The effects of post covid-19 in adults with regard to oxygen-carrying capacity after infection

by

Kidibone Mkhabela

DISSERTATION

Submitted in fulfilment of the requirements for the degree of

Master of Science
in
Physiology

in the

FACULTY OF SCIENCE AND AGRICULTURE
(School of Molecular and Life Science)

at the

UNIVERSITY OF LIMPOPO

Supervisor: Dr. YY Chetty
Co-Supervisor: Prof. M van Staden

2024

DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science (Physiology) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Mkhabela K (Miss)	29/10/2023
Surname, initials (title)	**Date:**

DEDICATION

This work is dedicated to:

- **God**, for the strength He gave through everything I was going and managing to complete my work.
- **My family**: who were going through as much as I was but still found it in themselves to be understanding, supportive and my sounding board all the time.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following people:

- My supervisor, Dr YY Chetty for her guidance throughout my pursuit of this Master of Science degree, and her invaluable support, as well as editing.
- My co-supervisor, Prof M van Staden, for the guidance through the long journey and editing.
- Zandile Maleka, Ishmael Phalana, and Haskly Mokoena for the huge help with data collection.
- Friends and family who made sure I had all I needed.
- The Department of Physiology and Environmental Health for the support and using their equipment.

TABLE OF CONTENTS **PAGE**

LIST OF TABLES

LIST OF FIGURES

LIST OF ADDENDA

LIST OF ABBREVIATIONS

ACE2	Angiotensin-converting Enzyme 2
ARB	Angiotensin receptor blocker
ARDS	Acute respiratory distress syndrome
BG	Blood glucose
BMI	Body mass index
BP	Blood Pressure
CAD	Coronary artery disease
CoV	Coronavirus
COVID-19	Coronavirus 2019
CoVs	Coronaviruses
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
Hb	Haemoglobin
Hct	Haematocrit
IBM SPSS	International Business Machines Statistical Packaging for the Social Sciences
IR	Insulin resistance
LV	Left ventricular
MERS	Middle East respiratory syndrome
MERS-CoV	Middle East respiratory syndrome-coronavirus
MetS	Metabolic syndrome
MRI	Magnetic Resonance Imaging
NICE	National Institute for Health and Care Excellence
PaO2	Oxygen partial pressure
PO	Partial pressure
RAAS	Renin-Angiotensin-Aldosterone System
RBCs	Red blood cells
RDA	Recommended Daily Allowance
RNA	Ribonucleic acid

RV	Right ventricular
SBP	Systolic blood pressure
SAMRC	South African Medical Research Council
SARS	Severe acute respiratory syndrome
SARS-like-CoV	Severe acute respiratory syndrome-like-coronavirus
SARS-CoV	Severe acute respiratory syndrome-coronavirus
SARS-CoV-2	Severe acute respiratory syndrome-coronavirus-2
SCAT	Subcutaneous adipose tissue
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
TREC	Turfloop Research Ethic Committee
VAT	Visceral adipose tissue
WHO	World Health Organisation

ABSTRACT

Aim and background: COVID-19 is a disease caused by SARS-CoV-2 that is characterised by ARDS and hyperinflammation. The lungs are the primary organs affected and this results in difficulty breathing. Invasion of cells induces cell apoptosis, and can lead to haemolysis, thereby altering the transport of gases. Individuals with obesity, diabetes, and hypertension have compromised innate immune systems, which is characterised by low-grade chronic inflammation that might aggravate the inflammation caused by COVID-19. This puts them at increased risk of severe infection and complications. COVID-19 symptoms can persist a few months after the viral load has decreased. There is an 80% prevalence of individuals having long-COVID. Therefore, the aim of this study was to investigate the effects of post COVID-19 on the oxygen-carrying capacity in these adults.

Method: This was a case-COVID-19 naïve cross-sectional study that included an COVID-19 positive (n=28) and a COVID-19 naïve group (n=196) with all participants older than18 years. Questionnaires were administered to acquire participant details. The oxygen carrying capacity (haemoglobin and haematocrit), and variables of COVID-19 risk factors such as blood glucose (BG), blood pressure (BP), and body mass index (BMI) were measured. Furthermore, a food frequency assessment was done to determine the daily intake of nutrients such as iron, folic acid, and vitamin B_{12}).

Results Males who had COVID-19 presented with significantly higher BG levels than those who never had COVID-19 (p=0,003). Males who had COVID-19 also presented with significantly high DBP (p=0,023), as well as significantly high HR (p=0,021). Obesity has a 47,6% prevalence in females who had COVID-19. Iron and folic acid intake was below the recommended daily allowance (RDA); however, that of vitamin B_{12} exceeded the RDA. The participants consumed less than the RDA iron and folic acid but consumed more than the RDA vitamin B_{12}.

Conclusion: Comorbidities decrease oxygen-carrying capacity in individuals who had COVID-19. Most comorbidities are more prevalent in males than females There was deficiency of iron and folic acid caused by participants not consuming RDA.

1. CHAPTER 1: BACKGROUND AND RATIONALE

1.1. Introduction

Chapter 1 focuses on introducing the dissertation and detailing the aim and the objectives of the research study. This chapter also provides a brief background on the research topic, as well as identifying the gaps in the knowledge on the topic.

1.2. Background

Coronavirus Disease 2019 (COVID-19) can be defined as an infectious disease that causes severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) (WHO, 2022). This variant of the virus was discovered late December 2019 in Wuhan, China and globally more than 600 million fatalities due to COVID-19 infections have been reported (WHO, 2022). It has spread worldwide at an unprecedented rate, resulting in moderate to severe infections that lead to hospitalisation and extreme fatality rates (Cascella *et al.,* 2023). The virus and disease were the cause of over-burdened health-care systems around the world and have had a negative impact on socio-economic activities (Arévalos *et al.,* 2021).

Since its discovery and the devastation of this virus, scientists have been working tirelessly to find solutions to reduce the spread. Clinically, COVID-19 can be asymptomatic, but it can also have life-threatening effects and it is confirmed by testing specimen acquired through swabbing the nose and/or mouth (Kaeuffer *et al.,* 2020). The most reported symptoms are fever, respiratory distress, and flu-like symptoms (Jarrott *et al.,* 2022). The primary entry of the virus, into the body, is via the respiratory system. This has caused some patients to develop acute respiratory distress syndrome (ARDS) and require mechanical ventilation to facilitate respiration (Sadhukhan *et al.,* 2020). Some of the risk factors that are associated with increased severity of the infection include chronic obstructive pulmonary diseases, diabetes, obesity, cardiovascular diseases, and sickle cell disease (Alberca *et al.,* 2021: Lopez-Leon *et al*., 2021).

Recovery from COVID-19 is possible, but there is a risk of reinfection (Ren *et al.,* 2022). There have been reports of several ongoing or persistent symptoms after "recovery", and it is now clinically referred to as "long-COVID", "long hauler", "post COVID syndrome" or just "post-COVID" (Pasini *et al.,* 2021). Post-COVID syndrome is the term that will be used in this dissertation.

1.3. Problem statement

COVID-19 is a global burden to the health-care system, economy, and social life (Pasini *et al.,* 2021). ARDS causes low blood oxygen which then causes shortness of breath and inability to breathe without support (Sadhukhan *et al*., 2020). This condition requires patients to be hospitalised and be administered invasive mechanical ventilation (Mattay *et al*., 2019). The SARS-CoV-2 virus interacts with haemoglobin (Hb) molecular receptors and, attacks the haeme group on the Hb 1-beta chain that culminates in erythrocyte lysis (Cavezzi *et al*., 2020). This can lead to an abnormal oxygen carrying capacity due to an abnormal erythrocyte count and Hb concentration, which may cause patients to develop severe hypoxia (Alayash, 2021). Some COVID-19 patients do recover, but approximately 80% of the patients report ongoing or persistent symptoms after testing negative with the most common reported symptoms being cough, fatigue and headaches which are some of the symptoms presented by patients with decreased blood oxygen (Lopez-Leon *et al*., 2021). This implies that the damage caused by the virus and its complications were severe enough to have long lasting effects, and with the possibility of more effects developing. There is a lack of substantial information on the possible longevity of the symptoms, their management and treatment.

1.3.1. Aim

The aim of the study was to investigate the oxygen carrying capacity of adults in the Mankweng area, Limpopo province, South Africa post COVID-19 infection.

1.3.2. Objectives

The objectives of this study were to:

i. assess factors associated with oxygen-carrying capacity such as Hb concentration and haematocrit (Hct) percentage.
ii. evaluate data on risk factors for COVID-19 such as blood glucose (BG), blood pressure (BP) and Body Mass Index (BMI) to assess risk of infection.
iii. assess dietary intake of nutrients such as iron, folic acid, and vitamin B_{12} concentration to rule out anaemia as a confounding factor.

1.3.3. Research questions

i. What is the prevalence of impaired oxygen carrying capacity in post-COVID-19 patients?
ii. What effect does post-COVID-19 have on Hb, Hct, BMI, BP and BG?
iii. What effect does post-COVID-19 have on iron, folic acid, and vitamin B_{12} concentration?

2. CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Chapter 2 focuses on the information found in the scientific literature. This includes the history of the topic and current knowledge. This chapter is divided into sections related to the objectives of the study, with the exception of 2.2 and 2.3 that is devoted to a historical reflection on the virus.

Since the beginning of the coronavirus pandemic in 2019, there have also been many recoveries from SARS-CoV-2 infection (Pasini *et al.,* 2021). Unfortunately, some of the recovered patients report persistent symptoms, such as fatigue and headache, and organ dysfunction, such as liver and kidney failure which forms part of symptoms exhibited by some patients (Lopez-Leon *et al.,* 2021). The purpose of this study is to investigate the influence on oxygen carrying capacity, and why some patients experience persistent symptoms after testing negative. This was achieved by first looking at the origin and pathology of SARS-CoV-2, followed by the factors associated with oxygen carrying capacity. Then lastly the comorbidities that might make individuals susceptible to the virus, and conditions that are caused by the virus, especially anaemia (Mohammad *et al.,* 2021).

2.2. Origin of coronaviruses

Coronaviruses (CoVs) date back to the 1960s, but a new SARS outbreak occurred in 2002 to 2003 in Guangdong, China, and spread to approximately 29 countries, reported 8089 infections and 774 fatalities (Kahn and McIntosh, 2005; Memish *et al*., 2020). In 2012, another outbreak called Middle Eastern respiratory syndrome-coronavirus (MERS-CoV) occurred in Saudi Arabia (Azhar *et al*., 2019). This was discovered in a patient who developed pneumonia and renal failure, which later caused his death (Yang *et al.,* 2020). The outbreak of 2019 is by far the most devastating CoV outbreak and thus the most publicised. These outbreaks may be related to other viruses with similar genetics that are found in bats and were speculated to have the largest number of CoV species (Chowdhury and Anwar, 2020). Other studies, such as the one by Hernandez-Aguilar *et al*. (2021), negates this theory with

the speculation that the CoVs could be transmitted to humans through other intermediate hosts such as domestic and wild animals; therefore, the virus might have a zoonotic origin.

After the 2002 outbreak, CoVs such as severe acute respiratory syndrome-like-coronavirus (SARS-like-CoV) were discovered in *Rhinolophus* bats from several Chinese provinces, and they initially lacked the capability to use the angiotensin-converting enzyme-2 (ACE2) receptors to infect humans (Morens *et al*., 2020; Platto *et al*., 2021). In 2013 another outbreak of SARS-like-CoV was found in a single colony of *Rhinolophus* bats in the Chinese province of Yunnan and some had the ability to infect host cells using ACE2 receptors, and were 95% identical to the SARS-CoV, showing that the virus might have evolved from the one found in 2002 (Morens *et al*. 2020). Platto *et al*. (2021) postulate that the spread of the virus leading to the pandemic began, or was aggravated, with the spread of flu in China that resulted in approximately 270 deaths at the beginning of 2019 and continued until it spread to other provinces, then countries, and finally the rest of the world.

Scientifically, CoVs are being classified by the type of organism, their characteristics, and properties. CoVs belong to the subfamily *Orthocoronavirinae* in the family *Coronaviridae* and the order *Nidovirales* (Khan *et al.,* 2021). CoVs have a large ribonucleic acid (RNA) genome of about 26–32 kilobases in size that codes for four structural proteins (S, M, N, and E) and sixteen non-structural proteins (Platto *et al.,* 2021). The envelope and membrane that coat the virus are formed by the E and M structural proteins, the N protein binds the RNA genome, and the S protein forms the characteristic protrusions from the surface of the virus which interacts with the plasma membrane receptor of the target cell and penetrates the cell (Platto *et al.,* 2021). The expanded size of the CoVs genome may be associated with increased replication fidelity (Wang *et al*., 2020). Its size facilitates the acquisition of genes that encode accessory proteins that promote the survival and adaptation in the specific host cell, thus supports the emergence of several variants of the virus (Fan *et al.,* 2019).

2.3. Pathology of coronaviruses

SARS-CoV-2 is spread directly by droplets of saliva and human-to-human transmission, and indirectly by contaminated objects and airborne contagion (Rauf *et al*., 2020). The transmission through respiratory droplets, expelled by coughing or sneezing, is achieved when an individual takes up aerosols through the mouth, nose, or eyes (Lotfi *et al.,* 2020). The droplets are unable to travel more than two metres, consequently, they are airborne for a short period of time (Lotfi *et al.,* 2020). The virus affects the respiratory system the most and causes respiratory illnesses, thus the name severe acute respiratory syndrome-coronavirus-2 (Alberca *et al.,* 2021). Once the virus has entered the body it can cause moderate to severe organ damage and induce the systemic disease, COVID-19 (Desai *et al*., 2022).

CoVs are RNA viruses and SARS-CoV-2 is the most infectious CoV documented. The virus depends on its receptor binding property and was found to be more compatible with the human ACE2 receptor when their protein sequences were compared. As such, it uses the ACE2 receptor to enter the host cell (Khan *et al.,* 2021). ACE2 receptors are reported to be expressed in the endothelial cells of the liver, lungs, stomach, kidneys, ileum, and colon; however, their expression is lower in the other organs than in the lungs (Wadman *et al.,* 2020). Considering the high infectivity of the virus it was speculated that the virus might depend on other potential receptors or mediating proteins to facilitate infection (Cantuti-Castelvetri *et al.,* 2020; Daly *et al.,* 2020). The binding of SARS-CoV-2 to ACE2 seems to be mediated by a spike protein (Shang *et al,* 2020; Wang *et al*., 2020). Furthermore, the CD147 receptor has been identified as another means for the virus to enter cells (Radzikowska *et al*., 2020; Wang *et al.,* 2020). This receptor is a transmembrane glycoprotein of the immunoglobulin superfamily, commonly expressed by erythrocytes (Radzikowska *et al.,* 2020).

The virus uses the host cells' genetic material to rapidly replicate, then exits the cell through exocytosis (Gazzaz, 2021). The stress caused by the virus triggers cell apoptosis which also induces an inflammatory response through the activation of proinflammatory cytokines, and their release in high concentrations. This excessive release induces a "cytokine storm" that leads to ARDS in the lungs, organ failure and hyper-inflammation (Gazzaz, 2021). This inflammatory response might continue to increase even when the viral load diminishes (Jain, 2020). These effects have been

observed in patients with severe cases who later developed sepsis due to vascular leakage, and who presented with damaged epithelial cells in the lungs, thrombosis, and hypercoagulation (Jain, 2020). Therefore, it can be concluded that this damage will decrease the gaseous exchange surface area, in turn decreasing the rate of gaseous exchange.

2.4. Transportation of oxygen by erythrocytes and Hb

Oxygen is carried in the blood by dissolving in the plasma and red blood cell water, and by reversibly binding to Hb (Pittman, 2011). Haemoglobin is the molecule that is responsible for carrying oxygen in the blood (Pittman, 2011). It is composed of four subunits each comprising of an iron-containing porphyrin ring (Mohanty *et al.,* 2014). The iron atoms are the binding site for oxygen; therefore, one Hb tetramer can bind to four oxygen molecules (Mohanty *et al.,* 2014). Any alterations in the synthesis or structure of erythrocytes, Hb, or the globin polypeptide chain can impair the oxygen-carrying capacity of the blood and lead to hypoxia (Kaufman *et al*., 2022).

The body is sufficiently oxygenated by responding to changes in oxygen partial pressure (PO) and the demand for oxygen, which is determined by the percentage of Hb saturated with oxygen (Rhodes *et al*., 2022). This is expressed on an oxygen dissociation curve that can shift to the left or to the right depending on the temperature, pH, and 2,3-bisphophoglycerate concentration, among other factors, which can increase or decrease the affinity of Hb for oxygen (Powers and Dhamoon, 2023).

There are physiological and pathological factors that could affect Hb concentration and RBC count such as age and gender (Gassmann *et al*., 2019). Change in altitude is one of the physiological determinants of blood elements (Alkhaldy *et al*., 2022). At high altitude the volume of blood plasma decreases, while erythropoiesis is increased due to hypoxia caused by erythrocytosis and increases Hb and Hct (Alkhaldy *et al*., 2022). This might boost the oxygen-carrying capacity, but a decrease in altitude might impair oxygen-carrying capacity.

2.5. Oxygen carrying-capacity

2.5.1. Gas exchange

COVID-19 was reported as a cluster of pneumonia before its effects were further investigated and understood (Rajanna *et al.,* 2021). Patients infected with SARS-CoV-2 developed ARDS due to the virus' main effect on the lungs. The symptoms vary, but mild cases may experience fever, cough, and fatigue, moderate cases may have difficulty breathing or mild pneumonia, and severe cases may have severe pneumonia, organ failure and possible death (Zayed *et al*., 2022). Some COVID-19 patients with severe SARS-CoV-2 infection were admitted to medical facilities and administered invasive mechanical ventilation as the syndrome caused difficulty in respiration and insufficient delivery of oxygen to the organs (Sadhukhan *et al.,* 2020). This insufficiency could be elicited by various conditions.

SARS-CoV-2 enters the host cell via ACE2 receptors expressed by pneumocytes in the epithelial alveolar lining to enter the host cell, which leads to injury of the epithelial layer of the lungs (Varga *et al.,* 2020). The virus causes disruption of the pulmonary endothelial and alveolar cells once it has gained entry (Barton *et al.,* 2020; Xu *et al.,* 2020). This disruption leads to an inflammatory response that causes alveolar and interstitial oedema; thus, disrupting gas exchange in the lungs (Copin *et al.,* 2020; Hariri *et al.,* 2020). During systemic circulation to the lungs, blood travels via the bronchial circulation and drains into the pulmonary vein, this is known as the left-to-left anatomic shunt which results in a slight decrease in oxygen partial pressure (PaO_2) from 100 mmHg at the end of pulmonary capillaries to 95 mmHg in the pulmonary vein (Powers and Dhamoon, 2023). Pulmonary oedema causes a right-to-left shunt in oxygen-Hb dissociation curve that may result in a severe decrease in PaO_2 that may result in hypoxemia (Powers and Dhamoon, 2023). This reduction in PaO_2 lowers the oxygen binding affinity in Hb, causing less oxygen to bind to it and be transported to tissues (Patel *et al*., 2023).

2.5.2. Haematocrit, Haemoglobin, and Iron

Haematocrit is the percentage of red blood cells (RBCs) or erythrocytes in the blood. Erythrocytes are important for the transport of gases between the lungs and tissues using Hb (Mohanty *et al.,* 2014). The production of erythrocytes requires iron for the

synthesis of Hb (Kronstein-Wiedemann *et al.,* 2022). The uptake of iron in erythrocytes depends on a glycoprotein with two sites to bind transferrin (iron III) which also has the function of preventing the formation of free radicals and the invasion of pathogens in erythrocytes (Muckenthaler *et al.,* 2017).

Disturbed iron metabolism could lead to hypoxia and anaemia (Lang *et al.,* 2020). Ferritin, a protein that stores iron, is released from macrophages, and can enter erythrocyte progenitor cells to support their differentiation in the absence of transferrin, but only *in vivo* (Habib *et al.,* 2021). *In vitro*, transferrin is required to acquire erythroid iron (Habib *et al.,* 2021). The virus causes an inflammatory response that leads to the increased secretion of ferritin by macrophages, which also causes an inflammatory response through the secretion of inflammatory cytokines (Cheng *et al.,* 2020; Shoenfeld, 2020).

Iron retention in ferritin could alter the passage of iron across absorptive enterocytes, which has been observed in COVID-19 patients (Suriawinata and Mehta, 2022). A study by Taneri *et al.* (2020) suggests that when viral replication occurs in a host cell, a boosted cellular metabolism and optimal iron levels are required. Therefore, the innate immune system will respond by decreasing iron bioavailability to restrict viral replication. This will lead to activation of hepcidin, removal of cellular iron, increased ferritin, and decreased Hb (Ganz and Nemeth, 2012; Taneri *et al.,* 2020). Therefore, increased ferritin, decreased Hb, hypoxia, and anaemia might be signs for SARS-CoV-2 infection and COVID-19. Anaemia will result from iron-restricted erythropoiesis caused by the disturbed or altered iron metabolism (Kernan and Carcillo, 2017), thus COVID-19 patients have a lower-than-normal Hct (Behl *et al*., 2022).

The expression of CD147, ORF8 proteins and other receptors and spike proteins, by erythrocytes increases the susceptibility for SARS-CoV-2 infection and the virus uses these receptors to invade the cells (Ragotte *et al.,* 2021). When the virus invades the host cells, the spike protein and cell receptors bind to porphyrin and attack the haeme on the 1-beta chain of Hb (Wenzlong and Hualan, 2020). Consequently, the virus will cause haemolysis or produce a dysfunctional Hb (Wenzlong and Hualan, 2020). An inflammatory response will be stimulated because of the invasion and cell destruction. The CD147 is highly expressed in tumour cells, pathogen infected cells and inflamed

cells (Kosugi *et al.,* 2015). Their expression was found to increase in inflammatory processes, which forms a positive feedback loop (Cavezzi *et al.,* 2020; Kong *et al.,* 2014). This results in a decrease in erythrocytes and a low and impaired oxygen carrying capacity, causing anaemia and hypoxia. It was also observed that hypoxia might upregulate the expression of CD147, further worsening the effects (Wang *et al.,* 2020). It is speculated that these receptors might be the entry-point of SARS-CoV-2 in the bone marrow immature cells, which might decrease the number of maturing cells, and the cells that do mature might be dysfunctional (Ulrich and Pillat, 2020).

Haemoglobin plays an important role in transporting oxygen to tissues by reversibly binding them (Mohanty *et al.,* 2014). Therefore, any alteration to Hb will lead to deficient oxygen carrying capacity. As a protein, Hb comprises of four subunits, each containing one polypeptide chain and one haeme group, and they have the same prosthetic haeme group bound iron protoporphyrin associated with a polypeptide chain (Marengo-Rowe, 2006). The ferrous iron of the haeme is linked to the nitrogen (N) of a histidine and binds oxygen reversibly for transportation and delivery to tissues (Pillai *et al.,* 2020). Therefore, iron is a vital component of Hb structure and its function. Low levels of iron cause anaemia, while high levels cause excessive oxidative stress that could lead to organ damage (Litton and Lim, 2019).

Litton and Lim (2019) observed low levels of iron in hospitalised COVID-19 patients. A deviation in iron concentration was observed in COVID-19 cases, from the mild to the severe cases, which contributed to the oxygen insufficiency (Alhazzani *et al.,* 2020). It was also observed that the deviation in iron concentration persisted for a period of a few months after the onset of the infection in some of the patients (Taneri *et al.,* 2020). This suggests that some of the COVID-19 patients might have low concentration of Hb and have a low oxygen carrying-capacity after the onset of the infection. Therefore, as part of this study, Hb, Hct and iron were investigated to see how these factors may have played a role in oxygen carrying capacity in previously infected patients.

2.6. Risk factors of COVID-19

COVID-19 was found to elicit complications and worsen some pre-existing conditions as seen in previous cases of SARS and MERS (Badawi and Ryoo, 2016). Some of these pre-existing conditions became risk factors for SARS-CoV-2 infection (Ranard *et al.,* 2020). Among those risk factors, comorbidities such as cardiovascular diseases (CVDs), hypertension, diabetes, and obesity were included (Chowdhury and Anwar, 2020; Wang *et al.,* 2020). Vascular risk factors of the mentioned comorbidities and age seem to be the cause of high fatality cases in COVID-19 patients (Mohammad *et al.,* 2021). Therefore, heart rate, blood glucose, BMI, and blood pressure were investigated in this study to see how they might have played a role in oxygen carrying capacity in previously infected patients.

2.6.1. Cardiovascular diseases

Cardiovascular diseases may arise with or without pulmonary complications. In previous cases of SARS and MERS, CVDs were a common comorbidity in patients (Badawi and Ryoo, 2016). Hypokalaemia in COVID-19 patients increases the risk of arrhythmias. The hypokalaemia might be caused by the virus invading the cells which can increase urinary potassium excretion due to increased availability of angiotensin II, which might also lead to hypertension (Chen *et al.,* 2020). The increase of angiotensin II will stimulate the renin-angiotensin-aldosterone system (RAAS) and will lead to destructive effects on the heart and lungs (Chen *et al.,* 2020).

SARS-CoV-2 uses the ACE-2 receptors for cell entry which are expressed in the lungs, myocytes, and vascular endothelial cells (Zhang *et al.*, 2020). A study by Varga *et al.* (2020), found evidence that the virus can affect endothelial cells directly and cause endothelial inflammation. Endothelial dysfunction is an important factor of microvascular dysfunction, and it leads to vasoconstriction followed by organ ischaemia, inflammation with associated tissue oedema, and a procoagulant state (Davignon and Ganz, 2004). Some COVID-19 patients developed endotheliitis, which led to a systemic impaired microcirculatory function due to the endothelial inflammation (Feldmann *et al.,* 2020).

An ischemic cardiac injury can develop due to known or unknown coronary artery disease (CAD), caused by plaque rupture and thrombosis (Jain, 2020). Viral infection

increases the risk of developing CAD instigated by inflammation-mediated plaque destabilisation (Ranard *et al.,* 2020). The cytokine storm and inflammation response elicited in COVID-19 patients might enhance myocardial stress and injury. This might lead to impaired cardiac function causing complications such as cardiomyopathy, as well as lead to elevated antiphospholipid antibodies which are associated with thrombosis (Pelliccia *et al.,* 2017; Zhang *et al.,* 2020). Zheng *et al.* (2020) speculated that the myocardial injury might be caused by inappropriate activation of the T-helper cells of the adaptive immune system in COVID-19 patients. The cytokine storm and inflammation might persist for a few months after viral load decreases and recovery, as such, the effects of these conditions might also continue until chronic complications develop (Jain, 2020).

2.6.2. Diabetes mellitus, hypertension, and obesity

Diabetes mellitus, hypertension and obesity are risk factors of each other. Metabolic syndrome (MetS) is a cluster of at least four risk factors: high BG, high BP, high serum triglycerides and abdominal obesity (Fahed *et al.,* 2022). These risk factors could lead to metabolic dysfunction and the development of diabetes, hypertension, obesity and/or cardiovascular diseases (Alberti *et al.,* 2009). Individuals with diabetes and individuals with obesity generally have a compromised innate and adaptive responses, which is characterised by chronic low-grade inflammation (Francisco *et al*., 2018). Low-grade inflammation is characterised by high pro-inflammatory adipokines and low anti-inflammatory adipokines and can result in systemic metabolic dysfunction (Andrade-Oliveira *et al.,* 2015; Francisco *et al.,* 2018).

Patients with diabetes mellitus (DM), hypertension and obesity are at higher risk of poor COVID-19 outcomes and the most affected by SARS-CoV-2 (Guan *et al.,* 2020). Studies by Huang *et al*. (2020) and Wu *et al*. (2020) have reported that hypertension, diabetes, and CVDs are the most frequent and common comorbidities in COVID-19 patients who developed ARDS. The virus manifests in the lower airways of the respiratory system and causes pneumonia and inflammation (Muniyappa and Gubbi, 2020).

The virus targets cells expressing the ACE2 receptor to infect the host. Infected cells undergo apoptosis which activates proinflammatory cytokines and recruit inflammatory cells, such as CD4+ T helper cells, causing an inflammatory response (de Wit *et al.,* 2016). The inflammatory cells should regulate antigen presentation and immunity against SARS-CoV-2, but they get infected and lymphocytopenia results (Khan *et al.,* 2021). Consequently, this worsens the effects and causes hyperinflammation and cytokine storm by stimulation of innate immunity. This leads to impaired innate and adaptive immune responses, which is characterised by chronic low-grade inflammation, a complication of DM and obesity (Andersen *et al.,* 2016).

Individuals with diabetes and hypertension are more vulnerable to SARS-CoV-2 infection, which might be due to the ACE inhibiting and angiotensin receptor blocking (ARB) medication they are using (Schiffrin *et al.,* 2020). It has been speculated that ACE inhibitors upregulate ACE2 receptors, which increases the binding of SARS-CoV-2 and increases the likelihood of infection (Furuhashi *et al.,* 2015). Studies by Batlle *et al*. (2020) and Zheng *et al*. (2020) report that ACE inhibitors can reduce the inflammatory response caused by angiotensin II, by forming angiotensin 1-7 which is a vasodilator agent affecting cardiovascular organs. This will cause a reduction in the circulating angiotensin II and reduce inflammation. Gurwitz (2020), presented similar arguments, and further observed that this could reduce the risk of the development of ARDS, myocarditis, and acute kidney injury in COVID-19 patients. Therefore, ACE inhibitors and angiotensin receptor blockers are not the risk factors of SARS-CoV-2 but could be used as a therapeutic approach to reduce the viral load in COVID-19 patients (Schiffrin *et al.,* 2020). Even with the studies by Batlle *et al*. (2020), Gurwitz (2020), and Zheng *et al*., 2020 that negates the theory of use of ACE inhibitors placing diabetics and hypertensives at high risk, the cause of high infection and severe outcomes of COVID-19 in these individuals is not yet determined, therefore it can only be assumed that it is due to related complications of diseases.

Individuals with obesity are amongst people at high risk of SARS-CoV-2 infection (Schiffrin *et al.,* 2020). Excessive nutrient intake and low energy expenditure cause adipose tissue to expand to accommodate the excess nutrients resulting in obesity (Booth *et al.,* 2016). It is associated with endothelial dysfunction of adipose tissue that causes chronic low-grade inflammation (Andersen *et al.,* 2016). Adipose tissue

hypertrophy is predominantly predisposed to activating endoplasmic reticulum and mitochondrial stress responses, which promotes the activation of an inflammatory response (Kawai *et al.,* 2021). The stress and inflammation lead to adipose tissue apoptosis and the release of macrophages, cytokines, and chemokines in the adipose tissue, which increases the inflammatory state (Kawai *et al.,* 2021; Kraakman *et al.,* 2014). This increases the susceptibility of obese individuals to diseases such as CVDs, DM, and certain types of cancers, and now COVID-19 as well (Mohammad *et al.,* 2021).

Systemic inflammation is a major complication for COVID-19 caused by the proinflammatory cytokine storm (Vepa *et al.,* 2020). Therefore, it can be speculated that an obese individual with chronic inflammation might be infected with SARS-CoV-2 and develop COVID-19, which might amplify the existing inflammation with its arising acute inflammation and result in increased mortality. Patients with higher-than-normal BMI seem to be more vulnerable to SARS-CoV-2, accounting for most of the severe cases where patients required hospital admittance and mechanical ventilation (Vassilopoulou *et al.,* 2022). Because inflammation induced by SARS-CoV-2 infection is persistent even after the viral load has reduced, especially if it is amplified by chronic inflammation caused by adipose tissue, long-COVID is a high possibility (Vassilopoulou *et al.,* 2022). A study by Vimercati *et al*. (2021) found that a higher-than-normal BMI is a risk factor for long-COVID with the symptoms lasting more than a month, which substantiates the link between post-COVID and obesity.

2.7. Folic acid and vitamin B_{12}

There are different types of anaemia named according to their causes. It could be caused by a loss of blood, parasitic infection, a deficiency of specific nutrients and congenital conditions (Pei *et al*., 2019). The most common type is the anaemia caused by deficiency of iron which was discussed in 2.5.2. However, this dissertation also includes folic acid and vitamin B_{12}, which are the leading causes of megaloblastic anaemia.

2.7.1. Folic acid

Folic acid (vitamin B9) is a water-soluble vitamin also used to manage and treat anaemia caused by folic deficiency (Sayar *et al*., 2020). This vitamin also plays an important role in deoxyribonucleic acid (DNA). It is not naturally synthesized by the body; thus, it must be ingested regularly in food or in supplements (Pei *et al*., 2019). Folic acid is the synthetic form of folate, which is important for normal development of the central nervous system: therefore, the high requirements during pregnancy (Pei *et al*., 2019).

The deficiency in folate results in macrocytic megaloblastic anaemia (Hariz and Bhattacharya, 2023). Macrocytosis refers to a condition where the erythrocytes are larger than normal, and that there are fewer but much larger erythrocytes in the peripheral blood (Aslinia *et al*., 2006). The best source of folate includes leafy green vegetables like spinach, broccoli, lettuce, meat (especially liver), eggs and milk (Shulpekova *et al*., 2021). The recommended daily allowance (RDA) of folic acid is 400 µg for adults according to the National Institute of Health.

It was found that vitamins A, B, C, D, and E play a vital role as supplemental medication for viral infections (Patti *et al.*, 2021). Patel and Sobczynska-Malefora (2017) demonstrates that in vitro, vitamin D suppresses the replication of Mycobacterium tuberculosis (TB), and vitamin C is associated with decreased inflammatory markers in COVID-19. Folic acid was found to inhibit SARS-CoV-2 entry into host cells (Chen *et al*., 2022), while some studies found that supplementing with folic acid for SARS-CoV-2 induced systemic inflammation might increase the risk of mortality after infection (Karakousis *et al*., 2023). A cohort study by Liu *et al*. (2022) and another by Meisel *et al.* (2021) found that both low serum levels of folate and both low and high serum levels of vitamin B12 are significantly associated with higher risk of bad outcomes in COVID-19 patients.

2.7.2. Vitamin B_{12}

Vitamin B_{12} works in concert with folic acid to support DNA synthesis required for cell growth and cell division (Sayar *et al*., 2020). Vitamin B_{12} (cobalamin) deficiency anaemia develops as a result of immature and dysfunctional erythrocytes (Langan and Goodbred, 2017). The deficiency affects vital processes such as erythropoiesis in

bone marrow suppression (Stabler, 2013). This could result in normal red blood cell count but the cells will be abnormal, resulting in hypoxemia and hypoxia (Langan and Goodbred, 2017).

The prevalence of vitamin B_{12} deficiency varies by age range, with individuals 60 years and older having the highest risk (Shipton and Thachil, 2015). Food such as meat, eggs and dairy products are great sources of vitamin B_{12}, and like folic acid, the amount consumed daily is recommended by national health professionals (Stabler, 2013). The RDA for vitamin B_{12} is 2,4 µg according to the NIH. This recommendation is there to prevent deficiencies caused by malnourishment (Hariz and Bhattacharya, 2023).

Vitamin B_{12} also regulates the immune system and antiviral activity, and it is highly recommended by the United States Food and Drugs Administration (FDA) to use as treatment for COVID-19 (Kandeel and Al-Nazawi, 2020). It contributes to improving the immune response by increasing CD8+ T cells and natural killer T cells, which can defend the body against pathogens (Yoshii *et al.*, 2019). There are various vitamin B_{12} symptoms that are similar to COVID-19 and post-COVID-19 symptoms, as such supplementing with vitamin B12 might alleviate some of the symptoms (Batista *et al.,* 2022). As such, one of the objectives of the study was to assess the intake of the nutrients to eliminate them as cofounding factors of anaemia in the participants who might be pre-anaemic.

3. CHAPTER 3: METHODOLOGY

3.1. Introduction

Chapter 3 focuses on how the study was conducted. This chapter is divided into sections including the type of study, sampling method, the research area, the inclusion and exclusion criteria, study population, the qualitative and quantitative methods used during data collection, and the statistical analysis.

3.2. Study design

The study was a case COVID-19 naïve cross-sectional study, comprising of an COVID-19 positive group of individuals previously diagnosed with COVID-19 and a COVID-19 naïve group of individuals who never tested positive for COVID-19. The study used quantitative and qualitative methods to collect data. Data collection was conducted over a period of two months; and include the weekdays of August and September 2023.

3.3. The research area

The selected place of data collection was the University of Limpopo, Turfloop campus, Mankweng, Limpopo province, South Africa. This area was chosen as there is a lack of information available on COVID-19 and its effects in this community or area.

3.4. Sampling and study population

The participants were selected using convenient sampling. The aim of this study was to investigate the oxygen carrying capacity of adults in the Mankweng area post COVID infection. Participants that were selected were given an extensive explanation on what the study entails and were further required to sign informed consent forms (Addendum D).

The study sample size was calculated using the prevalence of long COVID-19 in individuals who have been diagnosed with COVID-19 before. The principle of a case-

COVID-19 naïve study was utilised as participants were divided into two groups. The COVID-19 positive group consisted of participants who were confirmed COVID-19 positive at least once, and the COVID-19 naïve group consisted of participants who were either COVID-19 naïve or have never tested positive for COVID-19. Since COVID-19 can also present as asymptomatic, it is difficult to accurately ascertain the COVID status of the participant. Thus, where possible, preference was given to participants who had proof of their status.

The participants were selected using convenient sampling in the Mankweng area, University of Limpopo campus. All participants of the study met both the exclusion and inclusion criteria which are outlined in section **3.6**. Based on the study by Lopez-Leon *et al*. (2021) who reported 80% of patients experiencing long term effects of COVID-19 infection after the viral load decreases, the sample size was calculated using the $n= z^2 p (1-p)/e^2$ formula. The total sample population was calculated to be 246, in which we expected 80% to be individuals who were previously diagnosed with COVID-19; however, we were only able to obtain 223 participants at the end of the study. The number obtained for the COVID-19 positive group was 28, in which 7 were males and 21 females, while the number obtained for the COVID-19 naïve group was 195, in which 81 were males and 114 females. All participants signed informed consent forms to participate in the study before the administration of questionnaires and taking of measurements.

3.5. Questionnaires used in this study

3.5.1. Participant details questionnaire

This questionnaire (Addendum F) was designed to acquire socio-demographic information and was used to record anthropometric measurements, BG, BP, Hct and Hb.

3.5.2. Participant selection questionnaire

This questionnaire (Addendum E) was designed using most of the exclusion criteria which is a list of conditions and characteristics which place individuals at higher risk of COVID-19 or are vulnerable to COVID-19 and its complications due to an overwhelmed and compromised immune system. Individuals who met the inclusion ad exclusion criteria were included in the study. The questionnaire was also used to acquire COVID-19 information from study participants.

3.6. Inclusion and exclusion criteria

The inclusion and exclusion criteria list were used to recruit participants who did not already have the comorbidities that might develop secondary to COVID-19 infection. This allows us to investigate whether the comorbidities are complications of the infection.

Participants with any of the following characteristics were included in the study:

- 18 years of age and older,
- Individuals who have lived in the research area for three months or more,
- Individuals who tested negative for COVID-19 in the last three to six months post infection.

Participants with any of the following conditions or characteristics were excluded from the study:

- Chronic pulmonary obstructive disease,
- Pregnant, lactating, or menstruating,
- Smoking,
- Iron deficiency anaemia or are taking iron supplements,
- Stomach ulcers,
- Cancer or Hodgkin’s disease,
- HIV-positive,
- Chronic kidney disease, and
- Trauma experience or blood loss within the last three months (donation or otherwise).

3.7. Data collection procedure

A formal letter (Addendum B) seeking permission to collect data in the research area was sent to the registrar of the University of Limpopo, Prof JK Masha, and gatekeeper's permission was awarded (Addendum C). Potential convenient participants were approached and recruited. A formal informed consent form (Addendum D) was administered and signed prior to further questioning and taking of measurements. Thereafter, the participants were administered a participants selection form (Addendum E) to identify individuals who could be included in the study, and participant details form (Addendum F) to complete. Anthropometric measurements and postprandial glucose, BP, Hct and Hb were measured and recorded. Lastly, participants were administered a food frequency questionnaire (Addendum G), and the food frequency assessment was conducted. As the study was conducted only on the staff members and students at the University of Limpopo, translating of the questionnaires was unnecessary, and participants were given the freedom to ask for elaboration where they clarity was required.

3.7.1. Measuring haematocrit, haemoglobin, and blood glucose

An altered oxygen-carrying capacity can lead to hypoxia and hypoxemia. This study focused on the molecule and cells that are relevant in transporting oxygen to and from tissues. Hct percentage and Hb concentration were measured in the participants using standardized finger prick method (Billet,1990; Rovó *et al*., 2022). This method is regularly used with point of care devices to self-monitor levels of specific parameters. For this method, there were no prior prerequisites for the participants to follow, except that all participants need to have lived in the study area for more than 3 months to eliminate a change in altitude as a confounding factor for abnormal values of Hct and Hb. Billet, (1990), stated that Hct measures the volume of red blood cells compared to the total blood volume. The measurements were done with the FORA® 6 Connect, using specific test strips designed to measure Hct, Hb and BG. The Hct results were

classified by gender as normal if the value was within normal range displayed in **Table 1**, low if below the range, and high if the value was above the range.

Table 1: Normal Hct percentage and Hb concentration in adults according to WHO

	Normal Hct	Normal Hb
Male	40 to 54%	14 to 18 g/dL
Females	36 to 48%	12 to 16 g/dL

(Billett, 1990)

The Hb concentration was classified as normal if the value was within the normal range displayed in **Table 1,** low if below the normal range, and high if above the normal range.

The monitoring of BG is crucial in determining fluctuations during regular checkups that might occur and plays a vital role as part of diagnostic techniques for diabetes mellitus (Khadilkar *et al*., 2013). The standardized finger prick method was applied (Mathew *et al*., 2022) using the FORA® 6 Connect portable device. The study participants were recruited at a convenience, as such random BG tests were conducted, and results were classified as displayed in **Table 2**.

Table 2: Normal BG in adults according to the Centre for Disease COVID-19 naïve and Prevention (CDC) and National Institute for Health and Care Excellence (NICE)

	Normal BG
Fasted BG	3.5 – 5.5 mmol/L
Random BG	Less than 11.1 mmol/L

Adapted from https://www.cdc.gov/diabetes/basics/getting-tested.html and https://www.cdc.gov/diabetes/basics/getting-tested.html

3.7.2. Measuring blood pressure and HR

Blood pressure is monitored to assist in the diagnosis of hypertension and other BP related conditions, and such conditions usually have no signs and symptoms in the early stages (Pickering *et al*., 2004). For this reason, it is advisable to regularly monitor

BP. There are two ways to measure BP: using the conventional manual auscultation or the automated devices which does not require a health care professional. The automated device method was used in this study, in accordance with the standardized procedure (Pickering *et al.*, 2004; Rehman *et al.*, 2022). The OMRON M2 Basic that was used for this study computed the systolic BP (SBP), diastolic BP (DBP) and HR. For accuracy, three measurements were performed, and an average was calculated. The classification of the BP was in accordance with the categories in **Table 3** and **Table 4** for HR.

Table 3: Classification of BP according to Heart and Stroke foundation

Categories	SBP mmHg	DBP mmHg
Normal	Less than 120	Less than 80
Elevated	120 - 129	Less than 80
Hypertension stage 1	130 - 139	80 - 89
Hypertension stage 2	140 or higher	90 or higher

Adopted from: https://www.heart.org/en/health-topics/high-blood-pressure/understanding-blood-pressure-readings

Table 4: Classification of HR of the average adult according to the Heart and Stroke foundation

Categories	HR (bpm)
Low	Less than 60
Normal	60 - 99
High	100 or higher

(Avram *et al.*, 2019)

3.7.3. Measuring anthropometric indices

The anthropometric indices used in this study is the BMI. BMI is used to determine whether an individual is underweight, normal, overweight, or obese, by taking the

weight of the individual and dividing it with their height squared, represented by the SI unit kilograms per meter squared.

Weight was measured using a digital body weight scale DH-2008B in accordance with to the standardised method for measuring weight (Casadei and Kiel, 2022). The height of the participants was measured using Leicester Height Measure stadiometer. The measurements were conducted using the standardized method for measuring height (Casadei and Kiel, 2022). The BMI was then calculated, and participants were classified in accordance with the cutoff points by WHO as presented in table 5.

Table 5: BMI classification according to WHO

Classifications	Cut off points (kg/m^2)
Underweight	15 – 19,9
Normal	20 – 24,9
Overweight	25 – 29,9
Obese	Above 30

(Nutall, 2015)

3.7.4. Measuring dietary intake in adults

There are different methods to measure dietary intake depending on how extensive the required dietary intake information needs to be, such as the 24-hour recall and food frequency questionnaire.

In this study, the food frequency questionnaire was used to obtain dietary information of macronutrients and micronutrients (iron, folic acid, and vitamin B_{12}) of the participants. The questionnaire (Addendum F) took approximately 30 minutes per participant, as such it was administered last. With the understanding that remembering over a month's worth of information could be challenging, participants were required to recall their diet of the past 30 days beginning with the day the questionnaire was administered. Only food and beverages that was consumed more than once every week within the 30 days was recorded. The advantage of this type of questionnaire was that it accounts for food and beverages consumed on a regular basis, while the

24-hour accounts for the previous day's food and beverages which might vary to the food and beverages consumed on the regular basis.

Table 6: Recommended daily allowance of selected micronutrients and elements according to the National Institute of Health (NIH)

Micronutrients	RDA Male	RDA Female
Iron concentration	18 mg	18 mg
Folic acid	400 µg	400 µg
Vitamin B_{12}	2.4 µg	2.4 µg

Adapted from: https://ods.od.nih.gov/HealthInformation/nutrientrecommendations.aspx

This questionnaire was conducted in accordance with the South African Medical Research Council (SAMRC) dietary intake guidelines. The participants were given food cards and asked to pick cards that present food and beverages consumed for the past 30 days. These cards functioned as a prompt for the recall. Cooking methods and meal portions were asked and recorded. The results were captured and analysed using the SAMRC food finder software and IBM SPSS version 29.0. The information from the questionnaire was used to calculate the total daily intake of micronutrients and macronutrients for each individual and compared with males and females from the COVID-19 positive and COVID-19 naïve group. **Table 6**, which displays the recommended daily allowance (RDA) of iron, folic acid, and vitamin B_{12}, was used to determine if participants consumed at least the RDA for each of the nutrients.

3.8. Statistical analysis

The data collected was captured and analysed using International Business Machines Statistical Packaging for the Social Sciences (IBM SPSS) statistics version 29. A 95% confidence interval (CI) and 5% margin of error ($p \leq 0,05$) were used. The distribution of the data was tested for normality using the independent student t-test presented in a table form, histogram, and line graphs. The data that was not normally distributed was logistically transformed. Descriptive statistical analysis was conducted with participants' demographic information and presented in frequency and percentage tables and pie charts and were used to determine the prevalence of parameters of

oxygen-carrying capacity and risk factors of COVID-19. The Pearson Chi-square was used to determine the p-value of frequency, and the independent student t-test was used to determine the mean, standard deviation, and p-value of mean of the risk factors of COVID-19 against the parameter of oxygen-carrying capacity of males and females within the COVID-19 positive group and COVID-19 naïve group. The 95% CI was used, and statistical significance set at $p \leq 0,05$. The association between Hct and Hb against risk factors of COVID-19 was analysed using the two tailed Pearson correlation factor (*r*) to find a possible correlation between the two factors. For the dietary intake, the RDAs were generated by Food Finder, then captured in IBM SPSS version 29.0 to run descriptive statistical analysis of the nutrients, and the Pearson correlations factor and p-value of mean using the independent student t-test against Hct and Hb. The t-test was used to find a link between the nutrients consumed and the levels of Hb and Hct.

3.9. Ethical consideration

Ethics approval (Addendum A) was approved obtained for the Turfloop Research Ethics Committee (TREC) at the University of Limpopo (project number TREC/370/2022:PG). Gate keeper's permission (Addendum C) was granted by the University of Limpopo to conduct data collection within the university premises. All the participants of this study read and signed the informed consent form (Addendum D), which was also explained in simple terms to ensure understanding. The reason and way the study was conducted, and the storage and use of the data and their personal information was explained prior to the signing of the informed consent form.

3.10. Reliability, viability, and objectivity

All anthropometric and BP measurements were taken three times and an average of the three values were calculated.

3.11. Bias

The selection of participants was convenient which eliminated selection bias. The selected participants were given a participant selection questionnaire that helped determine whether they participate in the study based on the exclusion and inclusion

criteria, which could've caused stigmatism and introduced bias. The finger prick measurements for BG, Hb and Hct were done only once to avoid causing unnecessary discomfort, but the rest of the measurements (anthropometry and BP) were repeated twice to prevent bias. All participants received the same informed consent form and questionnaires to ensure consistency.

4. CHAPTER 4: PRESENTATION AND INTERPRETATION OF RESULTS

4.1. Introduction

Chapter 4 focuses on the presentation and interpretation of results. This chapter is divided into sections relating to the order of the objectives of the study, then further subdivided according to the subtopics in chapter 2, except the first section of the chapter which is devoted to demographic and descriptive statistics to provide an overview of the study population. Tables and graphs have been used to present the results.

4.2. Descriptive statistics

Two hundred and twenty-three (223 or 91,0%) individuals participated in the study out of two hundred and forty-six of the anticipated participants. This was satisfactory as it was more than 80% of our expected sample size (Lopez-Leon *et al.*, 2021). The COVID-19 positive group consisted of twenty-eight (28 or 13,0%) participants, 21 (75,0%) were females and the seven (25,0%) were males. The COVID-19 naïve group consisted of one hundred and ninety-five (195 or 87,0%) participants, 114 (58,5%) were females and 81 (41,5%) were males presented in **Figure 1** below. This shows that there was an unequal distribution of participants in each group, as well as an uneven distribution of males and females within the groups. This could be because of the sampling method used to recruit participants, as it didn't target a more specific group of people.

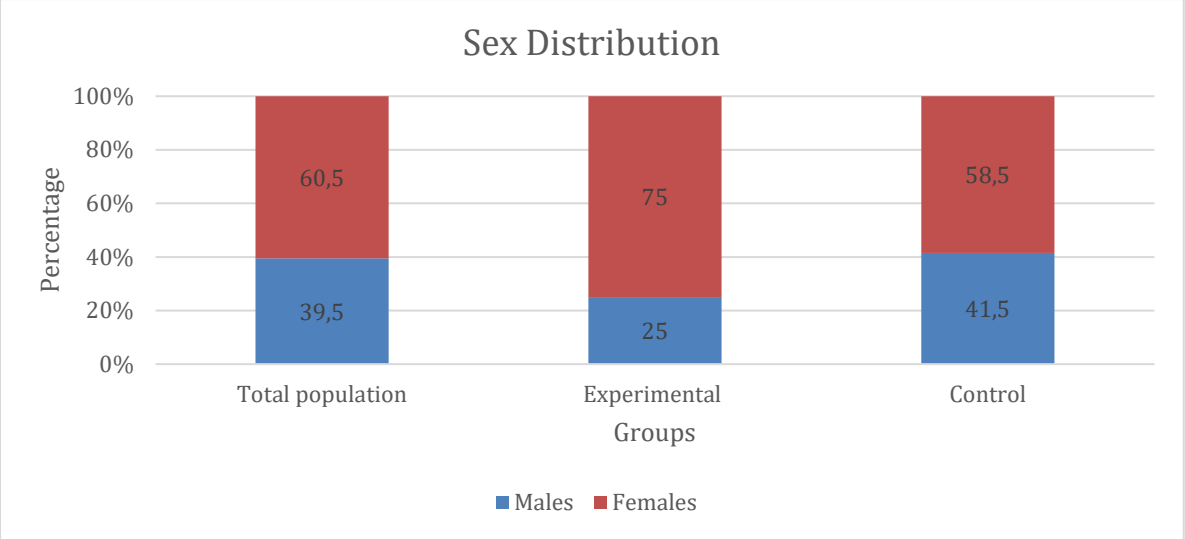

Figure 1: Distribution of males and females in the COVID-19 positive group and COVID-19 naïve group.

4.3. Age distribution participants

The age criteria of the study were eighteen years old and older, and that goal was achieved which was facilitated by the area in which data was collected. The research area is dominated by individuals within the age group of 18–25 years old. Majority of the study participants (92,8%) falls within that age group, while 4,9% of the study participants are within the 26–33 age group, 0,4% of the study participants is within the 34–41 age group, 0,4% of the study participants is within the 42–48 age group, and 0,9% of the study participants are within the 49–55 age group as demonstrated in **Figure 2** below.

4.3.1. Age distribution of study participants in COVID-19 positive group

The COVID-19 positive group consists of individuals who were confirmed positive for COVID-19 at least once. The number obtained for this group was 28 (13,0%) study participants out of a total of 223. Within that number, 24 (85,7%) study participants fell within the 18–25 age group, two (7,1%) study participants fell within the 26–33 age group, with one (3,6%) each in the 34–41 and 49–55 age groups (**Figure 2)**. For this study group, no study participant within the 42-48 age group was obtained.

The distribution of study participants according to their sex into age groups shows that most of the males and females fell within the 18–25 age group. The COVID-19 positive group consists of seven males and 21 females. Of those seven male participants, four (57,1%) participants fall within the 18–25 age group, one (14,3) participant falls within the 26–33 age group, one (14,3%) participant falls within the 34-41 age group, and one (14,3%) participant falls within the 49–55 age group (**Figure 3)**. No participants were obtained for the other age group. Of the 21 females in the COVID-19 positive group, 20 (95,2%) participants fall within the 18–25 age group and one (4,8%) participant falls within the 26–33 age group (**Figure 3)**. No participants were obtained for the other age groups.

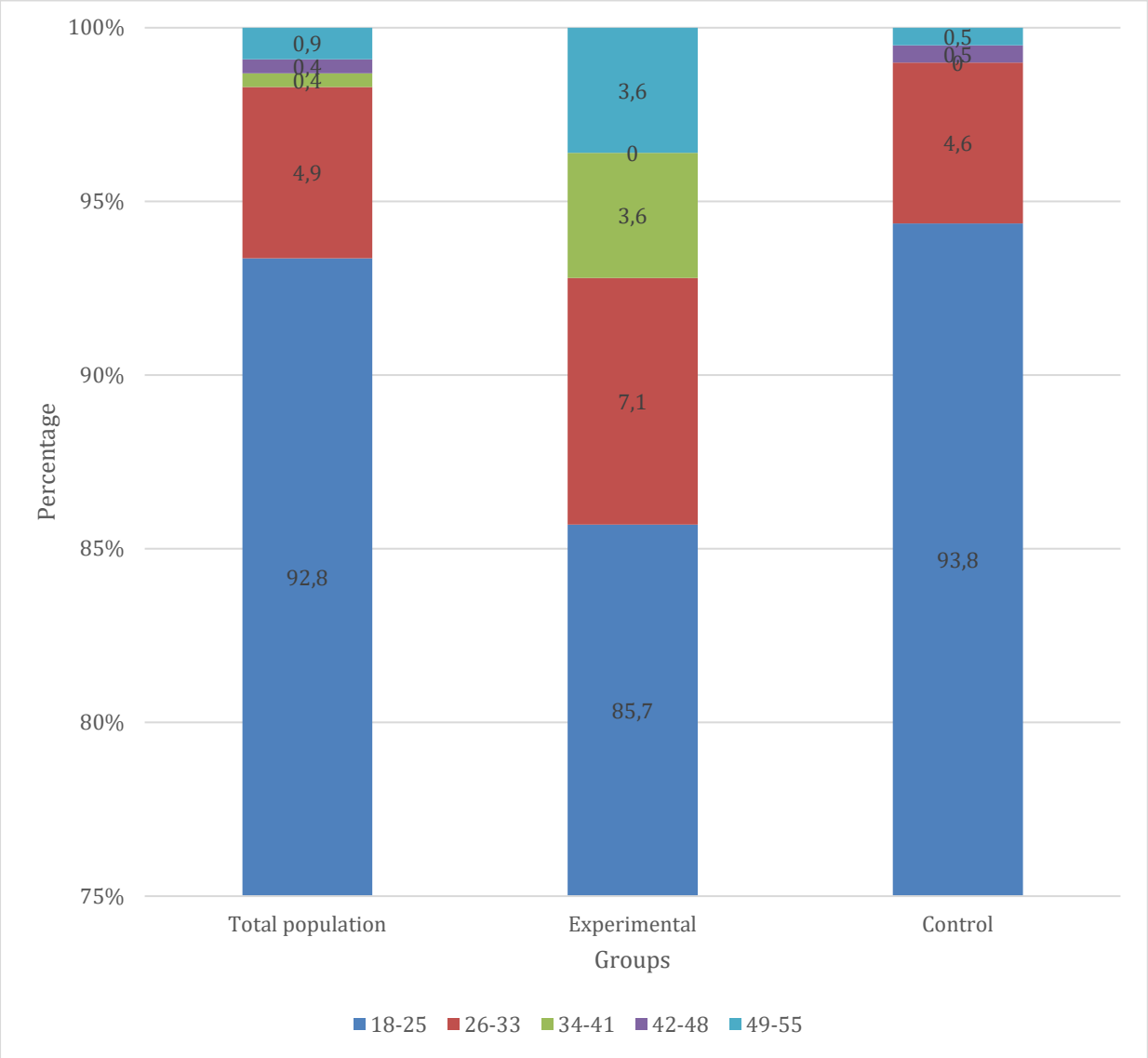

Figure 2: Age distribution of study participants in age groups.

4.3.2. Age distribution of study participants in COVID-19 naïve group

The COVID-19 naïve group consisted of individuals who were COVID-19 naïve or had never tested positive for COVID-19 before. The number of participants obtained for this group is 195 (87,0%). Of that number, 183 (93,8%) study participants fell within the 18–25 age group, nine (4,6%) within the 26–33 age group, and one (0,5%) each in the 42–48 and 49-55 age groups (**Figure 2)**.

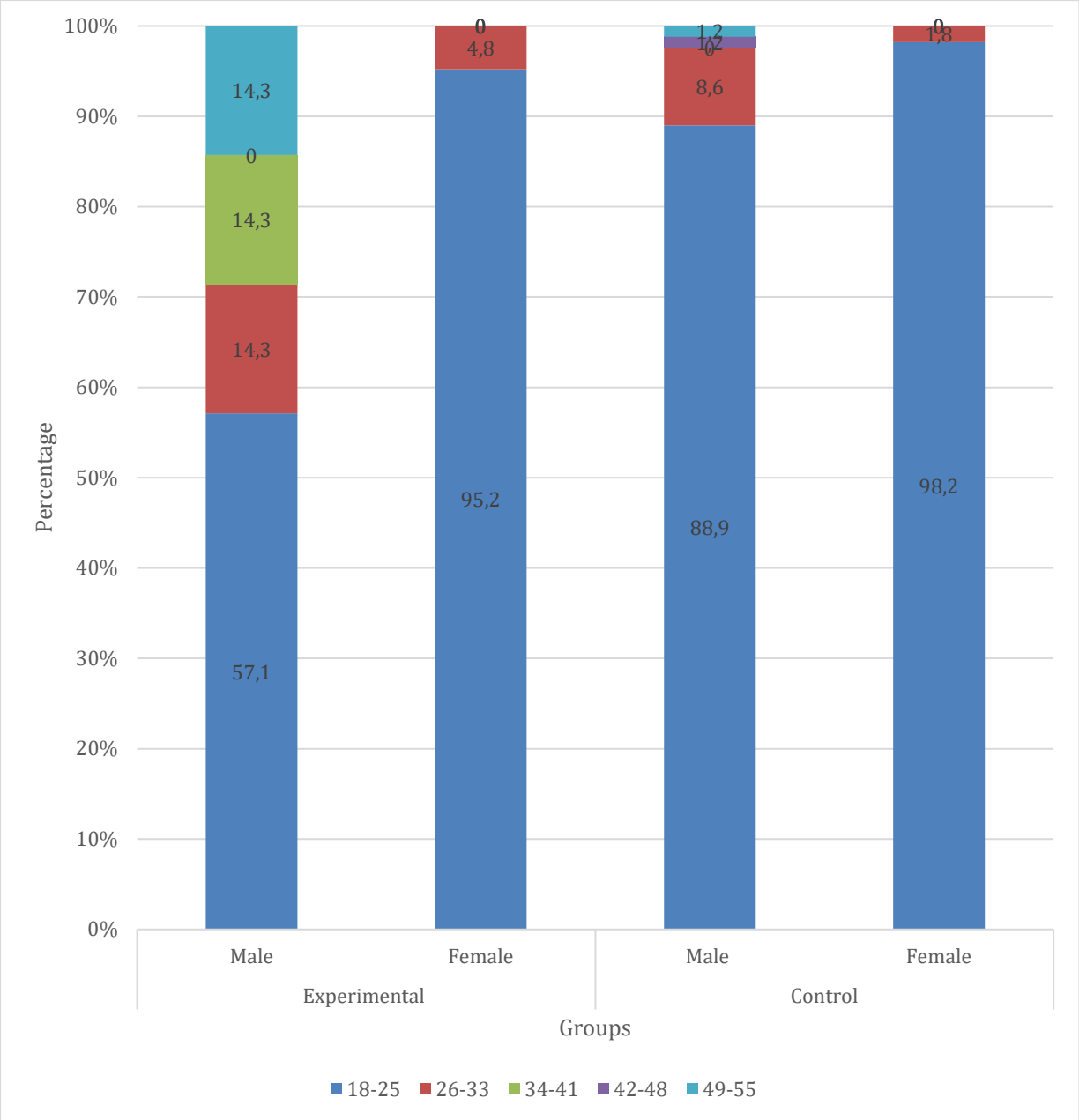

Figure 3: Percentage of males and females within the age groups in COVID-19 positive group and COVID-19 naïve group.

There is one missing regarding the age of the participants as there was no record of it. Therefore, the valid number of participants for the COVID-19 naïve group is 194 with respect to their age. No participants were obtained for the age group 34–48.

The COVID-19 naïve group consists of 195 participants, 81 (42,0%) males and 114 (58,0%) females. Of the 81 males, 72 (88,9%) fell within the 18–25 age group, seven (8,6%) participants fall within the 26–33 age group, and one (1,2%) each 42–48 and 49–55 age groups (**Figure 3)**. No participants were obtained for the other age group. Of the 114 females in the COVID-19 naïve group, 111 (98,2%) fell within the 18–25 age group and two (1,8%) participants within the 26–33 age group (**Figure 3)**. No participants were obtained for the other age groups.

4.4. Parameters associated with oxygen-carrying capacity

The first objective of the study was to assess parameters associated with oxygen-carrying capacity such as Hb concentration and Hct percentage. The collected raw data of Hb and Hct were categorized into low, normal, and high and the participants in the COVID-19 positive and COVID-19 naïve groups were distributed accordingly (**Table 7)**.

The number of males in the study sample of 223 was 88 (39%). Of that number 66 (75%) had normal Hct values, with three (3,4%) presenting with high Hct levels, and 19 (21,6%) with low Hct values. On the other hand, 74 (84,1%) of the male participants had normal levels of Hb, one (1,1%) had a high Hb level, and 13 (14,8%) had low Hb levels. This shows a similar trend where the frequency of males having either normal, high, or low percentage of Hct percentage is reflected in the Hb concentration, implying that there might be a correlation between Hct percentage and Hb concentration in the male participants.

Table 7: Prevalence of Hct and Hb of COVID-19 positive and COVID-19 naïve group based on sex.

Variables			Total population (N = 223)	COVID-19 positive group (n = 28)	COVID-19 naïve group (n= 195)	p-value
Males (n; %)			**88 (39)**	**7 (25,0)**	**81 (41,5)**	**0.050**
Females (n; %)			**135 (61)**	**21 (75,0)**	**114 (58,5)**	
Hct (n; %)	**Male**	**Normal**	66 (75,0)	6 (85,7)	60 (74,1)	0,754
		High	3 (3,4)	0	3 (3,7)	
		Low	19 (21,6)	1 (14,3)	18 (22,2)	
	Female	**Normal**	43 (32,6)	6 (28,6)	37 (33,3)	0,896
		High	7 (5,3)	1 (4,8)	6 (5,4)	
		Low	82 (62,1)	14 (66,7)	68 (61,3)	
Hb (n; %)	**Male**	**Normal**	74 (84,1)	7 (100,0)	67 (82,7)	0,487
		High	1 (1,1)	-	1 (1,2)	
		Low	13 (14,8)	-	13 (16,0)	
	Female	**Normal**	87 (65,9)	14 (66,7)	73 (65,8)	0,721
		High	8 (6,1)	2 (9,5)	6 (5,4)	
		Low	37 (28,0)	5 (23,8)	32 (28,8)	

The number of female participants in the study sample of 223 was 135 (61%). Of that 43 (32,6%) had normal Hct percentages, seven (5,3%) elevated Hct percentages, and 82 (62,1%) had low Hct percentages. On the other hand, 87 (65,9%) female participants had normal Hb concentrations, eight (6,1%) participants had high Hb concentrations, and 37 (28,0%) had low Hb concentrations. This implies an inverse correlation between Hct percentage and Hb concentration regarding prevalence in females.

Table 7 also indicates the prevalence of low Hct percentage and Hb concentration after COVID-19 infection across the study sample. Males have a prevalence of 21,6% for low Hct percentage and a prevalence of 14,8% for low Hb concentration, while females have a prevalence of 62,1% for low Hct percentage and a prevalence of 28,0% for low Hb concentration. This implies that females have a higher prevalence for low Hct percentage and Hb concentration after COVID-19 infection than males; therefore, might have a lower oxygen-carrying capacity.

Table 8 presents the means and p-value for means of Hb and Hct between the males from the COVID-19 positive group and the COVID-19 naïve, as well as means and p-value for means between the females from the COVID-19 positive group and COVID-19 naïve group.

Table 8: Descriptive statistics of Hct and Hb among males and females in the COVID-19 positive group and COVID-19 naïve group

Variables		COVID-19 positive group	COVID-19 naïve group	p-value
		Mean (±SD)	Mean (±SD)	
Hct	Male	45,86 (3,436)	43,48 (5,259)	0,301
	Female	33,29 (7,072)	34,17 (6,770)	0,771
Hb	Male	15,571 (1,163)	14,716 (1,829)	0,268
	Female	11,319 (2,444)	11,586 (2,302)	0,798

The mean for Hct and Hb between the COVID-19 positive group and COVID-19 naïve group show no statistical significance for either the males or the females as all p-values for mean are $p<0,05$ (**Table 8**).

4.4.1. Prevalence of low haematocrit after COVID-19 infection

The COVID-19 positive group consisted of 7 (25,0%) of male participants. Of this number, 6 (85,7%) had normal Hct percentages, and 1 (14,3%) had low Hct percentage. None of the male participants in the COVID-19 positive group had high Hct levels. The COVID-19 naïve group had 81 (41,5%) male participants and of that number 60 (74,1%) had normal Hct percentages, three (3,7%) had high Hct percentage, and 18 (22,2%) had low Hct percentages. This shows that males have normal Hct percentage regardless of whether they had COVID-19 or not. Male participants who had low and high Hct percentage are lower in the COVID-19 positive group than in the COVID-19 naïve group. **Table 7** shows a 14,3% prevalence of males having low Hct after COVID-19 infection. The p-value (p=0,754) of Hct percentage of males in the COVID-19 positive and COVID-19 naïve group shows no statistical significance (**Table 7**).

The COVID-19 positive group consisted of 21 (75,0%) female participants, of which 6 (28,6%) had normal Hct percentages, one (4,8%) had a high Hct percentage, and 14 (66,7%) had low Hct percentages. The COVID-19 naïve group consisted of 114 (58,5%) females; however, only 111 (56,9%) Hct percentages were recorded. Of these 37 (33,3%) were normal Hct, six (5,4%) were high Hct, and 68 (61,3%) were low Hct. The results show similar trends where majority of the female participants in both groups have low Hct percentages. **Table 7** displays a p-value of p=0.896, meaning that no statistical significance was found between the groups. **Table 7** also displays that there is a prevalence of 66,7% of having low Hct percentage for females after COVID-19 infection.

Comparing the prevalence of low Hct percentage for males (14,3%) and females (66,7%), it can be argued that females may have a higher prevalence than males, but the low Hct percentage in males might be due to the low number on male participants in the group. Therefore, have a compromised oxygen-carrying capacity post COVID-19 infection caused by the low percentage of erythrocytes.

4.4.2. Prevalence of low haemoglobin after COVID-19 infection

The COVID-19 positive group consisted of seven (25,0%) male participants, and their Hb concentrations were normal. The COVID-19 naïve group consisted of 81 (41,5%) male participants, of which 67 (82,7%) participants had normal Hb concentrations, one (1,2%) had an elevated concentration of Hb, and 13 (16,0%) had low Hb levels. The results show that all participants in the COVID-19 positive group had normal Hb concentrations, substantiated by the p-value of p=0,487 displayed by **Table 7** above. **Table 7** shows a prevalence of 0% of males having low Hb concentration after COVID-19 infection. This could be due to the low number of male participants in the COVID-19 positive group.

The COVID-19 positive group had 21 (75,0%) female participants, of which 14 (66,7%) participants had normal Hb concentrations, two (9,5%) participants had high Hb concentrations, and five (23,8%) participants had low Hb concentrations. The COVID-19 naïve group had 114 (58,5%) females, but only 111 (56,9%) had their Hb concentrations measured and recorded. Of them 73 (65,8%) had normal Hb concentrations, six (5,4%) had high Hb concentrations, and 32 (28,8%) had low Hb concentrations. The results obtained from both are very similar to each other, with most of the participants having normal Hb concentrations. **Table 7** shows the lack of statistical significance (p=0,721), and the prevalence of 23,8% for low Hb concentrations in females after COVID-19 infection. Comparing the prevalence of low Hb concentration for males (0,0%) and females (23,8%) shows that females are more prevalent to low Hb concentration than males: therefore, might have a lower oxygen-carrying capacity after COVID-19 infection.

4.5. Risk factors of COVID-19

4.5.1. Prevalence of diabetes

Table 9 displays the prevalence of diabetes between the male and female participants in the COVID-19 positive and COVID-19 naïve groups based on the random BG levels. To determine if the study participants might have an increased risk of becoming diabetic after COVID-19 infection, a random BG test was done. All participants were found to have a normal BG concentration. According to the NICE guidelines, a random BG tests with a value less than 11,1 mmol/L is considered to be normal, while more

than 11,1 mmol/L is classified as diabetic. The p-value displayed by **Table 9** is p= a which means no statistics are computed because all study participants had normal BG.

Table 9: Prevalence of diabetes after COVID-19 infection

Variables			Total population (N= 223)	COVID-19 positive group (n= 28)	COVID-19 naïve group (n= 195)	p-value
Males (n; %)			**88 (39)**	**7 (25,0)**	**81 (41,5)**	**0,050**
Females (n; %)			**135 (61)**	**21 (75,0)**	**114 (58,5)**	
BG (n; %)	**Males**	**Normal**	81 (100,0)	7 (100,0)	81 (100,0)	. [a]
	Females	**Normal**	132 (100,0)	21 (100,0)	111 (100,0)	. [a]

a. No statistics are computed because all participants have normal BG.

Table 9 presents the mean, standard deviation from the mean and p-value of mean of BG levels between male participants in the COVID-19 positive group and COVID-19 naïve group, and BG levels between female participants in the COVID-19 positive group and COVID-19 naïve group. The p-value of mean of **p=0,003** between the male participants from both groups shows statistical significance. BG levels were significantly higher in male participants in the COVID-19 positive group than in the COVID-19 naïve group. This suggests that males have higher glucose levels after COVID-19 infection. But this might be an incorrect conclusion as random BG tests were done to acquire this data and some participants were not fasted.

Table 10: Descriptive statistics for BG in male and female participants in the COVID-19 positive and COVID-19 naïve group

Variables		COVID-19 positive group	COVID-19 naïve group	p-value
		Mean (±SD)	**Mean (±SD)**	
BG	**Male**	5,500 (2,141)	4,793 (1,025)	**0,003**
	Female	4,281 (0,937)	4,476 (0,872)	0,922

4.5.2. Prevalence of hypertension

Table 11 displays the prevalence of hypertension amongst the male and female participants in the COVID-19 positive and COVID-19 naïve groups, based on their systolic and DBP values. In a study sample of 223 participants, 61 (69,7%) males out of 88 had an elevated SBP, while 23 (26,1%) had an elevated DBP and the remaining of the male participants had normal systolic and DBP. On the other hand, 35 (25,9%) of the 135 females had elevated SBP, and 23 (17,0%) had elevated DBP. The remaining female participants had normal systolic and DBPs. This implies that males are at risk of having elevated systolic and DBP in general.

A higher number of the female participants in the overall study sample had normal heart rate (HR) as compared to the male participants. Sixty-nine (83,1%) male participants had normal HR, none had high HRs, and 14 (16,9%) had low HR. On the other hand, 122 (91,0%) female participants had normal HRs, seven (5,2%) had high HRs and five (3,7%) had low HR. Females were at higher risk of elevated HR than males. This might because males are physically more active than females and so they would have a fit and healthy heart with lower HR than an unhealthy heart (Craft *et al*., 2014). The heart of females naturally beats faster than males because it is smaller in size and has a resting cardiac that is 10 – 20% less than a male heart, it must beat faster to compensate for the smaller volume of blood pumped with every beat (Prabhavathi *et al*., 2014).

In the COVID-19 positive group with seven male participants, four (57,1%) had normal SBP, and three (42,7%) had elevated SBPs. While in the COVID-19 naïve group (n=81), 23 (28,4%) had normal SBP, and 58 (71,6%) had elevated SBP. This implies that males who have been infected with COVID-19 might have an increased risk of elevated SBP. These findings must be interpreted with caution as the small size of the COVID-19 positive group participants is a concern for reliability. However, the SBP between the two male groups did not differ significantly (**Table 11,** p=0,114).

In the COVID-19 positive group with 21 female participants, 15 (71,4%) had normal SBP, and six (28,6%) had elevated SBP. While the COVID-19 naïve group (n=114) had 85 (74,6%) with normal SBP, and 29 (25,4%) with elevated SBP. This implies that females with a history of COVID-19 infection are likely to be at risk of high SBP than females who never had COVID-19. This implication might be inaccurate as the number of female participants was too low to be representative of the population. Similar to the males, the SBP differences between the two female groups were insignificant (**Table 11,** p= 0,763).

It was found that all male participants in the COVID-19 positive group (7; 100,0%) have normal DBP, while 58 (71,6%) male participants in the COVID-19 naïve group had normal DBP and 23 (28,4%) had elevated BP. This indicates that there is 0% prevalence of elevated DBP amongst the males after COVID-19 infection, but there is a prevalence of 28,4% of males having elevated DBP without the history of COVID-19 infection. **Table 11** displays a p-value of p= 0,101 which means there was no statistical difference found. This could be due to small sample size of the COVID-19 naïve and so this might not be accurate.

On the contrary, 17 (81,0%) female participants in the COVID-19 positive group had normal DBP and four (19,0%) had elevated DBP, while 95 (83,3%) female participants in the COVID-19 naïve group had normal DBP and 19 (16,7%) had elevated DBP. The results show that there is a 19,0% prevalence of females having elevated DBP after COVID-19 infection. These results might be inaccurate as there was a low number of female participants in the COVID-19 positive group to be representative of the population of females who tested positive for COVID-19 in the research area. **Table 11** displays a p-value of p= 0,790 which means there is no statistical significance.

Table 11: Prevalence of hypertension after COVID-19 infection

Variables			Total population (N= 223)	COVID-19 positive group (n= 28)	COVID-19 naïve group (n= 195)	p-value
Males (n; %)			**88 (39)**	**7 (25,0)**	**81 (41,5)**	**0,050**
Females (n; %)			**135 (61)**	**21 (75,0)**	**114 (58,5)**	
SBP (n; %)	**Males**	**Normal**	27 (30,7)	4 (57,1)	23 (28,4)	0,114
		Elevated	61 (69,3)	3 (42,9)	58 (71,6)	
	Females	**Normal**	100 (74,1)	15 (71,4)	85 (74,6)	0,763
		Elevated	35 (25,9)	6 (28,6)	29 (25,4)	
DBP (n; %)	**Males**	**Normal**	65 (73,9)	7 (100,0)	58 (71,6)	0,101
		Elevated	23 (26,1)	0 (0,0)	23 (28,4)	
	Female	**Normal**	112 (83,0)	17 (81,0)	95 (83,3)	0,790
		Elevated	23 (17,0)	4 (19,0)	19 (16,7)	
HR (n; %)	**Males**	**Normal**	69 (83,1)	7 (100,0)	62 (81,6)	0,213
		High	0 (0,0)	0 (0,0)	0 (0,0)	
		Low	14 (16,9)	0 (0,0)	14 (18,4)	
	Females	**Normal**	122 (91,0)	19 (90,5)	103 (91,2)	0,960
		High	7 (5,2)	1 (4,8)	6 (5,3)	
		Low	5(3,7)	1 (4,8)	4 (3,5)	

All the male participants in the COVID-19 positive group had a normal HR, while COVID-19 naïve 62 (81,6%) in the COVID-19 naïve group had a normal HR and 14 (18,4%) had a low HR. There was no significant difference between these two groups (p=0,213). The majority of the females from both groups had a normal HR, which did not differ significantly between the two groups (p=0,960) COVID-19 positive and COVID-19 naïve. However, female participants from the COVID-19 naïve group had a higher prevalence (5,3%) of an elevated HR than their counterparts from the COVID-19 positive group.

Table 12 presents the mean, standard deviation of the mean and p-value of mean of systolic and DBP, and HR between male participants in the COVID-19 positive group and COVID-19 naïve group, as well as the female participants in the COVID-19 positive group and COVID-19 naïve group. It shows that the DBP in the male participants of the COVID-19 naïve group was significantly higher (**p=0,023**) than in the male participants of the COVID-19 positive group. It also shows that the HR of male participants in the COVID-19 positive group is significantly higher (**p=0,021**) than in the male participants in the COVID-19 naïve group. This means that males who never had COVID-19 have a higher risk of elevated DBP, while males who had COVID-19 are at risk of higher HR.

Table 12: Descriptive statistics on systolic and diastolic BP and HR between males and females in the COVID-19 positive group and COVID-19 naïve group

Variables		COVID-19 positive group	COVID-19 naïve group	p-value
		Mean (±SD)	Mean (±SD)	
SBP	Males	118,76 (9,06)	126,38 (11,37)	0,61
	Females	115,53 (10,32)	114,10 (8,15)	0,17
DBP	Males	69,27 (4,16)	73,52 (9,42)	**0,02**
	Females	73,07 (7,59)	72,61 (7,39)	0,96
HR	Males	74,57 (4,96)	70,87 (11,46)	**0,02**
	Females	77,97 (13,64)	81,96 (12,47)	0,58

4.5.3. Prevalence of obesity

Table 13 displays the prevalence of high BMI in individuals who were diagnosed with COVID-19. Amongst 88 (39%) male participants, 61 (69,3%) had a normal BMI, 12 (13,6%) were underweight, and 15 (17,0%) were overweight. On the other hand, of the 135 (61%) female participants, 81 (60,0%) had a normal BMI, 14 (10,4%) were underweight, and 40 (29,6%) were overweight. The results show that there were more obese females (29,6%) than males (17,0%) in this study.

Table 13: Prevalence of a high BMI in individuals who were diagnosed with COVID-19

Variables			Total population (N= 223)	COVID-19 positive group (n= 28)	COVID-19 naïve group (n= 195)	p-value
Males (n; %)			**88 (39)**	**7 (25,0)**	**81 (41,5)**	**0,050**
Females (n; %)			**135 (61)**	**21 (75,0)**	**114 (58,5)**	
BMI (n; %)	**Males**	**Normal**	61 (69,3)	3 (42,9)	58 (71,6)	0,272
		Underweight	12 (13,6)	2 (28,6)	10 (12,3)	
		Overweight	15 (17,0)	2 (28,6)	13 (16,0)	
	Females	**Normal**	81 (60,0)	10 (47,6)	71 (62,3)	0,128
		Underweight	14 (10,4)	1 (4,8)	13 (11,4)	
		Overweight	40 (29,6)	10 (47,6)	30 (26,3)	

In the male COVID-19 positive group, three (42,9%) had a normal BMI, two (28,6%) were underweight, and two (28,6%) were overweight. While in the male COVID-19 naïve group, 58 (71,6%) had normal BMI, 10 (12,3%) were underweight and 13 (16,0%) were overweight. These results shows that there is a risk of 28,6% for males with high BMI after COVID-19 infection, but it was statistically insignificant (p=0,272).

In the female COVID-19 positive group, 10 (47,6%) had normal BMI, one (4,8%) was underweight, and 10 (47,6%) were overweight. While in the COVID-19 naïve group 71 (62,3%) had a normal BMI, 13 (11,4) were underweight, and 30 (26,3%) were overweight. These results show that there is no difference in risk of being normal or obesity in females after COVID-19 infection. These findings might not be accurate because of the low sample size of the COVID-19 positive compared to the COVID-19 naïve group. The p-value displayed by **Table 13** is p=0,128 which means there is no statistical significance.

Table 14: Descriptive statistics for BMI of males and females in the COVID-19 positive and COVID-19 naïve group

Variables		COVID-19 positive group	COVID-19 naïve group	p-value
		Mean (±SD)	**Mean (±SD)**	
BMI	**Males**	23,067 (6,149)	21,668 (3,696)	0,223
	Females	24,954 (5,412)	23,643 (5,535)	0,640

Table 14 displays the mean, standard deviation of mean and p-value of mean of BMI between male participants in the COVID-19 positive group and COVID-19 naïve, as well as female participants in the COVID-19 positive group and COVID-19 naïve group. There were no statistically significant differences noted for the BMI between the two male groups (**p=0223)** and the two female groups (**p=0,640**) (p=0,640).

4.6. Correlation of oxygen-carrying capacity and risk factors of COVID-19

The Pearson correlation analysis was run between oxygen-carrying capacity parameters (Hct and Hb) and the investigated COVID-19 risk factors for males and females. The correlation was run for males in the COVID-19 positive group against males in the COVID-19 naïve, and for females in the COVID-19 positive group against females in the COVID-19 naïve group.

Table 15: Pearson correlation between oxygen-carrying capacity parameters and COVID-19 risk factors in males.

	Hct		Hb	
	Pearson correlation factor (*r*)	**Significance (*p-value*)**	**Pearson correlation factor (*r*)**	**Significance (*p-value*)**
BG	0,038	0,722	0,047	0,661
SBP	0,037	0,735	0,026	0,811
DBP	0,077	0,475	0,093	0,387
HR	-0,117	0,292	-0,092	0,410
BMI	0.063	0,562	0,055	0,612

Table 15 displays the correlation factors between the parameters of oxygen-carrying capacity and the risk factors of COVID-19 among males of the COVID-19 positive and COVID-19 naïve groups. For both parameters, there was a positive correlation in all the risk factors except for HR which had a negative correlation. There was no statistically significant association for all the parameters and risk factors in males.

Table 16: Pearson correlation between oxygen-carrying capacity parameters and COVID-19 risk factors in females.

	Hct		Hb	
	Pearson correlation factor (*r*)	**Significance (*p-value*)**	**Pearson correlation factor (*r*)**	**Significance (*p-value*)**
BG	0,121	0,166	0,120	0,169
SBP	0,160	0,067	0,161	0,065
DBP	0,242	**0,005**	0,245	**0,005**
HR	-0,014	0,875	-0,017	0,845
BMI	-0,076	0,386	-0,073	0,406

Table 16 displays the Pearson correlation factors between oxygen-carrying capacity parameters and COVID-19 risk factor of females in the COVID-19 positive and COVID-19 naïve group. HR and BMI had a negative correlation against Hct, while SBP, DBP and BG had a positive correlation. For Hb, again HR and BMI had a negative correlation, while BG, DBP and SBP had a positive correlation. There was a statistically significant association between DBP and Hct and Hb.

4.7. Dietary intake of nutrients associated with anaemia.

Table 17 displays the mean and p-value of iron, folic acid, and vitamin B_{12} consumed by males daily in the COVID-19 positive group and COVID-19 naïve group. There are no statistically significant differences for dietary iron and folic acid between the two groups. However, for the males the Vit B_{12} levels differed significantly between the two groups ($p<0,001$); with the vitamin B_{12} intake significantly higher in the COVID-19 positive group than the COVID-19 naïve group. According to the RDA (**Table 6**), both groups consumed less than what is recommended for iron and folic acid, but all groups consumed more than what is recommended for vitamin B_{12}.

Table 17: Descriptive statistics of nutrients associated with anaemia between males in the COVID-19 positive and COVID-19 naïve group.

Variable	Groups		P-value
	COVID-19 positive Mean (±SD)	COVID-19 naïve Mean (±SD)	
Iron	6.943 (5,768)	7,021 (6,669)	0,895
Folic acid	90.978 (78,184)	107,036 (102,289)	0,466
Vitamin B_{12}	9.814 (12,240)	5,699 (5,982)	**0,001**

Table 18 displays the mean and p-value of iron, folic acid, and vitamin B_{12} consumed by females daily in the COVID-19 positive group and COVID-19 naïve group. There were no statistically significant differences noted for any of the consumed nutrients between the two groups. However, based on the RDA values (**Table 6**), both groups

consumed less than what is recommended for iron and folic acid, and more than what is recommended for vitamin B_{12}.

Table 18: Descriptive statistics of nutrients associated with anaemia of females in the COVID-19 positive group and COVID-19 naïve.

Variables	Group		P-value
	COVID-19 positive Mean (±SD)	COVID-19 naïve Mean (±SD)	
Iron	5,514 (3,125)	7,447 (6,405)	0,055
Folic acid	94,923 (74,874)	121,623 (107,272)	0,066
Vitamin B_{12}	4,576 (4,294)	4,868 (4,739)	0,546

4.8. Pearson correlation between daily dietary intake and parameters of oxygen-carrying capacity

Pearson correlation analysis was conducted to investigate the association between daily dietary intake of iron, folic acid, and vitamin B_{12}, and Hct and Hb (**Table 19)**. Dietary folic acid and vitamin B_{12} were negatively associated with both Hb and Hct, while iron was positively associated. There were no statistically significant differences observed between the consumed nutrients and the Hct and Hb levels.

Table 19: Pearson correlation of nutrients associated with anaemia and parameters of oxygen-carrying capacity.

Variables	Hct		Hb	
	Pearson Correlation factor (*r*)	Significance (p-value)	Pearson Correlation factor (*r*)	Significance (p-value)
Iron	0,018	0,794	0,018	0,793
Folic acid	-0,019	0.783	-0,014	0,835
Vitamin B_{12}	-0,027	0,691	-0,042	0,539

5. CHAPTER 5: DISCUSSION

5.1. Introduction

Chapter 5 focused on the discussion of the findings, and interpretation thereof. This chapter includes comparing the study's findings with similar previous research studies, while paying attention to the objectives and answering the research questions outlined in Chapter 1.

The results were discussed in the same order in which they were presented. The total study sample was divided into the COVID-19 positive and COVID-19 naïve groups, within those groups there was a group of males and another of females. The data was analysed, presented, and discussed comparing males from the COVID-19 positive group with males from the COVID-19 naïve group, as well as females from the COVID-19 positive group to females from the COVID-19 naïve group.

5.2. Prevalence of low haematocrit percentage and Hb concentration after COVID-19 infection

The first objective of the study was to assess Hb and Hct, associated with oxygen-carrying capacity. These factors play a vital role in transporting gases to and from tissues to ensure sufficient oxygenation. Therefore, any disruptions of these functions will alter the efficiency with which oxygen is transported, ultimately culminating in hypoxia and hypoxemia (Turner *et al*., 2023).

5.2.1. Haematocrit

There are conflicting reports on the effect of COVID-19 infection on Hct. Studies have reported haematological abnormalities after infection which could range from an altered morphological structure of haemocytes to haemolysis (Lazzaroni *et al*., 2021; Lechuga *et al*., 2023). Erythrocytes might be morphologically altered when they are invaded by SARS-CoV-2, which increased the risk of thrombosis (Russo *et al*., 2022). One characteristic of erythrocytes that makes it suitable to transport gases is deformability (Bateman *et al*., 2017), which decreases with COVID-19 infection leading

to haemolysis (Russo *et al*., 2022). Therefore, decreasing the volume of erythrocytes. The haemolysis of erythrocytes is speculated to be induced when SARS-CoV-2 binds to CD147 on the erythrocyte membrane and enters the cell via endocytosis (Ulrich and Pillat, 2020). This can lead to haemolytic anaemia which was found to be common in COVID-19 patients (Behl *et al*., 2022).

The results (**Table 7 and 8**) of this study suggest that there is no significant difference (males: p=0,754; females: p=0,896) in the Hct percentage of individuals who were infected and diagnosed with COVID-19, and those who never were infected. This might mean that COVID-19 infection does not alter the concentration of erythrocytes and might not be associated with a decrease in oxygen-carrying capacity, which stand to substantiate the study by (Mohanty *et al.,* 2014). It was reported that COVID-19 patients have an 80% prevalence of having persistent symptoms for approximately two months after the viral load (Lopez-Leon *et al*., 2021). The majority of participants in the COVID-19 positive group reported they were infected with COVID-19 in either 2020 or 2021; therefore, based on duration of persistent symptoms, the participants might have fully recovered. Lenehan *et al*. (2021) found that Hct and Hb were significantly lower before COVID-19 diagnosis and during the active infection.

The results might not be accurate as the number on participants within the two groups is not similar and lower than the ideal. The COVID-19 positive group, which consists of individuals who were infected and diagnosed with COVID-19, had a very low number of participants, and this might not be enough to be compared with a group with a high number of COVID-19 naïve participants. This might also mean that the participants are not big enough to be representative of a population.

Haemoglobin is the molecule found in erythrocytes which enables the binding of oxygen for transportation; and an erythrocyte is nonfunctional and not fully mature without Hb (Mohanty *et al.,* 2014), as such it was assumed that there might be positively correlated with each other. It was observed from results that most of the female participants had normal percentage of Hct but, most of the female participants also had low Hb concentration. This implies that Hct and Hb are independent variables of each other.

5.2.2. Haemoglobin

COVID-19 causes hyperinflammation, brought on by a cytokine storm, that leads to haemolysis (Mehta *et al*., 2020), and it is known to attack the oxygen binding sites on Hb, thereby decreasing oxygen-carrying capacity (Rapozzi *et al*., 2021). Yao *et al*. (2023) observed significantly lower Hb concentrations in COVID-19 patients, and a negative correlation between Hb concentration and severe COVID-19. This substantiates the speculation that COVD-19 affects oxygen-carrying capacity, in this regard by damaging Hb and decreasing the number of oxygen-binding sites.

It was observed that there was no statistical significance (male: p=0,487; females: p=0,721) between Hb concentration of participants who was infected with COVID-19, and those who were not infected. The same can be said regarding the reason for this observation as it was said above in **5.2.1**, as persistent symptoms were speculated to be tenacious for approximately two months, and the participants reported to have been infected in 2020 and 2021. Thus, it can be argued that enough time has lapsed for recovery to such an extent that the Hb levels have been restored to normal.

A normal Hct percentage does not guarantee normal Hb concentration, although the two are highly related and one can affect the others function and vice versa (Yoshida *et al*., 2019). An individual might have a high Hct but low Hb concentration. This phenomenon was noted in the current study where the majority of the female participants presented with low Hct levels, but most had normal Hb concentrations. Considering that they are female, there are numerous factors that could influence erythrocyte count such as diet, heavy menstruation and dehydration.

A study by Bergamaschi *et al*. (2021), found a high prevalence of anaemia in COVID-19 patients, especially those who were hospitalized. It is worthy to note that their study participants were of the ages between 41–65 years old, and older age is a known risk factor for most diseases and conditions, as such in comparison to the present study's findings whose participants are mostly within the age range of 18–25 years old, the prevalence of anaemia may be lower. Dinevari *et al*. (2021) also found that there is a high prevalence of anaemia in the older hospitalised COVID-19 patients and was associated with poor COVID-19 outcomes.

5.3. Prevalence of diabetes, hypertension, and obesity after COVID-19 infection.

5.3.1. Diabetes

Diabetes is one of the well-known risk factors for COVID-19 infection (Wang *et al.,* 2020). Individuals who had diabetes were accounted for having some of the more severe cases (Chowdhury and Anwar, 2020). It is speculated that having been diagnosed with COVID-19 makes individuals susceptible to developing hyperglycaemia and diabetes due the long-term effects of the infection (Fahed *et al.,* 2022). Diabetes, like hypertension and obesity, are characterised by low grade hyperinflammation which is also a characteristic of COVID-19 (Francisco *et al*., 2018). The invasion of the SARS-CoV-2 triggers an immune response that includes the stimulation of inflammatory mediators such as cytokines, resulting in a cytokine storm and hyperinflammation (Chowdhury and Anwar, 2020; Wang *et al.,* 2020). Hyperglycaemia, leading to diabetes, can develop due to this accumulation of activated innate immune cells, such as macrophages and natural killer cells, through promotion of insulin resistance (IR) and beta cell damage (Vepa *et al.,* 2020).

In the present study all participants had normal blood glucose, therefore there was no individuals at higher risk of diabetes among the study sample. There was no statistics computed as all participants had normal BG (**Table 9**). This could be due to the way in which participants were selected. The method of conveniently selecting participants made it challenging to inform potential participants to fast in the morning or 10–12 hours before voluntarily taking part in the study. With other selection methods participants would've been given the instructions and fasting BG or postprandial BG would've been measured and easily classified. This could've assisted with the determination of the prevalence of diabetes among the participants as there are reports of COVID-19 patients being diagnosed with diabetes during a high viral load.

A statistical significance of mean was found for BG in males who were diagnosed with COVID-19. Males who had COVID-19 had higher BG than males who never had COVID-19. This implies that males in the COVID-19 positive group are at higher risk

of diabetes than males in the COVID-19 naïve group, or that males with high BG are at higher risk of COVID-19 infection. This observation was expected as DM puts one at an increased risk for COVID-19. A study by Kautzky-Willer *et al.* (2023) reports the prevalence of T2D is increasing in both sexes but males are diagnosed at a young age and lower BMI that females.

Li *et al*. (2020), reported that 20,8% of their hospitalised COVID-19 patients were newly diagnosed with diabetes, and 28,4% were diagnosed with dysglycaemia and at high risk of diabetes, majority being elderly. This substantiates the hypothesis that comorbidities may develop due to COVID-19 infection. In addition, Unsworth *et al*. (2020), reported in London, U.K., that 30 children with COVID-19 of 23 months to 16,8 years were newly diagnosed with early onset type 1 diabetes (T1D). The risk of developing diabetes during hospitalisation due to COVID-19 infection is speculated to be provoked by various factors including bed rest and inactivity, which also results in disruption of glucose homeostasis (Ali Abdelhamid *et al*., 2016; Zahedi *et al*., 2023). Type 2 diabetes (T2D) is known to be accompanied by other cardiovascular risk factor such as hypertension and obesity (Vosko *et al*., 2023).

5.3.2. Hypertension

Hypertension is one of the common risk factors of COVID-19. A study by Hu *et al*. (2019) reported a prevalence of hypertension of between 15% and 25% in COVID-19 Chinese patients, on the other hand Garg *et al*. (2019) reported a prevalence of 49,7% among hospitalised COVID-19 patients in the United States. This condition is related to the severity and high mortality rate among COVID-19 patients (Guan *et al*., 2020).

The development of hypertension can be evaluated by monitoring the systolic and DBP. This study has found no statistically significant differences when comparing the prevalence of systolic and DBP males in the COVID-19 positive group and COVID-19 naïve, as well as that of females in the COVID-19 positive group and COVID-19 naïve group. However, a gender disparity was noted as it was found that males had a higher risk of increased systolic (69,3%) and DBP (26,1%) than females. This could be that high BP is more prevalent in males than females, that is until after menopause then females have a higher prevalence than males (Yanes and Reckelhoff, 2011). This is

corroborated by Ntuli *et al.* (2015), who studied the prevalence of hypertension in the Dikgale population. They found that the prevalence of hypertension was slightly higher in males than in females. But since 94% of the participants in the study sample are within the ages 18–25 years, then menopause is not accounted for, but premature menopause is plausible. In the current study none of the female participants reported to be in menopause.

There was statistical significance of the p-value of mean (±SD) (**p=0,023**) in the DBP between males in the COVID-19 positive group and COVID-19 naïve group. This implies that males from the COVID-19 positive group had higher DBP than males in the COVID-19 naïve group. Although there was no statistical difference or significance, males in the COVID-19 naïve group had a higher average SBP than males in the COVID-19 naïve group. Overall, this would imply that COVID-19 naïve males or males who were never diagnosed with COVID-19 would have higher SBP and DBP than males who were diagnosed. This contradicts all the studies that reported that COVID-19 patients have high BP or are at risk of having high BP. This could be due to the significant difference in the number in male participants within the COVID-19 positive and COVID-19 naïve groups.

Hypertension and cardiovascular diseases are interrelated comorbidities, along with diabetes (Guan *et al*., 2020). Therefore, the HR of participants was also included. There were statistically significant differences observed in the prevalence of high HR between males in the COVID-19 positive group and COVID-19 naïve group, as well as females in the COVID-19 positive group and COVID-19 naïve group. But this method is not the most efficient way to diagnose cardiovascular diseases as it can only determine the rate at which the heart beats, it is unable to determine the regularity of the heart beats. Even so, there was statistical significance or difference of mean (±SD) (**p=0,021**) in the HR between the males in the COVID-19 positive group and the COVID-19 naïve group. This implies that males who were diagnosed with COVID-19 have a higher HR than COVID-19 naïve males. Males naturally have a lower HR because they have a bigger heart than females, and so there is no need for their hearts pump as fast as their female counterparts (Prabhavathi *et al*., 2014). Males are assumed to be more active than females, and so they would have fit heart

(Prabhavathi *et al*., 2014). The findings suggest that the infection affects the male body so that the heart needs to pump faster to compensate the decrease in cardiac output.

After the onset of the pandemic, it was found that there was a link between the severity of the infection and coexisting CVDs and risk factors (Cheng *et al*., 2021). Using echocardiographic studies, Giustino *et al*. (2020) reported that there is an association between ventricular dysfunction and COVID-19. They observed that the most common pathologic findings in COVID-19 patients are right ventricular (RV) dysfunction (26,3%), followed by left ventricular (LV) wall abnormalities (23,7%), then global LV dysfunction at 18,4%, grade II diastolic dysfunction at 13,2%, and pericardial effusion at 7,2%. Another study by Tuvali *et al*. (2022) found that males have a higher prevalence of developing myocarditis or pericarditis a few days after infection, but they also observed similar findings in individuals who were never infected. Huang *et al*. (2020) reported that 58% of their recently recovering patients had myocardial oedema, fibrosis, and impaired right ventricular function. This substantiates the speculation that the effects of COVID-19 do linger during recovery and possibly longer.

Observing only the HR, without using more efficient methods such as electrocardiograph (ECG) and cardiac magnetic resonance imaging (MRI), our results could not determine any pathologic findings amongst the study participants. However, it could indicate whether participants had bradycardia or tachycardia, and the prevalence of those conditions. But even then, this information is not enough to support grounded speculations on the correlation of CVDs and COVID-19. Especially, when considering that there are many factors that could influence an individual's HR, such as anxiety.

5.3.3. Obesity

It was found that there is an increased prevalence of obesity in COVID-19 positive individuals and has been linked to worse clinical outcomes (Popkin *et al*., 2020). Popkin *et al*. (2020) reported an increase of approximately 48% in mortality rate of COVID-19 patients who were obese. High adipose tissue causes an inflammatory response, which results in an imbalance of T cell response and cause low-grade

inflammation which is a common characteristic of obesity (Frasca *et al*., 2022). This low-grade proinflammation might aggravate the hyperinflammation caused by COVID-19 infection and further impair immunity (Frasca *et al*., 2022).

There was no statistical significance in the prevalence of obesity among the study participants, but a higher number of the female participants were overweight/obese, than the male participants. This could be attributed to the physiological difference between males and females. Sex hormones play a vital role in body fat distribution (Frank *et al*., 2019). Oestrogen in females promotes increase in gluteofemoral subcutaneous adipose tissue (SCAT) deposits, especially during peak reproductive age of late teens to late 20s (Wells, 2007). On the other hand, the decline of testosterone in males promotes increase in abdominal visceral adipose tissue (VAT) accumulation (De Maddalena *et al*., 2012). Testosterone starts to decline after the age of 30 years, leading to an increase in VAT, and reaches its lowest at 70 years of age (De Maddalena *et al*., 2012). In comparison to the dominating age group (18-25 years old) in the current study, it can be assumed that since the female participants are within the reproductive age, they might have a higher BMI than the male participants who are well below the age of testosterone decline.

There is a 28,6% prevalence of obesity in males who tested positive for COVID-19, but this percentage has no statistical significance when compared to males who were COVID-19 naïve or were never infected with COVID-19 (16,0%) but it is higher. On the other hand, it also indicated that 47,6% of the females who tested positive for COVID-19 were overweight/obese. However, it did not differ significantly when compared to the COVID-19 naïve group. Which, although not statistically significant, but it corroborates the speculation of positive correlation. A study by Singh *et al*. (2022) found that obesity significantly increases and is the risk of severity and mortality in hospitalised COVID-19 patients. Nour *et al*., 2023 reported that in the US, the prevalence of obesity was 11% and increased to 25% during the pandemic. The current study's findings show a higher percentage.

5.4. Correlation between oxygen-carrying capacity and COVID-19 risk factors

Pearson correlation factor was used to determine any relationship between the parameters of oxygen carrying capacity and the risk factors on COVID-19. A study by Gandhi *et al*. (2017) reported that a high prevalence of anaemia was associated with advancing age and comorbidities such as DM, hypertension, and CVDs. A study by Alsayegh *et al*. (2022) found that patients with acute coronary syndrome (ACS) have a prevalence of 10%–43% of developing anaemia.

The correlation between male participants in the COVID-19 positive group and the COVID-19 naïve group, Hb and Hct was positive with all the risk factor variables, except for HR which had a negative correlation. This means that with an increase in Hb and Hct, which are the oxygen-carrying capacity parameters, there might be an increase in BG, BP, and BMI, but a decrease in HR. This could mean that in males, anaemia might not be a confounding factor of comorbidities such as hypertension, DM, and CVDs or vice versa, but they are commonly found in COVID-19 patients and associated with the severity of the infection (Tao *et al*., 2021). There was no significant difference in these correlations. High HCTHCT percentage and Hb concentration may cause blood vessels to constrict due to the increase in blood viscosity, which then increases the BP and the HR, and vice versa (Bazmandegan *et al*., 2023). As such, since SARS-CoV-2 may cause haemolysis, individuals who had COVID-19 might have decreased blood viscosity and have low BP and HR as a result. This was corroborated by Atsman *et al*. (2012) and Xuan *et al*. (2018) who reported a positive correlation between Hb concentration and BP.

Table 16 shows that in the correlation between female participants in the COVID-19 positive group and COVID-19 naïve group, Hb and Hct had a positive correlation with all the risk factor variable except for HR and BMI, which had a negative correlation. This implies that BP and BG increase with the increase of Hb and Hct, but there will be a decrease in BMI and HR. This could mean that in females, anaemia could not be risk factor for hypertension or DM and vice versa, but anaemia could be a risk factor for abnormal HR and obesity. The only relation is that COVID-19 infection has the potential to cause anaemia and the risk factors of COVID-19 in patients.

The risk factors for anaemia include nutritional deficiency of iron, B vitamins and folic acid, as well as chronic inflammation, parasitic infections, and congenital conditions

(Deivita *et al*., 2021). Chronic inflammation is a characteristic of DM, obesity, and hypertension (Francisco *et al*., 2018), so it is possible that an individual with any of these comorbidities is at risk of anaemia due to the chronic inflammation. Therefore, it could be speculated that there should be a negative correlation between Hb and Hct and the comorbidities. However, the leading cause of anaemia worldwide is iron deficiency (Deivita *et al*., 2021), but this still does not rule out the other possible risk factors.

5.5. Daily dietary intake

Inadequate dietary intake is associated with the risk of anaemia (Wang *et al*., 2015), which is commonly determined by monitoring Hb concentration (Paramastri *et al*., 2021). As previous studies have speculated and proved the association of COVID-19 and low Hb and Hct resulting in anaemia. The dietary intake assessment was included in this study to eliminate deficiency as a confounding factor of discrepancies with the results. A study by Litton and Lim (2019), reported high serum ferritin, which is an iron-storage protein, but low serum iron in COVID-19 patients within three days of admission, which placed patients at high risk of becoming anaemic.

The findings of this study (**Table 17, 18 and 19**) show a positive association of iron and Hb, as well as Hct, but a negative association of folic acid and vitamin B_{12} in participants who had COVID-19 and those who were never infected. The association of iron could substantiate the findings of other COVID-19 studies with similar results, especially since the COVID-19 positive group had a lower average iron concentration than the COVID-19 naïve group. But in this regard, it shows that the COVID-19 positive group consumed less dietary iron than what is recommended, and thus cause iron deficient anaemia. This could be the cause of low Hb and Hct.

Although folic acid and vitamin B_{12} are vital nutrients for a functional Hb molecule, the findings of this study show an inverse association implying that the fewer of these nutrients are consumed the higher the Hb and Hct. It could be said that these variables are independent to the parameters of oxygen-carrying capacity, as they don't seem to affect the outcome of Hb and Hct. The consumed vitamin B_{12} was above the RDA, but

still negatively associated with the parameters. Anaemia that is caused by vitamin B_{12} deficiency and folic acid deficiency increases with age and is less prevalent in the younger population irrespective of their dietary intake (Haris and Bhattacharya, 2022), which might be the reason for the negative correlation.

6. CHAPTER 6: CONCLUSION LIMITATIONS AND RECOMMENDATIONS

Chapter 6 focuses on concluding the discussed findings and answering the research questions outlined in chapter 1. The study limitations and recommendations are included.

6.1. Conclusion

The aim of the study was to investigate the effects of long-COVID with regards to oxygen-carrying capacity in the population of Mankweng, Limpopo province, South Africa. This was determined by assessing factors associated with oxygen-carrying capacity such as Hb; evaluating data on the risk factors for COVID-19; and assessing daily dietary intake of iron, folic acid, and vitamin B_{12}.

The study sample of this study had no statistical significance between the COVID-19 positive group and COVID-19 naïve. Most of the participants had normal Hct and Hb in both groups. The Hct mean was higher than Hb mean, showing that they are independent variables regardless of their related function. This is not substantiated in COVID-19 studies that found significant association between these factors and COVID-19 infection.

The findings showed no statistical significance in the prevalence between the factors associated with oxygen-carrying capacity and BG. Random glucose tests were conducted, and all participants had normal BG below 11.1 mmol/L (Mathew *et al*., 2023). However, there was statistical significance of mean (p=0,003) for BG in males between the two groups which showed that males who were had COVID-19 had BG that was statistically higher than males who never had COVID-19, implying that they might be at risk of diabetes. This was expected since T2D increases the risk of infection, and it supports other studies that had similar results.

The findings for BP and HR were statistically insignificant in the prevalence after COVID-19 infection, but DBP (p=0,023) of males who had COVID-19 was statistically higher than males who never had COVID-19, and HR (p=0,021) of males who never

had COVID-19 was statistically higher than that of males who had COVID-19, which negates the speculation of correlation between BP and HR with and Hb and Hct. There was no statistical significance found for BMI among participants in the COVID-19 positive group and COVID-19 naïve group. This negates studies that found COVID-19 patients having higher BMI than individuals who never had it. But there was a prevalence of obesity in females of 47,6% after COVID-19 infection. It was speculated that the inflammation caused adipose tissue will aggravate the inflammation caused by COVID-19 and therefore compromise the immune system making them more vulnerable, similarly with diabetes and hypertension.

The findings showed that the participants consumed less than the RDA for iron and folic acid but consumed more than the RDA for vitamin B_{12}. As such, it is possible that some participants were anaemic due to deficiency of these nutrients. This implies that low Hct and Hb might be due to the deficiency of iron and folic acid.

The aim of this study to investigate the effects of long-COVID with regard to oxygen-carrying capacity was achieved in some cases where it was observed that oxygen-carrying capacity is affected by COVID-19 risk factors. But there were some limitations that was disadvantageous to the study, such as the amount of time that has passed since the study participants had COVID-19. As it was found that long-COVID can last up to approximately two months, most of the participants who have been previously diagnosed with COVID-19 do not have long-COVID.

The results of this study bridged the knowledge gap of the period long-COVID might be experienced. They could be applied in the health sector as new variants of CoVs are still being discovered. They could also be used to direct the focus of prevention and treatment as the population that might be at higher risk has been clarified. The participants of the study were given immediate feedback about their BP, BG, Hct, and Hb, and made them more informed about their health without financial loss. Future studies might provide more information which will help with developing medication to mitigate the spread and ways of managing possible infection and long-COVID-19. This study can assist in directing researchers on what to focus on for the future.

6.2. Limitations

- This was a case-COVID-19 naïve cross-sectional study, progression could not be investigated from time of infection until total recovery of COVID-19, and beyond.
- The sample area was dominated by black people, as such there is a lack of diversity among the study sample, this can be changed by choosing a more diverse research area to sample participants.
- Convenient sampling was used to select participants and made it impossible to instruct participants to fast before glucose test.
- There was no way of determining honesty of the details on the administered questionnaires as all information was word of mouth without the provision of proof to what was said.
- The food frequency questionnaire assessment was quite lengthy which resulted in some participants leaving before completing. The questionnaire needs to be shortened or the way of administering them needs to be changed.
- Some participants were afraid of needles and therefore did not participate in the parts of the study that required a finger prick.
- The number of participants in the COVID-19 naïve group was too small to be representative of the population in Mankweng which made concluding difficult. For future studies, it would be advisable have almost the same number of participants in the control and experimental group for valid comparisons.
- Participants were trusted to be honest about with all given information, including COVID-19 status, without providing proof. Sampling participants from a health institution will be beneficial as the information will be valid and will even be specific on how long it has been since infection.
- The exclusion and inclusion criteria can be improved to either eliminate some of the factors on the list of to add on depending on what future studies will be investigating.

6.3. Recommendations

- Make the study a longitudinal study, which make it possible to investigate all the objectives using one group of participants over a period of study how they recover and investigate the cause of reinfection.
- Adding spirometry to study will assist to investigate how lung function is affected by COVID-19, and how they improve with recovery.
- Adding ECG can show how cardiac function is affected and can be studied extensively.
- This study can be conducted using animal models that will allow investigators to study the same participants before infection and after infection, as well as through recovery, if it is ethically possible.
- Choosing a research sample area that is more diverse to include different ethnicities and eliminate race bias.
- Measuring serum iron, folic acid, and vitamin B_{12} will help determine concentrations of these nutrients to determine anaemia.
- Future studies can replace blood glucose with HbA1c as it gives blood glucose of three months.

REFERENCES

Alberca, R.W., Orfali, R.L., Rigato, P.O., da Silva Duarte, A.J., Aoki, V. and Sato, M. N. 2021. Long-term effects of COVID-19 in sickle cell disease: A case report. *World Academy of Sciences Journal*, ***3***: DOI: 10.3892/wasj.2021.117.

Alberti, K.G., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., Fruchart, J.C., James, W.P., Loria, C.M., Smith, S.C., Jr, International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity. 2009. Harmonising the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*, ***120***(16): 1640–1645.

Alhazzani, W., Møller, M.H., Arabi, Y.M., Loeb, M., Gong, M.N., Fan, E., Oczkowski, S., Levy, M.M., Derde, L., Dzierba, A., Du, B., Aboodi, M., Wunsch, H., Cecconi, M., Koh, Y., Chertow, D.S., Maitland, K., Alshamsi, F., Belley-Cote, E., Greco, M., andRhodes, A. 2020. Surviving Sepsis Campaign: Guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). *Intensive care medicine*, ***46***(5): 854–887.

Ali Abdelhamid, Y., Kar, P., Finnis, M.E., Phillips, L.K., Plummer, M.P., Shaw, J.E., Horowitz, M., and Deane, A.M. 2016. Stress hyperglycaemia in critically ill patients and the subsequent risk of diabetes: a systematic review and meta-analysis. *Critical care (London, England)*, ***20***(1): 301. https://doi.org/10.1186/s13054-016-1471-6

Alkhaldy, H.Y., Awan, Z.A., Abouzaid, A.A., Elbahaey, H.M., Al Amoudi, S.M., Shehata, S.F., and Saboor, M. 2022. Effect of altitude on haemoglobin and red blood cell indices in adults in different regions of Saudi Arabia. *International journal of general medicine*, ***15***: 3559–3565.

Alsayegh, F., Alkhamis, M.A., Ali, F., Attur, S., Fountain-Jones, N.M., and Zubaid M. 2022. Anaemia or other comorbidities? using machine learning to reveal deeper

insights into the drivers of acute coronary syndromes in hospital admitted patients. *PLoS ONE,* ***17***(1): e0262997. https://doi.org/10.1371/journal.pone.0262997

Andersen, C.J., Murphy, K.E., and Fernandez, M.L. 2016. Impact of Obesity and Metabolic Syndrome on Immunity. *Advances in nutrition (Bethesda, Md.)*, ***7***(1): 66−75.

Andrade-Oliveira, V., Câmara, N.O., and Moraes-Vieira, P.M. 2015. Adipokines as drug targets in diabetes and underlying disturbances. *Journal of Diabetes Research*, ***2015***: 681612. https://doi.org/10.1155/2015/681612

Arévalos, V., Ortega-Paz, L., FernandezRodrı´guez, D., Alfonso Jime´nez-Dı´az, V., Rius, J.B., Campo, G.I. 2021. Long-term effects of coronavirus disease 2019 on the cardiovascular system, CV COVID registry: A structured summary of a study protocol. *Public Library of Science ONE*, ***16***(7): e0255263. https://doi.org/10.1371/journal.pone.0255263

Aslinia, F., Mazza, J.J., and Yale, S.H. 2006. Megaloblastic anaemia and other causes of macrocytosis. *Clinical Medicine and Research*, ***4***(3): 236–241.

Avram, R., Tison, G.H., Aschbacher, K., Kuhar, P., Vittinghoff, E., Butzner, M., Runge, R., Wu, N., Pletcher, M.J., Marcus, G.M., and Olgin, J. 2019. Real-world HR norms in the health eHeart study. *NPJ Digital Medicine*, ***2***(58). https://doi.org/10.1038/s41746-019-0134-9

Azhar, E.I., Hui, D.S.C., Memish, Z. A., Drosten, C., and Zumla, A. 2019. The Middle East Respiratory Syndrome (MERS). *Infectious Disease Clinics of North America*, ***33***(4): 891–905. https://doi.org/10.1016/j.idc.2019.08.001

Badawi, A. and Ryoo, S.G. 2016. Prevalence of comorbidities in the Middle East respiratory syndrome coronavirus (MERS-CoV): A systematic review and meta-analysis. *International Journal of Infectious Diseases,* ***49***: 129–133. doi: 10.1016/j.ijid.2016.06.015

Barton, L.M., Duval, E.J., Stroberg, E., Ghosh, S., and Mukhopadhyay, S. 2020. COVID-19 Autopsies, Oklahoma, USA. *American Journal of Clinical Pathology*, ***153***(6): 725–733.

Bateman, R. M., Sharpe, M. D., Singer, M. and Ellis, C. G. 2017. The effect of sepsis on the erythrocyte. *International Journal of Molecular Sciences*, ***18***(9): 19–32.

Batista, K.S., Cintra, V.M., Lucena, P.A.F., Manhães-de-Castro, R., Toscano, A.E., Costa, L.P., Queiroz, M.E.B.S., de Andrade, S.M., Guzman-Quevedo, O., and Aquino, J. S. 2022. The role of vitamin B12 in viral infections: a comprehensive review of its relationship with the muscle-gut-brain axis and implications for SARS-CoV-2 infection. *Nutrition reviews*, ***80***(3): 561–578.

Batlle, D., Wysocki, J., and Satchell, K. 2020. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clinical Science (London, England: 1979)*, ***134***(5): 543–545.

Bazmandegan, G., Abbasifard, M., Ostadebrahimi, H., Gholamrezapour, M., and Kamiab, Z. 2023. Positive association between haemoglobin concentration and BP in adults: A cross-sectional study based on Rafsanjan Cohort Study. *International Journal of Hypertension*, ***2023:*** 6283711. https://doi.org/10.1155/2023/6283711

Behl, T., Kaur, I., Aleya, L., Sehgal, A., Singh, S., Sharma, N., Bhatia, S., Al-Harrasi, A., and Bungau, S. 2022. CD147-spike protein interaction in COVID-19: Get the ball rolling with a novel receptor and therapeutic target. *The Science of the Total Environment*, ***808***: 152072. https://doi.org/10.1016/j.scitotenv.2021.152072

Bergamaschi, G., Borrelli de Andreis, F., Aronico, N., Lenti, M.V., Barteselli, C., Merli, S., Pellegrino, I., Coppola, L., Cremonte, E.M., Croce, G., Mordà, F., Lapia, F., Ferrari, S., Ballesio, A., Parodi, A., Calabretta, F., Ferrari, M. G., Fumoso, F., Gentile, A., Melazzini, F., andInternal Medicine Covid-19 Collaborators. 2021. Anemia in patients with Covid-19: Pathogenesis and clinical significance. *Clinical and COVID-19 positive Medicine*, ***21***(2): 239–246. https://doi.org/10.1007/s10238-020-00679-4

Billett, H.H. Haemoglobin and Haematocrit. *In*: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths; 1990. Chapter 151. Available from: https://www.ncbi.nlm.nih.gov/books/NBK259/

Booth, A., Magnuson, A., Fouts, J. and Foster, M. 2016. Adipose tissue: An endocrine organ playing a role in metabolic regulation. *Hormone Molecular Biology and Clinical Investigation*, ***26***(1): 25–42.

Cantuti-Castelvetri, L., Ojha, R., Pedro, L.D., Djannatian, M., Franz, J., Kuivanen, S., Van Der Meer, F., Kallio, K., Kaya, T., Anastasina, M., Smura, T., Levanov, L., Szirovicza, L., Tobi, A., Kallio-Kokko, H., Österlund, P., Joensuu, M., Meunier,

F.A., Butcher, S.J., Winkler, M.S., Mollenhauer, B., Helenius, A., Gokce, O., Teesalu, T., Hepojoki, J., Vapalahti, O., Stadelmann, C., Balistreri, G. and Simons, M. 2020, Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity, *American Association for the Advancement of Science (AAAS)*, ***370***(6518): 856–860.

Casadei K, Kiel J. Anthropometric Measurement. [Updated 2022 Sep 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537315/

Cascella M, Rajnik M, Aleem A, et al. Features, Evaluation, and Treatment of Coronavirus (COVID-19) [Updated 2023 Aug 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/

Cavezzi, A., Troiani, E. and Corrao, S. 2020. COVID-19: Hb, iron, and hypoxia beyond inflammation: A narrative review. *Clinics and Practice*, ***10***: doi: 10.4081/cp.2020.1271.

Chen, D., Li, X., Song, Q., Hu, C., Su, F., Dai, J., Ye, Y., Huang, J. and Zhang, X. 2020. Hypokalaemia and clinical implications in patients with coronavirus disease 2019 (COVID-19). *MedRxiv* .2020-02. doi: https://doi.org/10.1101/2020.02.27.20028530

Chen, L., Li, X., Chen, M., Feng, Y., and Xiong, C. 2020. The ACE2 expression in the human heart indicates a new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovascular Research*, ***116***(6): 1097–1100.

Chen, Y.M., Wei, J.L., Qin, R.S., Hou, J.P., Zang, G.C., Zhang, G.Y., and Chen, T.T. 2022. Folic acid: a potential inhibitor against SARS-CoV-2 nucleocapsid protein. *Pharmaceutical biology*, ***60***(1): 862–878.

Cheng, L., Li, H., Li, L., Liu, C., Yan, S., Chen, H. and Li, Y. 2020. Ferritin in the coronavirus diseases 2019 (COVID-19): A systematic review and meta-analysis. *Journal of Clinical Laboratory Analysis, **34***: e23618. DOI: 10.1002/jcla.23618

Cheng, S., Zhao, Y., Wang, F., Chen, Y., Kaminga, A.C., and Xu, H. 2021. Comorbidities' potential impacts on severe and non-severe patients with COVID-19: A systematic review and meta-analysis. *Medicine*, ***100***(12): e24971. https://doi.org/10.1097/MD.0000000000024971

Chowdhury, S.F. and Anwar, S. 2020. Management of Hb disorders during the COVID-19 Pandemic. *Frontiers in Medicine*. ***7***: 306. doi: 10.3389/fmed.2020.00306

Copin, M.C., Parmentier, E., Duburcq, T., Poissy, J., Mathieu, D., and Lille COVID-19 ICU and Anatomopathology Group. 2020. Time to consider the histologic pattern of lung injury to treat critically ill patients with COVID-19 infection. *Intensive Care Medicine*, ***46***(6): 1124–1126.

Craft, B.B., Carroll, H.A., and Lustyk, M.K. 2014. Gender differences in exercise habits and quality of life reports: Assessing the moderating effects of reasons for exercise. *International Journal of Liberal Arts and Social Science*, ***2***(5): 65–76.

Daly, J., Simonetti, B., Plagaro, C.A., Kavanagh-Williamson, M., Shoemark, D., Simon-Gracia, L., Klein, K., Bauer, M., Hollandi, R., Greber, U., Horvath, P., Sessions, R., Helenius, A., Hiscox, J., Teesalu, T., Matthews, D., Davidson, A., Cullen, P. and Yamauchi, Y. 2020, Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science*, ***370***(6518): 861–865.

Davignon, J. and Ganz, P. 2004. Role of endothelial dysfunction in atherosclerosis. *Circulation*, ***109***(23): III-27-27.

Deivita, Y., Syafruddin, S., Andi Nilawati, U., Aminuddin, A., Burhanuddin, B., and Zahir, Z. 2021. Overview of anaemia; risk factors and solution offering. *Gaceta Sanitaria*, ***35***(2): S235–S241.

De Maddalena, C., Vodo, S., Petroni, A., and Aloisi, A.M. 2012. Impact of testosterone on body fat composition. *Journal of Cellular Physiology*, ***227***(12): 3744–3748.

Desai, A.D., Lavelle, M., Boursiquot, B.C., and Wan, E.Y. 2022. Long-term complications of COVID-19. *American Journal of Physiology. Cell physiology*, ***322***(1): C1–C11.

de Wit, E., van Doremalen, N., Falzarano, D., and Munster, V.J. 2016. SARS and MERS: Recent insights into emerging coronaviruses. *Nature Reviews. Microbiology*, ***14***(8): 523–534.

Faghih Dinevari, M., Somi, M. H., Sadeghi Majd, E., Abbasalizad Farhangi, M., and Nikniaz, Z. 2021. Anaemia predicts poor outcomes of COVID-19 in hospitalized patients: a prospective study in Iran. *BMC infectious diseases*, ***21***(1): 170. https://doi.org/10.1186/s12879-021-05868-4

Fahed, G., Aoun, L., Bou Zerdan, M., Allam, S., Bou Zerdan, M., Bouferraa, Y., and Assi, H. I. 2022. Metabolic syndrome: Updates on pathophysiology and

management in 2021. *International Journal of Molecular Sciences*, ***23***(2): 786. https://doi.org/10.3390/ijms23020786

Fan, Y., Zhao, K., Shi, Z. and Zhou, P. 2019. Bat coronaviruses in China. *Viruses*, **11**(210): doi:103390/v11030210

Feldmann, M., Maini, R.N., Woody, J.N., Holgate, S.T., Winter, G., Rowland, M., Richards, D. and Hussell, T. 2020. Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed. *Lancet (London, England)*, ***395***(10234): 1407–1409.

Francisco, V., Pino, J., Campos-Cabaleiro, V., Ruiz-Fernández, C., Mera, A., Gonzalez-Gay, M.A., Gómez, R., and Gualillo, O. 2018. Obesity, fat mass, and immune system: Role for leptin. *Frontiers in Physiology*, ***9***: 640. https://doi.org/10.3389/fphys.2018.00640

Frank, A.P., de Souza Santos, R., Palmer, B.F., and Clegg, D.J. 2019. Determinants of body fat distribution in humans may provide insight about obesity-related health risks. *Journal of Lipid Research*, ***60***(10): 1710–1719.

Frasca, D., Reidy, L., Romero, M., Diaz, A., Cray, C., Kahl, K., and Blomberg, B.B. 2022. The majority of SARS-CoV-2-specific antibodies in COVID-19 patients with obesity are autoimmune and not neutralizing. *International Journal of Obesity (2005)*, ***46***(2): 427–432.

Furuhashi, M., Moniwa, N., Mita, T., Fuseya, T., Ishimura, S., Ohno, K., Shibata, S., Tanaka, M., Watanabe, Y., Akasaka, H., Ohnishi, H., Yoshida, H., Takizawa, H., Saitoh, S., Ura, N., Shimamoto, K., and Miura, T. 2015. Urinary angiotensin-converting enzyme 2 in hypertensive patients may be increased by olmesartan, an angiotensin II receptor blocker. *American Journal of Hypertension*, ***28***(1): 15–21.

Gandhi, S.J., Hagans, I., Nathan, K., Hunter, K., and Roy, S. 2017. Prevalence, comorbidity and investigation of anaemia in the primary care office. *Journal of Clinical Medicine Research*, ***9***(12): 970–980.

Garg, S., Kim, L., Whitaker, M., O'Halloran, A., Cummings, C., Holstein, R., Prill, M., Chai, S.J., Kirley, P.D., Alden, N.B., Kawasaki, B., Yousey-Hindes, K., Niccolai, L., Anderson, E.J., Openo, K.P., Weigel, A., Monroe, M.L., Ryan, P., Henderson, J., Kim, S., Fry, A. 2020. Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed Coronavirus Disease 2019 - COVID-NET,

14 States, March 1-30, 2020. *MMWR. Morbidity and Mortality Weekly Report*, ***69***(15): 458–464.

Ganz, T., and Nemeth, E. 2012. Hepcidin and iron homeostasis. *Biochimica et Biophysica Acta*, ***1823***(9): 1434–1443.

Gassmann, M., Mairbäurl, H., Livshits, L., Seide, S., Hackbusch, M., Malczyk, M., Kraut, S., Gassmann, N.N., Weissmann, N., and Muckenthaler, M.U. 2019. The increase in haemoglobin concentration with altitude varies among human populations. *Annals of the New York Academy of Sciences*, ***1450***(1): 204–220.

Gazzaz, Z.J. 2021. Diabetes and COVID-19. *Open Life Science*, ***16***: 297–302.

Giustino, G., Croft, L.B., Stefanini, G.G., Bragato, R., Silbiger, J.J., Vicenzi, M., Danilov, T., Kukar, N., Shaban, N., Kini, A., Camaj, A., Bienstock, S.W., Rashed, E.R., Rahman, K., Oates, C.P., Buckley, S., Elbaum, L.S., Arkonac, D., Fiter, R., Singh, R., Goldman, M.E. 2020. Characterization of myocardial injury in patients with COVID-19. *Journal of the American College of Cardiology*, ***76***(18): 2043–2055.

Guan, W.J., Liang, W.H., Zhao, Y., Liang, H.R., Chen, Z.S., Li, Y.M., Liu, X.Q., Chen, R.C., Tang, C.L., Wang, T., Ou, C.Q., Li, L., Chen, P.Y., Sang, L., Wang, W., Li, J.F., Li, C.C., Ou, L.M., Cheng, B., Xiong, S., China Medical Treatment Expert Group for COVID-19. 2020. Comorbidity and its impact on 1590 patients with COVID-19 in China: A nationwide analysis. *The European Respiratory Journal*, ***55***(5): 2000547. https://doi.org/10.1183/13993003.00547-2020

Guan, W.J., Ni, Z.Y., Hu, Y., Liang, W.H., Ou, C.Q., He, J.X., Liu, L., Shan, H., Lei, C.L., Hui, D.S.C., Du, B., Li, L.J., Zeng, G., Yuen, K.Y., Chen, R.C., Tang, C.L., Wang, T., Chen, P.Y., Xiang, J., Li, S. Y., China Medical Treatment Expert Group for Covid-19. 2020. Clinical characteristics of Coronavirus Disease 2019 in China. *The New England Journal of Medicine*, ***382***(18): 1708–1720.

Gurwitz D. 2020. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Development Research*, ***81***(5): 537–540.

Habib, H.M., Ibrahim, S., Zaim, A., and Ibrahim, W.H. 2021. The role of iron in the pathogenesis of COVID-19 and possible treatment with lactoferrin and other iron chelators. *Biomedicine and Pharmacotherapy = Biomedecine and Pharmacotherapy*, ***136***: 111228. https://doi.org/10.1016/j.biopha.2021.111228

Hariri, L., and Hardin, C.C. 2020. Covid-19, angiogenesis, and ARDS endotypes. *The New England Journal of Medicine*, ***383***(2): 182–183.

Hariz, A., Bhattacharya, P.T. Megaloblastic Anaemia. [Updated 2023 Apr 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537254/

Hernández-Aguilar, I., Lorenzo, C., Santos-Moreno, A., Naranjo, E.J., and/and Navarrete-Gutiérrez, D. Coronaviruses in bats: A review for the Americas. *Viruses* 2021, ***13***: 1226. https://doi.org/10.3390/v13071226

Hu, Y., Sun, J., Dai, Z., Deng, H., Li, X., Huang, Q., Wu, Y., Sun, L., and Xu, Y. 2020. Prevalence and severity of coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, ***127***: 104371. https://doi.org/10.1016/j.jcv.2020.104371

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Cao, B. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet (London, England)*, ***395***(10223): 497–506.

Huang, L., Zhao, P., Tang, D., Zhu, T., Han, R., Zhan, C., Liu, W., Zeng, H., Tao, Q., and Xia, L. 2020. Cardiac involvement in patients recovered from COVID-2019 identified using Magnetic Resonance Imaging. *JACC. Cardiovascular Imaging*, ***13***(11): 2330–2339.

Jain, U. 2020. Effect of COVID-19 on the organs. *Cureus*, ***12***(8): e9540. doi: 10.7759/cureus.9540

Jarrott, B., Head, R., Pringle, K.G., Lumbers, E.R., and Martin, J.H. 2022. "LONG COVID"- A hypothesis for understanding the biological basis and pharmacological treatment strategy. *Pharmacology Research and Perspectives*, ***10***(1): e00911. https://doi.org/10.1002/prp2.911

Kaeuffer, C., Le Hyaric, C., Fabacher, T., Mootien, J., Dervieux, B., Ruch, Y., Hugerot, A., Zhu, Y.J., Pointurier, V., Clere-Jehl, R., Greigert, V., Kassegne, L., Lefebvre, N., Gallais, F., Meyer, N., Hansmann, Y., Hinschberger, O., Danion, F., COVID Alsace Study Group. 2020. Clinical characteristics and risk factors associated with severe COVID-19: Prospective analysis of 1,045 hospitalised cases in North-Eastern France, March 2020. *Eurosurveillance*, ***25***(48): 2000895. doi: 10.2807/1560-7917.ES.2020.25.48.2000895

Kahn, J. and McIntosh, K. 2005. History and Recent Advances in Coronavirus Discovery. *The Paediatric Infectious Disease Journal,* ***24***(11): S223-S227. | DOI: 10.1097/01.inf.0000188166.17324.60

Kandeel, M., and Al-Nazawi, M. 2020. Virtual screening and repurposing of FDA approved drugs against COVID-19 main protease. *Life sciences*, ***251***: 117627. https://doi.org/10.1016/j.lfs.2020.117627

Karakousis, N.D., Gourgoulianis, K.I., and Kotsiou, O.S. (2023). The Role of Folic Acid in SARS-CoV-2 Infection: An Intriguing Linkage under Investigation. *Journal of personalized medicine*, ***13***(3). https://doi.org/10.3390/jpm13030561

Kaufman, D.P., Kandle, P.F., Murray, I.V., and Dhamoon, A.S. 2023. Physiology, oxyhaemoglobin dissociation curve. In *StatPearls*. StatPearls Publishing.

Kautzky-Willer, A., Leutner, M., and Harreiter, J. 2023. Sex differences in type 2 diabetes. *Diabetologia*, ***66***(6): 986–1002.

Kawai, T., Autieri, M.V., and Scalia, R. 2021. Adipose tissue inflammation and metabolic dysfunction in obesity. *American Journal of Physiology. Cell Physiology*, ***320***(3), C375–C391.

Kernan, K.F., and Carcillo, J.A. 2017. Hyperferritinaemia and inflammation. *International Immunology*, ***29***(9): 401–409

Khadilkar, K.S., Bandgar, T., Shivane, V., Lila, A., and Shah, N. 2013. Current concepts in BG monitoring. *Indian Journal of Endocrinology and Metabolism*, ***17***(3), S643–S649.

Khan, J., Al Asoom, L.I., Khan, M., Chakrabartty, I., Dandoti, S., Rudrapal, M. and Zothantluanga, J.H. 2021. Evolution of RNA viruses from SARS to SARS-CoV-2 and diagnostic techniques for COVID-19: A review. *Beni-Suef University Journal of Basic and Applied Sciences*, ***10***(60). https://doi.org/10.1186/s43088-021-00150-7

Kong, L.M., Liao, C.G., Zhang, Y., Xu, J., Li, Y., Huang, W., Zhang, Y., Bian, H., and Chen, Z.N. 2014. A regulatory loop involving miR-22, Sp1, and c-Myc modulates CD147 expression in breast cancer invasion and metastasis. *Cancer Research*, ***74***(14): 3764–3778.

Kosugi, T., Maeda, K., Sato, W., Maruyama, S., and Kadomatsu, K. 2015. CD147 (EMMPRIN/Basigin) in kidney diseases: From an inflammation and immune system viewpoint. *Nephrology,* ***30***(7): 1097–1103.

Kraakman, M.J., Murphy, A.J., Jandeleit-Dahm, K., and Kammoun, H.L. 2014. Macrophage polarisation in obesity and type 2 diabetes: weighing down our understanding of macrophage function? *Frontiers in Immunology*, ***5***: 470. https://doi.org/10.3389/fimmu.2014.00470

Kronstein-Wiedemann, R., Stadtmüller, M., Traikov, S., Georgi, M., Teichert, M., Yosef, H., Wallenborn, J., Karl, A., Schütze, K., Wagner, M., El-Armouche, A. and Tonn, T., 2022. SARS-CoV-2 infects red blood cell progenitors and dysregulates Hb and Iron metabolism. *Stem Cell Reviews,* ***18***(5): 1809–1821.

Lang, M., Som, A., Mendoza, D.P., Flores, E.J., Reid, N., Carey, D., Li, M.D., Witkin, A., Rodriguez-Lopez, J.M., Shepard, J.O., and Little, B.P. 2020. Hypoxaemia related to COVID-19: Vascular and perfusion abnormalities on dual-energy CT. *The Lancet. Infectious Diseases*, ***20***(12): 1365–1366.

Langan, R.C., and Goodbred, A.J. 2017. Vitamin B_{12} deficiency: Recognition and management. *American Family Physician*, ***96***(6): 384–389.

Lazzaroni, M.G., Piantoni, S., Masneri, S., Garrafa, E., Martini, G., Tincani, A., Andreoli, L., and/and Franceschini, F. 2021. Coagulation dysfunction in COVID-19: The interplay between inflammation, viral infection, and the coagulation system. *Blood Reviews*, ***46***: 100745, ISSN 0268-960X. https://doi.org/10.1016/j.blre.2020.100745

Lechuga, G.C., Morel, C.M., and De-Simone, S.G. 2023. Haematological alterations associated with long COVID-19. *Frontiers in Physiology*, ***14***: 1203472. doi: 10.3389/fphys.2023.1203472

Lenehan, P. J., Ramudu, E., Venkatakrishnan, A. J., Berner, G., McMurry, R., O'Horo, J. C., Badley, A. D., Morice, W., 2nd, Halamka, J., and Soundararajan, V. 2021. Anaemia during SARS-CoV-2 infection is associated with rehospitalization after viral clearance. *iScience*, ***24***(7): 102780. https://doi.org/10.1016/j.isci.2021.102780

Li, H., Tian, S., Chen, T., Cui, Z., Shi, N., Zhong, X., Qiu, K., Zhang, J., Zeng, T., Chen, L., and Zheng, J. 2020. Newly diagnosed diabetes is associated with a higher risk of mortality than known diabetes in hospitalized patients with COVID-19. *Diabetes, Obesity and Metabolism*, ***22***(10): 1897–1906.

Litton, E. and Lim, J. 2019. Iron metabolism: An emerging therapeutic target in critical illness. *Critical Care*, ***23***: 81.

Lotfi, M., Hamblin, M.R., and Rezaei, N. 2020. COVID-19: Transmission, prevention, and potential therapeutic opportunities. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, ***508***: 254–266.

Lopez-Leon, S., Wegman-Ostrosky, T., Perelman, C., Sepulveda, R., Rebolledo, P. A., Cuapio, A. and Villapol, S. 2021. More than 50 long-term effects of COVID-19: A systematic review and meta-analysis. *Scientific Reports*, ***11***: https://doi.org/10.1038/s41598-021-95565-8

Marengo-Rowe, A.J. 2006. Structure-function relations of human haemoglobins. *Proceedings (Baylor University. Medical Center)*, ***19***(3): 239–245.

Mathew, T.K., Zubair, M., and Tadi, P. BG Monitoring. [Updated 2023 Apr 23]. *In*: StatPearls [Internet]. Treasure Island (FL): *StatPearls Publishing*; 2023 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK555976/ [Accessed: 18 Oct. 23]

Mehta, P., McAuley, D.F., Brown, M., Sanchez, E., Tattersall, R.S., Manson, J.J., and HLH Across Speciality Collaboration, UK 2020. COVID-19: Consider cytokine storm syndromes and immunosuppression. *Lancet (London, England)*, ***395***(10229): 1033–1034.

Meisel, E., Efros, O., Bleier, J., Beit Halevi, T., Segal, G., Rahav, G., Leibowitz, A., and Grossman, E. 2021. Folate Levels in Patients Hospitalized with Coronavirus Disease 2019. *Nutrients*, ***13***(3). https://doi.org/10.3390/nu13030812

Memish, Z.A., Perlman, S., Van Kerkhove, M.D., and Zumla, A. 2020. Middle East respiratory syndrome. *Lancet (London, England)*, ***395***(10229): 1063–1077.

Mohammad, S., Aziz, R., Al Mahri, S., Malik, S.S., Haji, E., Khan, A.H., Khatlani, T.S., and Bouchama, A. 2021. Obesity and COVID-19: What makes obese host so vulnerable? *Immunity and Ageing: I and A*, ***18***(1): 1. https://doi.org/10.1186/s12979-020-00212-x

Mohanty, J.G., Nagababu, E. and Rifkind, J.M. 2014. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell ageing. *Frontiers in Physiology,* ***5***(84). doi: 10.3389/fphys.2014.00084.

Morens, D.M., Breman, J.G., Calisher, C.H., Doherty, P.C., Hahn, B.H., Keusch, G. T., Kramer, L.D., LeDuc, J.W., Monath, T.P., and Taubenberger, J.K. 2020. The

origin of COVID-19 and why it matters. *The American Journal of Tropical Medicine and Hygiene*, ***103***(3): 955–959.

Muckenthaler, M.U., Rivella, S., Hentze, M.W. and Galy, B. 2017. A red carpet for iron metabolism. *Cell,* ***168***(3): 344–361.

Muniyappa, R., and Gubbi, S. 2020. COVID-19 pandemic, coronaviruses, and diabetes mellitus. *American Journal of Physiology. Endocrinology and Metabolism*, ***318***(5), E736–E741.

Nour, T.Y., and Altintaş, K.H. 2023. Effect of the COVID-19 pandemic on obesity and it is risk factors: A systematic review. *BMC Public Health*, ***23***(1): 1018. https://doi.org/10.1186/s12889-023-15833-2

Nuttall, F.Q. 2015. Body Mass Index: Obesity, BMI, and Health: A Critical Review. *Nutrition today*, ***50***(3): 117–128.

Ntuli, S.T., Maimela, E., Alberts, M., Choma, S., and Dikotope, S. 2015. Prevalence and associated risk factors of hypertension amongst adults in a rural community of Limpopo province, South Africa. *African Journal of Primary Health Care and Family Medicine*, ***7***(1): 847. https://doi.org/10.4102/phcfm.v7i1.847

Paramastri, R., Hsu, C.Y., Lee, H.A., Lin, L.Y., Kurniawan, A.L., and Chao, J.C. 2021. Association between dietary pattern, lifestyle, anthropometric status, and anaemia-related biomarkers among adults: A population-based study from 2001 to 2015. *International Journal of Environmental Research and Public Health*, ***18***(7): 3438. https://doi.org/10.3390/ijerph18073438

Pasini, E., Corsetti, G., Romano, C., Scarabelli, T.M., Chen-Scarabelli, C., Saravolatz, L. and Dioguardi, F.S. 2021. Serum metabolic profile in patients with long-COVID (PASC) syndrome: Clinical implications. *Frontiers in Medicine*, ***8***: 714426. doi:10.3389/fmed.2021.714426

Patel, K. R., and Sobczyńska-Malefora, A. 2017. The adverse effects of an excessive folic acid intake. *European journal of clinical nutrition*, ***71***(2): 159–163. https://doi.org/10.1038/ejcn.2016.194

Patel, S., Jose, A., and Mohiuddin, S.S. 2023. Physiology, Oxygen Transport and Carbon Dioxide Dissociation Curve. In *StatPearls*. StatPearls Publishing.

Patti, G., Pellegrino, C., Ricciardi, A., Novara, R., Cotugno, S., Papagni, R., Guido, G., Totaro, V., De Iaco, G., Romanelli, F., Stolfa, S., Minardi, M.L., Ronga, L., Fato, I., Lattanzio, R., Bavaro, D.F., Gualano, G., Sarmati, L., Saracino, A., Palmieri, F., and Di Gennaro, F. 2021. Potential Role of Vitamins A, B, C, D and E in TB

Treatment and Prevention: A Narrative Review. *Antibiotics (Basel, Switzerland)*, ***10***(11): 1354. https://doi.org/10.3390/antibiotics10111354

Pei, P., Cheng, X., Yu, J., Shen, J., Li, X., Wu, J., Wang, S., and Zhang, T. 2019. Folate deficiency induced H2A ubiquitination to lead to downregulated expression of genes involved in neural tube defects. *Epigenetics and Chromatin*, ***12***(1): 69. https://doi.org/10.1186/s13072-019-0312-7

Pelliccia, F., Kaski, J.C., Crea, F. and Camici, P.G. 2017. Pathophysiology of Takotsubo Syndrome. *Circulation*, ***135***(24): 2426–2441.

Pickering, T.G., Hall, J.E., Appel, L.J., Falkner, B.E., Graves, J., Hill, M.N., Jones, D.W., Kurtz, T., Sheps, S.G., and Roccella, E.J. 2004. Recommendations for BP measurements in humans and COVID-19 positive animals. *Hypertension*, ***45***(1): 142–161.

Pillai, A.S., Chandler, S.A., Liu, Y., Signore, A.V., Cortez-Romero, C.R., Benesch, J.L.P., Laganowsky, A., Storz, J.F., Hochberg, G.K.A., and Thornton, J.W. 2020. Origin of complexity in Hb evolution. *Nature*, ***581***(7809), 480–485.

Pittman, R.N. Regulation of Tissue Oxygenation. San Rafael (CA): Morgan and Claypool Life Sciences; 2011. Chapter 4, Oxygen Transport. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54103/

Platto, S., Wang, Y., Zhou, J. and Carafoli, E. 2021. History of the COVID-19 pandemic: Origin, explosion, worldwide spreading. *Biochemical and Biophysical* Research Communications, ***538***: 14–23.

Popkin, B.M., Du, S., Green, W.D., Beck, M.A., Algaith, T., Herbst, C.H., Alsukait, R.F., Alluhidan, M., Alazemi, N., and Shekar, M. 2020. Individuals with obesity and COVID-19: A global perspective on the epidemiology and biological relationships. *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity*, ***21***(11): e13128. https://doi.org/10.1111/obr.13128

Powers, K.A. and Dhamoon, A.S. 2023. Physiology, Pulmonary Ventilation and Perfusion. [Updated 2023 Jan 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539907/

Prabhavathi, K., Selvi, K.T., Poornima, K.N., and Sarvanan, A. 2014. Role of biological sex in normal cardiac function and in its disease outcome - a review. *Journal of Clinical and Diagnostic Research: JCDR*, ***8***(8): BE01–BE4.

Radzikowska, U., Ding, M., Tan, G., Zhakparov, D., Peng, Y., Wawrzyniak, P., Wang, M., Li, S., Morita, H., Altunbulakli, C., Reiger, M., Neumann, A.U., Lunjani, N., Traidl-Hoffmann, C., Nadeau, K.C., O'Mahony, L., Akdis, C., and Sokolowska, M. 2020. Distribution of ACE2, CD147, CD26, and other SARS-CoV-2 associated molecules in tissues and immune cells in health and in asthma, COPD, obesity, hypertension, and COVID-19 risk factors. *Allergy*, ***75***(11): 2829–2845.

Ragotte, R.J., Pulido, D., Donnellan, F.R., Hill, M.L., Gorini, G., Davies, H., Brun, J., McHugh, K., King, L.D.W., Skinner, K., Miura, K., Long, C.A., Zitzmann, N., and Draper, S.J. 2021. Human basigin (CD147) does not directly interact with SARS-CoV-2 spike glycoprotein. *mSphere*, ***6***(4): e0064721. https://doi.org/10.1128/mSphere.00647-21

Rajanna, A.H., Sivasankaran, R. and Krishnegowda, R. 2021. Anaemia predicts poor outcomes of COVID-19 in hospitalised patients. *Journal of Medical Sciences*, ***12***(8). http://nepjol.info/index.php/AJMS

Ranard, L.S., Fried, J.A., Abdalla, M., Anstey, D.E., Givens, R.C., Kumaraiah, D., Kodali, S.K., Takeda, K., Karmpaliotis, D., Rabbani, L.E., Sayer, G., Kirtane, A.J., Leon, M.B., Schwartz, A., Uriel, N., and Masoumi, A. 2020. Approach to acute cardiovascular complications in COVID-19 infection. *Circulation. Heart failure*, ***13***(7): e007220. https://doi.org/10.1161/CIRCHEARTFAILURE.120.007220

Rapozzi, V., Juarranz, A., Habib, A., Ihan, A., and Strgar, R. 2021. Is haem the real target of COVID-19? *Photodiagnosis and Photodynamic Therapy*, ***35***: 102381. https://doi.org/10.1016/j.pdpdt.2021.102381

Rauf, A., Abu-Izneid, T., Olatunde, A., Ahmed Khalil, A., Alhumaydhi, F.A., Tufail, T., Shariati, M.A., Rebezov, M., Almarhoon, Z.M., Mabkhot, Y.N., Alsayari, A., and Rengasamy, K.R.R. 2020. COVID-19 pandemic: Epidemiology, aetiology, conventional and non-conventional therapies. *International Journal of Environmental Research and Public Health*, ***17***(21): 8155. https://doi.org/10.3390/ijerph17218155

Rehman, S., Hashmi, M.F., and Nelson, V.L. BP Measurement. [Updated 2022 Dec 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482189/

Ren, X., Zhou, J., Guo, J., Hao, C., Zheng, M., Zhang, R., Huang, Q., Yao, X., Li, R., and Jin, Y. 2022. Reinfection in patients with COVID-19: A systematic review. *Global Health Research and Policy*, **7**(1): 12. doi: 10.1186/s41256-022-00245-3

Rhodes, C.E., Denault, D., and Varacallo, M. Physiology, Oxygen Transport. [Updated 2022 Nov 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538336/

Rovó, A., Baierlein-Leimbach, C., Medri, C., Chanias, I., Errass, L., Fehr, T., Triemer, T., McCarthy-Pontier, D.B., and Lehmann, T. 2022. Haematocrit self-testing in patients with polycythemia vera and other haematological conditions: Assessing the accuracy of the StatStrip Xpress® 2 LAC/Hb/HctHct Device and user opinion about the device in Real-World Clinical Practice. *Journal of Clinical Medicine*, ***11***(14): 4234. https://doi.org/10.3390/jcm11144234

Sadhukhan, P., Ugurlu, M.T. and Hoque, M.O. 2020. Effect of COVID-19 on lungs: Focusing on prospective malignant phenotypes. *Cancers (Basel),* ***12***: Doi: 10.3390/cancers12123822

Sayar, E.H., Orhaner, B.B., Sayar, E., NesrinTuran, F., and Küçük, M. 2020. The frequency of vitamin B_{12}, iron, and folic acid deficiency in the neonatal period and infancy, and the relationship with maternal levels. *Turk Pediatri Arsivi*, ***55***(2): 139–148.

Schiffrin, E.L., Flack, J.M., Ito, S., Muntner, P., and Webb, R.C. 2020. Hypertension and COVID-19. *American Journal of Hypertension*, ***33***(5): 373–374.

Shang, J., Wan, Y., Liu, C., Yount, B., Gully, K., Yang, Y., Auerbach, A., Peng, G., Baric, R. and Li, F. 2020. Structure of mouse coronavirus spike protein complexed with receptor reveals mechanism for viral entry. *Public Library of Science Pathogens,* ***16***(3): e1008392. https://doi.org/10.1371/journal.ppat.1008392

Shipton, M.J., and Thachil, J. 2015. Vitamin B_{12} deficiency - A 21st century perspective. *Clinical Medicine (London, England)*, ***15***(2): 145–150.

Shoenfeld, Y. 2020. Corona (COVID-19) time musings: Our involvement in COVID-19 pathogenesis, diagnosis, treatment, and vaccine planning. *Autoimmunity Reviews*, ***19***(6): 102538. https://doi.org/10.1016/j.autrev.2020.102538

Shulpekova, Y., Nechaev, V., Kardasheva, S., Sedova, A., Kurbatova, A., Bueverova, E., Kopylov, A., Malsagova, K., Dlamini, J.C., and Ivashkin, V. 2021. The concept

of folic acid in health and disease. *Molecules (Basel, Switzerland)*, ***26***(12): 3731. https://doi.org/10.3390/molecules26123731

Singh, R., Rathore, S.S., Khan, H., Karale, S., Chawla, Y., Iqbal, K., Bhurwal, A., Tekin, A., Jain, N., Mehra, I., Anand, S., Reddy, S., Sharma, N., Sidhu, G.S., Panagopoulos, A., Pattan, V., Kashyap, R., and Bansal, V. 2022. Association of obesity with COVID-19 severity and mortality: An updated systemic review, meta-analysis, and meta-regression. *Frontiers in Endocrinology*, ***13***, 780872. https://doi.org/10.3389/fendo.2022.780872

Stabler S.P. 2013. Clinical practice. Vitamin B_{12} deficiency. *The New England Journal of Medicine*, ***368***(2): 149–160.

Suriawinata, E., and Mehta, K. J. 2022. Iron and iron-related proteins in COVID-19. *Clinical and COVID-19 positive medicine*, 1–23.. https://doi.org/10.1007/s10238-022-00851-y

Taneri, P.E., Gómez-Ochoa, S.A., Llanaj, E., Raguindin, P.F., Rojas, L.Z., Roa-Díaz, Z.M., Salvador, D., Jr, Groothof, D., Minder, B., Kopp-Heim, D., Hautz, W.E., Eisenga, M.F., Franco, O.H., Glisic, M., and Muka, T. 2020. Anaemia and iron metabolism in COVID-19: A systematic review and meta-analysis. *European Journal of Epidemiology*, ***35***(8): 763–773.

Turner, J., Parsi, M., and/and Badireddy, M. Anaemia. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): *StatPearls Publishing*; 2023 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499994/

Tuvali, O., Tshori, S., Derazne, E., Hannuna, R.R., Afek, A., Haberman, D., Sella, G., and George, J. 2022. The incidence of myocarditis and pericarditis in post COVID-19 unvaccinated patients - A large population-based study. *Journal of Clinical Medicine*, ***11***(8): 2219. https://doi.org/10.3390/jcm11082219

Ulrich, H., and Pillat, M.M. 2020. CD147 as a target for COVID-19 treatment: Suggested effects of azithromycin and stem cell engagement. *Stem Cell Reviews and Reports*, ***16***(3): 434–440.

Unsworth, R., Wallace, S., Oliver, N.S., Yeung, S., Kshirsagar, A., Naidu, H., Kwong, R.M.W., Kumar, P., and Logan, K.M. 2020. New-onset type 1 diabetes in children during COVID-19: Multicenter regional findings in the U.K. *Diabetes Care*, ***43***(11): e170–e171.

Varga, Z., Flammer, A.J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., Mehra, M.R., Schuepbach, R.A., Ruschitzka, F., and Moch, H. 2020.

Endothelial cell infection and endotheliitis in COVID-19. *Lancet (London, England)*, ***395***(10234): 1417–1418.

Vassilopoulou, E., Bumbacea, R.S., Pappa, A.K., Papadopoulos, A.N., and Bumbacea, D. 2022. Obesity and infection: What have we learned from the COVID-19 Pandemic. *Frontiers in Nutrition*, ***9***: 931313. https://doi.org/10.3389/fnut.2022.931313

Vepa, A., Bae, J.P., Ahmed, F., Pareek, M., and Khunti, K. 2020. COVID-19 and ethnicity: A novel pathophysiological role for inflammation. *Diabetes and Metabolic Syndrome*, ***14***(5): 1043–1051.

Vimercati, L., De Maria, L., Quarato, M., Caputi, A., Gesualdo, L., Migliore, G., Cavone, D., Sponselli, S., Pipoli, A., Inchingolo, F., Scarano, A., Lorusso, F., Stefanizzi, P., and Tafuri, S. 2021. Association between Long COVID and overweight/obesity. *Journal of Clinical Medicine*, ***10***(18): 4143. https://doi.org/10.3390/jcm10184143

Vosko, I., Zirlik, A., and Bugger, H. 2023. Impact of COVID-19 on cardiovascular disease. *Viruses*, ***15***(2): 508. https://doi.org/10.3390/v15020508

Wadman, M., Couzin-Frankel, J. and Kaiser, J. 2020. How does coronavirus kill? Clinicians trace a ferocious rampage through the body, from brain to toes. https://www.sciencemag.org/news/2020/04/how-does-coronavirus-kill-clinicians-trace-ferocious-rampage-through-body-brain-toes

Wang, H., Li, X., Li, T., Zhang, S., Wang, L., Wu, X., and Liu, J. 2020. The genetic sequence, origin, and diagnosis of SARS-CoV-2. *European Journal of Clinical Microbiology and Infectious Diseases* , ***39***(9): 1629–1635.

Wang, K., Chen, W., Zhang, Z., Deng, Y., Lian, J.Q., Du, P., Wei, D., Zhang, Y., Sun, X.X., Gong, L., Yang, X., He, L., Zhang, L., Yang, Z., Geng, J.J., Chen, R., Zhang, H., Wang, B., Zhu, Y.M., Nan, G., Jiang, J.L., Li, L., Wu, J., Lin, P., Huang, W., Xie, L., Zheng, Z.H., Zhang, K., Miao, J.L., Cui, H.Y., Huang, M., Zhang, J., Fu, L., Yang, X.M., Zhao, Z., Sun, S., Gu, H., Wang, Z., Wang, C.F., Lu, Y., Liu, Y.Y., Wang, Q.Y., Bian, H., Zhu, P., Chen, Z.N. 2020. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduct Target Ther*, ***5***(1): 283. doi: 10.1038/s41392-020-00426-x.

Wang, L., Duan, Y., Zhang, W., Liang, J., Xu, J., Zhang, Y., Wu, C., Xu, Y. and Li, H. 2020. Epidemiologic and clinical characteristics of 26 cases of COVID-19 arising

from patient-to-patient transmission in Liaocheng, China. *Clinical Epidemiology*, ***12***: 387–391.

Wang, X., Wu, Z., Chen, Y., Zhu, J., Dong, X., Fu, C., and Jiang, Q. 2015. Increased prevalence and incidence of anaemia among adults in transforming rural China: Two cross-sectional surveys. *BMC Public Health*, ***15***: 1302. https://doi.org/10.1186/s12889-015-2671-8

Wells J.C. 2007. Sexual dimorphism of body composition. *Best Practice and Research. Clinical Endocrinology and Metabolism*, ***21***(3): 415–430.

Wenzhong, L., and Hualan, L. (2020). COVID-19: Attacks the 1-Beta chain of Hb and captures the porphyrin to inhibit human haeme metabolism. ChemRxiv. doi:10.26434/chemrxiv.11938173.v7 This content is a preprint and has not been peer-reviewed.

World Health Organisation. 2022. Mean Fasting BG. https://www.who.int/data/gho/indicator-metadata-registry/imr-details/2380 (Accessed: 23 March 2022).

Wu, C., Chen, X., Cai, Y., Xia, J., Zhou, X., Xu, S., Huang, H., Zhang, L., Zhou, X., Du, C., Zhang, Y., Song, J., Wang, S., Chao, Y., Yang, Z., Xu, J., Zhou, X., Chen, D., Xiong, W., Xu, L., Song, Y. 2020. Risk factors associated with acute respiratory distress syndrome and death in patients with Coronavirus Disease 2019 pneumonia in Wuhan, China. *JAMA Internal Medicine*, ***180***(7): 934−943.

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., Zhu, L., Tai, Y., Bai, C., Gao, T., Song, J., Xia, P., Dong, J., Zhao, J., and Wang, F. S. 2020. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet. Respiratory Medicine*, ***8***(4): 420–422.

Xuan, Y., Zuo, J., Zheng, S., Ji, J., and Qian, Y. 2019. Association of haemoglobin and BP in a Chinese community-dwelling population. *Pulse (Basel, Switzerland)*, ***6***(3-4): 154–160.

Yanes, L.L., and Reckelhoff, J.F. 2011. Postmenopausal hypertension. *American Journal of Hypertension*, ***24***(7): 740–749.

Yang, Y., Peng, F., Wang, R., Guan, K., Jiang, T., Xu, G., Sun, J. and Chang, C. 2020. The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. *Journal of Autoimmunity*, ***109***: 102434. https://doi.org/10.1016/j.jaut.2020.102434

Yoshida, T., Prudent, M., and D'alessandro, A. 2019. Red blood cell storage lesion: causes and potential clinical consequences. *Blood Transfusion = Trasfusione del Sangue*, ***17***(1): 27–52.

Yoshii, K., Hosomi, K., Sawane, K., & Kunisawa, J. 2019. Metabolism of Dietary and Microbial Vitamin B Family in the Regulation of Host Immunity. *Frontiers in nutrition*, ***6***: 48. https://doi.org/10.3389/fnut.2019.00048

Zahedi, M., Kordrostami, S., Kalantarhormozi, M., and Bagheri, M. 2023. A Review of hyperglycaemia in COVID-19. *Cureus*, ***15***(4): e37487. https://doi.org/10.7759/cureus.37487

Zayed, N.E., Abbas, A., and Lutfy, S.M. 2022. Criteria and potential predictors of severity in patients with COVID-19. *The Egyptian Journal of Bronchology*, ***16***(1), 11. https://doi.org/10.1186/s43168-022-00116-y

Zhang, H., Penninger, J.M., Li, Y., Zhong, N. and Slutsky, A.S. 2020. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: Molecular mechanisms and potential therapeutic target. *Intensive Care Medicine*, ***46***(4): 586–590.

Zhang, Y., Xiao, M., Zhang, S., Xia, P., Cao, W., Jiang, W., Chen, H., Ding, X., Zhao, H., Zhang, H., Wang, C., Zhao, J., Sun, X., Tian, R., Wu, W., Wu, D., Ma, J., Chen, Y., Zhang, D., Xie, J., Yan, X., Zhou, X., Liu, Z., Wang, J., Du, B., Qin, Y., Gao, P., Qin, X., Xu, Y., Zhang, W., Li, T., Zhang, F., Zhao, Y., Li, Y. and Zhang, S. 2020. Coagulopathy and antiphospholipid antibodies in patients with Covid-19. *New England Journal of Medicine*, ***382***(17): e38. doi: 10.1056/NEJMc2007575

Zheng, Y.Y., Ma, Y.T., Zhang, J.Y., and Xie, X. 2020. COVID-19 and the cardiovascular system. *Nature Reviews. Cardiology*, ***17***(5): 259–260.

Zheng, Y.Y., Ma, Y.T., Zhang, J.Y., and Xie, X. 2020. Reply to: 'Interaction between RAAS inhibitors and ACE2 in the context of COVID-19'. *Nature Reviews. Cardiology*, ***17***(5): 313–314.

ADDENDUM A: TREC APPROVAL

University of Limpopo
Department of Research Administration and Development
Private Bag X1106, Sovenga, 0727, South Africa
Tel: (015) 268 3935, Fax: (015) 268 2306, Email: anastasia.ngobe@ul.ac.za

TURFLOOP RESEARCH ETHICS COMMITTEE

ETHICS CLEARANCE CERTIFICATE

MEETING: 22 August 2022

PROJECT NUMBER: TREC/370/2022: PG

PROJECT:

Title:	The effect of post COVID-19 in adults with regards to oxygen carrying capacity after infection.
Researcher:	K Mkhabela
Supervisor:	Dr Y Chetty
Co-Supervisor/s:	Prof M Van Staden
School:	Molecular and Life Science
Degree:	Master of Science in Physiology

PROF D MAPOSA
CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE

The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: REC-0310111-031

Note:

i) This Ethics Clearance Certificate will be valid for one (1) year, as from the abovementioned date. Application for annual renewal (or annual review) need to be received by TREC one month before lapse of this period.

ii) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee, together with the Application for Amendment form.

iii) PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Finding solutions for Africa

University of Limpopo
Department of Research Administration and Development
Private Bag X1106, Sovenga, 0727, South Africa
Tel: (015) 268 4713, Fax: (015) 268 2306, Email: moore.hutamo@ul.ac.za

TURFLOOP RESEARCH ETHICS COMMITTEE

ETHICS CLEARANCE CERTIFICATE

MEETING: 20 June 2023

PROJECT NUMBER: TREC/370/2022: PG- Amended

PROJECT:

Title:	The effect of post COVID-19 in adults with regards to oxygen carrying capacity after infection.
Researcher:	K Mkhabela
Supervisor:	Dr Y Chetty
Co-Supervisor/s:	Prof M Van Staden
School:	Molecular and Life Science
Degree:	Master of Science in Physiology

PROF D MAPOSA
CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE

The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: REC-0310111-031

Note:

i) This Ethics Clearance Certificate will be valid for one (1) year, as from the abovementioned date. Application for annual renewal (or annual review) need to be received by TREC one month before lapse of this period.

ii) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee, together with the Application for Amendment form.

iii) PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Finding solutions for Africa

ADDENDUM B: REQUEST FOR PERMISSION TO CONDUCT RESEARCH

To Prof Masha

Occupation: Registrar

Place of employment: University of Limpopo

Dear sir/madam

REQUEST FOR PERMISSION TO CONDUCT RESEARCH

I am a registered Master of Science student at the University of Limpopo in the Department of Physiology and Environmental Health. My supervisors are Dr Yvette Chetty and Prof Marlise van Staden. As part of my academic course, I am doing a research project on the proposed topic of ***The Effects of Post COVID-19 in Adults with Regard to Oxygen-Carrying Capacity After Infection.***

I am hereby seeking your consent to conduct research in your vicinity and interview and collect data from the students and staff members. Rest assured that the data that will be collected will remain absolutely confidential and to be used for academic purposes only. To assist you in reaching a decision, I have attached to this letter:

a) A copy of the certificate issued by the University
b) A copy of the research questionnaires which we intend to use for data collection

Should you require any further information, please do not hesitate to contact me or my supervisors. Our contact details are as follows:

Kidibone Mkhabela: kidibone.mkhabela@ul.ac.za

Dr Yvette Chetty: yvette.chetty@ul.ac.za

Prof Marlise van Staden: marlise.vanstaden@ul.ac.za

We hope for your positive response on the matter. Your approval will be greatly appreciated.

Sincerely yours,

Kidibone Mkhabela and Supervisors

ADDENDUM C: GATEKEEPER PERMISSION TO CONDUCT RESEARCH

University of Limpopo
Office of the Registrar
Private Bag X1106, Sovenga, 0727, South Africa
Tel: (015) 268 2407, Fax: (015) 268 3048, Email: Kwena.Masha@ul.ac.za/Retha.Balie@ul.ac.za

02 August 2023

K Mkhabela

Email: 201605662@keyaka.ul.ac.za

Dear K Mkhabela,

GATEKEEPER PERMISSION TO CONDUCT RESEARCH

TITLE: THE EFFECTPF POST COVID-19 IN ADULTS WITH REGARDS TO OXYGEN CARRYING CAPACITY

Researcher:	**K Mkhabela**
Supervisor/s:	**Dr. Y Chetty**
Co-Supervisor/s:	**Prof. M Van Staden**
School:	**Molecular and Life Science**
Degree:	**Master of Science in Physiology**

Kindly be informed that Gatekeeper permission is granted to you to conduct research at the University of Limpopo entitled: **"The effect of post COVID-19 in adults with regards to oxygen carrying capacity".**

Regards,

PROF. JK MASHA
UNIVERSITY REGISTRAR

Cc. Prof. RJ Singh: Deputy Vice-Chancellor; Research, Innovation and Partnerships
Prof. RN Madadzhe: Deputy Vice-Chancellor: Teaching and Learning
Dr. T Mabila, Director: Research Development and Administration
Prof. D Maposa – Chairperson: Research and Ethics Committee
Ms. M Hutamo – Assistant: Ethics Secretarist
Ms. C Ngobeni – Research Administration and Development

Finding solutions for Africa

ADDENDUM D: INFORMED CONSENT FORM

FACULTY OF SCIENCE AND AGRICULTURE
SCHOOL OF MOLECULAR AND LIFE SCIENCES
DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL HEALTH

CONSENT FORM

My name is Kidibone Mkhabela, a master sciences student from the Department of Physiology and Environmental Health, School of Molecular and Life Sciences at the University of Limpopo. I am conducting this research for the fulfilment of the master science degree in physiology as an independent research project titled "**the effects of post covid-19 in adults with regard to the oxygen carrying capacity after infection**". The questionnaire will be used as a research tool to firstly determine if you meet the requirements to participate in the study and to record health data required for the study. By signing this document, you will be agreeing to the conditions below:

1. Your identity and all data obtained from you will remain anonymous
2. All information and procedures regarding the study will be fully explained to you before the initiation of the survey.
3. No monetary or any reward shall be given for your participation in this research.
4. You may withdraw at any time during participation if you feel uncomfortable with the questions or procedure.
5. You understand the meaning and purpose of this study and agree to participate voluntarily.

I, _____have read and fully understood the above information and willing to participate in the study voluntarily.

Signature: _____

Date: _____

ADDENDUM E: PARTICIPANT SELECTION QUESTIONNAIRE

Code:

PARTICIPANT SELECTION QUESTIONNAIRE								
Have you ever tested positive for COVID-19?	1	No	2	Yes				
How long has it been since you tested negative after infection?								
Are you vaccinated?	1	No	2	Yes				
Type/Name of vaccine								
Did you get the booster shot?	1	No	2	Yes				
Do you smoke?	1	No	2	Yes				
Have you recently experienced trauma or blood loss?	1	No	2	Yes				
Do have chronic renal disease?	1	No	2	Yes				
Do you have diabetes?	1	No	2	Yes				
Reproductive stage	0	Male	1	Pre-menopausal	2	Post-menopausal		
Are you on your periods?	0	Male	1	No	2	Yes		
Are you lactating?	0	Male	1	No	2	Yes		
Do medicate with anti-retro viral drugs?	1	No	2	Yes				
Have you ever been diagnosed with cancer?	1	No	2	Yes				
Have you ever been diagnosed with Hodgkin's disease?	1	No	2	Yes				
Have you ever been diagnosed with stomach ulcers?	1	No	2	Yes				
History of lung conditions								
History of heart conditions								
Additional information								

ADDENDUM F: PARTICIPANT DETAILS

Code:

PARTICIPANT DETAILS											
Sex	1	Male	2	Female	3	Prefer not to say					
Age											
Ethnicity	1	Black	2	White	3	Indian	4	Coloured	5	Other	
Occupation											
Blood pressure (mmHg)	Systolic/Diastolic				Average						
Heart rate (beats per min)				Average							
BMI (kg/m^2)											
Weight (kg)				Ave (kg)		**Height (m)**				Ave (m)	
Haemoglobin concentration (g/dL)											
Haemoglobin (mmol/L)											
Haematocrit (percentage)											
Do you take medication? (if yes, please indicate what they are and what they are for)											
Any additional information											

ADDENDUM G: FOOD FREQUENCY QUESTIONNAIRE

FOOD FREQUENCY QUESTIONNAIRE RECORDING SHEET

Name of participant: .....

Participant code:

Date of birth:

D	D	M	M	Y	Y	Y	Y

Interviewer name and code: .....

A. Food items (with FPM)	B. Description of food item	C. Item code	D. Amount usually eaten (g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten at least once a month
				Times / day	Days / week	Times / month
DAIRY						
1. Tea						
1.Coffee						
1.Sugar in tea/coffee						
2.Milk in tea/coffee						
2.Milk in porridge						
3.Buttermilk/maas						
4.Milk drinks						
5.Yoghurt						
6.Cottage cheese						
7.Hard cheese						
8.Processed cheese						
9.Ice cream & ice-lollies						
STARCH						
1.Brown bread/rolls						
1.White bread/rolls						
2.Traditional bread/roti						
2.Fat cakes						
3.Breakfast cereals						
4.Maize porridge soft						
4.Maize porridge stiff (pap)						
4.Mabele/maltabella soft						
4.Mabele stiff (pap)						
4.Oats						
5.Pasta without sauce						
6.Pasta dish						
7.Rice						
7.Samp/mealie rice						
7.Wheat rice						
8.Pizza & savoury tart						
FATS						
1.Brick margarine						
1.Tub margarine						
1.White margarine						
1.Butter						
2.Animal fat, i.e., lard						

3.Cream & substitutes						
4.Oils						
5.Salad dressing						
5.Mayonnaise						
SPREADS						
Cheese spread						
Fish paste						
Honey/syrup						
Jam						
Marmite						
Meat spread i.e., Bovril						
Peanut butter						
Sandwich spread						
EGGS						
Boiled						
Fried						
Omelette						
Scrambled						
FRUIT						
1.Apples						
2.Bananas						
3.Berries						
4.Figs/prickly pears						
5.Fruit salad						
6.Grapes						
7.Guavas						
8.Mangos/Paw paw						
9.Melons						
10.Naartjies						
11.Oranges						
12.Peaches						
13.Pears						
14.Pineapples						
15.Plums						
16.Dry fruit						
17.Fruit juice						
SOUP, LEGUMES & NUTS						
1.Soups						
2.Beans & lentils						
3.Nuts & seeds						
FISH & SEAFOOD						
1.Fried fish						
2.Grilled/smoked/dried fish						
3.Pilchard & sardines						
3.Tuna						
MEAT						
1.Beef & ostrich						
2.Patties & mince						
3.Burgers & take-aways						
4.Chicken & turkey						
5.Cold meat						
6.Meat fillings						
7.Meat pies						
8.Mutton						
9.Pork						
10.Sausage & Vienas						

11.Traditional & organ meats (e.g., tripe)						
12.Vegetarian products						
13.Dry sausage & biltong						
VEGETABLES						
1.Asparagus						
2.Avocado						
3.Baby marrow						
4.Beetroot						
5.Butternut & pumpkin						
6.Broccoli/cauliflower						
7.Cabbage						
8.Carrots						
9.Gem squash						
10.Green beans						
11.Mealies						
12.Mixed vegetables						
13.Mushrooms						
14.Peas						
15.Potatoes						
16.Potato chips						
17.Salad vegetables						
18.Spinach/morogo						
19.Sweet potatoes						
20.Tomatoes						
BISCUITS, CAKES & PUDDING						
1.Biscuits/cookies						
2.Biscuits/savoury						
3.Buns/muffins/scones						
4.Cakes & Tarts						
5.Doughnuts/eclairs						
6.Pancakes/waffles						
7. Puddings & custard						
8.Rusks						
9.Special breads						
SNACKS, SWEETS & COLD DRINKS						
1.Carbonated cold drinks						
1.Diet cold drinks						
2.Energy drinks						
2.Slashes						
3.Crisps & popcorn						
4.Sweets/chocolates						
SAUCES & CONDIMENTS						
1.Cheese & white sauces						
2.Chakalaka/Atchar						
2.Tomato sauce & other						
3.Salt, spices & seasoning						
ALCOHOLIC DRINKS						
1.Beer & cider & coolers						
2.Wine						
3.Spirits						
4.Liqueurs & fortified wine						
Other:						