001; Basnyat and Murdoch, 2003).

Although high altitude seems to modulate systemic arterial pressure (Mingji *et al.,* 2015; Song *et al.,* 2020), it induces insulin resistance (Woolcott *et al.,* 2014), dyslipidemia (Deng *et al.,* 2012; Lopez-Pascual *et al.,* 2018), and renal injury (Swenson, 2001; Laustsen *et al.,* 2014). In a study by Song *et al.* (2020), high altitude reduced hypertension prevalence from 40.6% to 20.4%, while Mingji *et al.* (2015) observed a 2% rise in hypertension prevalence. The variation in the observed effects of high altitude on blood pressure may be due to the cardiovascular consequences of divergent adaptation (Beall, 2000), including the activation of hypoxia- inducible factors-led hypoxia-responsive gene transcription (Mallet *et al.,* 2021). More so, high altitude is associated with high levels of cholesterol, triglycerides, and low levels of high-density lipoproteins (HDL-C) (Sherpa and Deji, 2011; Deng *et al.,* 2012), however, the underlying mechanism is yet to be reported. Furthermore, high altitude induces renal injury by modulating renal metabolism (Laustsen *et al.,* 2014) and nitric oxide (NO) generation (Swenson, 2001).

Excessive number generation has been shown to promote the reaction of NO with superoxide radicals to produce peroxynitrite which in turn causes endothelial nitric oxide synthase (eNOS) uncoupling, inducible nitric oxide synthase (iNOS) uncoupling and depletion of tetrahydrobiopterin (BH_4) and results in endothelial dysfunction (Otani, 2011; Akhigbe and Ajayi, 2021), a known cause of renal injury. Peroxynitrite from NO may also exert pro-oxidant activity, resulting in increased ROS generation (Akhigbe and Ajayi, 2021), resulting in renal damage. Also, eNOS may upregulate ROS generation and promote the oxidation of low-density lipoproteins (LDL) to yield oxidized LDL (OxLDL) and the activation of pro-inflammatory cytokines which both contribute to incident atherosclerosis and renal injury (Moore and Freeman, 2006; Akhigbe and Ajayi, 2021).

Despite the role of inflammatory cytokines in the development of renal damage, there is a scarcity of data on their role in high-altitude-induced renal damage. This study therefore investigated whether or not high-altitude exposure induces dyslipidemia and renal damage in the participants. Also, the likely roles of inflammation, specifically, C-reactive protein (CRP)/tumor necrosis factor-α (TNF- α)/interleukin-1β (IL-1 β) signaling pathway and oxidative stress were explored in lowlanders who were high-altitude travelers.

5.3 Materials and Methods

5.3.1 Study design

This is a cross-sectional study that examined the possible ways in which prolonged exposure to high altitude affects the cardiovascular system and biochemical components in pilots and other crew members.

5.3.2 Study population and setting

Participants in this study are professionals in the aviation sector who were chosen from among aircrew members (pilots and cabin crew) who have received the necessary certification from the Federal Airports Authority of Nigeria (FAAN), ground handlers (SAHCOL and NAHCO), Nigerian Airspace Management Agency (NAMA), and Nigerian Civil Aviation Authority (NCAA). The study concentrated on both male and female aviation employees.

5.3.3 Research Setting

The study was conducted at Kupa Aviation Medical Laboratory and Clinic, an approved and certified Aeromedical facility by the Civil Aviation of Nigeria, the Aeromedical Standard Department of NCAA, and some designated local and international airports in Nigeria such as Lagos, Abuja, Kano, and Ilorin Airports. The above areas serve as the Aviation environment in which workers and aircrew members operate primarily.

5.3.4 Sampling Method

The random sampling approach was utilized to choose the sample, and a statistician who was assigned to the project provided support. The Nigerian Civil Aviation Authority's aeromedical assessor and a qualified nurse assisted in contacting participants to gather their consent and fairly identify those who fit the study's inclusion criteria.

5.3.5 Sample Size

A total number of 300 participants were randomly selected into 3 groups. Determination of the sample size was done using the Guilford and Flrucher formula.

$$\frac{N}{1 + Q^2 N}$$

Where N= population size 1200

Q= 0.05

$$\frac{1200}{1 + (0.05)^2 x 1200}$$

$$sample\ size = \frac{1200}{1+3}\ 300$$

Sample Size = 300

Participants were grouped into three, namely.

Group A (Control)**:** 100 aviation workers who are non-aircrew and have no flight experience for at least two (2) years.

Group B: 100 local aircrew members from local airlines and have been traveling for not less than five years; PHA-L

Group C: 100 aircrew members who have traveled for not less than five years internationally; PHA-I.

Table 1:

Group	Group Name	Number of subjects (n)	Flying duration
A	Control – non crew member	100	0
B	Local aircrew	100	≥5years
C	International aircrew	100	≥5years

Inclusive criteria: Adults between the ages of 30 and 45 who gave their consent, worked in the aviation sector for at least five years, were not pregnant, had no history of cardiovascular or lipid disorders, were free from known infections or cancers, and were not dependent on cigarettes or alcohol (optional).

Exclusive criteria: participants who are less than 30 years and older than 45 years of age, non-consenting participants, non-aviation workers, participants with infections, disorders, known to have cardiovascular or lipid disorders were all excluded from the study.

5.3.6 Data collection tools

Before sample collection, the participants completed an open-ended structured questionnaire designed to gather sociodemographic information. The open-ended structured questionnaire contains two sections of twenty-two (22) questions and lasted for about 30 minutes to complete.

Section one contained bio data and industry experience of the participants while the other section focused on their demographics and lifestyles.

5.3.7 Participant Recruitment

The researcher and the team informed the participants about the research study before commencement after which they all gave written informed consent for participation in the study. Appointments were scheduled for consenting participants after they were told about the nature and objective of the study.

5.3.8 Data and sample collection

A structured pilot-tested questionnaire was used to obtain socio-demographic data from the participants. Anthropometric data including weight, height, blood pressure, waist, and hip circumference, as well as biochemical parameters such as lipid profile, kidney function, and inflammatory and oxidative stress markers, were determined. Participants were fasted overnight for 12 hours and then blood samples were obtained from them through the medial cubital vein into appropriate sample bottles. The obtained blood samples were centrifuged at 3000 rpm for 10 minutes to obtain the serum/ plasma that was kept frozen at -80^0C at the KUPA Aviation Medical Clinic, Lagos, Nigeria until they were needed for analysis.

5.3.9 Ethical consideration

Approval for the study was duly obtained from the faculty research committee of the Faculty of Clinical Sciences College of Health Sciences, Osun state university, Osogbo Nigeria. Ethical approval was also sought and obtained from the Aviation regulatory body in Nigeria, The Nigerian Civil Aviation Authority (NCAA) after KUPA medical center in Lagos, an Aviation Medical Laboratory and Clinic also gave approval for the study in premises. Ethnical clearance

was also sought and obtained from the research ethnics committee (REC) of the Faculty of Health and Wellness Sciences, Cape Peninsula University of technology, Bellville, South Africa (HW-REC) registration number NHREC: REC-230408-014 of Cape Peninsula University of Technology. The nature of the study was explained to the participants prior to administering questionnaires. International journal of innovative research and scientific studies, 6(4) 2023, pages: 753-761 756.

Biochemical Parameters and Analysis

Plasma lipids were determined by colorimetric method using standard laboratory reagents (Randox Laboratory Ltd., UK). Castelli risk index-I (CR-I), also known as cardiac risk ratio and a pointer to the formation of coronary plaques was determined as the ratio of total cholesterol (TC) to high-density lipoprotein (HDL), while Castelli risk index-II (CR-II) was determined as the ratio of low-density lipoprotein (LDL) to HDL (Akhigbe *et al.,* 2021; Akhigbe and Hamed, 2021). Also, non-HDL was determined as non-HDL=TC-HDL, atherogenic coefficient (AC) determined as AC= (TC-HDL)/HDL, and atherogenic index of plasma (AIP) determined as AIP= Log TG/HDL-C (Akhigbe *et al.,* 2021; Akhigbe and Hamed, 2021).

Urea and creatinine levels in serum were evaluated using standard enzymatic colorimetric techniques and laboratory reagents obtained from Randox Laboratory Ltd. (Antrim, UK). Blood urea nitrogen (BUN) was determined as BUN= Urea/2.1428 (Akhigbe *et al.,* 2021).

The plasma activities of Na+-K+-ATPase and Ca2+-ATPase were assayed spectrophotometrically, as previously documented (Akhigbe *et al.,* 2020). Plasma levels of C- reactive protein, tumor necrosis factor-alpha (TNF-α), and interleukin-1beta (IL-1β) were

determined using ELISA kits (Elabscience, USA) according to the manufacturers‟ guidelines. Furthermore, the level of malondialdehyde (MDA), an index of lipid peroxidation and a marker of oxidative stress was assayed by a colorimetric method by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation (Akhigbe *et al.*, 2020).

5.3.10 Statistical Analysis

Data was analyzed and bar charts were obtained using GraphPad Prism (version 9). Comparisons across the groups were done by one-way analysis of variance (ANOVA), while Tukey‟s posthoc test was conducted for pair-wise comparison. Results are expressed as the means + standard deviation (SD). *P* values less than 0.05 ($P \leq 0.05$) were considered as statistically significant.

5.4 Results

Plasma lipids and atherogenic indices

Exposure to high altitude at PHA-L led to a marked rise in TC, triglyceride, LDL, and very low-density lipoprotein (VLDL) when compared to the control. Also, high altitude exposure at PHA-I led to a significant rise in TC, triglyceride, LDL, and VLDL when compared with the control and PHA-L. Furthermore, people who traveled at high altitude locally (PHA-L) had a significantly low level of HDL when compared with the control, while people who traveled at high altitude internationally (PHA-I) had a significantly low level of HDL when compared with the control and PHA-L (Figure 1).

More so, high altitude exposure locally caused a significant increase in CR-I, CR-II, NHC, AC, and AIP when compared to the control. In addition, high altitude exposure internationally led to a marked rise in CR-I, CR-II, NHC, AC, and AIP when compared to the control and those

exposed to high altitude locally, suggesting that exposure to high exposure increases the risk of coronary artery disease (Figure 2).

Renal function

High altitude exposure locally significantly increased the serum concentrations of urea and creatinine as well as BUN when compared to the control. In addition, high altitude exposure internationally significantly increased the serum levels of urea, creatinine, and BUN when compared with the control and PHA-L (Figure 3).

Plasma transmembrane protein

High altitude exposure locally markedly reduced plasma Ca2+-ATPase and Na+/K+-ATPase activities when compared to the control. In addition, high altitude exposure internationally significantly reduced plasma Ca2+-ATPase and Na+/K+-ATPase activities when compared with the control and PHA-L (Figure 4).

Inflammatory and oxidative stress markers

Exposure to high altitude at PHA-L led to a marked rise in CRP, TNF-α, and IL-1β when compared to the control. In addition, high altitude exposure at PHA-I led to a significant rise in CRP, TNF-α, and IL-1β when compared with the control and PHA-L (Figure 5). Furthermore, people who traveled at high altitude locally (PHA-L) had a significantly higher level of MDA when compared with the control, while people who traveled at high altitude internationally (PHA-I) had a significantly higher concentration of MDA when compared with the control and PHA-L (Figure 6).

5.5 Discussion

Dyslipidaemia and renal injury induced by high altitude exposure remain a disturbing complication for highlanders and lowlanders who are high altitude travelers. Although the

mechanisms associated with high altitude-induced dyslipidemia is yet to be fully explored, high altitude-induced renal damage has been demonstrated to be due to the modulation of renal metabolism and increased NO generation (Swenson *et al.,* 2001; Laustsen *et al.,* 2014). The present study provides compelling evidence confirming that high altitude exposure led to dyslipidemia and renal injury evidenced by a marked increase in TC, triglyceride, LDL, and VLDL, and serum urea and creatinine and BUN respectively. These findings were accompanied by a decline in transmembrane protein (Na^+/K^+-ATPase and Ca^{2+}-ATPase) activities, upregulation of CRP/TNF-α/IL-1β signaling pathway, and increased MDA generation. Hence, the main finding of the present study is that high altitude induces dyslipidemia and renal damage through the induction of oxidative stress and inflammatory response.

Incident hypertension is one of the deleterious effects of high-altitude exposure. This has been linked to the activation of hypoxia-inducible factor-led hypoxia-responsive gene transcription. (Mallet *et al.,* 2021) and induction of dyslipidemia (Sherpa and Deji, 2011; Deng *et al.,* 2012). In addition to confirming that exposure to high altitudes causes dyslipidemia, this work adds to the body of literature by showing that exposure to high altitudes raises atherogenic indices. This study found that exposure to high altitude increased the atherogenic indices and markers of coronary plaque formation, CR-I, CR-II, NHL, AC, and AIP. The observed hypercholesterolemia and dyslipidemia may be due to unhealthy dietary lifestyles and reduced energy expenditure reported among highlanders (Mallet *et al.,* 2021). However, this may not be the case in the present study because PHA-L and the control subjects in the present study were exposed to similar dietary habits, yet PHA-L had higher levels of TC, triglycerides, and LDL. Although the present findings may corroborate some previous findings, they do not agree with some findings that documented a reduced cardiovascular risk at high altitudes (Baibas et al.,

2005; Faeh et al., 2009). Also, it does not align with the study of Hochachka *et al.* (1996) and Sasaki *et al.* (2002) that demonstrated that hypoxia at high altitude stimulates angiogenesis via activation of vascular endothelial growth factor (VEGF) and the switch in myocardial energy substrate from free fatty acids to glucose.

Also, the present study confirms previous studies (Laustsen *et al.*, 2014; Haditsch *et al.*, 2015) that reported that exposure to high altitude induced renal injury. Although high altitude-induced renal damage has been associated with the modulation of renal metabolism (Laustsen *et al.,* 2014) and nitric oxide (NO) generation (Swenson, 2001), the observed dyslipidemia may also contribute to altered renal metabolism, and by extension renal injury. High altitude-induced dyslipidemia likely promoted renal fatty accumulation, resulting in impaired renal metabolism. Our results corroborate previous findings that reported impaired renal function at high altitudes and attributed it to high altitude-induced reduced renal plasma flow (Scheinfeldt *et al.,* 2012), increased uric acid generation (Schouten *et al.,* 1983), and reduced glomerular filtration rate (Sanchez-Lozada *et al.,* 2002).

Given that hypertension is a constituent of high-altitude renal syndrome, it is probable that the dyslipidemia observed at high altitudes contributes to renal damage and hypertension by stimulating atherosclerotic processes. Also, given that high altitude has been associated with increased NO (Swenson, 2001) and uric acid (Schoutsen *et al.,* 1983) generation, it is possible that NO reacts with superoxide radicals to generate peroxynitrite which in turn reacts with uric acid to induce oxidative stress (Akhigbe and Hamed, 2021). A major finding of this study is increased MDA generation in individuals exposed to high altitude. MDA is an established index of lipid peroxidation and oxidative stress. Hence, it is plausible to infer that high altitude-induced lipid peroxidation oxidized LDL to increase the levels of oxidized LDL (OxLDL) (Akhigbe and

Ajayi, 2021) which may be transported with macrophages by CD36 to form foam cells that undergo apoptosis and yield a necrotic lipid core that finally transforms into an unstable atheromatous plaque (Wainwright, 2004; Hung *et al.,* 2006; Libby, 2008; Akhigbe and Ajayi, 2021). This plaque narrows the tunica intima and increases the intraluminal pressure (Akhigbe and Ajayi, 2021), resulting in hypertension and reduced renal plasma flow.

Another novel finding of this study is the upregulation of inflammatory cytokines, CRP, TNF-α, and Il-1β, at high altitudes. Since oxidative stress could be a cause as well as an outcome of inflammation (Akhigbe and Ajayi, 2020), the observed upsurge in inflammatory cytokines may likely be due to the observed rise in MDA generation. It is also possible that the rise in inflammatory cytokines may trigger MDA generation. The presence of oxidative stress and inflammatory response at high altitudes may also account for the observed decrease in Ca2+-ATPase and Na+/K+-ATPase activity generated by high altitudes. Na+/K+-ATPase and Ca2+-ATPase are membrane-associated proteins that use energy to transport sodium, potassium, and calcium across the cell membrane. They achieve this by harnessing the energy released from the hydrolysis of adenosine triphosphate within the cell (Clausen *et al.,* 1991; Therien *et al.,* 1997). The optimal performance of these pumps maintains a normal electrochemical gradient across the cell membrane and optimal cellular metabolism (Therien *et al.,* 1997). The observed decline in the activities of these pumps at high altitude may be due to oxidative and inflammatory injury (Akhigbe and Ajayi, 2021) and may lead to calcium accumulation and apoptosis (Akhigbe *et al.,* 2020), resulting in impaired renal metabolism.

It is important to note that although high altitude exposure locally (PHA-L) and internationally (PHA-I) caused dyslipidemia and renal damage, the impact from international travels was more

evidenced by higher TC, triglyceride, LDL, VLDL, atherogenic indices, and serum urea, creatinine, and BUN. These findings may be ascribed to the duration of time spent at high altitudes since PHA-I spends more time at high altitudes than PHA-L.

5.6 Conclusion

Finally, the current investigation shows that exposure to high altitudes causes kidney damage and dyslipidemia. High altitude exposure-induced dyslipidemia and renal injury may be partly due to the activation of oxidative stress and upregulation of inflammatory cytokines. Nonetheless, it is pertinent to explore other possible mechanisms associated with high altitude exposure and dyslipidemia and renal injury. Furthermore, it is recommended to conduct clinical trials to investigate the possible advantages of antioxidants and anti-inflammatory drugs in treating dyslipidemia and renal injury caused by high altitude.

Declarations

Ethical Approval

Approval for the study was obtained from the Faculty Research Committee of the Faculty of Clinical Sciences College of Health Sciences, Osun State University, Osogbo Nigeria and Ethical Review Committees of the Aviation regulatory body in Nigeria, The Nigerian Civil Aviation Authority (NCAA), KUPA Medical Center in Lagos, an Aviation Medical Laboratory and Clinic. Ethnical clearance was also obtained from the Research Ethnics Committee (REC) of the Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa (HW-REC) (Approval number: NHREC: REC-230408-014).

Competing Interests

The authors have no conflicts of interest.

Authors' Contributions

Conceptualization and design: IPO and OOO. Funding acquisition: AMA and OOO. Investigation: AMA, IPO, GYA, and OOO. Methodology: IPO, GYA, and OOO. Project administration: AMA. Supervision: IPO and OOO. Validation: OOO. Writing-original draft: AMA. Writing-review and editing and final approval: AMA, IPO, GYA, and OOO.

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Data Availability

The data used to support the findings of the present study are available from the corresponding author upon request.

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Figures

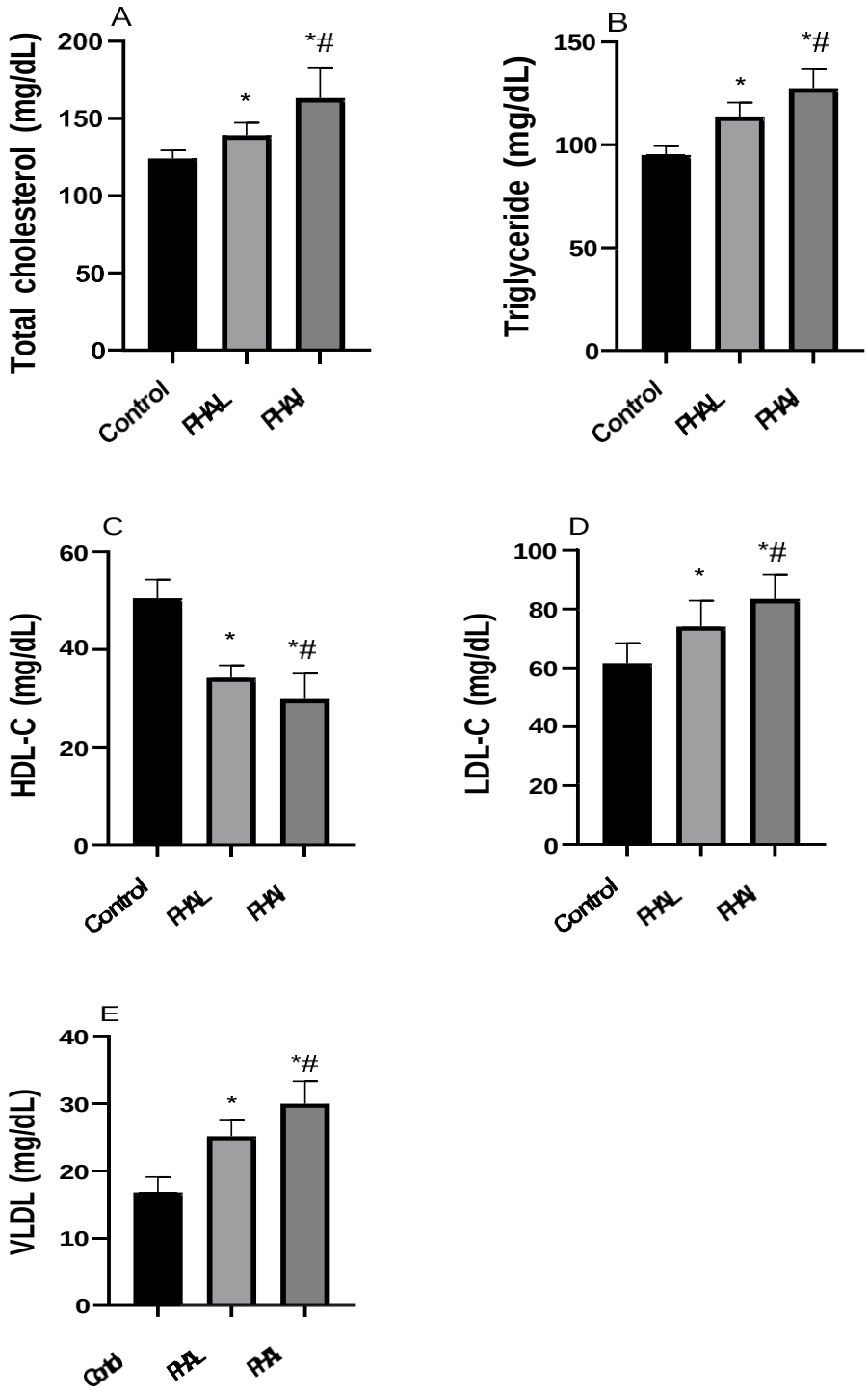

Figure 1: Effect of high-altitude exposure on serum lipid profile

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; # $P < 0.05$ vs. PHA-L

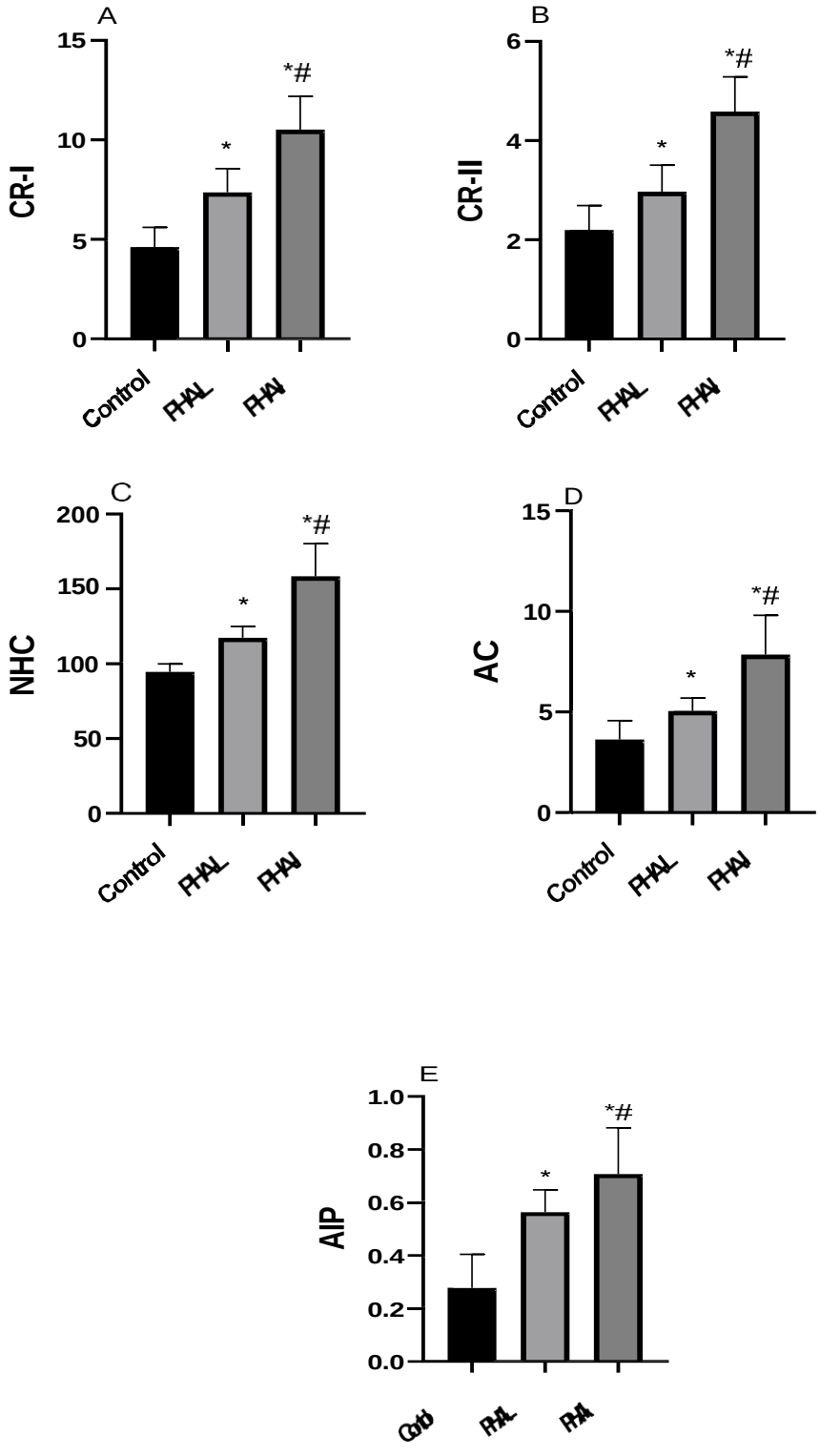

Figure 2: Effect of high-altitude exposure on atherogenic indices

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; # $P < 0.05$ vs. PHA-L

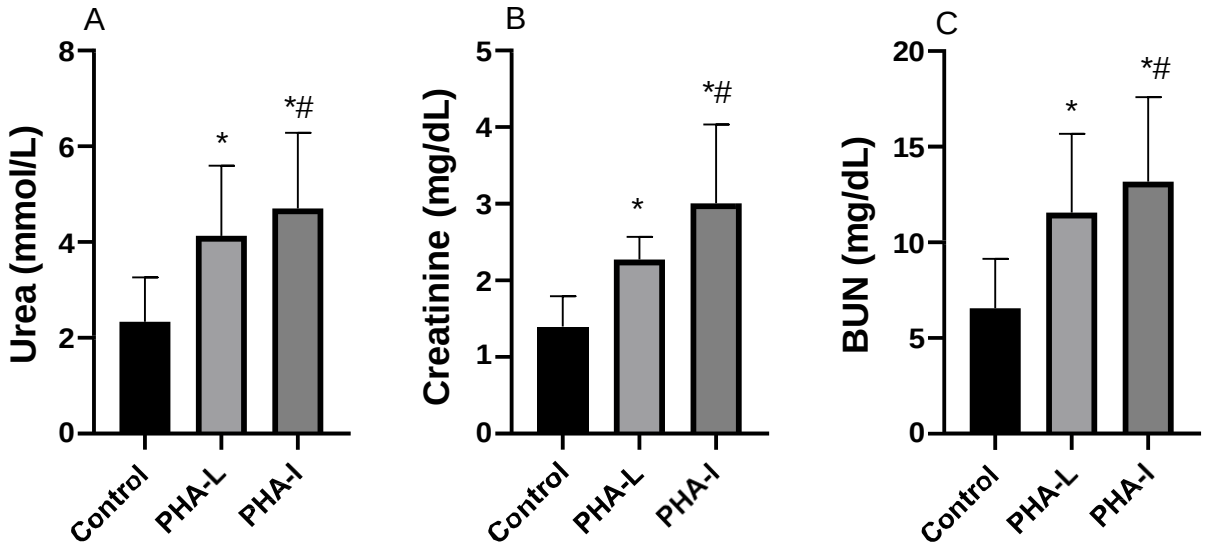

Figure 3: Effect of high-altitude exposure on renal function markers

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; # $P < 0.05$ vs. PHA-L

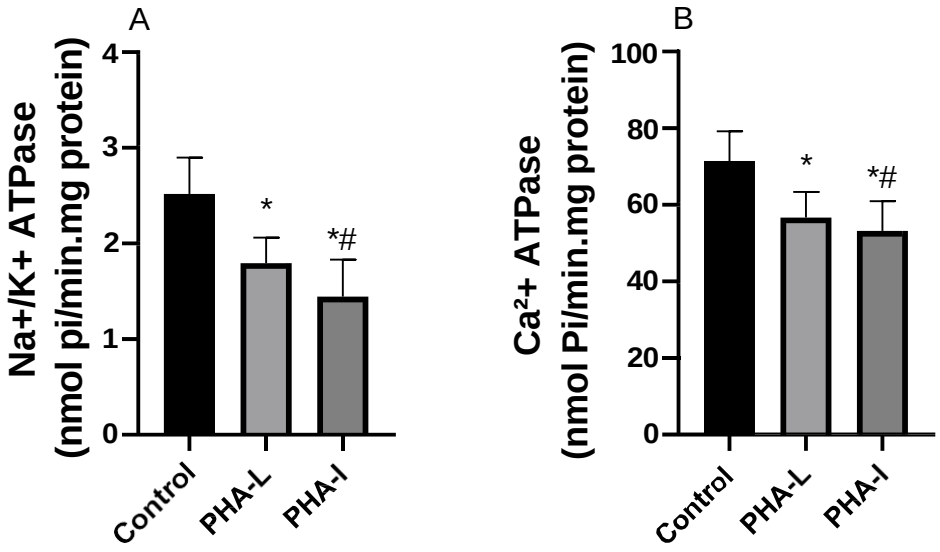

Figure 4: Effect of high altitude exposure on serum transmembrane proteins

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; # $P < 0.05$ vs. PHA-L

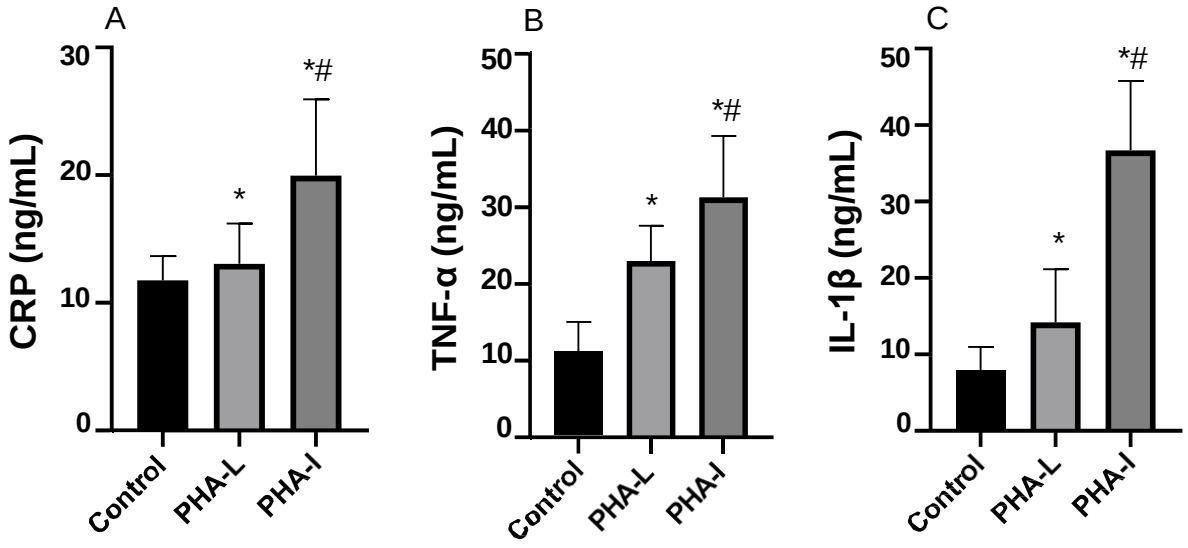

Figure 5: Effect of high-altitude exposure on inflammatory markers

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; # $P < 0.05$ vs. PHA-L

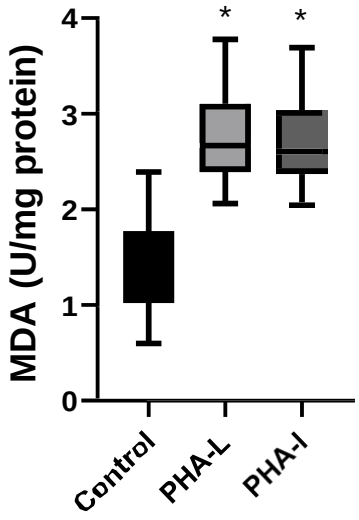

Figure 6: Effect of high-altitude exposure on malondialdehyde (MDA) generation

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; # $P < 0.05$ vs. PHA-L

Chapter 6

Title: Exposure to high altitude induces insulin resistance and hepatic injury via an oxidative stress-dependent signaling pathway

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Running title: High altitude alters antioxidant and inflammatory biomarkers in humans.

This chapter has been submitted and undergoing peer-review for possible publication in Frontiers in Physiology (Scopus- and Pubmed-indexed).

6.1 Abstract

Background: There are contradicting reports about how exposure to high altitude affects insulin resistance and hepatic function. Some studies reported the beneficial role of high-altitude exposure in obesity-induced non-alcoholic fatty liver disease, while some reported that it induces hepatic injury and insulin resistance. **Aim:** In this study, lowlanders who traveled to high altitudes had their exposure to high altitude affect insulin sensitivity and liver function assessed.

Materials and methods: A total of 300 aviation workers were allotted to 3 groups (n= 100 subjects per group); the control who were non-crew members and did not travel by air, the PHA- L group who were crew members and traveled by air locally for at least 5 years, and the PHA-I who were crew members and traveled by air internationally for at least 5 years. **Results:** Subjects exposed to high altitude had impaired hepatic function evidenced by a marked reduction in total protein and elevated AST, ALT, and ALP when compared with the control. Additionally, in comparison to the control group, individuals exposed to high altitude had noticeably higher plasma levels of glucose and insulin resistance, as indicated by the TyG index. Furthermore, in comparison to the control group, the high altitude participants exhibited increased levels of triglycerides, total cholesterol, low-density lipoprotein, TC/HDL, and LDL/HDL, but decreased levels of high-density lipoprotein. Accompanying these findings were a significant decrease in GSH levels, GPx, GST, SOD, catalase, Ca2+-ATPase, and Na+/K+-ATPase activities, as well as an increase in malondialdehyde, TNF-α, IL-1β, and C-reactive protein levels. The perturbations were substantially higher in PHA-I than in PHA-L.

Conclusion: Exposure to high altitude increases insulin resistance, which was followed by altered glucose and lipid metabolism through the production of oxidative stress and inflammation.

Keywords: Insulin resistance; hypoxia; inflammation; lipids; oxidative stress; hepatic injury.

6.2 Introduction

The liver is the primary metabolic organ in mammals, and it is involved in several essential physiological processes such as detoxification, decomposition of red blood cells, hematopoiesis, lymph production, protein and hormone synthesis, metabolism and digestion, immunological response, storage of substances like vitamin A, B12, D, E, and K (*Abdel-Misih et al., 2010*). It

also serves as an accessory digestive gland that produces bile, which emulsifies and aid the breakdown of fat (Tortora and Derrickson, 2008). However, due to its anatomical position and functions, it is prone to damage (*Vos et al., 2016*). Sources of hepatic injury include exposure to air pollution, smoke, environmental toxicants such as heavy metals and pesticides (Saka *et al.,* 2021), infections (Schweitzer *et al.,* 2015), drugs (Akhigbe *et al.,* 2020; Akhigbe *et al.,* 2022; Younossi *et al.,* 2023), intestinal ischaemia/reperfusion injury (Afolabi *et al.,* 2022a; Afolabi *et al.,* 2022b), and exposure to high altitude (Yang *et al.,* 2023).

Although the findings of Song *et al.* (2020) revealed that chronic hypoxia following high altitude exposure ameliorated obesity-induced non-alcoholic fatty liver diseases and improved liver functions while Kametas *et al.* (2003) reported that high altitude exposure did not alter liver marker enzymes, some studies documented impaired liver functions in high altitude exposure (Ou, 1974; Yel *et al.,* 2022; Yang *et al.* 2023). Yeh *et al.* (2022) reported that high altitude mountain climbers developed rhabdomyolysis and acute liver injury evidenced by a marked rise in the circulatory levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin as well creatinine phosphokinase and lactate. Yang *et al.* (2023) demonstrated that high altitude exposure induced liver damage by upregulating caspase 1 activity, high- mobility group protein B1 (HMGB1), gasdermin D N- terminal (GSDMD- N), and nuclear factor-kappa B (NF-kB)-dependent pro-inflammatory cytokines and oxidative stress.

In addition, insulin sensitivity and glucose regulation are perturbed by exposure to high altitude, although available data on these variables are conflicting. High altitude exposure at short term has been reported to increase fasting blood glucose, which is normalized within a week of exposure and then reduced in the second week via a catecholamine-dependent mechanism

(Koufakis *et al.*, 2019). Young *et al.* (1992) and Braun *et al.* (2000) reported no significant change in blood glucose levels at long-term high-altitude exposure, while Woolcott *et al.* (2015) documented a significant decline in fasting blood glucose.

Despite well-documented impacts of high-altitude exposure on physiological processes, its effect on hepatic function, glucose regulation, and insulin resistance is yet unclear. Hence, this study evaluated the effect of high-altitude exposure on hepatic function, glucose regulation, and insulin sensitivity; the roles of oxidative stress and inflammation were also explored.

6.3 Materials and Methods

6.3.1 Study design

This study examined the effect of high attitude exposure on hepatic function, glucose regulation and insulin sensitivity in pilots and other crew members.

6.3.2 Study population and setting

The study includes participants who are employed in the aviation sector and have been chosen from among aircrew members (pilots and cabin crew) who have been duly certified by the Federal Airports Authority of Nigeria (FAAN), ground handlers (SAHCOL and NAHCO), Nigerian Airspace Management Agency (NAMA), and Nigerian Civil Aviation Authority (NCAA). The study specifically focused on male and female Aviation workers.

6.3.3 Research Setting

6.3.4 Sampling Method

Using the random sampling approach, the study's designated statistician assisted in the selection of the sample. To get consent and fairly choose individuals who fit the study's inclusion criteria,

participants were contacted with the help of a certified nurse and an aeromedical assessor from the Nigerian Civil Aviation Authority.

6.3.5 Sample Size

A total of 300 individuals were divided into 3 groups by random selection. The Guilford and Flrucher formula was used to get the sample size.

$$\frac{N}{1 + Q^2 N}$$

Where N= population size 1200

Q= 0.05

$$\frac{1200}{1 + (0.05)^2 x 1200}$$

$$sample\ size = \frac{1200}{1+3}\ 300$$

Sample Size = 300

Participants were divided into three groups, namely:

Group A (Control)**:** this consists of 100 participants randomly selected from aviation workers who are non-aircrew and had no flight experience for at least two (2) years. n=100

Group B: 100 local aircrew members randomly selected from the aircrew of local airlines and have been traveling for not less than five years. PHA-L

Group C: 100 participants randomly selected from aircrew that has traveled for not less than five years internationally. PHA-I

Table 1:

Group	Group Name	Number of subjects (n)	Flying duration
A	Control – non crew member	100	0
B	Local aircrew	100	≥5years
C	International aircrew	100	≥5years

Inclusive criteria: Participants in the study were consenting adults between the ages of 30 and 45 who have worked in the aviation sector for at least five years, are not pregnant, have no history of cardiovascular or lipid disorders, are free from known infections, cancers, or addictions to alcohol or cigarettes (optional).

Exclusive criteria: participants who are less than 30 years and older than 45years of age, non-consenting participants, non-aviation workers, participants with infections, disorders, known to have cardiovascular or lipid disorder were all excluded from the study.

6.3.6 Data collection tools

An open-ended structured questionnaire was given to the participants before sample collection to get socio-demographic data from the participants. The open-ended structured questionnaire contains two sections of twenty-two (22) questions and lasted for about 30 minutes to complete. Section one contained bio data and industry experience of the participants while the other section focused on their demographics and lifestyles.

6.3.7 Participant Recruitment

Participants were informed about the research study before commencement after which all of them gave written informed consent for participation in the study. Consenting participants were informed about the nature and purpose of the study and, appointment was scheduled for their participation.

6.3.8 Ethical consideration

Approval for the study was duly obtained from the faculty research committee of the Faculty of Clinical Sciences College of Health Sciences, Osun state university, Osogbo Nigeria. Ethical approval was also sought and obtained from the Aviation regulatory body in Nigeria, The Nigerian Civil Aviation Authority (NCAA) after KUPA medical center in Lagos, an Aviation Medical Laboratory and Clinic also gave approval for the study in premises. Ethnical clearance was also sought and obtained from the research ethnics committee (REC) of the Faculty of Health and Wellness Sciences, Cape Peninsula University of technology, Bellville, South Africa (HW-REC) registration number NHREC: REC-230408-014 of Cape Peninsula University of Technology. The nature of the study was explained to the participants prior to administering questionnaires.

6.3.9 Data and sample collection

A well-structured pilot-tested questionnaire was used to obtain socio-demographic information from the participants. Anthropometric data including weight, height, blood pressure, waist, and hip circumference, as well as other parameters such as plasma level of total protein, fasting blood glucose and lipids, hepatic function makers as well as inflammatory and oxidative stress markers were determined. Participants were fasted over the night for 12 hours and then blood samples

were gotten from them through the medial cubital vein into applicable sample bottles. The obtained blood samples were centrifuged at 3000 rpm for 10 minutes to obtain the serum/ plasma that were kept frozen at -80^{0}C at the KUPA Aviation Medical Clinic, Lagos, Nigeria until they were needed for analysis.

6.3.10 Sample Analysis

Plasma level of total protein and the activities of AST, ALT, and alkaline phosphatase (ALP) were determined as hepatic function markers by spectrophotometry as earlier documented by Saka *et al.* (2011).

Fasting blood glucose and lipids were determined by the colorimetric method using standard laboratory reagents (Randox Laboratory Ltd., UK). Atherogenic indices such as the ratios of total cholesterol (TC) to high-density lipoprotein (HDL) and low-density lipoprotein (LDL) to HDL were determined (Akhigbe *et al.*, 2021; Akhigbe and Hamed, 2021). Insulin resistance was also determined as the triglyceride-glucose ratio (TyG index) using the formula: Ln [Fasting triglyceride (mg/dl) × Fasting glucose (mg/dl)] / 2 (Akhigbe *et al.*, 2021).

The concentration of malondialdehyde (MDA), which is an index of lipid peroxidation and also an indicator of oxidative stress, was determined by a colorimetric method by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation (Akhigbe and Ajayi, 2020), while reduced glutathione (GSH) level (Beutler *et al.*, 1963; Akhigbe and Ajayi, 2020), glutathione peroxidase (GPx) (Rotruck *et al.*, 1973), glutathione-S-transferase (Habig *et al.*, 1974), superoxide dismutase (SOD) (Fridovich and Misra, 1972), and catalase (Euler *et al.*, 1972) activities were assayed as markers of antioxidants by colorimetric method as previously reported.

Tumor necrotic factor-alpha (TNF-α), interleukin-1beta (IL-1β), and C-reactive protein (CRP) were assayed as inflammatory markers using ELISA kit (Elabscience, Biotechnology Co., Ltd, USA) following the guidelines of the manufacturers. Also, plasma activities of Na+-K+-ATPase and Ca2+-ATPase were assayed by spectrophotometry (Akhigbe *et al.,* 2020).

6.3.11 Statistical Analysis

Data was analyzed and bar charts were obtained using GraphPad Prism (version 9). One-way analysis of variance (ANOVA) and Tukey‟s posthoc test were employed for comparisons across the groups and pair-wise comparison respectively. Results are expressed as the means + standard deviation (SD). Values of P less than 0.05 ($P \leq 0.05$) were considered statistically significant.

6.4 Results

Effect of high-altitude exposure on liver function markers

Although the total protein level was similar between the control and individuals exposed to high altitude at PHA-L, those exposed to high altitude at PHA-I showed markedly reduced total protein levels. Also, high altitude exposure at PHA-L and PHA-I showed comparable AST and ALP activities; however, there was a significant rise in AST and ALP activities in individuals exposed to high altitude at PHA-L and PHA-I when compared with the control. Furthermore, people who traveled at high altitude locally (PHA-L) had a significantly higher ALT activity when compared with the control, while people who traveled at high altitude internationally (PHA-I) had significantly higher ALT activity when compared with the control and PHA-L (Figure 1).

Effect of high-altitude exposure on fasting glucose level and insulin resistance

Exposure to high altitude at PHA-L led to a marked rise in fasting blood glucose levels when compared to the control. Also, high altitude exposure to PHA-I caused a significant rise in the fasting blood glucose level when compared with the control and PHA-L. More so, people who traveled at high altitude locally (PHA-L) had a significantly increased insulin resistance, evidenced by raised TyG index, when compared with the control, while people who traveled at high altitude internationally (PHA-I) had significantly increased insulin resistance when compared with the control and PHA-L, suggesting that exposure to high altitude impaired glucose metabolism and possibly increases the chance of incident diabetes (Figure 2).

In addition, high altitude exposure locally (PHA-L) significantly increased the plasma fasting levels of TC, TG, and LDL-C, but markedly reduced the level of HDL-C when compared with the control. At PHA-I, the rise in TC, TG, and LDL-C and the decline in HDL-C were significantly higher when compared with the control and PHA-L. Moreover, high altitude exposure locally led to a significant increase in TC/HDL and LDL/HDL when compared to the control. High altitude exposure internationally led to a significant rise in TC/HDL and LDL/HDL when compared to the control and those exposed to high altitude locally, suggesting that exposure to high altitude impaired lipid metabolism and possibly increased the risk of incident dyslipidemia and fatty liver disease (Table 1).

Oxidative stress and inflammatory markers

People who traveled at high altitudes had a significantly higher level of MDA when compared with the control; however, the level of MDA was comparable in PHA-L and PHA-I. Also, GSH levels and GPx and GST activities were significantly higher in high-altitude travelers when compared with the control, Nonetheless, these antioxidants were similar in PHA-L and PHA-I. Furthermore, high altitude exposure locally resulted in a significant decline in SOD and catalase

activities when compared with the control; PHA-I had marked reductions in SOD and catalase activities when compared with the control and PHA-L (Figure 3).

Besides, exposure to high altitude led to a marked rise in TNF-α, IL-1β, and CRP when compared to the control. The rise in these inflammatory markers was significantly higher in PHA-I than in PHA-L (Table 2).

Effect of high-altitude exposure on plasma transmembrane protein

High altitude exposure locally reduced plasma Ca2+-ATPase and Na+/K+-ATPase activities markedly when compared to the control. Also, high altitude exposure internationally significantly down-regulated plasma Ca2+-ATPase and Na+/K+-ATPase activities when compared with the control and PHA-L (Table 3).

6.5 Discussion

The impact of high-altitude exposure on hepatic function and glucolipid regulation was investigated in local and international travelers. Compared to the control, high altitude exposure resulted in impaired hepatic function, altered glucose control, insulin resistance, and lipid dysmetabolism. The effect of high altitude was more pronounced in international travelers when compared with local travelers. These perturbations were associated with oxidative stress, inflammatory response, and impaired transmembrane activities, which suggested that the impact of high altitude on hepatic function and glucolipid regulation could be mediated by oxidative stress-dependent signaling pathways.

Non-alcoholic fatty liver disease (NAFLD) is a growing pandemic with a prevalence of 24% globally (Younossi *et al.*, 2018). The development of NAFLD is influenced by multiple factors such as lifestyle, diet, and environment (Danganaa *et al.*, 2019), including air travel.

Dyslipidaemia with ectopic lipid deposition in the liver is intimately associated with insulin resistance (Samuel and Shulman, 2019), which has been reported to play a major role in the development of NAFLD (Hamed *et al.,* 2022). Insulin resistance promotes the hepatic uptake and synthesis of free fatty acids. The findings of this study show that high altitude exposure led to increased TG and glucose levels as well as TyG index, which is reflected in hepatic insulin resistance (Tsuchiya *et al.,* 2013). A growing number of studies has shown that hepatic insulin resistance facilitates glycogenolysis, gluconeogenesis, and increased glucose output from the liver, which is associated with dyslipidemia and hepatic TG accumulation (Omolekulo *et al.,* 2019; Hamed *et al.,* 2022); hence, it is likely that the observed insulin resistance in PHA-L and PHA-I, evidenced by an increased TyG index, may be accountable for the increased level of fasting blood glucose and dyslipidemia in high altitude travelers. Since these alterations were accompanied by an impaired hepatic function, evidenced by altered levels of total protein and hepatic enzyme markers, it is also possible that these findings may reflect on hepatic TG accumulation and hepatic injury.

The role of increased TG levels in NAFLD and hepatic damage is well documented. Free fatty acid is picked up from the circulation and re-esterified in the liver into TG which is then released back into the circulation as LDL (Postic and Girard, 2008; Yki-Jarvinen, 2010). Hence, increased levels of LDL and insulin resistance are associated with hepatic TG deposition, NAFLD, and hepatic injury.

Fasting blood glucose is not just a diagnostic tool for glucose dysmetabolism, it is also a feature of metabolic diseases (Hamed *et al.,* 2022). Insulin resistance markers such as TyG index, TC/HDL, and LDL/HDL are also predictors of incident metabolic disorders and a pointer of

NAFLD development (Hamed *et al.*, 2022). Contrary to the report of Song *et al.* (2020), the present study demonstrates that high altitude exposure may lead to glucose deregulation, a negative energy balance, and insulin resistance-associated dysmetabolism. These findings agreed with the previous reports of Yang *et al* (2023).

High altitude-driven TG accumulation in the present study may enhance the formation of toxic metabolites like diacylglycerol and ceramides, which are known triggers of oxidative stress, inflammation, and hepatic injury (Kasumov *et al.*, 2015; Mota *et al.*, 2016, Lou et al., 2023). The present findings of increased MDA level and reduced antioxidant level and activities as well as increased pro- inflammatory cytokines along with insulin resistance, glucolipid dysmetabolism, and hepatic injury suggest that oxidative stress-sensitive signaling pathways may mediate high altitude- induced insulin resistance and hepatic injury. Dyslipidaemia promotes reactive oxygen species (ROS) generation, inflammatory response, and incomplete fatty acid oxidation, which conversely enhances mitochondrial ROS generation in the liver (Shin *et al.*, 2018; Akhigbe and Ajayi, 2021), leading to increased MDA generation, a marker of oxidative stress. Under physiological conditions, the hepatic antioxidants curtail excessive ROS and maintain redox balance. However, persistent ROS generation may overwhelm the antioxidant buffering capacity, leading to oxidative stress, inflammation, impaired hepatic electrolyte handling due to depressed transmembrane pumps, and hepatic injury (Akhigbe *et al.*, 2020). Consistent with previous findings in experimental animals (Song *et al.*, 2020; Yang *et al.*, 2023), the current study revealed that high altitude exposure provoked oxidative stress, evidenced by elevated MDA levels and reduced GSH levels, and GPx, GST, SOD, and catalase activities, which may trigger the upregulation of pro-inflammatory cytokines and suppression of transmembrane protein (Akhigbe *et al.*, 2020), leading to insulin resistance, dyslipidemia, and hepatic injury.

6.6 Conclusion

This study revealed that high altitude exposure impaired glucose and lipid metabolism by the induction of insulin resistance through the activation of oxidative stress and inflammation. However, despite the convincing data presented in this study, studies in other populations using different models of high-altitude exposures are important in validating the present findings.

Declarations

Ethical Approval

Approval for the study was obtained from the Faculty Research Committee of the Faculty of Clinical Sciences College of Health Sciences, Osun State University, Osogbo Nigeria and Ethical Review Committees of the Aviation regulatory body in Nigeria, The Nigerian Civil Aviation Authority (NCAA), KUPA Medical Center in Lagos, an Aviation Medical Laboratory and Clinic. Ethnical clearance was also obtained from the Research Ethnics Committee (REC) of the Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa (HW-REC) (Approval number: NHREC: REC-230408-014).

Competing Interests

The authors have no conflicts of interest.

Authors' Contributions

Conceptualization and design: IPO and OOO. Funding acquisition: AMA and OOO. Investigation: AMA, IPO, GYA, and OOO. Methodology: IPO, GYA, and OOO. Project

administration: AMA. Supervision: IPO and OOO. Validation: OOO. Writing-original draft: AMA. Writing-review and editing and final approval: AMA, IPO, GYA, and OOO.

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Data Availability

The data used to support the findings of the present study are available from the corresponding author upon request.

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Figures

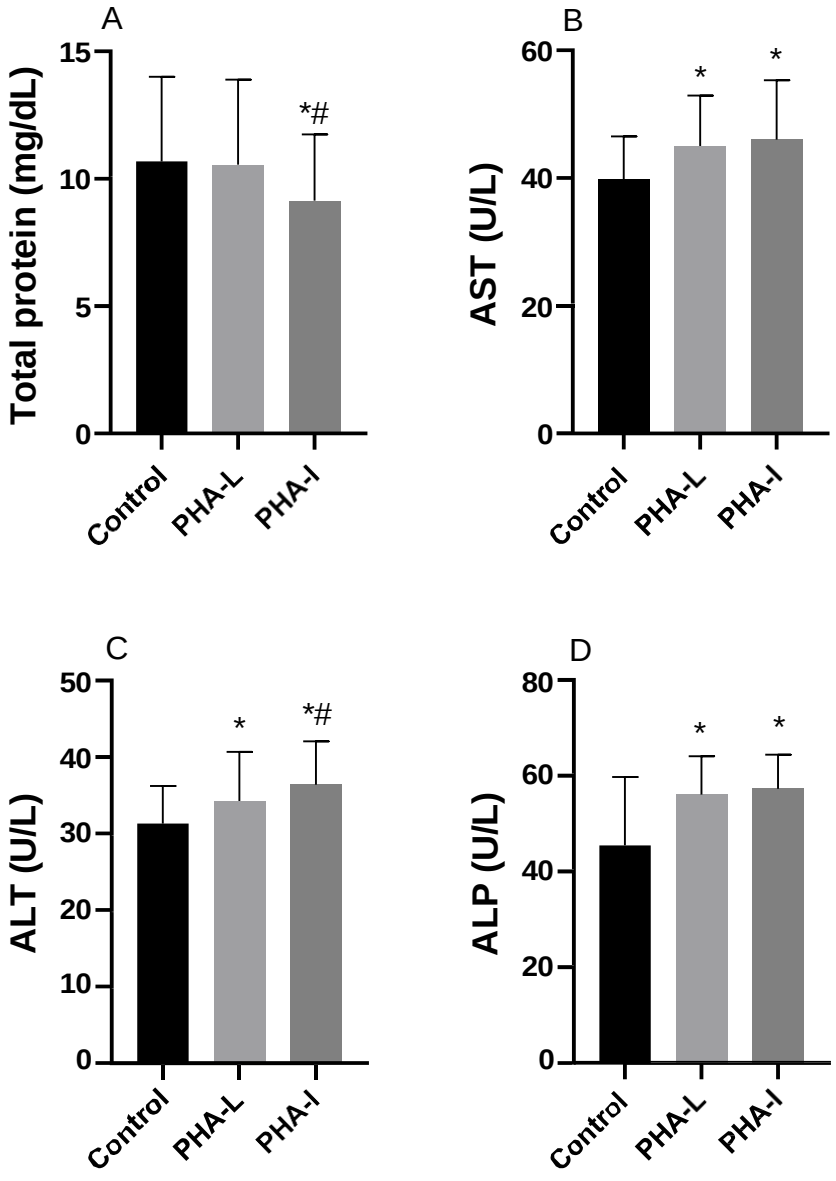


Figure 1: Effect of high altitude exposure on liver function markers

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; $^{\#}$ $P < 0.05$ vs. PHA-L

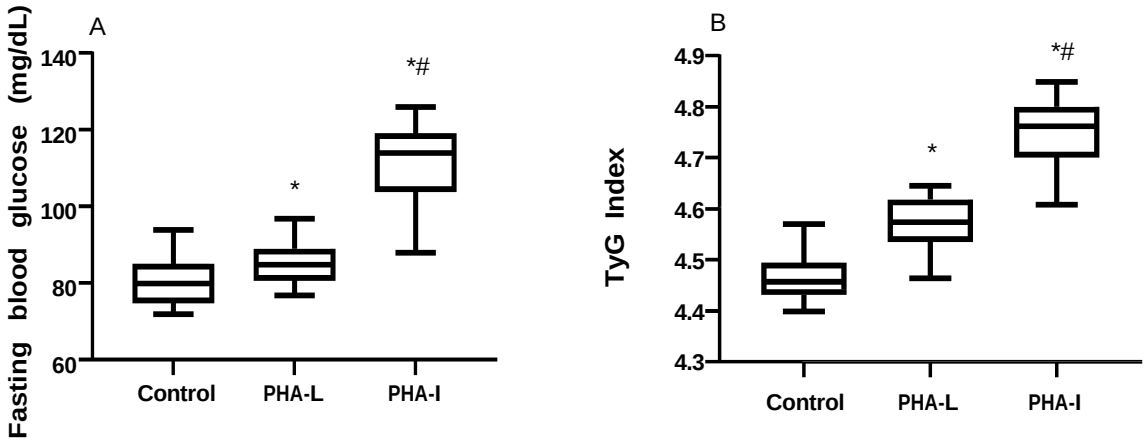
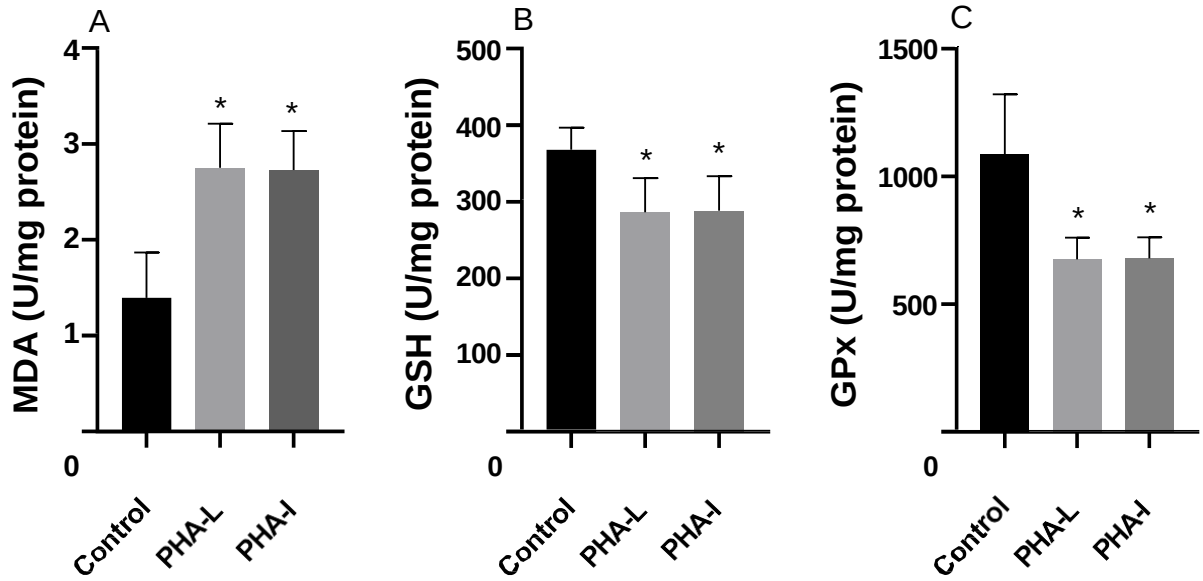


Figure 2: Effect of high altitude exposure on fasting blood glucose (A) and insulin resistance (B)

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; # $P < 0.05$ vs. PHA-L



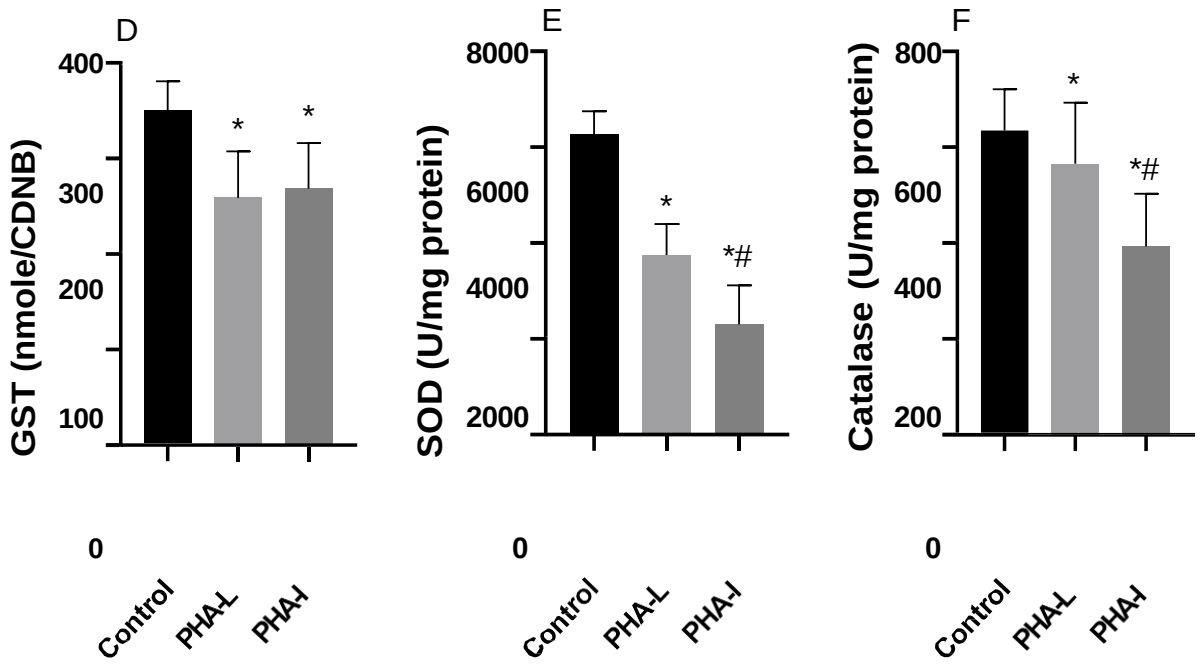


Figure 3: Effect of high altitude exposure on oxidative stress markers

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; $^{\#}$ $P < 0.05$ vs. PHA-L

TABLES

Table 1: Effect of high altitude exposure on serum lipid profile and atherogenic indices

Variables/Groups	Control	PHA-L	PHA-I
TC (mg/dL)	124.30±5.12	139.20±8.07*	163.20±19.40$^{*\#}$
TG (mg/dL)	95.24±4.21	113.80±6.79*	127.60±9.21$^{*\#}$
HDL-C (mg/dL)	50.47±3.84	34.29±2.50*	29.86±5.21$^{*\#}$
LDL-C (mg/dL)	61.65±6.76	74.11±8.79*	83.42±8.32$^{*\#}$
TC/HDL	4.60±0.98	7.36±1.19*	10.52±1.67$^{*\#}$
LDL/HDL	2.20±0.48	2.97±0.53*	4.59±0.70$^{*\#}$

TC: total cholesterol, TG: triglyceride, HDL: high-density lipoprotein, LDL: low-density lipoprotein

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; $^{\#}$ $P < 0.05$ vs. PHA-L

Table 2: Effect of high altitude exposure on inflammatory markers

Variables/Groups	Control	PHA-L	PHA-I
TNF-α (ng/mL)	11.23±3.84	23.02±4.57*	31.32±7.97*#
IL-1β (ng/mL)	7.91±3.07	14.17±6.97*	36.68±9.11*#
CRP (ng/mL)	11.76±1.89	13.05±3.17*	19.95±5.97*#

TNF-α: tumour necrosis factor-alpha, IL-1β: interleukin-1beta, CRP: C-reactive protein

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; # $P < 0.05$ vs. PHA-L

Table 3: Effect of high altitude exposure on plasma transmembrane proteins

Variables/Groups	Control	PHA-L	PHA-I
Na+/K+ ATPase (nmol pi/min.mg protein)	2.52±0.37	1.79±0.27*	1.45±0.38*#
Ca^{2+} ATPase (nmol Pi/min.mg protein)	71.48±7.69	56.75±6.63*	53.25±7.73*#

Data are mean ± SD of 100 replicates. * $P < 0.05$ vs. control; # $P < 0.05$ vs. PHA-L

CHAPTER 7

General Discussion and Conclusion

Studies reporting the effects of high-altitude exposure on physiological processes have been inconsistent and results remain inconclusive. The disparity in previous findings may be due to the study design, sample size, and duration of exposure. The present study was designed to circumvent the limitations presented in earlier studies. First, in the present study, exposure to high altitude was graded and compared with matched control. One hundred local aircrew members randomly selected from the aircrew of local airlines and another one hundred international aircrew members randomly selected from the aircrew of international airlines were included in this study. Additionally, the studied aircrew members were exposed to high altitudes for at least five years.

7.1 Impact of high altitude on BMI: an evaluation of cardiometabolic risk in exposed individuals

Exposure to high altitude markedly increased the incidence of obesity independent of the dietary patterns of the subjects when compared with the control. In addition, high altitude exposure triggered a rise in the levels of serum total cholesterol, triglyceride, and low-density lipoprotein, but reduced levels of high-density lipoprotein when compared with the control. These findings were associated with increased Castelli risk indices I and II and elevated TNF-α and IL-1β. The observed perturbations were markedly higher in PHA-I than in PHA-L. This demonstrates that high altitude exposure is a predisposing risk factor to cardiometabolic disorder evidenced by increased obesity development, lipid deregulation, and elevated Castelli risk indices. This may be mediated by the upregulation of pro-inflammatory cytokines.

In summary, the data presented here demonstrate that high altitude exposure causes lipid deregulation and obesity development, which is associated with increased cardiovascular risk evidenced by elevated SBP, MAP, HR, CR-I, and CR-II, and upregulated pro-inflammatory factors which are triggers of atherosclerosis. These findings revealed that high altitude exposure may be a predisposing factor to the development of cardiometabolic disorder.

7.2 Effect of high-altitude exposure on lipid profile and renal function

The current study reveals that high altitude exposure causes dyslipidemia and renal injury, as evidenced by significant increases in TC, triglycerides, LDL, and VLDL, as well as serum urea, creatinine, and BUN. These findings were associated with a reduction in transmembrane protein (Na+/K+-ATPase and Ca2+-ATPase) activities, upregulation of CRP/TNF-α/IL-1β signaling pathway, and increased MDA generation. The current study not only confirms that high altitude exposure causes dyslipidemia, but it also adds to the existing literature by revealing that high altitude exposure raises atherogenic indices (Mallet *et al.,* 2021). This study found that exposure to high altitude raised CR-I, CR-II, NHL, AC, and AIP, all of which are atherogenic indices and predictors of coronary plaque formation.

While high altitude-induced renal damage has been linked to changes in renal metabolism (Laustsen *et al.,* 2014) and nitric oxide (NO) generation (Swenson, 2001), the observed dyslipidemia may potentially contribute to altered renal metabolism and, as a result, kidney injury. It is possible that high altitude-induced dyslipidemia facilitated renal fatty buildup, resulting in decreased renal metabolism. Our findings support prior study findings that found impaired renal function at high altitudes due to reduced renal plasma flow (Scheinfeldt *et al.,* 2012), and decreased glomerular filtration rate (Sanchez-Lozada *et al.,* 2002). A striking discovery of this study is that high altitude induces oxidative stress and enhances inflammatory

cytokines such as CRP, TNF-α, and Il-1β. Since oxidative stress can be both a cause and a result of inflammation (Akhigbe and Ajayi, 2020), the observed increase in inflammatory cytokines is most likely owing to the observed increase in MDA production. It is also possible that an increase in inflammatory cytokines stimulates MDA production. The observed oxidative stress and inflammatory response at high altitudes could potentially explain the decrease in Na+/K+- ATPase and Ca2+-ATPase activity, resulting in impaired renal metabolism and renal injury as shown in schematic illustration in figure 4.

7.3 Effect of high-altitude exposure on insulin sensitivity and hepatic function

In comparison to the control group, high altitude exposure impaired hepatic function and glucose regulation, and induced insulin resistance and lipid dysmetabolism. These changes were linked with oxidative stress, inflammatory response, and reduced transmembrane functions, implying that the effects of high altitude on hepatic function and glucolipid regulation may be mediated via oxidative stress-dependent signaling pathways. This study found that high altitude exposure increased TG and glucose levels, in addition to the TyG index, indicating hepatic insulin resistance (Tsuchiya *et al.,* 2013). A growing number of studies has shown that hepatic insulin resistance facilitates glycogenolysis, gluconeogenesis, and increased glucose output from the liver, which is associated with dyslipidemia and hepatic TG accumulation (Omolekulo *et al.,* 2019; Hamed *et al.,* 2022); thus, it is possible that the noted insulin resistance in PHA-L and PHA-I, evidenced by an increased TyG index, may be responsible for the increased level of fasting blood glucose and dyslipidemia in high altitude exposure. Considering these changes were associated with reduced hepatic function, as demonstrated by modified levels of total protein and hepatic enzyme indicators, it is likely that these findings are indicative of hepatic TG buildup and injury.

In this study, high altitude-driven TG accumulation may increase the synthesis of hazardous metabolites such as diacylglycerol and ceramides, which are known to cause oxidative stress, inflammation, and liver damage (Kasumov *et al.*, 2015; Mota *et al.*, 2016). The current findings of increased MDA levels and reduced antioxidant levels and activities, as well as increased pro-inflammatory cytokines along with insulin resistance, glucolipid dysmetabolism, and hepatic injury, suggest that oxidative stress-sensitive signaling pathways may mediate high altitude-induced insulin resistance and hepatic injury as shown in schematic illustration in figure 4.

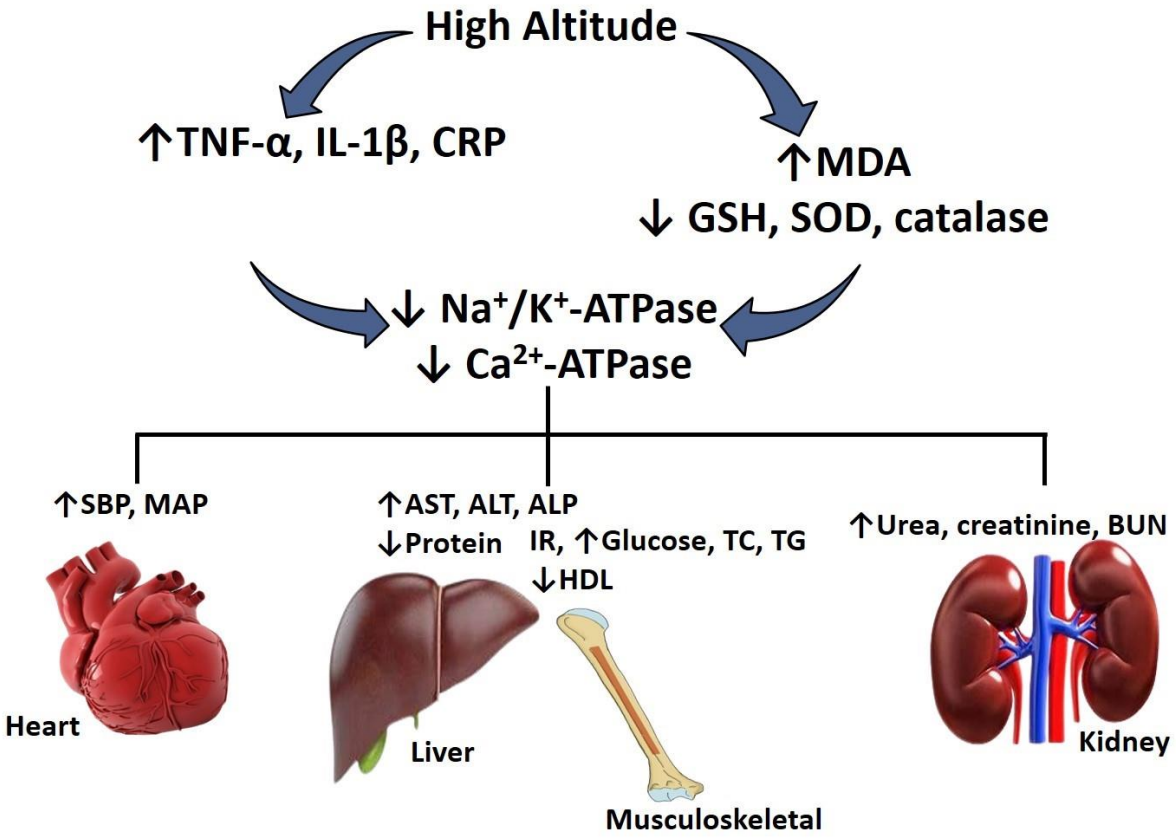


Figure 4: Schematic illustration showing the effect and associated mechanisms of action of high altitude exposure on cardiovascular and hepatorenal functions

Prolong high altitude exposure impairs Na^+/K^+-ATPase and Ca^{2+}-ATPase via the induction of oxidative stress by promoting malondialdehyde (MDA) generation and down-regulating reduced glutathione (GSH), superoxide dismutase (SOD), and catalase. This was accompanied by upregulation of tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and C - reactive protein (CRP). This resulted in alteration in cardiovascular variables evidenced by increased systolic blood pressure (SBP) and mean arterial pressure (MAP), impaired hepatic function as depicted by elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), reduced protein, insulin resistance and gluco-lipid deregulation, and impaired renal function as shown by elevated serum urea, creatinine and blood urea nitrogen (BUN).

7.4 Conclusion

This thesis presents multiple congruent studies that highlighted novel mechanisms underlying the deleterious impact of high-altitude exposure to hepatorenal system and insulin sensitivity. From the present findings, it can be concluded that high altitude exposure exerts multiple pathological effects and the specific ones identified in this work include the following:

1. High altitude exposure-induced dyslipidemia and renal injury due to the activation of oxidative stress and upregulation of inflammatory cytokines. This is presented in chapter four.

2. High altitude exposure impaired glucose and lipid metabolism by the induction of insulin resistance through the activation of oxidative stress and inflammation. This is presented in chapter five.

7.5 Further Studies

Although the present study provides convincing data on the deleterious effect of high-altitude exposure, many more aspects of this topic are yet unresolved. Further studies exploring other possible mechanisms associated with high altitude exposure hepatorenal injury and gluco-lipid dysmetabolism are necessary. This will reveal other pathways involved and open a window of possible prophylactic and therapeutic opportunities.

1. Studies in other populations using different models of high-altitude exposures are important in validating the present findings.

2. In addition, clinical trials exploring the potential benefits of antioxidants and anti-inflammatory agents in high altitude-induced hepatorenal injury and gluco-lipid dysmetabolism are recommended.

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