Investigating the relationship between iron deficiency and depressive symptoms in relation to the body compositions of students residing in the Western Cape

---

UNIVERSITY *of the*
WESTERN CAPE

By Kiyara Govender

Submitted in fulfilment for the degree

Magister Scientiae

(Medical Bioscience)

In the department of Medical Bioscience, Faculty of Science

At the University of the Western Cape

Bellville

South Africa

Supervisor: Dr Juley De Smidt

Date: June 2022

Declaration

I, Kiyara Govender, student number: 3981498, at this moment declare that the work in this thesis titled **"Investigating the relationship between iron deficiency and depressive symptoms in relation to the body compositions of students residing in the Western Cape"** is my work and has not previously been submitted for any degree or examination at any university. All the sources that I have used or quoted have been indicated and acknowledged by utilising complete references.

K Govender — 1 June 2022

K Govender — Date

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor, Dr Juley De Smidt. Your ongoing support throughout this unpredictable journey has been steadfast and a light during the moments of chaos. To my colleagues Ammaarah Gamieldien and Tammy Hartel, your help has been an essential part of this journey, and I am so thankful for you both.

Secondly, to my family, who have encouraged me from the very beginning. Your understanding and listening ears have been a great source of comfort and inspiration throughout the difficult moments. To my partner, Drew James Joseph, thank you for your unwavering support. You have kept me motivated and engaged more times than you know.

Lastly, to all my participants, thank you for being a part of this study. Without you, this study would not be possible.

Abstract

Iron deficiency is the consequence of a prolonged diminished iron balance, whereby iron stores are no longer sufficient to meet the requirements of regular iron turnover. It is the most ubiquitous single nutrient deficiency, accounting for most cases of anaemia globally. Iron deficiency has been associated with cognitive impairments, including attention span, intelligence, emotions, and behaviour. Subsequently, the association between iron deficiency and depression was investigated. Iron is indirectly involved in monoamine metabolism, the homeostasis of glutamate and γ-aminobutyric acid (GABA), as well as the myelination of the spinal cord and white matter of the cerebellar folds. Iron-related deviations from these processes have been observed in individuals in depressive states. Additionally, iron deficiency can cause symptoms of fatigue, issues regulating body temperature and inadequate physical endurance. As such, the association between iron deficiency and obesity was assessed. These two conditions are related through a complex inflammatory state and symptoms of fatigue and depression that can potentially hinder weight management initiatives. Therefore, these relationships must be assessed in a South African student population to understand the possible implications of iron deficiency on students. This research study aimed to investigate the relationship between iron deficiency and depressive symptoms in a student population and investigate the relationship between obesity and iron-deficient students with moderate to severe depressive symptoms.

This research study comprised fifty-one (n = 51) participants who answered standardised depressive scale questionnaires and a sleep quality questionnaire. The participants were then scheduled for their physical measurements, which consisted of anthropometric measurements such as weight, height, waist, and hip circumferences. The second study measure comprised of approximately 5.00 mL of venous blood drawn from each participant, and the samples were transported to PathCare, N1 City, Goodwood, for iron status analysis. The five iron status indicators analysed were serum iron, transferrin, transferrin saturation (TSAT), ferritin and total iron-binding capacity (TIBC). After statistical analysis using IBM SPSS™ Statistics 27, it was determined that no statistical significance existed between iron deficiency and depressive symptoms. It was also revealed that no statistical significance was observed between obesity and iron-deficient students that experienced moderate to severe depressive symptoms. Lastly, significance was observed between sleep quality and depressive symptoms.

Table of Contents

Abbreviations

5-TTLPR – serotonin transporter gene

ABCB1 – ATP-binding cassette sub-family B member 1

AGP – alpha-1-acid-glycoprotein

ARDS – acute respiratory syndrome

BDI – Beck's Depression Inventory

BMI – body mass index

CMIA – chemiluminescent microparticle immunoassay

CNS – central nervous system

COPD – chronic obstructive pulmonary disease

CRP – C-reactive protein

DALY – disability-adjusted life years

GABA – γ-aminobutyric acid

GDP – gross domestic product

GWAS – genome-wide association studies

HC – hip circumference

HDAC6 – histone deacetylase

HDRS – Hamilton Depression Rating Scale

IDA – iron deficiency anaemia

iPAH – idiopathic pulmonary artery hypertension

MADRS – Montgomery-Asberg Depression Rating Scale

MCH – mean corpuscular haemoglobin

MCV – mean cell volume

MDD – major depressive disorder

NAFLD – non-alcoholic fatty liver disease

NCDs – non-communicable diseases

NMDA – N-methyl-D-aspartate

NREM – non-rapid eye movement

OHS – obesity hypoventilation syndrome

RBC – red blood cells

REM – rapid eye movement

RNA – ribonucleic acid

SADAG – South African Depression and Anxiety Group.

SANHANES -1 – South African National Health and Nutrition Examination Survey

SDS – self-rating depression scale

TfR – transferrin receptor

TIBC – total iron-binding capacity

TSAT – transferrin saturation

UIBC – unsaturated iron-binding capacity

WC – waist circumference

WHO – World Health Organization

WHR – waist-to-hip ratio

WHtR – waist-to-height ratio

List of figures

List of Tables

Chapter 1

Introduction

1.1 Background

Iron status exists as a continuum, ranging from replete iron stores to depleted iron stores, iron deficiency, iron deficiency anaemia (IDA), and iron overload (Beck, et al., 2014) & (Daru, et al., 2017). Iron deficiency is defined as a disorder whereby no utilisable iron stores are identified, resulting in signs of inadequate iron supply to both the tissues and the red blood cells (RBCs). When iron-deficient erythropoiesis ensues, haemoglobin concentrations are diminished to below optimal levels and, in severe stages, are associated with anaemia (World Health Organisation, 2001). IDA is the most severe consequence of iron deficiency, occurring when an imbalance among iron consumption, iron stores, and the body's iron loss arises. Consequently, this can result in inadequate production of RBCs that do not meet the body's physiological needs (Bailey, et al., 2015) & (Miller, 2013). Common symptoms relating to IDA consist of tiredness and fatigue, which can reduce human productivity, physical performance, and cognitive functions of affected individuals. Consequently, IDA can jeopardise our economy by way of reduced performance (Ross & Horton, 1998), (Hunt, 2002) & (Blank, et al., 2019). Iron is essential for metabolic processes that allow oxygen transport and storage and electron transport in energy metabolism. Should iron deficiency prevail, it could negatively affect our economy (Ross & Horton, 1998). IDA is a severe consequence of iron deficiency, and it is imperative to assess the prevalence of iron deficiency and its implications within our sample population.

Globally, iron deficiency is the most common micronutrient deficiency and one of the foremost contributors to the global burden of disease (Clark, 2008). It is most prevalent in children, pre-menopausal women, and individuals residing in low-income and middle-income countries (Pasricha, et al., 2020). Iron deficiency is the most substantial contributor to the onset of anaemia, affecting 33.00 % of the world's population. Of these anaemia cases, approximately half are due to iron deficiency (Pasricha, et al., 2020) & (World Health Organization, 2008). A report published by the South African National Health and Nutrition Examination Survey (SANHANES -1), a series of surveys to assess South African individuals' health and nutritional status, observed that anaemia was prevalent in 17.50 % of the population. This statistic reflects approximately half of the global prevalence (Human Sciences Research Council, 2014). Regarding a study conducted by Phatlhane et al., anaemia was prevalent in 12.60 % of their

South African sample population, with 78.00 % of these anaemic participants being iron-deficient. Additionally, 39.80 % of the total population was considered iron-deficient (Phatlhane, et al., 2016).

While the frequency of anaemia was lower when compared with the global majority, the prevalence of iron deficiency observed in the anaemic patients was substantially elevated. These studies suggest that while anaemia may be considered a minor health concern in South Africa, many individuals with iron deficiency present without anaemia and therefore go untreated. As previously mentioned, iron is essential for various processes such as monoamine metabolism, glutamate and GABA homeostasis and typical myelination of the spinal cord and white matter of the cerebellar folds (Hare, et al., 2013), (Kim & Wessling-Resnick, 2014) & (Noorazar, et al., 2015). Iron-related deviations from these highly controlled processes have been observed in individuals in depressive states (Murat, et al., 2015), (Sarawagi, et al., 2021) & (Williams, et al., 2019).

Consequently, the increased prevalence of iron deficiency in a South African adult population can increase the frequency of depressive symptoms experienced by the population. According to a report published by the World Health Organization (WHO), the total number of individuals living with depression worldwide was 322 million in 2015, approximately 4.40 % of the global population. In South Africa, the prevalence of depressive disorders was estimated to be 2 402 230 total cases, approximately 4.60 % of the population (World Health Organization, 2017). Furthermore, around 9.80 % of the adult population in South Africa were estimated to experience major depression at some point in their life, with only 25.00 % of these individuals have sought treatment and care for their mental health disorder, despite treatment being available (Cuadros, et al., 2019). Additionally, iron deficiency may exacerbate what is already being experienced by South African students. Visser and Law-van Wyk assessed both the mental health and emotional wellbeing of South African university students during the pandemic following the national lockdown. This study reported that one-third of the sample student population experienced difficulties coping with psychological stressors during the lockdown, with 22.10 % of these individuals describing the experience as traumatic. Moreover, 45.60 % of the students reported feelings of anxiety, with 35.00 % of the students experiencing depressive symptoms (Visser & Law-van Wyk, 2021).

Iron deficiency is also related to obesity. Obesity is considered an inflammatory condition whereby the secretion of pro-inflammatory cytokines by the adipose tissue results in hepcidin

synthesis. Hepcidin then inhibits the export of iron into circulation, thus implicating obesity in iron deficiency (Ellulu, et al., 2017), (Castro, et al., 2017) & (Alshwaiyat, et al., 2021). The WHO reported approximately 39.00 % of adults 18 years and older worldwide were considered overweight. Of these overweight individuals, over 13.00 % were regarded to be obese. These statistics reflect a three-fold increase in the prevalence of obesity between 1975 and 2016 (World Health Organization, 2021). In South Africa, rapid urbanisation has brought about a health transition characterised by non-communicable diseases (NCDs), including obesity (Pisa & Pisa, 2017). A study conducted by Chukwudi observed that 20.00 % of students attending the University of Venda in Limpopo, South Africa, were considered overweight, and 9.50 % of the student population were obese (Chukwudi, 2016). While these statistics are slightly decreased compared to the global frequency, overweight and obesity are still substantially prevalent within our student population. This increased prevalence could exacerbate the prevalence of iron deficiency.

This introduction describes the current global and national issues regarding the prevalence of iron deficiency, depression, and obesity. The following sub-section consists of the problem statement, which describes how the incidence of these three conditions can affect the economy.

1.2 Problem Statement

According to the WHO, South Africa is plagued by a quadruple burden of disease. The first burden consists of communicable diseases like HIV/AIDS and tuberculosis. The second burden is maternal and child mortality. The third burden comprises of non-communicable diseases (NCDs) such as hypertension and cardiovascular diseases, diabetes, cancer, mental illnesses, and chronic lung diseases. Lastly, the fourth burden of disease consisted of injury and trauma (World Health Organization , 2018). More recently, the WHO published an African multi-country study that quantified the gross domestic product (GDP) associated with disability-adjusted life years (DALYs) in specific countries. This measurement of total disease burden is expressed as the number of years lost due to ill health, disability, or premature death. The results revealed that diseases and illnesses prevalent in the WHO African regions, including South Africa, are responsible for significant losses in health and current and future economic productivity (World Health Organization, 2019).

While iron deficiency, depression, and obesity are not directly mentioned as burdens of disease in South Africa, these conditions relate directly or indirectly to one or more NCDs discussed above. Firstly, regarding depression, it has been reported that South Africa carries a

considerable burden of mental illnesses. Approximately one in six South Africans suffer from anxiety, depression, or a substance abuse disorder, therefore implicating the condition as one of the NCDs prevalent in South Africa (Matlala, et al., 2018) & (Nguse & Wassenaar, 2021).

Secondly, iron is an imperative component needed for oxygen utilisation and transport. Therefore, oxygen transport to the heart and skeletal muscles can decrease when iron deficiency ensues, potentially leading to cardiovascular diseases (Ruiter, et al., 2011). As a result, many studies have been conducted to determine whether an association exists between iron deficiency and idiopathic pulmonary artery hypertension (iPAH) (Ramakrishnan, et al., 2018). While a few studies have found no possible association between the two conditions, many studies have found iron deficiency to be highly prevalent in individuals with iPAH (van Empel, et al., 2014), (Ruiter, et al., 2015), (Kim, et al., 2012) & (Tilea, et al., 2021). Additionally, both anaemia and iron deficiency have been associated with chronic obstructive pulmonary disease (COPD) and potentially increased morbidity and mortality (Nunes & Tata, 2017), (Pizzini, et al., 2020).

Thirdly, obesity affects various tissues and can be a direct or indirect consequential risk factor for acquiring cardiovascular diseases, cancer, and respiratory diseases. The condition significantly contributes to cardiovascular risk factors, namely dyslipidaemia, type 2 diabetes, hypertension, and sleep disorders. These risk factors can ultimately lead to the development of cardiovascular diseases (Powell-Wiley, et al., 2021), (Cerato & Fonseca, 2019), (Csige, et al., 2018). Also, obesity increases the risk of developing cancer. According to the World Cancer Research Fund, endometrial, colorectal, prostate, renal, postmenopausal breast and oesophageal adenocarcinoma cancers have the most significant association with obesity (De Pergola & Silvestris, 2013) & (Stone, et al., 2018). There is also a significant association between tumours of the gastrointestinal tract and obesity, whereby being overweight increases the risk of developing cancer by 1.50 – 2.40-fold (Stone, et al., 2018). Lastly, obesity is considered a significant risk factor in the acquirement and exacerbation of asthma, obstructive sleep apnoea, obesity hypoventilation syndrome (OHS), and pulmonary hypertension (Peters & Dixon, 2019) & (Brock, et al., 2020). These conditions ultimately affect the outcome of acute respiratory syndrome (ARDS) and COPD (Peters & Dixon, 2019).

As previously mentioned, iron deficiency, depression and obesity are not directly mentioned as burdens of disease in South Africa. However, if they are not treated promptly, they can result in specific NCDs discussed above, thus causing significant losses to our people's health and

our country's economic productivity. This research study intended to investigate the relationship between iron deficiency and depressive symptoms in a South African university student population. It also proposed to evaluate obesity concerning increased depressive symptoms. Assessing these relationships could provide the basis for novel ways these pathologies are treated in the future. The following sub-section will discuss the research questions, hypotheses, aims and objectives of this study.

1.3 Research Questions

Deriving from the problem statement, the research questions are as follows: Does a correlation exist between iron deficiency and moderate to severe depressive symptoms in students residing in the Western Cape? Is there an association between obesity and iron-deficient students experiencing moderate to severe depressive symptoms? Lastly, is there an association between sleep quality and depressive symptoms in a university student population?

1.4 Research Hypotheses

- Iron deficiency will positively correlate with moderate to severe depressive symptoms.
- Obesity will positively associate with iron deficiency and moderate to severe depressive symptoms.
- Poor sleep quality will positively associate with moderate to severe depressive symptoms.

1.5 Research Aims and Objectives

1.5.1 Aim

This research study aimed to investigate the relationship between iron deficiency and depressive symptoms related to specific body composition classifications in students.

- Each one of these conditions relate to one another and their inter-relationships are represented in a Venn diagram presented in Figure 1.5.1.1. These relationships and will be discussed further in Chapter Two.

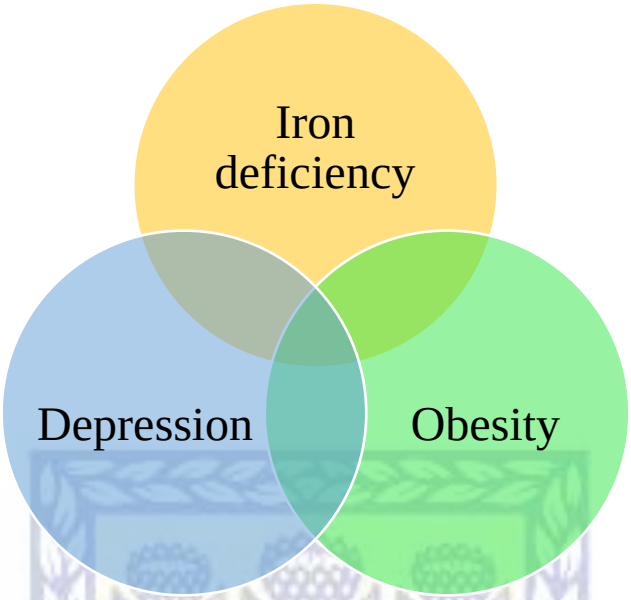


Figure 1.5.1.1: A Venn diagram showing the inter-relationships among iron deficiency, depression, and obesity.

1.5.2 Objectives

Primary objectives

- To assess the relationship between iron deficiency and depressive symptoms within the sample population. This relationship was assessed in three ways. Firstly, by identifying whether significant differences existed among the depressive scoring of the iron-deficient and iron-replete participants. Secondly, by using correlation analyses of the MADRS and BDI continuous variable scores against the iron status indicator continuous measurements. Thirdly, by assessing the relationship using categorical data based on whether the participants were iron-deficient and depressed.
- To assess the relationship between iron deficiency and obesity within the sample population. This relationship was also assessed in three ways. Firstly, by identifying whether significant differences existed among the BMI, WC, WHR, and WHtR measurements of the iron-deficient and iron-replete participants. Secondly, by using correlation analyses of the BMI, WC, WHR and WHtR continuous variable measurements against the iron status indicator continuous variable measurements. Thirdly, by evaluating the relationship using categorical data based on whether the participants were iron-deficient and obese.
- To assess the association between obesity and iron-deficient participants experiencing moderate to severe depressive symptoms. This was investigated by using the

categorical data based on whether the participants were obese and iron-deficient with depressive symptoms.

Secondary objective

- To assess the relationship between sleep quality and depressive symptoms within the sample population. This relationship was investigated in three ways. Firstly, by identifying whether significant differences were observed among the sleep quality questionnaire results between the depressed and non-depressed participants. Secondly, by association analyses using the ordinal questionnaire results variable and the depressive scoring. Thirdly by assessing the relationship using categorical data based on the sleep quality and depressive symptoms.

1.6 Relevance of the Research

As previously mentioned, there are bodies of research that have found an association between depression and iron deficiency through iron's intricate role in the synthesis and signalling of certain neurotransmitters involved in emotion and behaviour. Furthermore, researchers have found that abnormal iron statuses, observed with iron deficiency, are frequently detected in overweight and obese individuals (Cepeda-Lopez, et al., 2010), (Aigner, et al., 2014) & (Zhao, et al., 2015). However, very few studies thus far have investigated the correlations between these three pathologies and how they potentially influence each other. This research study sought to add to the existing knowledge regarding the relationships between iron deficiency, depression, and obesity and their potentially comorbid associations to establish a novel basis whereby these three conditions can be regulated or prevented in the future.

1.7 Thesis outline

This dissertation consists of five chapters. Chapter One introduced the research study by providing background information regarding iron deficiency, depression, obesity and how rampant these conditions are at a global and national level. It also briefly described the relationship between iron deficiency and both depression and obesity. Chapter One also discussed the problem statement, research questions, aims and objectives, and lastly, the relevance of this research study. Next, Chapter Two presented the literature review whereby the topics discussed in the introduction are explained further. Thereafter, Chapter Three navigated the methodology and research design utilised throughout this research study. Chapter Four presented the study results, and Chapter Five discussed these results and considered the

main findings with existing literature. Chapter Five also addressed the study's strengths and limitations and made recommendations for future research.

1.8 Chapter summary

Chapter One consisted of the background information on iron deficiency, depression, and obesity at a national and global level. Thereafter, the problem statement was discussed, and points were made that if iron deficiency goes untreated, it can result in specific NCDs and affect our people's health and our country's economic productivity. Next, the research questions, hypotheses, aims, and objectives were deliberated. These topics were then followed by the relevance of the research and the thesis outline.

Chapter 2

Literature Review

2.1 Introduction

This chapter highlights the current literature concerning the study's major themes. The first theme discussed iron and how prevalent iron deficiency is withing a university student population. The iron status indicators utilised as part of this study are also discussed and a summary was presented in Table 2.3.1. To sum up the iron section, the iron deficiency definition employed in this study was explained. The second theme discussed was depression and its prevalence within a student population. This section also reflects on the relationship between iron deficiency and depression, as well as sleep quality and depression. The last theme discussed was obesity within a student population and how it relates to both iron deficiency and depression. To end, the relationships between iron deficiency, depression, and obesity were discussed.

2.2 Iron

Iron is an essential trace element found in the body and is vital for oxygen transport, delivery, and utilisation (Saito, 2014) & (Ueda, et al., 2021). Iron primarily exists in complex forms bound to proteins as haem compounds, such as haemoglobin and myoglobin, in the human body. These compounds are directly responsible for oxygen binding, transport, and metabolism and indirectly responsible for electron transport and mitochondrial respiration (Yiannikourides & Latunde-Dada, 2019). Iron also exists as haem enzymes, or non-haem iron-containing compounds such as flavin-iron enzymes, transferrin, and ferritin, which are essential in DNA synthesis, cell proliferation and differentiation and gene regulation (Abbaspour, et al., 2014), (McDermid & Lonnerdal, 2012) & (Yiannikourides & Latunde-Dada, 2019). The iron transport is regulated by transferrin and is predominantly stored in ferritin and hemosiderin in the liver, spleen, marrow, and various other anatomical areas (McDermid & Lonnerdal, 2012) (Saito, 2014). Approximately two-thirds of bodily iron are found in circulating RBCs as haemoglobin. An additional 25 % are readily available in the body. The remaining 15 % is bound to myoglobin in muscle tissue and various enzymes involved in the oxidative metabolism and other cell functions, as shown in Figure 2.2.1 (Abbaspour, et al., 2014). Once iron is absorbed, there is no available physiological process for the excretion of excess iron from the body other than blood loss, pregnancy, menstruation, or exfoliation of dead skin cells or mucosal cells.

Due to this unregulated means of iron excretion, the iron balance is maintained through the regulated control of iron absorption. This controlled absorption is influenced by two essential factors, including the degree of erythropoietic activity and the level of body iron stores (Means Jr, 2014).

A high level of circulating iron and iron storage promotes the production of hepcidin, which in turn inhibits dietary iron absorption and further iron storage (Nemeth, 2010) & (D'Angelo, 2013). Hepcidin is a 25-amino acid long peptide hormone secreted by hepatocytes into circulating blood and is a fundamental regulator of systemic iron homeostasis (Nemeth, 2010) & (Ginzburg & Shaz, 2013). The released hepcidin acts on its receptor ferroportin, a transmembrane iron exporter protein located on the basolateral surface of duodenal enterocytes. These receptors export absorbed dietary iron into the blood. Ferroportin are also found on macrophages specialising in the recycling of worn RBCs and other cells involved in iron storage, which then export stored iron into circulation (Pagani, et al., 2019) & (Guindi, 2018). The interaction between hepcidin and ferroportin controls the absorption and distribution of iron (Nemeth & Ganz, 2021). When circulating iron levels are elevated, hepcidin is secreted by hepatocytes and binds with ferroportin, causing it to degrade. This process inhibits further iron export into circulation. Hepcidin has an additional role in innate immunity and acts as an antimicrobial agent in host defence against gram-negative bacteria and specific fungi that induce inflammation (Barton & Acton, 2019), (Nemeth & Ganz, 2021) & (Girelli, et al., 2016). The hormone reduces iron levels present in the plasma and extracellular fluids, thus limiting bacterial proliferation (Barton & Acton, 2019).

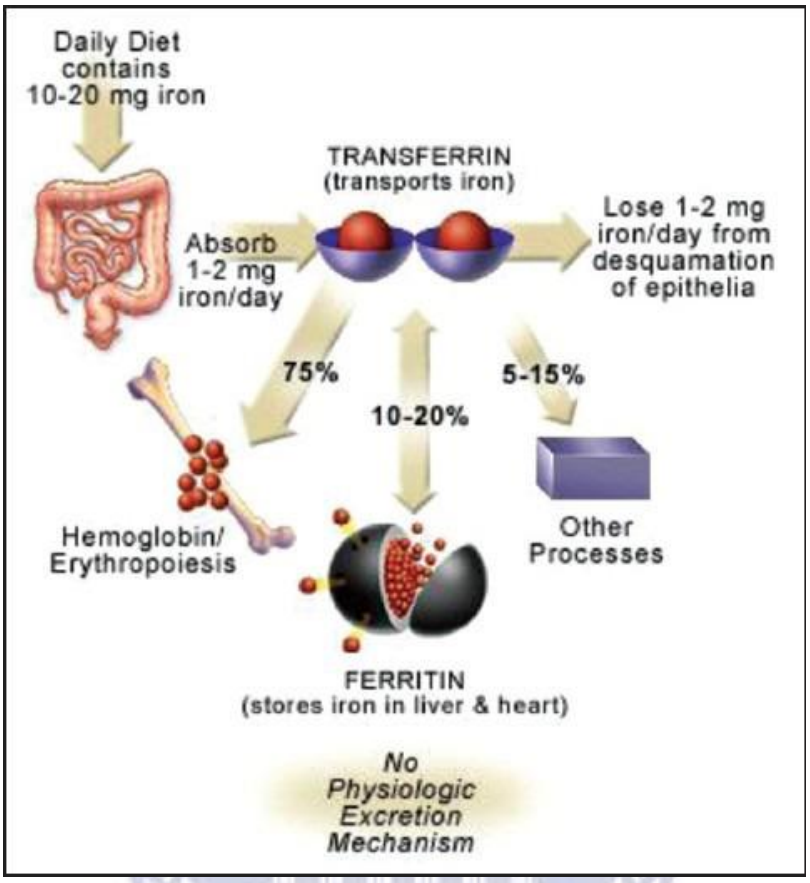


Figure 2.2.1: A schematic representation of how iron is transported and stored in the body.

As previously mentioned, iron deficiency is defined as a disorder whereby no utilisable iron stores are identified, resulting in signs of inadequate iron supply to both the tissues and the RBCs and accounts for approximately half of the world's anaemia burden (World Health Organisation, 2001). Approximately two billion people that are nutritionally iron-deficient are affected with IDA, globally. Subsequently, the health burden of iron deficiency can be inferred from the global prevalence of anaemia (Miller, 2013).

Those at risk of iron deficiency are individuals with increased iron demands such as children, menstruating women, pregnant women, and adolescents (Camaschella, 2019). However, this study's focus is on South African university students, therefore the literature assessed will relate to iron deficiency observed in a student sample population. For example, Alkindi et al. aimed to evaluate the prevalence of IDA and iron deficiency in Omani university students and observed that 26 % and 38 % of the sample population presented with these conditions, respectively (Alkindi, et al., 2018). Similarly, Al-Alimi et al. aimed to assess the prevalence of IDA among Yemeni university students and observed that 30.4 % of their sample population presented with IDA. This result was slightly higher when compared to the Omani sample

population (Al-Alimi, et al., 2018). Much like the Yemeni student population, Al-Jamea et al. observed that 35.3 % of their female Saudi Arabian college student sample population were affected with IDA (Al-Jamea, et al., 2019). The results of these studies express the need for iron status evaluation among university students as the prevalence of IDA, and therefore iron deficiency remains relatively high.

2.3 Iron status indicators

Evaluating iron status and thus iron deficiency requires multiple essential laboratory measures. There are three ways iron levels can be estimated in plasma and serum. The total iron content can be measured per unit volume in μg/dL, the total iron-binding capacity (TIBC) can be measured in $\mu g/dL^2$, or the transferrin saturation percentage can be estimated. Additional iron status indicators are serum ferritin, zinc protoporphyrin, transferrin receptors (TfR) and haemoglobin (Abbaspour, et al., 2014).

Serum ferritin is a good indicator of iron in body stores under typical circumstances with no concurrent infection. At the same time, zinc protoporphyrin represents the diminished iron stores in the end stages of haemoglobin synthesis, as zinc replaces iron and is inserted into the protoporphyrin at this stage. On the other hand, TfRs indicate the intensity of erythropoiesis and the demand for iron. The concentration of the TfRs increase when iron stores are depleted, signifying severe iron deficiency in iron deficiency anaemia. Lastly, haemoglobin concentration measures anaemia and reflects end-stage iron deficiency (Abbaspour, et al., 2014) & (World Health Organization, 2007).

The World Health Organization recommends the following five measurements as suitable iron status indicators in assessing iron statuses: haemoglobin, zinc protoporphyrin, mean corpuscular volume, TfR or serum ferritin (World Health Organization, 2007). When evaluating the iron status in a population, the World Health Organization recommends using the ferritin model, which utilises serum ferritin and TfR measurements. Ferritin is an acute-phase response protein that increases in concentration in instances of inflammation, whereas the concentration of the TfRs do not rise under inflammatory conditions. Thus, combining these two measurements allows researchers to distinguish between iron deficiency and inflammation (World Health Organization, 2007) & (Pfeiffer & Looker, 2017). While these are the recommendations, many studies examining iron deficiency have used only a few indicators mentioned above, with the ferritin levels being the most commonly used indicator (Pfeiffer & Looker, 2017). Additionally, other studies have used various combinations of these indicators

or even additional indicators not noted. Therefore, a clear protocol for assessing iron deficiency needs to be established. Recently, Frater performed a bibliometric analysis examining the 100 most cited articles published in the field of iron deficiency and found that chemistry-based assays tended to be more sensitive in the diagnosis of iron deficiency when compared to red blood cell indices such as the MCV, as a significant proportion of individuals with iron deficiency do not present with a reduced MCV. The most employed indicators for iron deficiency were serum ferritin and transferrin saturation (TSAT). Furthermore, the ferritin model was a potentially helpful tool in evaluating whole-body iron stores; however, the lack of standardisation of serum TfR assays limited its usage (Frater, 2021).

For this research study, the iron status indicators used to assess iron status were ferritin, transferrin, and iron concentrations. From these measurements, we calculated the TIBC and the TSAT. These indicators provided adequate information in assessing iron deficiency and will be discussed further. A summary of these iron status indicators is presented in Table 2.3.1.

2.3.1 Ferritin

Iron stores exist in the body mainly in the form of ferritin, an iron storage protein discovered in 1937 by the French scientist Laufberger, who had isolated a novel protein from horse spleen that consisted of up to 23 % by dry weight of iron (World Health Organization, 2011) & (Wang, et al., 2010). The ferritin molecule is an intracellular hollow protein shell composed of 24 sub-units encompassing an iron core that may contain up to 4000.00 – 4500.00 iron atoms (World Health Organization, 2011). It is regulated post-transcriptionally by cellular iron status via iron-responsive elements in its messenger RNA. Consequently, high intracellular iron concentrations increase ferritin concentrations, while iron deficiency inhibits expression (Daru, et al., 2017).

The concentration of serum ferritin, ferritin secreted into the plasma, is positively correlated with the size of the total body iron stores in the absence of inflammation. A reduced serum ferritin result reflects depleted iron stores but not necessarily the severity of the depletion as it progresses (World Health Organization, 2011). While serum ferritin is considered the gold standard for assessing absolute iron deficiency, it is essential to note that ferritin is a positive acute-phase response protein (Camaschella, 2019) & (World Health Organization, 2011). Ferritin concentrations increase during inflammation, therefore no longer reflecting the actual size of the iron store but rather the degree of acute or chronic inflammation (World Health Organization, 2011), (Sciacqua, et al., 2020) & (Bouri & Martin, 2018).

2.3.2 Transferrin

Human serum transferrin is part of a group of homologous single-chained glycoproteins with a molecular weight of approximately 80.00 kDa that binds and transports iron in circulation (Wiencek, et al., 2020), (Rodriguez-Garcia, et al., 2021) & (Elsayed, et al., 2016). The amount of transferrin present in the blood is reflective of the amount of iron in the body, subsequently allowing the assessment of iron status to be its primary clinical use (Ogun & Adeyinka, 2021) & (Wiencek, et al., 2020). Transferrin is mainly produced in the liver, after which it is secreted into the blood, where it circulates and binds to iron. It delivers approximately 70 % of iron to the bone marrow, where it integrates into haemoglobin within red blood cells, while the residual iron is stored in tissue (Kundrapu & Noguez, 2018). Transferrin levels increase in instances of iron deficiency to maximise iron transport and can decrease when the iron status normalises or in protein-energy cases of malnutrition, infection, liver damage or kidney injury (Litchford, 2008) & (Ogun & Adeyinka, 2021). Unlike ferritin, transferrin is a negative acute-phase response protein that decreases in concentration in response to instances of inflammation (Jain, et al., 2011). Therefore, we ruled out inflammatory conditions that can exacerbate iron deficiency by monitoring ferritin and transferrin concentrations.

2.3.3 Transferrin saturation

An additional way to assess iron status is through transferrin saturation (TSAT), an index that describes the iron-binding capacity of transferrin. It is expressed as the percentage of binding sites on all transferrin molecules occupied with iron molecules, indicating the adequacy of iron supply (Sato, et al., 2019) & (Pfeiffer & Looker, 2017). It is a frequently used laboratory measure of iron deficiency, but more so of iron overload due to its high sensitivity. It is typically elevated before serum ferritin levels increase and iron overload develops (Stack, et al., 2014) & (Pietrangelo, 2018). Approximately one-third of circulating transferrin is saturated with iron. The saturation decreases while the transferrin concentration increases before the onset of anaemia (Evans, et al., 2013). Transferrin saturation can be calculated in various ways and can be observed in Figure 2.3.3.1 (Pfeiffer & Looker, 2017). It can be calculated as the ratio between serum iron to transferrin or serum iron to total iron-binding capacity (TIBC) (Pfeiffer & Looker, 2017). It is important to note that while serum transferrin concentrations remain relatively stable, serum iron concentrations fluctuate diurnally and are significantly influenced by dietary iron intake. Consequently, TSAT results are affected and are not intended to be interpreted in isolation but rather in conjunction with other laboratory tests (Elsayed, et al., 2016).

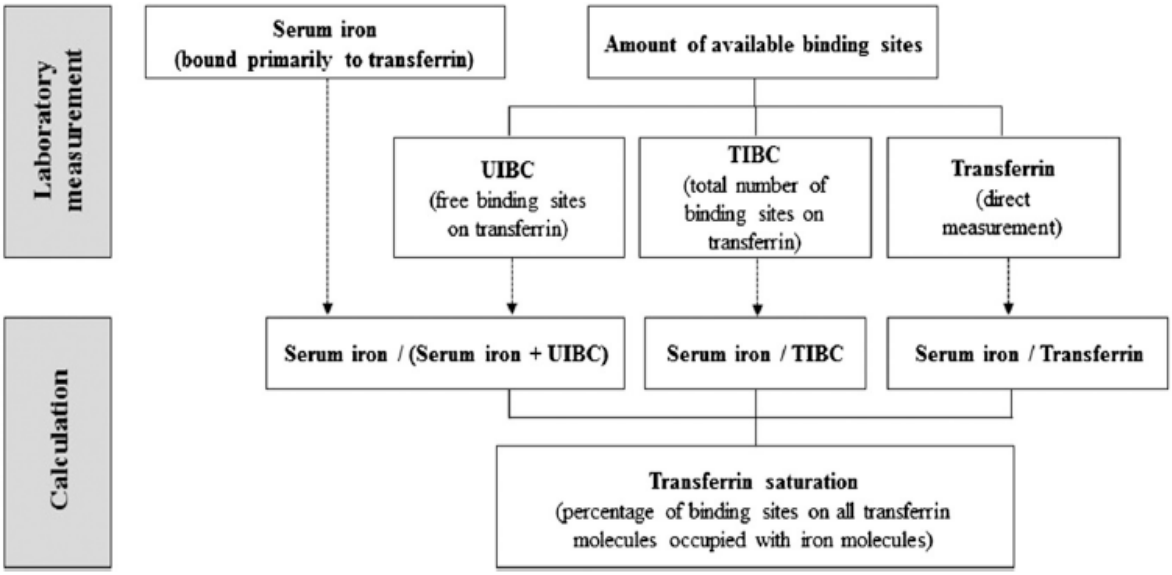


Figure 2.3.3.1: A diagram showing the laboratory measurements needed to calculate the transferrin saturation.

2.3.4 Serum iron

Serum iron concentration represents the amount of ferric iron (Fe^{3+}) primarily bound to serum transferrin, not including the divalent iron observed in serum as haemoglobin. Serum iron is not considered a good indicator of iron stores due to its daily fluctuation affected by dietary intake. Therefore, it alone is a good measure of iron deficiency (Centers for Disease Control and Prevention , 2008) & (Peng & Uprichard, 2017). However, serum iron measurements can be combined with TIBC measurements to provide enhanced utility for increased sensitivity. As previously mentioned, serum iron and TIBC measurements can indicate transferrin saturation. Low iron levels observed with increased TIBC levels that yield a transferrin saturation of less than 16 % are indicative of iron deficiency anaemia (Centers for Disease Control and Prevention , 2008).

2.3.5 Total iron-binding capacity

Iron binding capacity is the term used to describe the ability of transferrin to bind with iron and is represented in two ways (Faruqi & Mukkamalla, 2021). The first is the unsaturated iron-binding capacity (UIBC), which measures the transferrin binding sites unoccupied by iron and is considered the reserve iron-binding capacity (Kundrapu & Noguez, 2018). The second representation of iron-binding capacity is the TIBC which is the total sum of iron that serum proteins can bind (Faruqi & Mukkamalla, 2021) & (Collaborative Laboratory Services, 2007).

It represents the excess amount of iron needed to saturate transferrin thoroughly and is considered a proxy measure of transferrin (Elsayed, et al., 2016) (Peng & Uprichard, 2017). While TIBC is elevated in iron deficiency, its sensitivity towards the condition is less so when compared with serum ferritin. It is also reduced in patients with anaemia of chronic disease. Therefore, it can be used to assess an increased serum ferritin concentration that potentially masks iron deficiency. This indicator is not suitable for assessment alone but with ferritin and other iron parameters (Collaborative Laboratory Services, 2007) & (Peng & Uprichard, 2017).

Table 2.3.1: Summary of the iron status indicators used in this research study.

Iron indicator	Description	Advantages	Disadvantages	Response to iron deficiency	References
Ferritin	Ferritin is a positive acute phase response protein that reflects the iron stores without inflammation.	Serum ferritin positively correlates with the size of the total body iron stores and is considered the gold standard for assessing absolute iron deficiency.	Due to ferritin being an acute phase response protein, it increases in instances of inflammation, therefore no longer reflecting the actual size of the iron store but rather the degree of acute/chronic inflammation.	Decreases.	(Camaschella, 2019), (World Health Organization, 2011) & (Bouri & Martin, 2018).
Transferrin	A protein that binds and transports iron in circulation.	The amount of circulating transferrin reflects the amount of iron in circulation.	Transferrin is a negative acute phase response protein that decreases inflammation.	Increases.	(Elsayed, et al., 2016) , (Ogun & Adeyinka, 2021) & (Jain, et al., 2011).
Transferrin saturation	Transferrin saturation is an index that describes the iron-binding capacity of transferrin.	Transferrin saturation is a highly sensitive indicator of iron overload.	The Transferrin saturation requires using serum iron level as part of its calculation, and serum iron fluctuates diurnally, influencing the TSAT result.	Decreases.	(Sato, et al., 2019), (Pietrangelo, 2018) & (Elsayed, et al., 2016).
Serum iron	Serum iron represents the amount of ferric iron primarily bound to transferrin, excluding the iron observed in serum as haemoglobin.	If used in combination with other iron indicator tests, serum iron concentrations can be helpful.	Serum iron is not a good indicator of iron deficiency due to its daily fluctuations and sensitivity toward dietary iron intake.	Decreases.	(Centers for Disease Control and Prevention , 2008) & (Peng & Uprichard, 2017).
TIBC	The total concentration of iron that serum proteins can bind.	It indicates iron deficiency.	It needs to be interpreted with other iron indicator tests.	Increases.	(Collaborative Laboratory Services, 2007) & (Peng & Uprichard, 2017).

2.3.6 Iron deficiency definition

Defining iron deficiency proved challenging, as many studies utilised various iron parameters and combinations of these parameters to define the condition. For instance, Jankowich et al., defined iron deficiency based on sex-specific cut-offs using serum ferritin concentrations as follows: females (< 15 ng/mL); males (< 30 ng/mL). In a separate analysis sex-specific cut-offs for serum iron concentrations were used to define the condition as follows: females (< 30 µg/dL); males (< 45 µg/dL) (Jankowich, et al., 2016). Whereas Okan et al. only utilised serum ferritin in their definition of iron deficiency (< 30 ng/mL), and Motonishi et al. defined iron deficiency by the presence of both a reduced TSAT (< 20 %) and serum ferritin concentration (< 100 ng/mL) (Okan, et al., 2019) & (Motonishi, et al., 2018). Sanad et al. defined iron deficiency as one or more abnormal age-corrected iron parameters such as iron, ferritin, transferrin, and transferrin saturation (Sanad, et al., 2011). Similarly, Portugal-Nunes defined the condition as having a low serum ferritin concentration (< 15 ng/mL) or presenting with two or more biomarkers indicating iron deficiency elsewhere. This included serum iron (< 71 µg/dL), TSAT (< 20 %), and TIBC (⩾ 360 µg/dL) to name a few (Portugal-Nunes, et al., 2020).

Based on previous iron-related studies, this research study defined iron deficiency as the presence of both a reduced serum ferritin concentration (< 30 µg/L) as well as a reduced TSAT percentage (< 20 %) (PathCare Reference Laboratory, 2021), (Okan, et al., 2019), (Motonishi, et al., 2018) & (Portugal-Nunes, et al., 2020). The participants were organised into iron-deficient and iron-replete groups, whereby the iron-replete participants had iron stores adequate to meet the body's functional needs at a level above the iron deficiency and below that defined as iron excess (Taylor & Brannon, 2017).

2.4 Depression

Depressive disorders are characterised by sadness, poor concentration, loss of interest or pleasure, disturbed sleep or appetite, feelings of guilt or low self-esteem, and feelings of tiredness. Depression, also known as Major Depressive Disorder (MDD), can be long lasting or periodic, significantly debilitating an individual's ability to function at work or school or cope with daily activities (World Health Organization, 2017). The diagnostic criteria for MDD consist of the experience of a low mood, the inability to feel pleasure in previously pleasurable activities and an abnormal lack of energy, lasting for a period no sooner than two weeks (Parekh, et al., 2017).

The development of depression can be brought about by various factors, of which approximately one-third is inherited, and two-thirds are environmental (Saveanu & Nemeroff, 2012). Historically, environmental threats to mental health were viewed in the context of infectious agents, pollutants, and various exogenous factors that affect the individual's physical surroundings. Today, these traditional parameters are still considered environmental threats, in conjunction with pharmaceutical and illicit drugs, injuries, nutritional deficiencies, and psychosocial conditions that describe the individual's perception of the social and physical world (Schmidt, 2007). The growing brain is influenced by social deprivation factors and poor social environments, such as economic instability and unemployment, increasing the risk of developing depression. Recently, research has been done that proposes that physical aspects of the environment, such as noise and chemical pollutants, may also have a neurobiological impact and influence the risk of depression, especially among those genetically susceptible (van den Bosch & Meyer-Lindenberg, 2019).

In the context of genetically inheritable factors for the risk of developing depression, twin research studies have shown that the rate of heritability for depression is 37 %. Additionally, family studies showed a two to three-fold increase in the risk of depression in the biological offspring of patients with depression (Shadrina, et al., 2018) & (Lohoff, 2010). Studies were performed on the post-mortem brains of patients with depression to determine genes suspected of causing the disorder. Animal models of depression showed the ATP-binding cassette sub-family B member 1 (ABCB1), histone deacetylase (HDAC6), neuritin, a promoter region related to serotonin transporter gene transcription (5-TTLPR) and disruption in schizophrenia 1 (DISC1) had been implicated as candidate genes (Nabeshima & Kim, 2013). More recently, a breakthrough in genome-wide association studies (GWAS) for depression has identified 102 independent depression-associated genetic variants, of which 87 replicated in an independent

sample. Furthermore, a further eight novel independently repeated depression loci were identified using GWAS findings on multiple psychiatric phenotypes (Ormel, et al., 2019).

As previously discussed, South African students are at risk for severe depressive symptoms. In 2020, Pillay et al. aimed to investigate the depressive symptoms experienced by first-year psychology students in a rural-based university in KwaZulu-Natal, South Africa. Symptoms of low self-esteem, hopelessness and loss of interest were present in approximately one-fifth of the participants (Pillay, et al., 2020). Additionally, Janse van Vuuren assessed the mental well-being of postgraduate accounting students in a South African university and observed that 42.4 % and 17.2 % of the students presented with mild to moderate and severe depression, respectively (Janse van Vuuren, et al., 2021). The burden of depression and anxiety was also assessed among medical students at the University of Cape Town, South Africa. It was observed that 25 %, 20.5 % and 28.1 % of the students were diagnosed with depression and anxiety and were receiving psychotropic medication, respectively (van der Walt, et al., 2020). These statistics demonstrate the increased prevalence of depressive symptoms among university students. Should these symptoms not be treated at this tertiary level, they could affect the South African economy as these students' progress into the job market.

In South Africa, it has been reported that the economic burden of depression remains high, with the stigma surrounding the disease preventing employees from seeing help. Moreover, individuals that experienced cognitive symptoms associated with their depressive episodes had significantly lower job performance scores when compared to those that did not experience cognitive symptoms during their episodes (Stander, et al., 2016). This result suggests that more severe depressive symptoms affect job performance, thus burdening the economy. In 2019, Saonatse et al. investigated how MDD influenced the employment of South African citizens and found that the participants experienced distress at having an extended sick leave period after being hospitalised for MDD. This distress stemmed from the workload, job content concerns, and social concerns regarding meeting financial demands and responsibilities. Many individuals tended to resume work regardless of whether they experienced residual symptoms. The experience of residual feelings while at work could again affect the job performance. (Saonatse, et al., 2019). Depressive symptoms experienced by students at a tertiary level can affect the South African economy if they go untreated.

2.5 Iron deficiency and depression

Iron is essential for regular neuronal and glial energy metabolism, neurotransmitter production and myelination. As a result, iron deficiency has a negative effect on these brain processes, potentially leading to behavioural abnormalities, cognitive dysfunction or impairment (Georgieff, 2011), (Miller, 2013) & (Jauregui-Lobera, 2014). This sub-section will discuss the role of iron in the processes mentioned above and examine studies that have sought to determine the association between iron deficiency and depression.

Firstly, iron plays a vital role in monoamine metabolism. It synthesises and signals the neurotransmitters dopamine, noradrenaline, adrenaline, and serotonin implicated in emotion, attention, reward, and other conditions. Iron is a cofactor for phenylalanine hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase, iron-dependent enzymes responsible for synthesising the neurotransmitters mentioned above. While the levels of these enzymes are strictly maintained even in instances of brain iron deficiency, reduced neuronal uptake of dopamine, adrenaline and noradrenaline have been observed in brain iron deficiency models. This decreased neuronal uptake suggests that iron also plays a role in neurotransmitter signalling (Hare, et al., 2013). Interestingly, these neurotransmitters have been associated with the traditional monoamine hypothesis of depression, which states that the concentrations of these neurotransmitters are reduced in the synaptic gaps of individuals in depressive states (Richardson, et al., 2015) & (Boku, et al., 2018). Consequently, symptoms of lethargy, tiredness, and irritability due to the diminished monoamine oxidase activity are often observed in iron-deficient patients. As such, these patients can display signs and symptoms associated with those seen in individuals experiencing depressive symptoms (Murat, et al., 2015).

Secondly, iron is an essential cofactor in the homeostasis of both glutamate and γ-aminobutyric acid (GABA), neurotransmitters responsible for maintaining the balance between excitation and inhibition in the body (Kim & Wessling-Resnick, 2014), (Sarawagi, et al., 2021) & (Behuet, et al., 2019). Changes in the GABAergic neurotransmission have been observed in individuals with depression. These changes can reduce the regulation of principal neurons, resulting in an excessive release of glutamate in the synapse. Consequently, the N-methyl-D-aspartate (NMDA) receptors specific to glutamate undergo prolonged overstimulation, leading to the potential atrophy of glutamatergic neurons by excitotoxicity which has been observed in depressed subjects (Sarawagi, et al., 2021) & (Duman, et al., 2020).

Thirdly, iron is essential for the standard myelination of the spinal cord and white matter of the cerebellar folds (Noorazar, et al., 2015). Oligodendrocytes are specialised lipid-rich glial cells responsible for myelinating central nervous system (CNS) cells. Due to the precise and coordinated nature of their proliferation, migration and differentiation, oligodendrocytes are considered one of the more vulnerable cells of the CNS. Therefore, any deviations from their pathway can result in hypomyelination in early brain development (Bradl & Lassmann, 2010) & (Noorazar, et al., 2015). Iron is a crucial element in oxidative metabolism occurring in oligodendrocytes and is indirectly involved in myelin production. Iron is also a cofactor for cholesterol and lipid biosynthesis and is directly involved in myelin production (DeMaman, et al., 2010). As such, iron deficiency affects myelination. One aetiological pathway of depression is said to be caused by atypical myelin. Post-mortem studies have shown depression-related abnormalities in several myelin related proteins and transcription factors, particularly in the prefrontal cortex. Furthermore, animal models of major depressive disorder have yielded results showing a reduction in gene expression and altered morphology of myelin support cells. Reduced myelin is also observed in the prefrontal cortex (Sacchet & Gotlib, 2017). At the same time, human post-mortem studies showed an apparent decrease in the myelin of both the callosal splenium in the corpus callosum and dorsolateral prefrontal cortexes of individuals that had previously suffered from major depressive disorder (Williams, et al., 2019) & (Regenold, et al., 2007).

Lastly, when investigating studies that have explored the relationship between iron deficiency and iron deficiency anaemia and depression, many have used different methods and have produced conflicting results. A few of these studies have analysed the relationship between ferritin concentrations and depression. In 2007, Shariatpanaahi et al. aimed to examine the relationship between depression and serum ferritin concentrations in female medical students situated in Tehran, Iran. The researchers measured depressive symptoms using the BDI and measured each participant's haemoglobin, serum ferritin, erythrocyte sedimentation rate, C-reactive protein, folic acid, and vitamin B12 concentrations concurrently. This study observed that while the serum ferritin concentrations of both the depressed and healthy groups were within a normal range, it was 11.00 μg/l lower in students with depression when compared with the healthy students (Shariatpanaahi, et al., 2007). A subsequent study conducted by Yi et al. sought to explore the association between serum ferritin concentrations and depressive symptoms in a Japanese population. While no significant association was observed in women, depressive symptoms in men were significantly associated with a reduced serum ferritin

concentration. This observation suggested that middle-aged male Japanese workers may be prone to adverse psychological effects implicated in iron deficiency (Yi, et al., 2011). A more recent study performed by Su et al. aimed to determine the association between serum ferritin concentrations and depressive symptoms among Chinese adults using the Chinese version of the Zung Self-Rating Depression Scale (SDS) to measure the symptoms. They observed no significant association between both variables in multiple analyses (Su, et al., 2016).

Other studies explored the association between iron deficiency anaemia and depression. Noorazar et al. sought to investigate the relationship between the severity of depressive symptoms and iron deficiency anaemia in women with MDD by conducting a complete blood count and measuring haemoglobin, ferritin and TIBC. Depressive symptoms were measured using the Hamilton Depression Rating Scale (HDRS). They observed no significant relationship between females with iron deficiency anaemia and depressive symptoms. However, an inverse relationship was observed between haemoglobin concentrations and depressive symptoms (Noorazar, et al., 2015). Thereafter, Vulser et al. sought to examine the association between anaemia and depression in a large-scale French population consisting of participants that were considered generally healthy and free from chronic disease. Depressive symptoms were measured using the Questionnaire of Depression 2nd version, while anaemia was assessed by measuring haemoglobin concentrations. The researchers observed a strong association between depression and anaemia as depressed participants were significantly more likely to be anaemic when compared to the non-depressed participants. Furthermore, the frequency of anaemia tended to increase according to depression severity (Vulser, et al., 2016). While this study had a large population sample, utilising haemoglobin as the sole indicator of anaemia is not a true reflection of the potential anaemic conditions and could have affected the study results. Subsequently, Shafi et al. aimed to determine the relationship between iron deficiency anaemia and depression in a Pakistani population. The HDRS was also used to assess depressive symptoms, while haemoglobin concentration and a peripheral film analysis assessed iron deficiency anaemia. The film analysis provided the mean corpuscular volume of the participant. Researchers determined whether microcytic anaemia was present, as it is a diagnostic standard for iron deficiency anaemia. It was observed that the HDRS scores increased with a decrease in haemoglobin concentrations, implicating iron in essential brain functions (Shafi, et al., 2018).

Finally, researchers investigated the association between psychiatric disorders and iron deficiency anaemia. Chen et al. aimed to explore this association among children and adolescents living in Taiwan. Using the National Health Insurance Database from 1996 to 2008, it was observed that iron deficiency increased the risk of mood disorders, autism spectrum disorder, attention deficit hyperactivity, as well as developmental disorders among children. Furthermore, females with IDA had an increased risk of bipolar disorder or tic disorder (Chen, et al., 2013). More recently, Lee et al. aimed to explore the prevalence of psychiatric disorders in participants with iron deficiency anaemia compared with non-anaemic participants. The researchers used the same Taiwanese database as in the previous study. However, they focused on adults with newly diagnosed IDA enrolled in the database from 2000 to 2012. Individuals were included in the study if they had at least two diagnoses of iron deficiency anaemia in their medical claims before the index date. Serum iron, ferritin and TIBC measurements further confirmed iron deficiency anaemia. It was revealed that the incidence of psychiatric disorders, such as anxiety disorders, depression, psychotic disorders, and sleep disorders, were higher in the iron-deficiency anaemia group when compared with the non-anaemic participants (Lee, et al., 2020). Similarly, Korkmaz et al. aimed to analyse the prevalence of anaemia in chronic psychiatric patients residing in Turkey. It was observed that the condition was more frequent among those with chronic psychiatric diseases when compared with the general population, with 22 % of the 378 patients being diagnosed with a depressive disorder. Unfortunately, this study primarily focused on haematological parameters in its assessment of anaemia therefore, no information regarding the iron status of the participants was obtained (Korkmaz, et al., 2015).

2.6 Sleep quality and depression

There is a prominent association between sleep disturbance and major depressive disorder, as it is one of the most consistent symptoms related to the disorder (Nutt, et al., 2008) & (Murphy & Peterson, 2015). These disturbances are not secondary to the disorder, but often precede the depressive episode and can be characterised by the interruption of wake periods into the sleep process; the altered proportion and pattern of the sleep stages; and the enhancement of phasic phenomena within the REM stage of sleep (Murphy & Peterson, 2015) & (Borbely & Wirz-Justice, 1982).

Decreased sleep duration and quality have been associated with changes in lifestyle. Increased used of technological devices, and increased work and social demands. Subsequently,

university students have been identified as a group that is vulnerable to these sleep disruptions (Lemma, et al., 2012). Lemma et al. observed that prevalence of poor sleep quality among Ethiopian university students to be 55.8 %, with female students and second- and third-year students having a significantly higher odd of poor sleep (Lemma, et al., 2012). Çelik et al. noted an inverse relationship between sleep quality and depressive symptoms within their Turkish university student population in health sciences. Moreover, poor sleep quality increased the risk of depressive symptoms within the population by 3.28-fold (Çelik, et al., 2018). More recently, Kaya et al. demonstrated that a positive association existed between smart phone usage, Pittsburgh sleep Quality Index (PSQI) score and BDI score among Turkish university students (Kaya, et al., 2021). Similarly, Bhandari et al. observed that 35.4 %, 35.4 % and 21.2 % of their Nepalese university student population presented with poor sleep quality, internet addition and depression, respectively (Bhandari, et al., 2017). These studies highlight the impact of sleep quality on the mental health of university students.

2.7 Obesity

Obesity is a chronic disease generally defined as excess body fat (Ogden, et al., 2007). It can occur when an imbalance is established between calories derived from food consumption and calories expended through physical activity. The excess energy is stored in adipocytes resulting in the hyperplasia and hypertrophy of these cells, a defining pathological lesion of obesity (Bray, 2004) & (Hruby & Hu, 2015). However, this simple definition does not reflect the aetiological complexities associated with excess adiposity that can be established metabolically and not only in body size (Hruby & Hu, 2015). Psychosocial and behavioural factors, including genetics, socioeconomic status, and cultural influences, also have a significant role in the cause and preservation of obesity (Apovian, 2016).

Furthermore, adiposity is a continuous trait that does not have a defined marker distinguishing normal from abnormal, making it difficult to measure. Consequently, obesity is often described as excess body weight as an alternative to excess fat (Ogden, et al., 2007). As a result, BMI indexes were created to classify different weight ranges and were used as screening tools for overweight and obese individuals (Centers for Disease Control and Prevention, 2021).

Obesity is associated with various chronic NCDs that directly or indirectly affect different aspects of the affected individual's physiology (Fruh, 2017). The most common diseases are presented in Figure 2.7.1. Obesity is associated with type 2 diabetes, hypertension, dyslipidaemia, non-alcoholic fatty liver disease (NAFLD), stroke, pancreatitis, sleep apnoea,

cancer and various other disorders that can lead to chronic disability (Pantalone, et al., 2017), (Pi-Sunyer, 2009) & (Ogden, et al., 2007). More recently, it has also been determined that an overweight or obese individual that contracts COVID-19 is more likely to become severely ill, potentially needing to be hospitalised or put on a ventilator to aid breathing (Centers for Disease Control and Prevention , 2021).

Obesity burdens population health and can also be detrimental to the economy. The treatment of the chronic diseases associated with obesity strains the country's health care system. Furthermore, the consequences of obesity affect human productivity in the workplace, thus affecting the labour market and eventually the GDP of a country (Vuik, et al., 2019). In South Africa, rapid urbanisation has brought about a health transition characterised by the appearance of NCDs such as obesity (Pisa & Pisa, 2017). According to Statistics South Africa, approximately 68 % of women and just 31 % of men are considered overweight or obese. Additionally, obesity ranks fifth as a risk factor for early death and years of life lived with disability (Statistics South Africa, 2016) & (Department of Health: Republic of South Africa, 2015). It is therefore of the utmost importance to curb this fast-spreading disease.

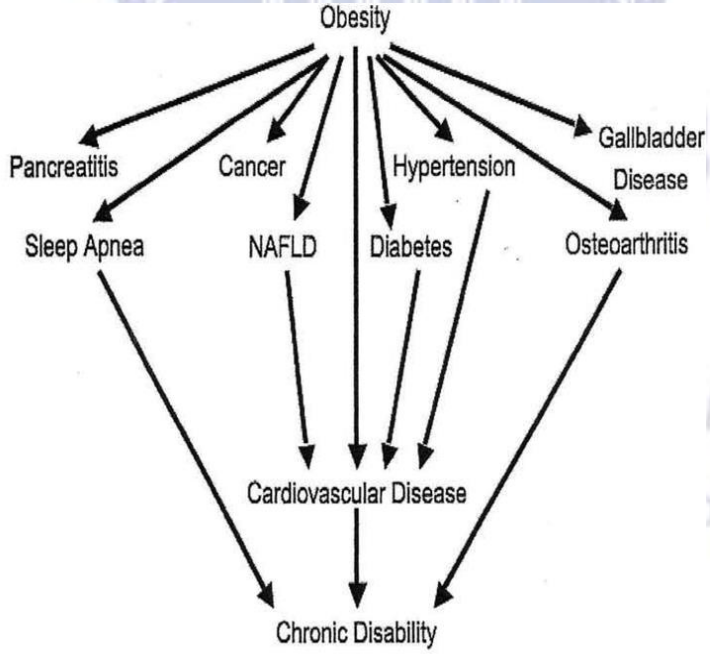


Figure 2.7.1: A diagram showing the non-communicable diseases associated with obesity that led to chronic disability.

Students entering university or college face challenges when adjusting to their new environments and workloads and are prone to weight gain (Oh, et al., 2016). Associations have been observed between overweight and obese university students regarding high preferences

for high-fat diets; a lack of physical activity; shorter sleep duration; and tobacco use (Telleria-Aramburu & Arroyo-Izaga, 2021). Therefore, many studies have aimed to further assess the relationship between obesity and university students.

Peltzer et al. assessed the prevalence of overweight and obesity among university students across 22 countries and observed 22 % of the participants were either overweight or obese (Peltzer, et al., 2014). Similarly, 21 % of a Moroccan female university sample population and 26.8 % of an Indian university student population were considered overweight (Boukrim, et al., 2021) & (Pengpid & Peltzer, 2014). Regarding a South African university student population, Peltzer and Pengpid demonstrated that 30.5 % and 8.7 % of the female and male students 8.7 % considered overweight or obese, respectively (Peltzer & Pengpid, 2012). These studies highlight the moderate prevalence of overweight and obesity among university students and identify a need for weight management strategies within the population.

2.8 Iron deficiency and obesity

Obesity and iron deficiency are two of the most common forms of nutritional disorders worldwide. Recently, various studies have investigated whether a correlation exists between these two disorders, and many conflicting results have emerged (Rad, et al., 2019). Abnormal iron statuses are frequently observed in overweight and obese individuals, with elevated iron stores and iron deficiency causing damage to health and exacerbating obesity-related conditions (Cepeda-Lopez, et al., 2015) & (Aigner, et al., 2014). Iron deficiency may worsen the burden of obesity through symptoms of fatigue and depression that are frequently experienced in iron-deficient individuals. These symptoms hinder weight management initiatives by reducing the willingness and capacity of the individual to exercise (Monteiro, et al., 2018). Furthermore, iron deficiency and anaemia may weaken cellular and mitochondrial energy homeostasis, further increasing sedentariness and fatigue of overweight individuals (Aigner, et al., 2014).

One school of thought regarding the relationship between iron deficiency and obesity lies with inflammation. Chronic inflammation, characterised by inflammatory cytokines and the proliferation of blood vessels and adipose tissue, is a principal constituent of metabolic syndrome. Moreover, one of the critical characteristics of metabolic syndrome is obesity, and obesity is therefore considered an inflammatory condition (Ellulu, et al., 2017). In addition to storing energy, adipose tissue secretes many proinflammatory cytokines that induce chronic inflammation and can be a catalyst in developing different diseases (Castro, et al., 2017). This

inflammatory state causes the synthesis of hepcidin via hepatocytes, which will cause the degradation of ferroportin and inhibit iron export into circulation (Alshwaiyat, et al., 2021) & (Nemeth & Ganz, 2021). The relationship between iron deficiency and obesity is shown in Figure 2.8.1 (Alshwaiyat, et al., 2021).

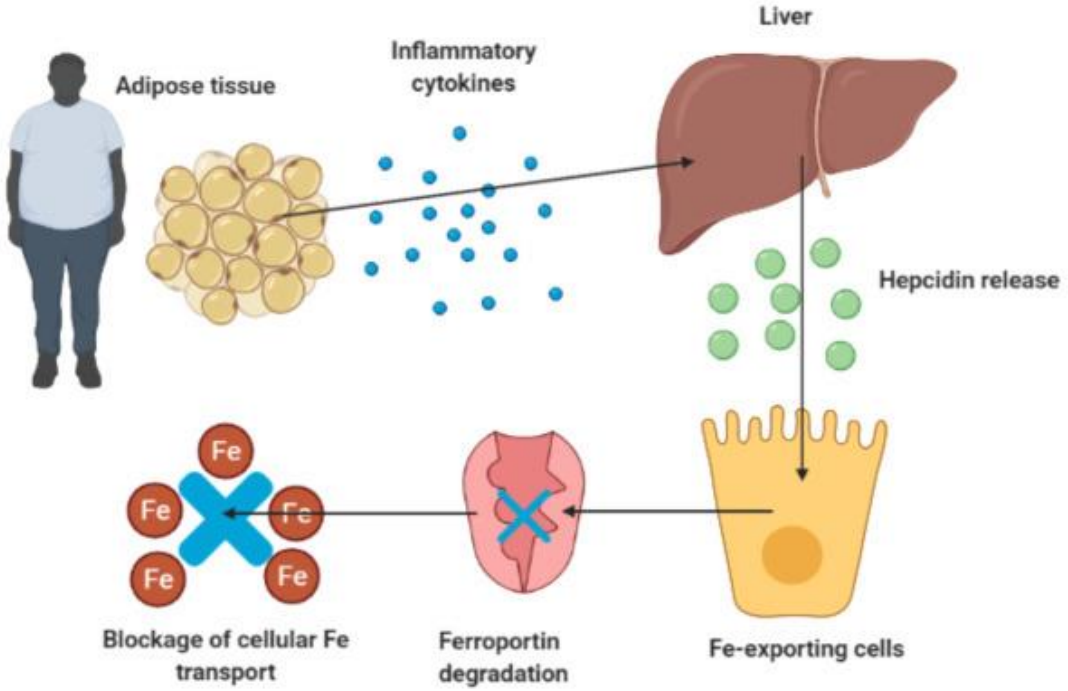


Figure 2.8.1: A schematic diagram showing how inflammation associated with obesity can cause iron deficiency.

Zeid et al. sought to investigate the variables that affect the iron status of obese rats. The iron status indicators assessed were haemoglobin, haematocrit, TIBC, TSAT and ferritin concentrations. Furthermore, leptin, interleukin-6 and systemic hepcidin were also measured to determine the inflammatory conditions of the rats. It was observed that significant differences existed between the obese and control rat groups. Increased hepcidin, interleukin-6, ferritin, leptin, and decreased serum iron, TIBC and TSAT were reported in the obese rat group compared with the control group. Furthermore, a positive correlation was observed between hepcidin and body weight (Abo Zeid, et al., 2014). Later, Stoffel et al. investigated the effect of central obesity on inflammation, hepcidin and iron metabolism in young women, and similar trends were observed. A significant positive correlation was reported between the ratio of android fat to total body fat and C-reactive protein (CRP), alpha-1-acid glycoprotein (AGP), hepcidin and TIBC. Furthermore, a significant inverse correlation was observed between the ratio and TSAT and the ferritin to hepcidin ratio (Stoffel, et al., 2020).

Kerkadi et al. investigated the association between central obesity and iron deficiency in Qatari adults. They observed that WC measurements were inversely correlated to serum iron and transferrin in women. Additionally, WC measurements were positively associated with haemoglobin concentration in men (Kerkadi, et al., 2021). Jordaan et al. aimed to assess the prevalence of anaemia and iron deficiency in women residing in the rural Free State in a South African context. Jordaan reported that significant inverse correlations existed between the BMI categories and the MCV, mean corpuscular haemoglobin (MCH) and TSAT levels, remaining in the normal ranges of the women (Jordaan, et al., 2020). Additionally, while CRP levels were normal, they were elevated in 45 % of the study population. These studies show that body fat distribution and body weight affect iron metabolism. Increased body fat or weight increased inflammatory markers and decreased iron status markers. To observe the correlations between the five iron status indicators and obesity, the participants' BMI, WC, WHR and WHtR were measured.

2.9 Depression and obesity

Both depression and obesity remain rife and have profound implications globally for the economy and disease burden. Their high prevalence may indicate a relationship between them, and many studies have been conducted further to understand this relationship (Dolatian, et al., 2017). Subsequently, a bidirectional link was established between depression and obesity; hence the presence of one condition increased the risk of developing the other (Luppino, et al., 2010) & (Milaneschi, et al., 2019). The strength and direction of this link were investigated and often produced inconsistent results. One study found that the intensity of the association was more pronounced, leading from depression to obesity. Additionally, this link was primarily observed in young and middle-aged women. In contrast, another study found that the bidirectional association was only observed in middle-aged and older women (Mannan, et al., 2016) & (Pan, et al., 2012). Researchers have also proposed that depression and obesity may synergise with other non-communicable diseases such as type 2 diabetes, hypertension, and high cholesterol (Ning, et al., 2020) & (Haregu, et al., 2020). Consequently, it became crucial to understand the associations between depression and obesity by focusing on their shared biological pathways (Milaneschi, et al., 2019).

One school of thought noted that depression and obesity were considered conditions with a tremendous inflammatory influence. The conditions have several overlapping factors, such as the sensitised and extended response of the innate immune system and proinflammatory

cytokines and the stimulation of the cortico-adrenal axis. These combined factors can potentially produce a common proinflammatory state between these pathologies (Milano, et al., 2020). Additionally, research suggests that the gut microbiota, symbiotic microorganisms that cohabit with the human body, are stimulated by high-fat diets that lead to obesity and chronic systemic inflammation (Schachter, et al., 2018). These microbiota facilitate the effects of a high-fat diet on the body's physiology through their ability to regulate the mechanism of extracting energy from food consumed by the host. They are, therefore, able to affect the body's nutritional and metabolic equilibrium and may also affect the mood and behaviour of the host (Milano, et al., 2020) & (Schachter, et al., 2018). A study conducted by Bruce-Keller et al. explored whether obesity-associated changes in gut microbiota hinder neurocognitive behaviour in mice. Gut microbiota transplanted from mice subjected to a high-fat diet to mice on a standard diet showed disturbances in neurotypical behaviour compared to the mice with the control diet microbiota in the absence of significant changes in body weight (Bruce-Keller, et al., 2015). These results suggest that manipulating the gut microbiota through either diet or pharmaceutical methods can potentially lessen the effects of depressive symptoms typically associated with obesity.

2.10 Iron deficiency, depression, and obesity

As previously mentioned, there are bodies of research that have found an association between iron deficiency and depression through the intricate role of iron in the synthesis and signalling of certain neurotransmitters involved in emotion and behaviour (Georgieff, 2011), (Miller, 2013) & (Jauregui-Lobera, 2014). Furthermore, iron deficiency relates to obesity through adipose tissue, which secretes proinflammatory cytokines that inhibit iron absorption (Alshwaiyat, et al., 2021). Lastly, research has found a bidirectional association between depression and obesity, possibly through a shared proinflammatory state (Luppino, et al., 2010) & (Milano, et al., 2020). However, very few studies have investigated the correlations between these three pathologies and how they may influence each other.

One such study, conducted by Lee and Park, aimed to investigate the association between serum ferritin concentrations and depression with regards to the BMI values of Korean men. They observed that the average depression score and serum ferritin concentration were significantly higher in the obesity group when compared to the control group. Moreover, BMI seemed to be notably associated with both depression and increased serum ferritin concentrations, providing evidence of a correlation existing between ferritin and depression

with obesity (Lee & Park, 2019). While this study investigated ferritin's role in depression and obesity, it did not thoroughly investigate the effects of iron deficiency. Furthermore, ferritin is an acute phase response protein that increases in instances of inflammation and infection (World Health Organization, 2011). Since both depression and obesity can be proinflammatory pathologies, the increased serum ferritin concentrations observed in the obesity group and its association with depression are consistent with current literature.

To reiterate, bodies of research have observed a relationship between iron deficiency and depression, iron deficiency and obesity, and depression and obesity. These relationships can be visually observed Chapter One, in Figure 1.5.1.1, a Venn diagram showing the inter-relationships between the three conditions. This research study aimed to concurrently explore the relationships among these three conditions within a young adult South African population. Much like the current bodies of research, this study assessed the relationships between iron deficiency and depression, and iron deficiency and obesity. However, this study also investigated the association between obesity and iron deficient participants experiencing moderate to severe depressive symptoms to provide more insight into the potential influences these three pathologies exert on each other.

2.11 Chapter summary

This chapter discussed the current literature concerning the major themes of this research study. The relationship between iron deficiency and depression was marked by iron's essential role in regular neuronal and glial metabolism (Georgieff, 2011), (Miller, 2013). It was also established that iron deficiency may exacerbate the burden of obesity through symptoms of fatigue and depression commonly associated with iron-deficient individuals (Monteiro, et al., 2018).. Moreover, a bidirectional link was discovered between depression and obesity, with the presence of one condition increasing the likelihood of developing the other (Luppino, et al., 2010) & (Milaneschi, et al., 2019). Lastly, poor sleep quality is a consistent symptom of depression and the relationship between these two conditions were discussed within a university student population (Nutt, et al., 2008) & (Murphy & Peterson, 2015). This chapter demonstrated that current literature has observed a relationship between iron deficiency and depression, iron deficiency and obesity, and depression and obesity. However, very few have sought to investigate these conditions concurrently.

Chapter 3

Research Methodology

3.1 Introduction

Chapter Three discusses the methodology utilised during this research study. A cross-sectional comparison study design was employed to compare the depressive symptoms and obesity measures between iron-deficient and iron-replete participants to address the primary objectives of this study. Sleep quality was an additional variable assessed in relation to depressive symptoms as a secondary objective. The participants comprised of students registered at the University of the Western Cape (UWC) that were aged between 18 – 52 years. The testing was conducted at UWC where the participants completed a MADRS questionnaire, had anthropometric measurements taken, and had 5 mL of venous blood drawn by a registered medical doctor. Any participants that presented with health challenges were issued a referral letter to either their general practitioner of choice, or to the campus doctor. All statistical analyses were conducted using the IBM Statistical Package for the Social Sciences version 27.

3.2 Research design

This study utilised a cross-sectional comparison study design with a control and examination group. Both standardised and general questionnaires were used to collect quantitative and qualitative data on each participant. The study's dependent variables comprised of depressive symptoms (using the MADRS and BDI to quantify the symptoms), anthropometric measures (comprising of the BMI, WC, WHR and WHtR measurements), and sleep quality. These variables were assessed concerning the iron status of each participant. This study aimed to investigate whether significant variance in depressive symptoms or body composition measurements can be explained by the variance observed in iron status. Therefore, the iron status remained the only independent variable.

An initial sample size calculation yielded a result of 64 participants using G*Power 3 software. This result was based on the Cohen Power Analysis formula at a significance level (α) of 0.05, a medium effect size of 0.3, and statistical power (1-β) of 0.80 (Faul, et al., 2007). These values were based on a similar study by Lee and Park. They aimed to assess the relationship between serum ferritin concentrations and depression in Korean male adults concerning the prevalence of obesity (Lee & Park, 2019).

In total, 71 participants were included in this study, and each were scheduled to arrive on campus for their measurements to be taken over three weeks. Eight of these participants were lost to follow-up and did not attend their allocated timeslots. Therefore, a total of 63 participants were included in this study. Each participant was emailed a link that provided access to the questionnaires they would need to complete before attending their appointments. This link included the MADRS, BDI, sleep quality, and a basic demographic questionnaire. During the meeting, each participant had their height (cm), weight (kg), waist circumference (cm) and hip circumference (cm) measured. These measurements allowed for the calculations of the BMI, WTH, and WHtR used to assess the participants' body compositions. Thereafter, each participant had 5 mL of whole venous blood drawn by a registered medical professional. These blood samples were delivered to PathCare, N1 City, Goodwood, where the iron profile was analysed. The three iron status parameters measured were serum iron, serum ferritin, and transferrin. These iron parameters allowed for the calculations of the TSAT and the TIBC, which were also used as iron status parameters in this study.

Of the 63 participants, five were initially excluded from this study due to the consumption of iron supplements throughout the data collection period. Furthermore, 12 participants could not have their iron status analysed due to laboratory technical difficulties. Due to this complication, the five participants initially excluded for iron supplementation were included in the study so as not to reduce further the sample number, which stood at 51 participants.

The 51 participants were then grouped according to their iron status, either iron-deficient or iron-replete. Iron deficiency was defined as having a ferritin concentration of less than 30 μg/L as well as a TSAT percentage of less than 20 % (PathCare Reference Laboratory, 2021), (Okan, et al., 2019), (Motonishi, et al., 2018) & (Portugal-Nunes, et al., 2020). Twenty-two participants comprised of the iron-deficient group (n =22), and 29 participants formed part of the iron-replete group (n = 29). The iron-deficient and iron-replete groups were then compared according to their depressive scores and obesity measures in order to meet the primary objectives of this study. For the secondary objective, the participants were grouped according to their depressive symptoms. The depressed (n = 24) and non-depressed (n = 27) groups were then compared against their sleep quality questionnaire results.

3.3 Research Setting

The study took place at UWC, with all participants being registered students at the University.

3.4 Participants and Sampling

The participants in this study included 51 adults aged 18-52 years. The study sample comprised of 11 males and 40 females (this demonstrated a male to female ratio of approximately 1:4). The participants were recruited online via a mass email sent out across the University, using its primary news bulletin, UWC Communication. The email stated the title of this research study and provided a brief description of what measurements would be conducted should they choose to participate. A link to the MADRS was also included in the email for the potential participant to complete should they decide to partake. A diagram demonstrating the sample screening process is shown in Figure 3.4.1.

3.5 Inclusion criteria

Registered students between the ages of 18-60 years old attending the University of the Western Cape were included in this research study.

3.6 Exclusion criteria

Participants that consumed iron supplements or multivitamins with a high ferrous content at least one week before their allocated timeslot were excluded from this research study.

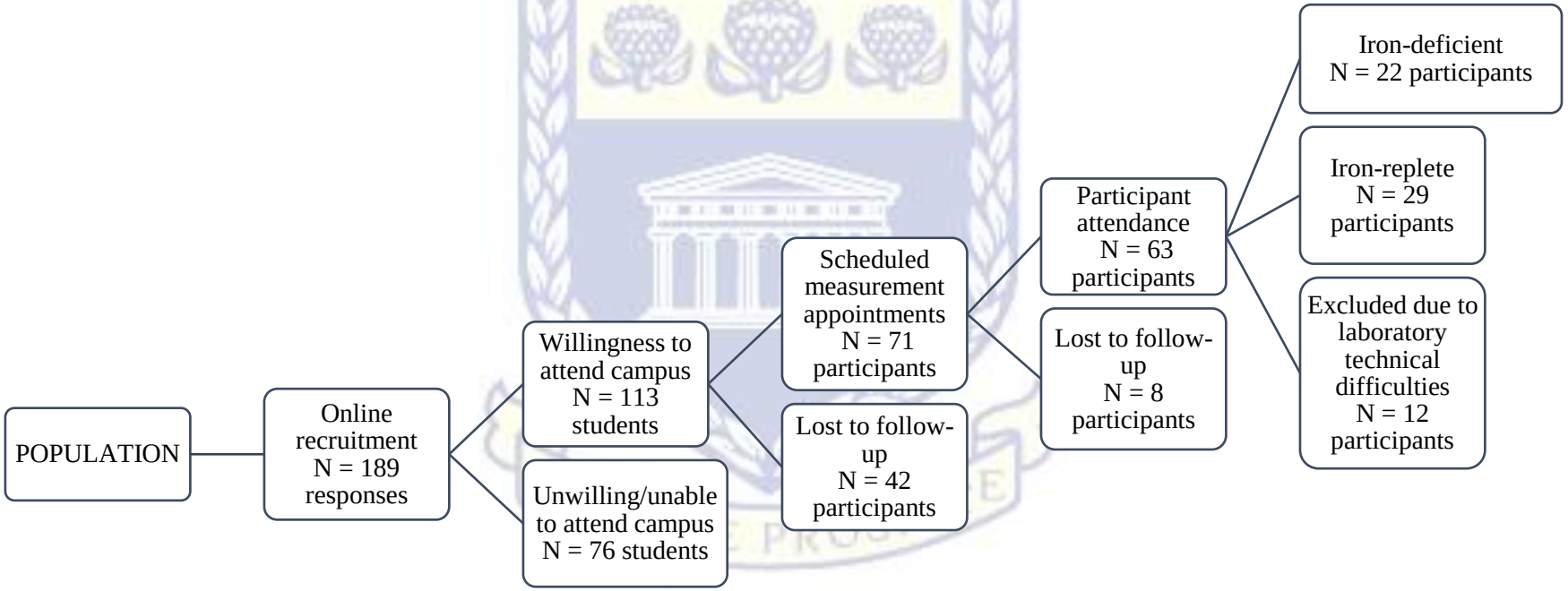


Figure 3.4.1: A schematic diagram showing the sample screening process.

3.7 The procedure

This section describes the process taken from the time of contact with the participant to arriving at the university to have their measurements taken.

3.7.1 Questionnaires

A student who had chosen to participate was sent an electronic copy of the information sheet that stated the nature of the study and what measurements would be conducted during the scheduled appointment after completing the MADRS and a basic demographic questionnaire attached to the invitation email. Additionally, the participant was sent a link to the supplementary questionnaires comprising of the BDI and sleep quality questionnaires. The demographic questionnaire questions can be found in Appendix A.

3.7.2 Montgomery-Asberg Depression Rating Scale (MADRS)

The MADRS is a depression rating scale constructed in 1979 by Montgomery and Asberg to sensitively record the effects of treatment the patient in question was undergoing at the time. It can differentiate between the patients responding to the treatment and those that are not (Montgomery & Asberg, 1979). The MADRS consists of 10 variables operationalised on four scale steps ranging from 0-6. This rating scale has clear, comprehensive definitions of its variables and scale steps related to its increased reliability (Svanborg & Asberg, 2001). The MADRS has been used by many studies to assess depressive symptoms (Knapskog, et al., 2011) & (Ntini, et al., 2020). For this research study, the participants that formed part of the depressed group had a MADRS score greater than 19, and the participants that comprised of the non-depressed group presented with MADRS scores of 19 and lower. A copy of the MADRS can be found in Appendix B.

The rating scale of the MADRS is as follows:

- 0-6: typical/absence of symptoms
- 7-19: mild depression
- 20-34: moderate depression
- >34: severe depression

3.7.3 Beck's Depression Inventory (BDI)

The BDI was constructed in 1961 by Beck et al. and was developed to provide a quantitative assessment of the intensity of depression and reflect changes in the intensity of depression after some time (Beck, et al., 1961). Several studies have widely authenticated the BDI, and three principal factors have become apparent. These include reflecting cognitive-affective symptoms and attitudes, impaired performance, and somatic symptoms (Svanborg & Asberg, 2001). The inventory consists of 21 variables describing four possible scale steps ranging from 0-3. No comprehensive definitions of the variables are given, and the patients can select more than one variable. However, the highest rating will be counted when the total score is calculated (Beck, et al., 1961). The total score calculated, which is the sum of all the answers, will determine the severity of the depressive symptoms experienced. The BDI was used in many studies to assess depressive symptoms (Shariatpanaahi, et al., 2007) & (Park, et al., 2020). The BDI was used as a secondary measure of depressive symptoms within this sample population. The participants that presented with BDI scores greater than 19 formed the depressed groups, and those with scores of 19 and lower formed part of the non-depressed group. A copy of the BDI can be found in Appendix C.

The rating scale of the BDI is as follows:

- 0-13: minimal depression
- 14-19: mild depression
- 20-28: moderate depression
- 29-63: severe depression

3.7.4 Sleep quality questionnaire

The sleep-quality questionnaire is used to address the secondary objective of this study, and therefore the participants were no longer grouped according to their iron status, but rather their depressive symptoms. The MADRS scale was used to quantify the symptoms and a depressed (n = 24) and non-depressed (n = 27) group was formed.

The sleep quality questionnaire also known as the Sleep Condition Indicator (SCI), was developed by Colin Espie, a professor of sleep medicine at the University of Oxford and co-founder of the sleep-education app, Sleepio (Huffington, 2017). The SCI was found to have

the potential to be a diagnostic tool for insomnia. This is a vital tool in this research study as insomnia and depression are closely related, suggesting overlapping neurobiology (Espie, et al., 2014) & (Benca & Peterson, 2008). This questionnaire consists of 9 questions that deal with participants' concerns on getting to sleep, remaining asleep, sleep quality, daytime personal functioning, daytime performance, duration of sleep problem, nights per week having a sleep problem and extent troubled by poor sleep (Espie, et al., 2014). The SCI has shown to reliably assess sleep quality and insomnia in patients (Lin, et al., 2020). A copy of the sleep-quality questionnaire can be found in Appendix D.

3.8 Data collecting stations

The participants were scheduled for one-hour appointments at the University, whereby anthropometric measurements and venipuncture occurred. A maximum of five participants were booked per hour. On arrival, each participant was provided with an informed consent form, MADRS questionnaire and a data collection sheet. The study and the measurements were summarised for each participant. They were asked to complete the MADRS questionnaire again to enable a real-time capturing of their current wellbeing. Once completed, the participant was directed to the first of two data collecting stations. A copy of the consent form and data collection sheet can be found in Appendices E and F, respectively.

3.8.1 Station one - Anthropometric measurements

The following anthropometric measurements were used to assess each participant's weight classification and metabolic risk factors.

3.8.1.1 Height and weight measurements

The height (cm) of the participant was measured using two fixed meter sticks, provided by the University of the Western Cape, that were attached to a wall, one on top of the other. The participant was instructed to remove their footwear and any head or hair accessories, excluding religious garments. Thereafter, they were to stand with their back against the meter stick, knees straight, feet together with their heels against the wall. The participant was to look straight ahead without tilting their head, breathe in and stand tall (World Health Organization, 2017). The height was read in centimetres by placing a ruler on the topmost part of the participant's head and reading the corresponding height on the meter stick. This process was repeated two additional times, and an average height was recorded.

The weight (kg) of the participant was measured using a portable digital weighing scale (Safeway, South Africa). Before the weighing occurred, the participant was instructed to

remove any jackets or heavy clothing and mobile phone and wallets from their pockets. Thereafter, the participant would step onto the scale with one foot on each side of the scale and was asked to stand still, face forward, keep their arms at their side and wait until asked to step off (World Health Organization, 2017). This process was repeated two additional times, and the average weight was recorded.

The BMI (kg/m^2) was calculated as the weight (kg) of the participant divided by the square of their height (m). Originally known as the Quetelet Index, BMI was developed by Adolphe Quetelet in 1832 after trends between weight and cardiovascular disease were investigated due to a rise in mortality of obese insurance policyholders. Adolphe created the index to define the characteristics of a typical man and fit this distribution around the norm in a general population. He concluded that other than periods of rapid growth occurring after birth and during puberty, weight increased as the square of the height (Eknoyan, 2008). It is important to note that while BMI is an easily obtained measure frequently used to categorise individuals into different nutritional statuses, it has significant limitations. It is not an accurate indicator of body fat mass percentage and is of more value as an indicator of cardiometabolic risk (Nuttall, 2015) & (Wells, 2014). The classification of overweight and obesity according to the BMI is shown in Table 3.8.1.1.1. A BMI of 25 – 29.9 kg/m^2 indicates overweight or pre-obesity, while a BMI of ⩾ 30 kg/m^2 reflects obesity. An increase in obesity classifications ranging from obesity class I to obesity class III indicates an increased risk of co-morbidities (World Health Organization, 1998). A BMI value of 30 kg/m^2 or greater was used to define obesity in the study population.

Table 3.8.1.1.1: The BMI classifications and their respective risk of co-morbidities for adults.

BMI (kg/m^2)	**Classification**	**Risk of co-morbidities**
< 18.5	Underweight	Low
18.5 – 24.9	Normal range	Average
25 – 29.9	Overweight	Increased
30 – 34.9	Obesity class I	Moderate
35 – 39.9	Obesity class II	Severe
⩾ 40	Obesity class III	Very severe

[1]

[1] BMI: Body Mass Index

3.8.1.2 Waist and hip circumference measurements

The participants' waist and hip circumferences (cm) were measured using a constant tension tape measure supplied by the University of the Western Cape. Regarding the waist circumference (WC) measurement, the participant was instructed to stand with their feet together and their weight evenly distributed between both feet. Additionally, the participant was to keep their arms relaxed and positioned on both sides and breathe normally. The measuring tape was then securely placed at the midpoint of the last palpable rib and the top of the hip bone, taking care not to cause skin compression. The measurement was read at the tape level to the nearest 0.1 cm (World Health Organization, 2017). This process was repeated two additional times, and the average WC was recorded. A consensus statement proposed by Ross et al. indicated that BMI measurements alone were not adequate in assessing or managing cardiometabolic risk associated with increased adiposity. They concluded that WC should be included in the assessment as it provides supplementary information for patient management and offers the best approach for arranging patients according to their risk (Ross, et al., 2020). Therefore, a WC of 94 cm or greater for males and 80 cm or greater for females was used to define central obesity and can be observed in Table 3.8.1.2.1.

Regarding the hip circumference (HC), the participant was instructed to stand with their feet and their weight evenly distributed across both feet and their arms relaxed at their sides. The constant tension measuring tape was then placed around the maximum circumference of the buttocks, taking care not to cause skin compression. The measurement was read at the tape level to the nearest 0.1 cm (World Health Organization, 2017). This process was repeated two additional times, and the average hip circumference was recorded.

The WHR was calculated by dividing the WC (cm) of the participant by their HC (cm). In 1985, Ashwell et al. examined 28 women using body composition measurements and tomography that found that the ratio of the intra-abdominal fat to the area of subcutaneous fat was significantly correlated to the ratio of the waist to hip circumference. It was also observed that metabolic complications, such as insulin resistance, hypertension, dyslipidaemia, and abdominal obesity, were related to a high ratio of waist to hip circumference (Ashwell, et al., 1985) & (Pedersen , 2013). Therefore, it was proposed that the waist-to-hip circumference ratio, the division of the WC by the hip circumference (HC), was related to metabolic complications through its association with intra-abdominal fat, and the WHR was developed (Ashwell, et al., 1985). The classifications of the WHR are shown in Table 3.8.1.2.1. A WC of ⩾ 94 cm for males and ⩾ 80 cm for females was indicative of an increased risk of metabolic

complications, including cardiovascular disease, type 2 diabetes, and hypertension. This increased risk was also associated with a WHR of ⩾ 0.9 cm for males and ⩾ 0.85 cm for females (World Health Organization, 2011). A WHR value of 0.9 cm for males and 0.85 cm was used to signify metabolic health risk within the study population.

Table 3.8.1.2.1: The classifications of the WC and WHR and their respective risk of metabolic complications.

Metabolic risk factors	**WC (cm)**		**WHR (cm)**	
	Males	Females	Males	Females
Low	< 94	< 80	-	-
High	94 – 102	80 – 88	-	-
Very high	> 102	> 88	⩾ 0.9	⩾ 0.85

[2, 3]

The WHtR, much like the WHR, was calculated by dividing the WC (cm) of the participant by their height (cm). Ashwell et al. conducted further research into body composition. They found that the WHtR, obtained through the division of the WC by the height, had the highest correlation with intra-abdominal fat compared to waist circumference, BMI and WHR. It was proposed that the WHtR was the best anthropometric measure of intra-abdominal fat for both men and women (Ashwell, et al., 1996). While most studies tended to use WC and WHR to indicate central obesity, WHtR has been proposed to predict visceral adipose tissues and can be used as a marker for early health risk. Ashwell and Gibson suggested that a boundary value of 0.50 may be a suitable indicator used to identify those at risk for conditions associated with central obesity (Ashwell & Gibson, 2016). Currently, the World Health Organization have not standardised the ranges for the WHtR. However, studies have found it to be a valuable screening tool when used together with other anthropometric indices such as BMI and WC, particularly in the screening of hypertension and obesity (Caminha, et al., 2017), (Rezende, et al., 2018) & (Ugochukwu, et al., 2020). According to the classifications of the WHtR, which are presented in Table 3.8.1.3.1, a value ⩾ 0.5 is indicative of an increased health risk. Subsequently, a value that is 0.6 ⩾ substantially increases that risk (Ashwell & Gibson, 2016). A WHtR value greater than 0.5 was used to signify general health risk within the study population.

[2] WC: Waist circumference
[3] WHR: Waist to Hip Ratio

Table 3.8.1.3.1: The classifications of the WHtR and its associated health risk.

WHtR	Health risk
< 0.5	No increased risk
0.5 to < 0.6	Increased risk
⩾ 0.6	Very high risk

[4]

3.8.2 Station two – Venepuncture for iron analysis

The following section describes the process of venepuncture used to obtain the blood samples needed for the iron analysis and the protocols used for this analysis. The samples were stored in a sealed ice box and were collected by a PathCare representative approximately three hours after it was drawn. This section also describes the iron status indicators used for analysis and their classifications.

3.8.2.1 Venepuncture site and blood draw

The participant was guided to the blood collection station, where they were instructed to sit and provide the doctor with their non-dominant arm. The incision site, located at the antecubital fossa of the forearm, was disinfected using a 70 % alcohol swab and allowed to dry for 30 seconds. The doctor sited the median cubital vein and applied a tourniquet approximately five finger widths above the venepuncture site. The participant was asked to form a fist before the doctor inserted the needle attached to an evacuated tube at a 30-degree angle (World Health Organization , 2010). The resulting blood was collected into a 5 mL gold-capped vacutainer and was delivered to PathCare N1 City, Goodwood, for analysis.

3.8.2.2 Iron status analysis

The three primary iron status indicators, namely serum iron, transferrin, and ferritin, were analysed using the Abbott Alinity m automated molecular diagnostic analyser (Abbott Laboratories, Chicago). Firstly, serum iron (µmol/L) was analysed using the Alinity c Iron Assay (Abbott Laboratories, Chicago), using direct colourimetric determination of iron without deproteinisation in human serum (Abbott, 2018). Secondly, transferrin (g/L) was analysed using the Alinity c Transferrin Assay to quantify transferrin in human serum (Abbott, 2018). Lastly, ferritin (µg/l) was analysed using the Alinity i Ferritin Assay, a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of ferritin in

[4] WHtR: Waist to Height Ratio

human serum (Abbott, 2018). Subsequently, the TSAT (%) was calculated by the division of serum iron by transferrin, and the TIBC (μmol/L) was calculated by multiplying transferrin by 22.50 (Pfeiffer & Looker, 2017) & (Podmore, et al., 2016).

3.8.2.3 Iron status indicators

The iron status indicators used allowed for an overall reflection of the iron status in a healthy population. Tables 3.8.2.3.1 and 3.8.2.3.2 indicate the reference ranges used in the classification of each iron indicator. These ranges were obtained from the PathCare reports containing each participant's results (PathCare Reference Laboratory, 2021).

Table 3.8.2.3.1: The reference ranges for serum iron, transferrin and TSAT and their respective classification.

Indicator classifications	**Reference ranges for serum iron, transferrin and TSAT**					
	Serum iron (µmol/L)		Transferrin (g/L)		TSAT (%)	
	Males	Females	Males	Females	Males	Females
Normal	12 – 31.00	9 – 30	1.74 – 3.64	1.8 – 3.82	20 – 55	16 – 50
Low	< 12	< 9	< 1.74	< 3.82	< 20	< 16
High	> 31	> 30	> 3.64	> 3.82	> 55	> 50
Critically low	< 7	< 5	-	-	< 16	< 12
Critically high	-	-	> 4	> 4	-	-

[5]

Table 3.8.2.3.2: The reference ranges for ferritin and TIBC and their respective classification.

Indicator classifications	**Reference ranges for ferritin and TIBC**			
	Ferritin (µg/L)		TIBC (µmol/L)	
	Males	Females	Males	Females
Normal	30 – 275	30 – 200	44.8 – 80.6	44.8 – 80.6
Low	< 30	< 30	< 44.8	< 44.8
High	> 275	>200	> 80.6	> 80.6
Critically low	< 15	< 15	-	-
Critically high	-	-	-	-

[6]

[5] TSAT: Transferrin Saturation
[6] TIBC: Total Iron Binding Capacity

3.9 Ethical considerations

Ethical clearance was obtained from the University of the Western Cape from both the UWC Senate Research Committee and the Biomedical Research Ethics Committee (Ethics reference number: BM21/9/15).

During the recruitment process, prospective participants were briefed on the nature of the study, the duration, what measurements will be taken during the data collection period, and its benefits. All questions and concerns were addressed via email, and an electronic copy of the information sheet was sent to each potential participant. Upon arrival, each participant was briefed on the study once more and informed that their participation was strictly voluntary. They could discontinue their involvement during the investigation if they wish. Anonymity was stressed throughout the brief. The participants were notified that their unique identification numbers would be used in place of their names throughout the study. Each participant signed a consent form before being directed to the data collection stations. All the information captured, including the MADRS questionnaire, consent forms and data collection sheets, were stored in locked filing cabinets and password-protected computers. All the information obtained was strictly used for data analysis.

Should the health of any of the participants be challenged, a referral letter to their general practitioner of choice or the campus doctor will be issued.

3.10 Data analysis

The data collected was analysed using the IBM Statistical Package for the Social Sciences version 27 (IBM SPSS™ Statistics 27 for Windows, Armonk, NY, USA: IMB Corp.). The sample population was not normally distributed; therefore, non-parametric tests were employed to analyse the data. The data analysis consisted of four main parts, namely the demographic, significant differences, continuous variables, and categorical variables assessment.

Frequencies were used to organise and present the demographic variables of the sample population in terms of their iron status. In addition, Kruskal-Wallis H tests were used to determine whether significant differences were observed among these variables between the iron-deficient and iron-replete groups. Thereafter, frequencies were used to display the prevalence of iron deficiency, depression, and obesity across the sample population in terms of sex. Kruskal-Wallis H tests were also used to identify significant differences between the two

groups. Next, descriptive statistics were employed to demonstrate the minimum, maximum, mean, and standard error values of the iron status indicators, depressive scales, and body composition measurements for both the iron-deficient and iron-replete participants.

Mann-Whitney U tests were used to assess the continuous variables of the MADRS and BDI depressive scales to determine whether there were significant differences among the scores between the iron-deficient and iron-replete participants. Similarly, Mann-Whitney U tests were used to determine whether significant differences existed among the BMI, WC, WHR, and WHtR continuous variable values between the two groups. Lastly, Kruskal-Wallis H tests were employed to assess significant differences among the sleep quality questionnaire results between the depressed and non-depressed participants.

The continuous variables were assessed as the first step in achieving the study's objectives. Spearman's rank-order correlation analyses were performed across the iron status indicators (serum iron, ferritin, transferrin, TSAT, and TIBC), the depressive scales (MADRS and BDI) and the body composition measurements (BMI, WC, WHR, and WHtR). Age was also included in this analysis. A linear regression analysis was performed to further assess any significance observed. Lastly, a Kendall's tau-b correlation analysis was run to assess the association among the depressive scoring between the depressed and non-depressed participants.

To end, an alternative way of achieving the study's objectives was to assess the relationships using categorical data. Fisher's Exact tests were used to determine the association between iron deficiency and depression; iron deficiency and obesity; obesity and iron-deficient participants experiencing moderate to severe depressive symptoms; and sleep quality and depressive symptoms. All analyses employed a confidence interval of 95 %, and a *p*-value of less than 0.05 indicated statistical significance.

3.11 Chapter summary

This chapter discussed the research design and setting procedures used to recruit the study participants and the tools used to assess their iron status, depressive symptoms, sleep quality, and body composition. The ethical considerations and the multiple tests used employed in the data analysis were also discussed.

Chapter 4

Results

4.1 Introduction

The main results of the study are presented in this chapter. This chapter begins with the presentation of the general characteristics of the sample population. Next, significant differences among the depressive scales and body composition measurements were compared between the iron-deficient and iron-replete participants using Mann-Whitney U tests. Thereafter, continuous variable analysis was performed among the iron indicators results, depressive scores, and body composition measurements, using Spearman's correlation tests. A Kendall-tau analysis was executed to assess the relationship among the sleep quality ordinal variables between the depressed and non-depressed participants. Lastly, categorical variable analyses were performed using Fisher's exact tests to understand the association between iron deficiency and depression; obesity and iron-deficiency in depressed participants; and sleep quality and depression.

4.2 Characteristics of the study population according to their iron status

In Table 4.2.1, the demographic profile of the sample study population according to their iron status is displayed. The sample comprised of 51 adults who were registered students from the University of the Western Cape. These participants consisted mainly of women (40 of 51, 78.43 %) and the majority of the sample were Black (26 of 51, 50.98 %), followed by Coloured (22 of 51, 43.14 %) participants. In addition, the largest proportion was 18-23 years of age (40 of 51, 78.43 %) in their first year of study (21 of 51, 41.18 %), not taking any iron supplements (46 of 51, 90.20 %), and without inflammatory conditions (30/51, 58.82 %). Figure 4.2.1 presents this information in a bar graph for visual interpretation.

Additionally, Table 4.2.1 also presents the p-values of the Kruskal-Wallis H tests used to identify and significant differences of the demographic variables between the iron-deficient and iron-replete groups. Those in the iron-deficient group were more likely to be women (p = 0.001) but no differences were found between the iron-deficient and iron-replete groups with regards to race, age, year of study, being on iron supplements, and having inflammatory conditions.

Table 4.2.1: Demographic variables of the study population according to their iron status.

Demographic variables		Iron-deficient		Iron-replete		P-value
		Frequency	Percentage	Frequency	Percentage	
Gender	Males	0	0.00 %	11	37.90 %	0.001
	Females	22	100.00 %	18	62.10 %	
Race	Black	10	45.50 %	16	55.20 %	0.403
	Coloured	10	45.50 %	12	41.40 %	
	Indian	0	0.00 %	1	3.40 %	
	White	1	4.50 %	0	0.00 %	
	Other	1	4.50 %	0	0.00 %	
Age group	18 – 23 years	18	81.80 %	22	75.90 %	0.569
	24 – 30 years	4	18.20 %	3	10.30 %	
	Over 31 years	0	0.00 %	4	13.80 %	
Year of study	First	8	36.40 %	13	44.80 %	0.706
	Second	4	18.20 %	4	13.80 %	
	Third	5	22.70 %	5	17.20 %	
	Fourth	2	9.10 %	2	6.90 %	
	Honours	1	4.50 %	3	10.30 %	
	Masters	1	4.50 %	2	6.90 %	
	PhD	1	4.50 %	0	0.00 %	
Iron supplements	Yes	2	9.10 %	2	6.90 %	0.803
	No	20	90.90 %	26	89.70 %	
	Unknown	0	0.00 %	1	3.40 %	
Inflammatory conditions	None	13	59.10 %	17	58.60 %	0.991
	Allergies	4	18.20 %	6	20.70 %	
	Polycystic ovary syndrome	1	4.50 %	0	0.00 %	
	Urinary tract infection	1	4.50 %	0	0.00 %	
	Sinusitis	0	0.00 %	1	3.40 %	
	Asthma	1	4.50 %	2	6.90 %	
	Psoriasis	0	0.00 %	1	3.40 %	
	Type 2 diabetes	0	0.00 %	1	3.40 %	
	Dermatitis	2	9.10 %	0	0.00 %	
	Unknown	0	0.00 %	1	3.40 %	
	Total	**22**	**100.00 %**	**29**	**100.00%**	

[7]

[7] Kruskal-Wallis H tests were used to generate the *p*-values for the demographic ordinal variables.

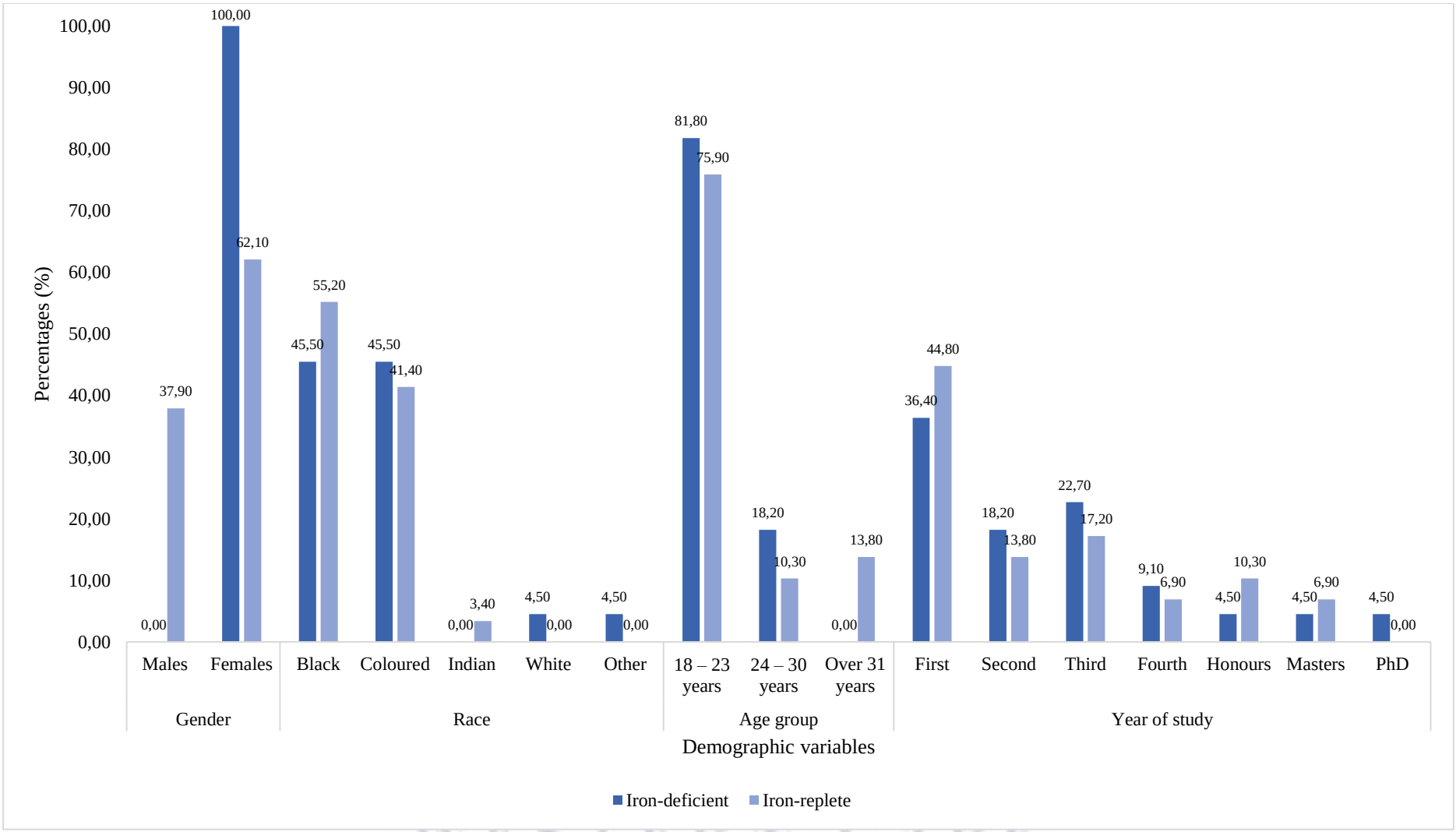


Figure 4.2.1: Bar graph presenting the percentage distribution of the demographic variables between the iron-deficient and iron-replete participants.

Three specific study variables were analysed during this study, namely iron deficiency, depression, and body composition. These variables were first assessed across the sample population in terms of sex and the results are presented in Table 4.2.2. A visual representation of the distribution of these conditions between the male and female participants can be seen in Figure 4.2.2. Regarding the male participants, the largest proportion comprised of iron replete (11/11, 100.00 %) and non-depressed (MADRS: 7/11, 63.60 %; BDI: 7/11, 63.60 %) individuals with the absence of obesity (8/11, 72.70 %), central obesity (8/11, 72.70 %), metabolic health risk (8/11, 72.70 %), and health risk (8/11, 72.70 %). Contrastingly, the largest proportion of female participants consisted of iron-deficient (22/40, 55.00 %) and mostly depressed individuals (MADRS: 20/20, 50.00 %; BDI: 19/40, 47.50 %), with the absence of obesity (31/40, 77.50 %), central obesity (25 of 40, 62.50 %), metabolic health risk (35 of 40, 87.50 %) and general health risk (25 of 40, 62.50 %). Table 4.2.2 also presents the *p*-values generated by Mann-Whitney U tests used to determine whether significant differences existed among iron deficiency, depression, and obesity between the male and female participants. As previously mentioned, the only significant difference between the two groups was their iron status ($p = 0.001$).

Table 4.2.2: The prevalence of iron deficiency, depression, and obesity among male and female participants.

Study variables	Categories	Males (N = 11)		Females (N = 40)		P-value
		N	%	N	%	
Iron status	Iron-deficient	0	0.00 %	22	55.00 %	0.001
	Iron-replete	11	100.00 %	18	45.00 %	
Depressive symptoms	Depressed (MADRS)	4	36.40 %	20	50.00 %	0.427
	Control	7	63.60 %	20	50.00 %	
	Depressed (BDI)	3	27.30 %	19	47.50 %	0.293
	Control	7	63.60 %	20	50.00 %	
	Unknown	1	9.10 %	1	2.50 %	
Body composition measures	Obese	3	27.30 %	9	22.50 %	0.743
	Control	8	72.70 %	31	77.50 %	
	Central obesity	3	27.30 %	15	37.50 %	0.534
	Control	8	72.70 %	25	62.50 %	
	Metabolic health risk	3	27.30 %	5	12.50 %	0.237
	Control	8	72.70 %	35	87.50 %	
	General health risk	3	27.30 %	15	37.50 %	0.777
	Control	8	72.70 %	25	62.50 %	
	Total	**11**	**100.00 %**	**40**	**100.00 %**	

8, 9, 10

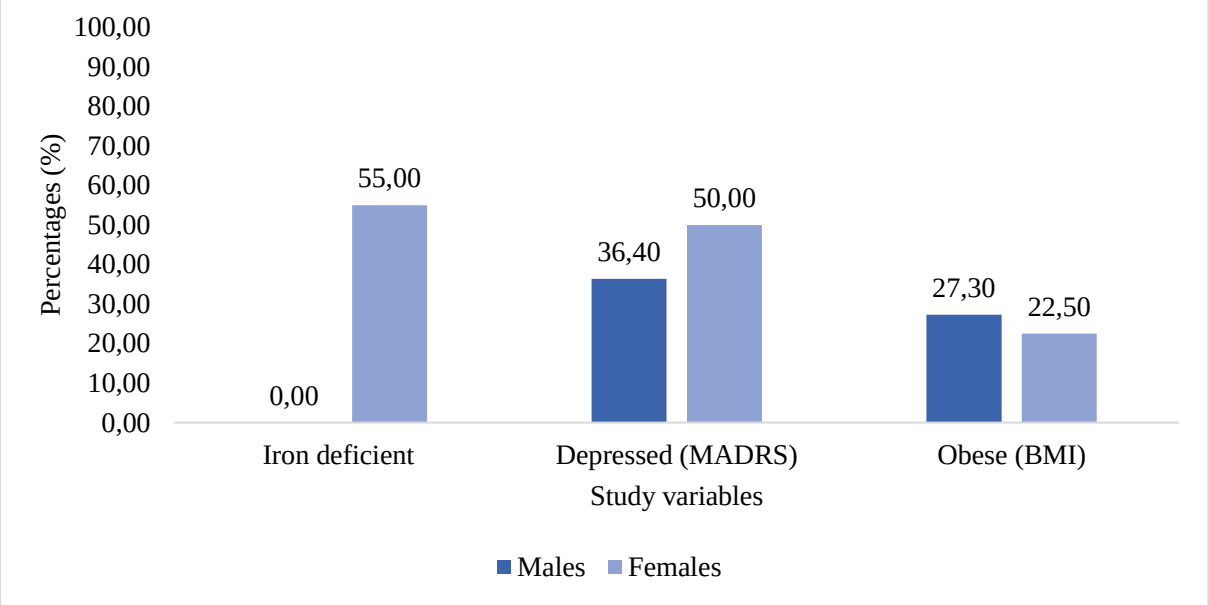


Figure 4.2.2: Bar graph presenting the percentage distribution of iron deficiency, depression, and obesity between the male and female participants.

[8] Kruskal-Wallis H tests were used to generate the *p*-values of the study's ordinal variables.
[9] MADRS: Montgomery-Asberg Depression Rating Scale
[10] BDI: Beck's Depression Inventory

Table 4.2.3 summarises the descriptive statistics (sample number, minimum, maximum, mean, and standard error (SE)) of the study variables (age, iron status indicators, depressive scales, and body composition measurements) and the age of the 22 iron-deficient (n = 22) participants. The mean age of the study population was approximately 21.050 years (SE = 0.673). Furthermore, the iron status indicators were individually assessed, and the mean concentrations of serum iron and transferrin were 7.636 µmol/L (Min. = 0.900 µmol/L; Max. = 17.400 µmol/L; SE = 1.015) and 3.561 g/L (Min. = 2.660 g/L; Max. = 4.320 g/L; SE = 0.096), respectively. Moreover, the mean TSAT, ferritin and TIBC concentrations were 8.680 % (Min. = 1.000 %; Max. = 19.000 %; SE = 1.133), 11.500 µg/L (Min. = 2.000 µg/L; Max. = 25.000 µg/L; SE = 1.437), and 81.009 µmol/L (Min. = 60.500 µmol/L; Max. = 98.300 µmol/L; SE = 2.195). Next, the depressive scales were individually assessed, and the mean MADRS and BDI scores were 21.230 (Min. = 2.000; Max. = 36.000; SE = 1.633), and 23.090 (Min. = 4.000; Max. = 45.000; SE = 2.389), respectively. Lastly, the body composition measurements were assessed, and the mean BMI and WC measurements were 24.012 kg/m^2 (Min. = 16.467 kg/m^2, Max. = 37.038 kg/m^2; SE = 1.101), and 75.367 cm (Min. = 56.800 cm; Max. = 105.333 cm; SE = 2.798), respectively. Additionally, the mean WHR and WHtR values were 0.053 (Min. = 0.630; Max. = 0.913; SE = 0.014) and 0.467 (Min. = 0.352; Max. = 0.642; SE = 0.018), respectively.

Table 4.2.3: The descriptive statistics of the age, iron status indicators, depressive scales and body composition measurement continuous variables for the iron-deficient participants.

Study variables	Variables	Sample number	Minimum	Maximum	Mean	Standard error
Age	Age	22	18.000	30.000	21.050	0.673
Iron status indicators	Serum iron	22	0.900	17.400	7.636	0.941
	Transferrin	22	2.660	4.320	3.561	0.096
	TSAT	22	1.000	19.000	8.680	1.133
	Ferritin	22	2.000	25.000	11.500	1.437
	TIBC	22	60.500	98.300	81.009	2.195
Depressive scales	MADRS	22	2.000	36.000	21.230	1.633
	BDI	22	4.000	45.000	23.090	2.389
Body composition measurements	BMI	22	16.467	37.038	24.012	1.101
	WC	22	56.800	105.333	75.367	2.798
	WHR	22	0.630	0.913	0.753	0.014
	WHtR	22	0.352	0.642	0.467	0.018

11, 12, 13, 14, 15, 16, 17, 18

Table 4.2.4 summarises the descriptive statistics (sample number, minimum, maximum, mean, and standard error (SE)) of the study variables (age, iron status indicators, depressive scales, and body composition measurements) and the age of the 29 iron-replete (n = 29) participants. The mean age of the study population was approximately 23.970 years (SE = 1.663). Furthermore, the iron status indicators were individually assessed, and the mean concentrations of serum iron and transferrin were 16.914 µmol/L (Min. = 7.100 µmol/L; Max. = 35.400 µmol/L; SE = 1.187) and 2.842 g/L (Min. = 2.230 g/L; Max. = 3.600 g/L; SE = 0.057), respectively. Moreover, the mean TSAT, ferritin and TIBC concentrations were 23.550 % (Min. = 10.000 %; Max. = 48.000 %; SE = 1.743), 97.000 µg/L (Min. = 9.000 µg/L; Max. = 400.000 µg/L; SE = 15.792), and 64.652 µmol/L (Min. = 50.700 µmol/L; Max. = 81.900

[11] TSAT: Transferrin Saturation
[12] TIBC: Total Iron Binding Capacity
[13] MADRS: Montgomery-Asberg Depression Rating Scale
[14] BDI: Beck's Depressive Inventory
[15] BMI: Body Mass Index
[16] WC: Waist Circumference
[17] WHR: Waist to Hip Ratio
[18] WHtR: Waist to Height Ratio

μmol/L; SE = 1.289). Next, the depressive scales were individually assessed, and the mean MADRS and BDI scores were 17.790 (Min. = 2.000; Max. = 53.000; SE = 2.069), and 18.190 (Min. = 2.000; Max. = 50.000; SE = 2.303), respectively. Lastly, the body composition measurements were assessed, and the mean BMI and WC measurements were 26.040 kg/m^2 (Min. = 19.148 kg/m^2, Max. = 41.070 kg/m^2; SE = 1.123), and 81.867 cm (Min. = 61.600 cm; Max. = 140.330 cm; SE = 3.633), respectively. Additionally, the mean WHR and WHtR values were 0.804 (Min. = 0.677; Max. = 1.064; SE = 0.020) and 0.503 (Min. = 0.364; Max. = 0.793; SE = 0.020), respectively.

Table 4.2.4: The descriptive statistics of the age, iron status indicators, depressive scales, and body composition measurement continuous variables for the iron-replete participants.

Study variables	Variables	Sample number	Minimum	Maximum	Mean	Standard error
Age	Age	29	18.000	52.000	23.970	1.663
Iron status indicators	Serum iron	29	7.100	35.400	16.914	1.187
	Transferrin	29	2.230	3.600	2.842	0.057
	TSAT	29	10.000	48.000	23.550	1.743
	Ferritin	29	9.000	400.000	97.000	15.792
	TIBC	29	50.700	81.900	64.652	1.289
Depressive scales	MADRS	29	2.000	53.000	17.790	2.069
	BDI	27	2.000	50.000	18.190	2.303
Body composition measurements	BMI	29	19.148	41.070	26.040	1.123
	WC	29	61.600	140.330	81.867	3.633
	WHR	29	0.677	1.064	0.804	0.020
	WHtR	29	0.364	0.793	0.503	0.020

19, 20, 21, 22, 23, 24, 25, 26

[19] TSAT: Transferrin Saturation
[20] TIBC: Total-Iron Binding Capacity
[21] MADRS: Montgomery-Asberg Depression Rating Scale
[22] BDI: Beck's Depressive Inventory
[23] BMI: Body Mass Index
[24] WC: Waist Circumference
[25] WHR: Waist to Hi Ratio
[26] WHtR: Waist to Height Ratio

4.3 Significant differences between the iron-deficient and iron-replete groups

4.3.1 Depressive symptom measures in the iron-deficient and iron-replete groups

Table 4.3.1.1 demonstrates the ranges of depressive symptoms and the number of iron-deficient and iron-replete participants within these specific ranges. According to the MADRS, the greatest proportion of iron-deficient participants presented with moderate depressive symptoms (12 of 22, 54.50 %), while the greatest proportion of iron-replete participants presented with mild depressive symptoms (13 of 29, 44.80%). This trend was reflected when using the BDI, where the majority of the iron-deficient participants also presented with moderate depressive symptoms (7 of 22, 31.80 %). However, unlike the MADRS, most of iron-replete participants had minimal depressive symptoms (10 of 29, 34.50 %) according to the BDI. Additionally, according to both the MADRS and BDI scales, the iron-deficient participants presented with higher mean depressive scores (MADRS – 21.23; BDI – 23.09) when compared to the iron-replete participants (MADRS – 17.79; BDI – 18.19). Despite this increase, no significant differences were observed between the MADRS and BDI scores of the iron-deficient and iron-replete participants.

The distributions of the MADRS and BDI depressive scores for the iron-deficient and iron-replete participants were similar, as assessed by visual inspection. The median MADRS depressive score for the iron-deficient participants (21.50) and the iron-replete participants (16.00) was not statistically significantly different, $U = 404.5$, $z = 1.628$, $p = 0.103$. Similarly, regarding the BDI, the median depressive score for the iron-deficient participants (22.50) and the iron-replete participants (15.00) was also not statistically significantly different, $U = 392$, $z = 1.911$, $p = 0.056$. These p-values are also presented in Table 4.3.1.1.

Table 4.3.1.1: The depressive symptom ranges, frequencies of their respective categories and the corresponding *p*-values observed in the iron-deficient and iron-replete groups.

	Depressive scale ranges	Iron-deficient (N = 22)				Iron-replete (N = 29)				P-value
		N	%	Mean	SD	N	%	Mean	SD	
Depressive symptoms	**MADRS**									
	Minimal	1	4.50 %	21.23	7.66	5	17.20 %	17.79	11.14	0.103
	Mild	8	36.40 %			13	44.80 %			
	Moderate	12	54.50 %			9	31.00 %			
	Severe	1	4.50 %			2	6.90 %			
	BDI									
	Minimal	4	18.20 %	23.09	11.20	10	34.50 %	18.19	11.97	0.056
	Mild	5	22.70 %			8	27.60 %			
	Moderate	7	31.80 %			5	17.20 %			
	Severe	6	27.30 %			4	13.80 %			
	Unknown	0	0.00 %			2	6.90 %			
	Total	**22**	**100.00%**			**29**	**100.00%**			

27, 28

4.3.2 Body composition measures in the iron-deficient and iron-replete groups

Table 4.3.2.1 demonstrates the ranges of specific body composition measurements and the number of iron-deficient and iron-replete group participants that fell within these ranges. Regarding the iron-deficient participants, the largest proportion of individuals had BMI (13 of 22, 59.10 %), WC (15 of 22, 68.20 %), WHR (20 of 22, 90.90 %), and WHtR (15 of 22, 68.20%) values that fell within the normal, low risk, low risk and no increased risk categories, respectively. This trend was also observed among the iron-replete participants, whereby the greatest proportion of individuals had BMI (18 of 29, 62.10 %), WC (18 of 29, 62.10 %), WHR (23 of 29, 79.30 %), and WHtR (18 of 29, 62.10 %) values that fell within the above-mentioned categories. In addition, the mean BMI (26.04 kg/m^2), WC (81.87 cm), WHR (0.80), and WHtR (0.50) values of the iron-replete participants were marginally higher compared to the mean BMI (24.01 kg/m^2), WC (75.37 cm), WHR (0.75), and WHtR (0.47) values of the iron-deficient participants. However, all four body composition measurements were not statistically

[27] Mann-Whitney U tests were used to generate the *p*-values for the MADRS (Montgomery-Asberg Depression Rating Scale) and BDI (Beck's Depression Inventory) continuous variables.
[28] SD: Standard Deviation

significantly different between the two groups and the p-values are also presented in Table 4.3.2.1.

The distributions of the WC measurements for the iron-deficient and iron-replete group participants were similar, as assessed by visual inspection. The median WC measurement for the iron-deficient participants (72.60 cm) and the iron-replete group participants (74.57 cm) was not statistically significantly different, $U = 268$, $z = -0.970$, $p = 0.332$. The distributions of the BMI, WHR and WHtR measurements for the iron-deficient and iron-replete group participants were not similar, as assessed by visual inspection. The BMI measurements for the iron-deficient participants (mean rank = 23.41 kg/m^2) and iron-replete group participants (mean rank = 27.97 kg/m^2) were not statistically significantly different, $U = 262$, $z = -1.084$, $p = 0.278$. Similarly, the WHR measurements for the iron-deficient (mean rank = 22.61 cm) and iron-replete group (mean rank = 28.57 cm) were also not statistically significantly different, $U = 244.5$, $z = -1.417$, $p = 0.156$. Lastly, the differences between the WHtR measurements of the iron-deficient (mean rank = 23.55 cm) and iron-replete participants (mean rank = 27.86 cm) were not statistically significant, $U = 265$, $z = -1.027$, $p = 0.304$.

Table 4.3.2.1: The body composition frequencies observed in the iron-deficient and iron-replete groups and their respective *p*-values.

	Body composition measurement ranges	**Iron deficient (N = 22)**				**Iron-replete (N = 29)**				**P-value**
		N	%	Mean	SD	N	%	Mean	SD	
Body composition measurements	**BMI**									
	Normal	13	59.10 %	24.01	5.16	18	62.10 %	26.04	6.05	0.278
	Overweight	5	22.70 %			2	6.90 %			
	Obesity class I	2	9.10 %			6	20.70 %			
	Obesity class II	1	4.50 %			2	6.90 %			
	Obesity class III	0	0.00 %			1	3.40 %			
	Underweight	1	4.50 %			0	0.00 %			
	WC									
	Low risk	15	68.20 %	75.37	13.13	18	62.10 %	81.87	19.56	0.332
	High risk	4	18.20 %			3	10.30 %			
	Very high risk	3	13.60 %			8	27.60 %			
	WHR									
	Low risk	20	90.90 %	0.75	0.07	23	79.30 %	0.80	0.11	0.156
	High risk	0	0.00 %			0	0.00 %			
	Very high risk	2	9.10 %			6	20.70 %			
	WHtR									
	No increased risk	15	68.20 %	0.47	0.08	18	62.10 %	0.50	0.11	0.304
	Increased risk	4	18.20 %			4	13.80 %			
	Very high risk	3	13.60 %			7	24.10 %			
	Total	**22**	**100.00 %**			**29**	**100.00 %**			

29, 30

[29] Mann-Whitney U tests were used to generate the *p*-values for the BMI (Body Mass Index), WC (Waist Circumference), WHR (Waist to Hip Ratio), and WHtR (Waist to Height Ratio) continuous variables.
[30] SD: Standard Deviation

4.3.3 Sleep quality measures in the depressed non-depressed groups

In order to meet the secondary objective of assessing the relationship between sleep quality and depression, the participants had to be grouped according to their depressive symptoms. The MADRS scale was used to quantify the depressive symptoms. Table 4.3.3.1 presents the results of the sleep quality questionnaire, as well as the number of depressed and non-depressed participants that fell within the specified categories. Regarding the depressed group (n = 24), the largest proportion of participants took longer than 60 minutes to fall asleep (12 of 24, 50.00 %), experienced problematic sleep approximately 6 – 7 times a week (10 of 24, 41.70 %), had average sleep quality (11 of 24, 45.80 %), and sometimes slept during the day (11 of 24, 45.80 %). Moreover, most depressed participants either sometimes or always felt drained after waking (9 of 24, 37.50 %), felt that poor sleep somewhat affected their mood, energy, or relationships (9 of 24, 37.50 %), and felt that poor sleep very much affected their concentration or productivity (8 of 24, 33.30%). In comparison, the non-depressed group (n = 27), the largest proportion of the participants took 15 – 30 minutes to fall asleep (10 of 27, 37.00 %), had problematic sleep for only 1 – 2 nights per week (11 of 27, 40.70 %), had average sleep quality (12 of 27, 44.40 %), and sometimes slept during the day (13 of 27, 48.10 %). Additionally, most non-depressed participants sometimes felt drained after waking (17 of 27, 63.00%), and felt that poor sleep somewhat affected their mood, energy (7 of 27, 25.90 %), or relationships, and their concentration or productivity (9 of 27, 33.30 %).

Table 4.3.3.1 also presents the *p*-values that signify whether a significant difference existed between the sleep quality questionnaire results of the depressed and non-depressed groups. As assessed by visual inspection, the distributions of the sleep quality questions were similar across both depressed and non-depressed participants. No significant differences were found among the time it takes to fall asleep ($p = 0.091$), the number of nights of problematic sleep ($p = 0.269$), daytime sleeping ($p = 0.770$), and whether poor sleep affects their mood, energy, or relationships ($p = 0.455$), and concentration or productivity ($p = 0.139$) results between the depressed and non-depressed participants. However, significant differences were observed among the sleep quality ($p = < 0.001$) and feeling drained ($p = 0.027$) results between the two groups.

Table 4.3.3.1: The results of the sleep quality questionnaire between the iron-deficient and iron-replete group participants and their respective *p*-values.

Sleep quality questions	Categories	Depressed (N = 24)		Non-depressed (N = 27)		P - value
		N	%	N	%	
How long does it take you to fall asleep?	15 – 30 minutes	4	16.70 %	10	37.00 %	0.091
	30 – 45 minutes	4	16.70 %	7	25.90 %	
	45 – 60 minutes	4	16.70 %	2	7.40 %	
	> 60 minutes	12	50.00 %	6	22.20 %	
	Unknown	0	0.0%	2	7.40 %	
How many nights a week do you have a problem with your sleep?	1 – 2 times	5	20.80 %	11	40.70 %	0.269
	3 – 4 times	6	25.00 %	6	22.20 %	
	4 – 5 times	3	12.50 %	2	7.40 %	
	6 – 7 times	10	41.70 %	5	18.50 %	
	Unknown	0	0.0%	3	11.1 %	
Rate your sleep quality from very good to very poor.	Very good	0	0.00 %	3	11.10 %	<0.001
	Good	0	0.00 %	8	29.60 %	
	Average	11	45.80 %	12	44.40 %	
	Poor	10	41.70 %	2	7.40 %	
	Very poor	0	0.00 %	0	0.00 %	
	Unknown	3	12.50 %	2	7.40 %	
Do you sleep during the day?	Never	7	29.20 %	7	25.90 %	0.770
	Sometimes	11	45.80 %	13	48.10 %	
	Mostly	3	12.50 %	3	11.10 %	
	Always	3	12.50 %	2	7.40 %	
	Unknown	0	0.00 %	2	7.40 %	
Do you feel drained after waking up?	Never	0	0.00 %	1	3.70 %	0.027
	Sometimes	9	37.50 %	17	63.00 %	
	Mostly	6	25.00 %	6	22.20 %	
	Always	9	37.50 %	1	3.70 %	
	Unknown	0	0.00 %	2	7.40 %	
Has poor sleep affected your mood, energy, or relationships?	Not at all	0	0.00 %	4	14.80 %	0.455
	A little	3	12.50 %	4	14.80 %	
	Somewhat	9	37.50 %	7	25.90 %	
	Much	7	29.20 %	6	22.20 %	
	Very much	5	20.80 %	4	14.80 %	
	Unknown	0	0.00%	2	7.40 %	
Has poor sleep affected your concentration or productivity?	Not at all	0	0.00 %	2	7.40 %	0.139
	A little	3	12.50 %	4	14.80 %	
	Somewhat	6	25.00 %	9	33.30 %	
	Much	7	29.20 %	8	29.60 %	
	Very much	8	33.30 %	2	7.40 %	
	Unknown	0	0.00 %	2	7.40 %	
Total		**24**	**100%**	**27**	**100.0%**	

[31]

[31] Kruskal-Wallis H tests were used to generate the *p*-values for the sleep quality questionnaire ordinal variables.

4.4 Correlation analysis of the iron status indicators, depressive scales, and body composition continuous variables

A Spearman's rank-order correlation was run to assess the relationships between all the study's continuous variables. Firstly, Table 4.4.1.1 presents the age and five iron indicator correlations against the continuous variables. Significant correlations among the age, WC, and WHtR values were then further assessed by linear regression analysis. Next, Table 4.4.2.1 displays the correlations of the MADRS and BDI depressive symptom scores against the study's continuous variables. The correlations of the four body composition measurements against the study's continuous variables were demonstrated in Table 4.4.3.1. A linear regression analysis assessed a significant correlation between the WHR measurements and ferritin concentration. Lastly, a Kendall's tau-b correlation analysis that was run to determine the associations between the responses obtained from the sleep quality questionnaire against the study's continuous variables. Significant correlations were observed between the sleep quality results and the MADRS and BDI scores, which is presented in Table 4.4.4.1.

4.4.1 Iron status indicators versus depressive scales and body composition measurements

Firstly, a Spearman's correlation was run to analyse the relationships between the five iron status indicators, serum iron, transferrin, TSAT, ferritin, and TIBC, against the age, iron status indicators, depressive symptoms, and body composition measurements. These results are presented in Table 4.4.1.1. All 51 participants were included in this analysis.

A preliminary analysis showed that all five iron status indicators had a monotonic relationship with one another, as assessed by visual inspection of a scatterplot. Furthermore, each iron status indicator had a statistically significant correlation with one another, $p = < 0.001$. There was a strongly negative correlation between the participants' serum iron (μmol/L) and transferrin (g/L) concentrations, r_s (49) = -0.496, $p = < 0.001$. Additionally, a strongly negative correlation was also observed between the serum iron, and TIBC (μmol/L) concentrations, r_s (49) = -0.495, $p = < 0.0005$. In contrast, a strongly positive correlation was found between serum iron concentrations and TSAT (%) percentages, r_s (49) = 0.967, $p = < 0.001$. Similarly, a statistically significant, strongly positive correlation was also observed between serum iron (μmol/L) and ferritin (μg/L) concentrations, r_s (49) = 0.651, $p = < 0.001$. Regarding transferrin, a strongly negative correlation was reported between transferrin concentrations and TSAT percentages, r_s (49) = -0.666, $p = < 0.001$, as well as transferrin and ferritin concentrations, r_s (49) = -0.693,

p = < 0.001. However, a strongly positive correlation was observed between transferrin and TIBC concentrations, r_s (49) = 1.000, p = < 0.001. In contrast to transferrin, there was a strongly negative correlation between TSAT and TIBC values, r_s (49) = -0.665, p = < 0.001, and a strongly positive correlation between TSAT and ferritin values, r_s (49) = 0.716, p = < 0.001. Lastly, a strongly negative correlation was reported between ferritin and TIBC concentrations, r_s (49) = -0.694, p = < 0.001.

Additional correlations among the other variables were also reported in Table 4.4.1.1. When evaluating the relationship between the age (years) of the participants and their respective WC (cm) measurements, a preliminary investigation showed the relationship to be monotonic, as assessed by visual inspection of a scatterplot. Furthermore, there was a statistically significant, positive correlation between the age of the participants and their WC measurement, r_s (49) = 0.277, p = 0.049. Similarly, a visual inspection of a scatterplot showed the relationship between the participants' age (years) and WHtR (cm) was also monotonic. There was a statistically significant, positive correlation between the age and the WHtR of the participants, r_s (49) = 0.287, p = 0.041.

Table 4.4.1.1: Spearman's rho correlation analysis of age and the iron status indicators against the study's continuous variables.

	Spearman's rho	Age	Serum iron	Transferrin	TSAT	Ferritin	TIBC
Age	Correlation Coefficient	1.000	0.240	-0.164	0.264	0.172	-0.163
	P-value	-	0.090	0.249	0.061	0.229	0.254
Serum iron	Correlation Coefficient	0.240	1.000	-0.496	0.967	0.651	-0.495
	P-value	0.090	-	< 0.001	< 0.001	< 0.001	< 0.001
Transferrin	Correlation Coefficient	-0.164	-0.496	1.000	-0.666	-0.693	1.000
	P-value	0.249	< 0.001	-	< 0.001	< 0.001	< 0.001
TSAT	Correlation Coefficient	0.264	0.967	-0.666	1.000	0.716	-0.665
	P-value	0.061	< 0.001	< 0.001	-	<0.001	< 0.001
Ferritin	Correlation Coefficient	0.172	0.651	-0.693	0.716	1.000	-0.694
	P-value	0.229	< 0.001	< 0.001	<0.001	-	< 0.001
TIBC	Correlation Coefficient	-0.163	-0.495	1.000	-0.665	-0.694	1.000
	P-value	0.254	< 0.001	< 0.001	< 0.001	< 0.001	-
MADRS	Correlation Coefficient	-0.079	-0.138	0.100	-0.151	-0.186	0.101
	P-value	0.583	0.334	0.483	0.289	0.192	0.481
BDI	Correlation Coefficient	-0.130	-0.150	0.161	-0.161	-0.168	0.161
	P-value	0.374	0.303	0.270	0.270	0.249	0.269
BMI	Correlation Coefficient	0.275	0.012	-0.090	0.038	0.182	-0.090
	P-value	0.051	0.932	0.531	0.792	0.200	0.528
WC	Correlation Coefficient	0.277	0.068	-0.121	0.095	0.253	-0.121
	P-value	0.049	0.633	0.398	0.506	0.073	0.396
WHR	Correlation Coefficient	0.236	0.185	-0.240	0.227	0.350	-0.240
	P-value	0.095	0.193	0.090	0.108	0.012	0.089
WHtR	Correlation Coefficient	0.287	0.104	-0.139	0.127	0.202	-0.140
	P-value	0.041	0.467	0.330	0.376	0.154	0.328

[32]

[32] Spearman's rho analysis of age, serum iron, TSAT (Transferrin Saturation), transferrin, ferritin and TIBC (Total Iron Binding Capacity) against age, serum iron, transferrin, TSAT, ferritin, TIBC, MADRS (Montgomery-Asberg Depression Rating Scale), BDI (Beck's Depression Inventory), BMI (Body Mass Index), WC (Waist Circumference), WHR (Waist to Hip Ratio) and WHtR (Waist to Height Ratio).

4.4.1.1 Linear regression analysis of the age and WC measurements of the participants

As mentioned, significant correlations were observed among all five iron status indicators with one another. This result was to be expected, and further analysis was not employed. However, a linear regression was run to assess further the relationship between the ages of the participants and their WC measurements. A scatterplot of the age (years) against the WC (cm) measurements with a superimposed regression line was plotted to assess the linearity as seen in Figure 4.4.1.1.1. Visual inspection of these two plots indicated a linear relationship between the two variables. There was homoscedasticity and normality in the residuals. One participant was an outlier with a WC measurement of 140.33 cm. However, they were not removed as their standardised residual value of 3.301 deviated slightly from the cut-off of 3 standard deviations.

The prediction equation was: WC = 47.540 + [1.388 x age (years)]. The age of the participants statistically significantly predicted the WC measurement, F (1, 49) = 24.383, p = < 0.001, accounting for 33.2 % of the variation observed in the WC, with an adjusted R^2 = 31.9 %, a medium size effect according to Cohen (Cohen, 1988). An increase of 1 year of age in results in a 1.388 cm (95% CI, 0.823 to 1.953) increase in WC.

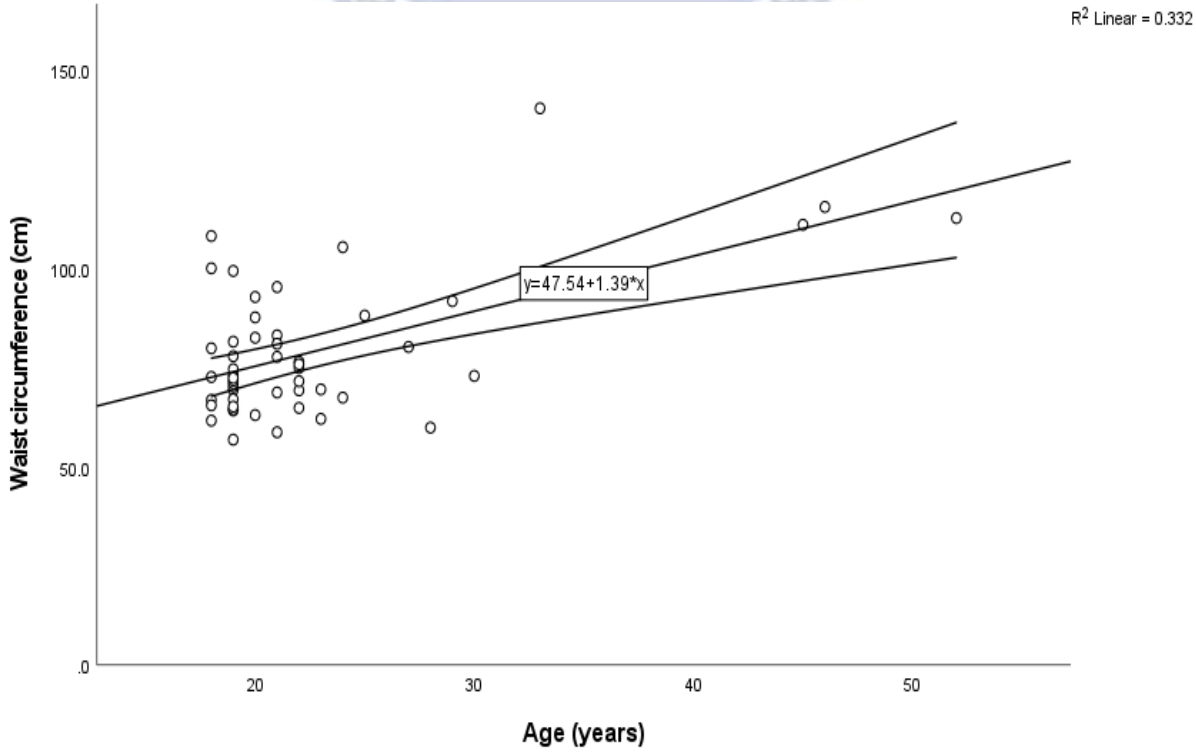


Figure 4.4.1.1.1: A scatterplot of the linear relationship between the age and WC measurements of the participants.

4.4.1.2 Linear regression analysis of the age and the WHtR measurements of the participants

Additionally, the relationship between the ages of the participants and their WHtR measurements was further analysed using linear regression. Figure 4.3.1.2.1 shows the scatterplot of WHtR (cm) against the age (years) with a superimposed regression line plotted to assess the linearity. Visual inspection of these two plots indicated a linear relationship between the two variables. There was homoscedasticity and normality of the residuals.

The prediction equation was: WHtR = 0.315 + [0.008 x age (years)]. The age of the participants statistically significantly predicted the WHtR measurement, F (1, 49) = 20.431, p = < 0.001, accounting for 29.4 % of the variation observed in the WHtR, with an adjusted R^2 = 28.0 %, a medium size effect according to Cohen (Cohen, 1988). An increase of 1 year of age in results in a 0.008 cm (95% CI, 0.009 to 0.011) increase in WHtR.

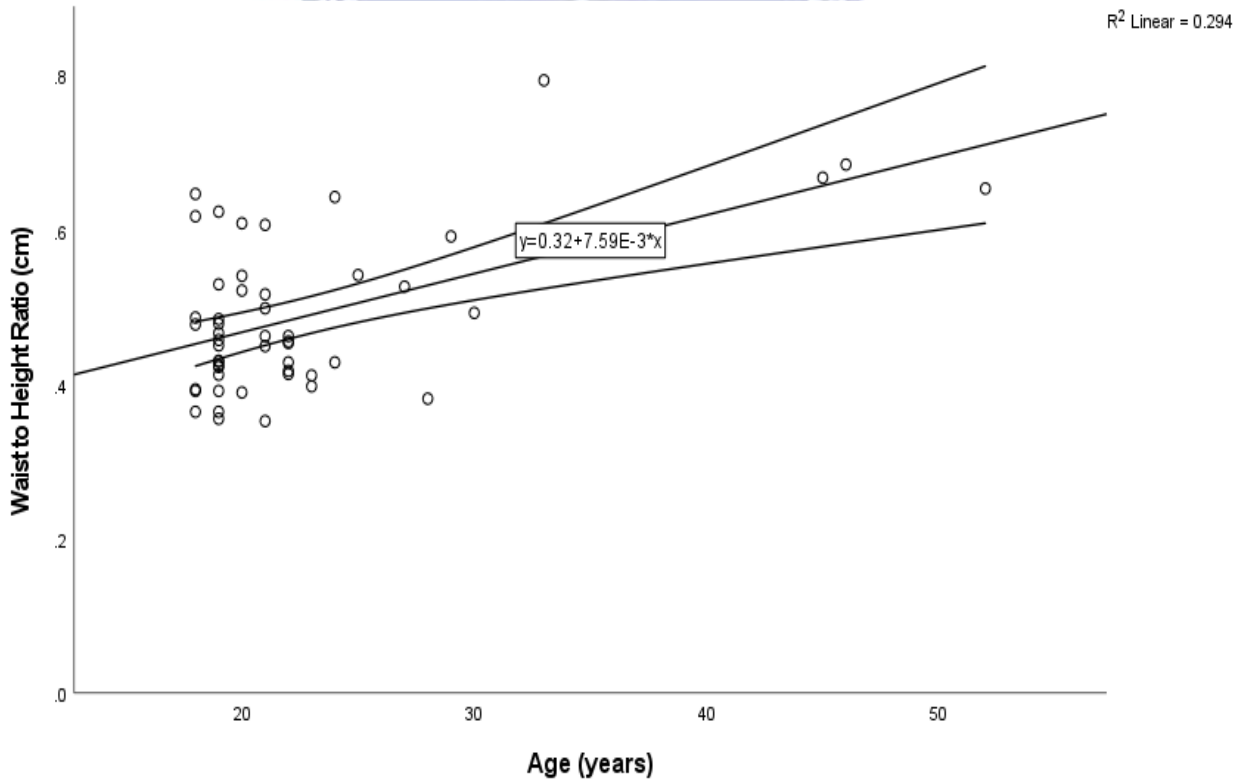


Figure 4.4.1.2.1: A scatterplot of the linear relationship between the age and WHtR measurements of the participants.

4.4.2 Depressive symptoms versus the iron status indicators and body composition measurements

Secondly, a Spearman's correlation was run to assess the relationships between the depressive scores of the MADRS and BDI questionnaires against the age, iron status indicators and body composition variables, as presented in Table 4.4.2.1. Forty-nine participants were included in this analysis.

A preliminary analysis showed the relationship between the MADRS and BDI scores to be monotonic, as assessed by visual inspection of a scatterplot. There was a statistically significant, strongly positive correlation between the MADRS and BDI scores, r_s (47) = 0.817, p = < 0.001. This result was the only considerable correlation observed. Furthermore, a significant correlation between the MADRS and BDI scores was expected, and further analysis was not employed.

Table 4.4.2.1: Spearman's rho correlation analysis results run on the MADRS and BDI scores against the age, iron status indicators, and body composition continuous variables.

	Spearman's rho	MADRS	BDI
Age	Correlation Coefficient	-0.079	-0.130
	P-value	0.583	0.374
Serum iron	Correlation Coefficient	-0.138	-0.150
	P-value	0.334	0.303
Transferrin	Correlation Coefficient	0.100	0.161
	P-value	0.483	0.270
TSAT	Correlation Coefficient	-0.151	-0.161
	P-value	0.289	0.270
Ferritin	Correlation Coefficient	-0.186	-0.168
	P-value	0.192	0.249
TIBC	Correlation Coefficient	0.101	0.161
	P-value	0.481	0.269
MADRS	Correlation Coefficient	1.000	0.817
	P-value	-	< 0.001
BDI	Correlation Coefficient	0.817	1.000
	P-value	< 0.001	-
BMI	Correlation Coefficient	-0.061	-0.067
	P-value	0.669	0.648
WC	Correlation Coefficient	-0.123	-0.090
	P-value	0.389	0.539
WHR	Correlation Coefficient	-0.105	-0.050
	P-value	0.464	0.732
WHtR	Correlation Coefficient	-0.046	-0.049
	P-value	0.750	0.737

[33]

[33] Spearman's rho analysis of the MADRS (Montgomery-Asberg Depression Rating Scale) and BDI (Beck's Depression Inventory) scores against age, serum iron, transferrin, TSAT, ferritin, TIBC, MADRS, BDI BMI (Body Mass Index), WC (Waist Circumference), WHR (Waist to Hip Ratio) and WHtR (Waist to Height Ratio).

4.4.3 Body composition measurements versus the iron status indicators and body composition measurements

Thirdly, a Spearman's rho correlation was run to analyse the relationships between the body composition measurements, including the BMI, WC, WHR and WHtR measurements, against the age, iron status indicators and depressive symptoms. These results are presented in Table 4.4.3.1. All 51 participants were included in this analysis.

As expected, each body composition measurement correlated with one another. A preliminary analysis showed the relationship between each measurement to be monotonic, as assessed by visual inspection of a scatterplot. Furthermore, each measurement had a statistically significant correlation with one another, $p = < 0.001$. A strongly positive correlation was reported between the BMI (kg/m^2) and WC (cm) measurements, r_s (49) = 0.891, $p = < 0.001$, as well as the BMI and WHR (cm) measurements, r_s (49) = 0.665, $p = < 0.001$. A strongly positive correlation was also reported between the BMI and WHtR (cm) measurements, r_s (49) = 0.923, $p = < 0.001$. Similarly, a strongly positive correlation was observed between the WC and WHR measurements, r_s (49) = 0.892, $p = < 0.001$, as well as the WC and WHtR measurements, r_s (49) = 0.954, $p = < 0.001$. Lastly, a strongly positive correlation was reported between the WHR and WHtR measurements, r_s (49) = 0.843, $p = < 0.001$.

An additional correlation was observed in Table 4.4.3.1. The relationship between the participants' WHR measurements and ferritin concentrations (µg/L) was analysed, and a preliminary investigation confirmed the relationship to be monotonic, as assessed by visual inspection of a scatterplot. A statistically significant, positive correlation was observed between WHR measurements and ferritin concentrations, r_s (49) = 0.350, $p = < 0.001$.

Table 4.4.3.1: Spearman's rho correlation analysis results run on the BMI, WC, WHR and WHtR measurements against the iron status indicators and depressive scales continuous variables.

	Spearman's rho	BMI	WC	WHR	WHtR
Age	Correlation Coefficient	0.275	0.277*	0.236	0.287
	P-value	0.051	0.049*	0.095	0.041
Serum iron	Correlation Coefficient	0.012	0.068	0.185	0.104
	P-value	0.932	0.633	0.193	0.467
Transferrin	Correlation Coefficient	-0.090	-0.121	-0.240	-0.139
	Sig. (2-tailed)	0.531	0.398	0.090	0.330
TSAT	Correlation Coefficient	0.038	0.095	0.227	0.127
	P-value	0.792	0.506	0.108	0.376
Ferritin	Correlation Coefficient	0.182	0.253	0.350	0.202
	P-value	0.200	0.073	0.012	0.154
TIBC	Correlation Coefficient	-0.090	-0.121	-0.240	-0.140
	P-value	0.528	0.396	0.089	0.328
MADRS	Correlation Coefficient	-0.061	-0.123	-0.105	-0.046
	P-value	0.669	0.389	0.464	0.750
BDI	Correlation Coefficient	-0.067	-0.090	-0.050	-0.049
	P-value	0.648	0.539	0.732	0.737
BMI	Correlation Coefficient	1.000	0.891	0.665	0.923
	P-value	-	<0.001	<0.001	<0.001
WC	Correlation Coefficient	0.891	1.000	0.892	0.954
	P-value	<0.001	-	<0.001	<0.001
WHR	Correlation Coefficient	0.665	0.892	1.000	0.843
	P-value	<0.001	<0.001	-	<0.001
WHtR	Correlation Coefficient	0.923	0.954	0.843	1.000
	P-value	<0.001	<0.001	<0.001	-

[34]

[34] Spearman's rho analysis of the BMI (Body Mass Index), WC (Waist Circumference), WHR (Waist to Hip Ratio) and WHtR (Waist to Height Ratio) measurements against age, serum iron, transferrin, TSAT, ferritin, TIBC, MADRS (Montgomery-Asberg Depression Rating Scale), BDI (Beck's Depression Inventory), BMI, WC, WHR and WHtR.

4.4.3.1 Linear regression analysis of the ferritin concentrations and the WHR measurements of the participants

As mentioned previously, each of the four body composition measurements significantly correlated with one another. This result was to be expected, and no further analysis was employed. However, a linear regression was run to understand further the relationship between the ferritin concentrations of the participants and their WHR measurements. Figure 4.4.3.1.1 presents the scatterplot of WHR (cm) against ferritin concentrations (µg/L) with a superimposed regression line plotted to assess the linearity. Visual inspection of these two plots indicated a linear relationship between the two variables. There was homoscedasticity and normality of the residuals. One participant was one outlier with a WHR value of 1.047. However, they were not removed as their standardised residual value of 3.051 deviated slightly from the cut-off of 3 standard deviations.

The prediction equation was: WHR = 0.751 + [0.001 x ferritin concentration (µg/L)]. The ferritin concentration statistically significantly predicted the WHR, $F(1, 49) = 10.805$, $p = 0.002$, accounting for 18.1% of the variation observed in the WHR, with an adjusted $R^2 = 16.4\%$, a medium size effect according to Cohen (Cohen, 1988). An increase of 1 µg/L in ferritin concentration results in a 0.001 (95% CI, < 0.0005 to 0.001) increase in WHR.

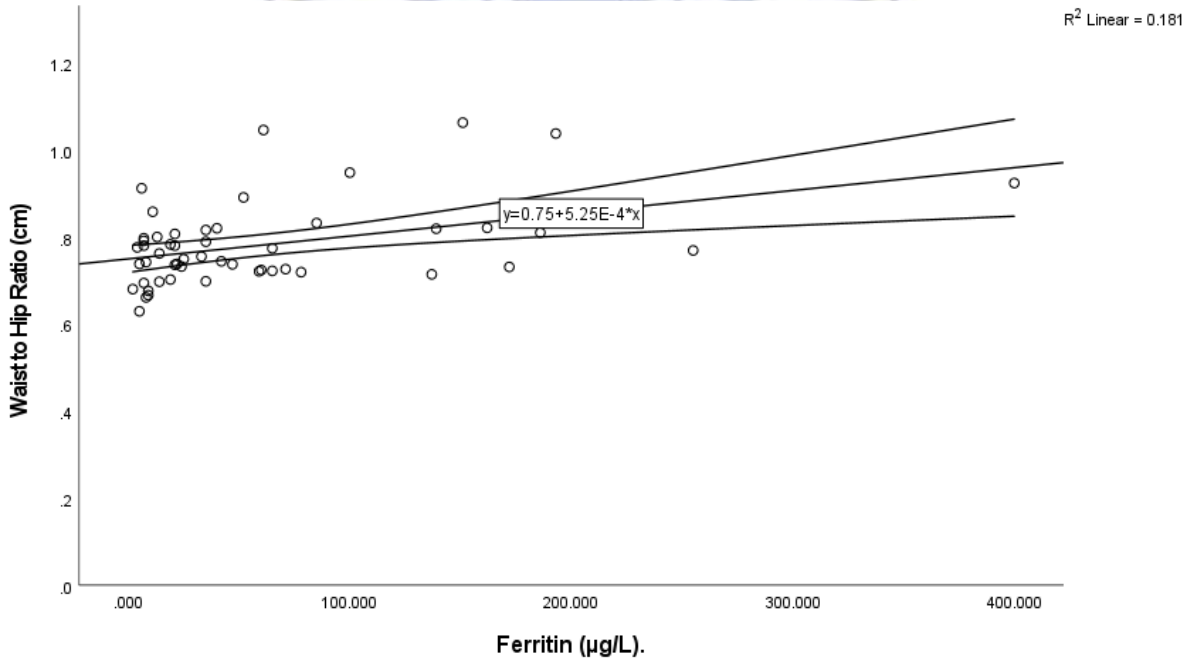


Figure 4.4.3.1.1: A scatterplot of the linear relationship between the ferritin concentrations and the WHR measurements of the participants.

4.4.4 Sleep quality versus depression symptoms

Significant results were reported between the sleep quality questions and the depressive scores of both the MADRS and BDI questionnaires, observed in Table 4.4.4.1. When a Kendall tau-b correlation was run to determine the relationship between the time it takes for participants to fall asleep and their BDI scores, a statistically significant, strongly positive association between the two variables was observed, $\tau_b = 0.316$, $p = 0.004$. A statistically significant, positive correlation was observed between the number of nights the participants had sleep problems and their MADRS score, $\tau_b = 0.213$, $p = 0.049$. While a statistically significant, strongly positive correlation was reported between the number of nights of problematic sleep and the BDI score, $\tau_b = 0.320$, $p = 0.004$. Additionally, statistically significant, strongly positive correlations were observed between the participants' sleep quality and both their MADRS, $\tau_b = 0.399$, $p = < 0.001$ and BDI scores, $\tau_b = 0.595$, $p = < 0.001$. Regarding feeling drained after waking, a statistically significant, strongly positive correlation was reported between this feeling and the MADRS, $\tau_b = 0.328$, $p = < 0.001$ and BDI scores, $\tau_b = 0.431$, $p = < 0.001$ of the participants. A statistically significant, strongly positive correlation was observed between the poor sleep affecting relationships question and the BDI score, $\tau_b = 0.360$, $p = 0.001$. Lastly, statistically significant, strongly positive correlations were reported between the poor sleep affecting concentration question and both the MADRS, $\tau_b = 0.309$, $p = 0.004$ and BDI scores, $\tau_b = 0.505$, $p = < 0.001$.

Table 4.4.4.1: Kendall's tau-b correlation analysis on the MADRS and BDI scores against the sleep quality questionnaire results.

Sleep quality questionnaire	Kendall's tau-b	MADRS	BDI
How long does it take you to fall asleep?	Correlation Coefficient	0.181	0.316
	P-value	0.095	0.004
How many nights a week do you have a problem with your sleep?	Correlation Coefficient	0.213	0.320
	P-value	0.049	0.004
Rate your sleep quality from very good to very poor.	Correlation Coefficient	0.399	0.595
	P-value	< 0.001	< 0.001
Do you sleep during the day?	Correlation Coefficient	-0.097	-0.024
	P-value	0.378	0.830
Do you feel drained after waking up?	Correlation Coefficient	0.328	0.431
	P-value	0.003	< 0.001
Has poor sleep affected your mood, energy, or relationships?	Correlation Coefficient	0.131	0.360
	P-value	0.221	0.001
Has poor sleep affected your concentration or productivity?	Correlation Coefficient	0.309	0.505
	P-value	0.004	< 0.001

35, 36

4.5 Categorical data analysis

Fisher's exact tests were run to analyse the relationships between the categorical data. The analysis explored the relationships between iron deficiency and depression and iron deficiency and obesity. Next, tests were run to assess the relationship between obesity and iron deficiency in depression. Lastly, the association between sleep quality and depression was examined.

4.5.1 Iron deficiency versus depression

Iron deficiency (ferritin < 30 µg/L; TSAT < 20.00 %), and depression, (MADRS > 19), were assessed in 51 participants. As discussed, 22 (43.10 %) participants were iron-deficient, and 29 (56.90 %) were iron-replete of the total study population. The results are presented in Table 4.5.1.1 and Figure 4.5.1.1. Of those iron-deficient participants, 13 (59.10 %) were depressed, and 9 (40.90 %) had mild to minimal depressive symptoms. Regarding the iron-replete participants, 11 (37.80 %) were depressed, and 18 (62.10 %) had only mild to minimal depressive symptoms. As assessed by Fisher's exact test, there was no statistically significant

[35] MADRS: Montgomery-Asberg Depression Rating Scale
[36] BDI: Beck's Depression Inventory

association between iron deficiency and depression, $p = 0.164$. A Fisher's exact test was rerun in assessing iron deficiency and depression, BDI > 19. However, the depression scale used was the BDI, and 49 participants were included in this analysis. Much like the previous analysis, 13 (59.10 %) of the iron-deficient participants were depressed, and 9 (40.90 %) had mild to minimal depressive symptoms. Regarding the iron-replete participants, 9 (33.30 %) were depressed, and 18 (66.70 %) had only mild to minimal depressive symptoms. Again, there was no statistically significant association between iron deficiency and depression, as assessed by Fisher's exact test, $p = 0.090$. Based on the research question of whether a correlation exists between iron deficiency and depressive symptoms in students residing in the Western Cape, no association exists between the two variables in terms of categorical data. As a result, we cannot reject the null hypothesis and cannot accept the alternative hypothesis.

Table 4.5.1.1: The association between iron status and depressive symptoms using Fisher's Exact tests.

Depressive scales	**Depressive symptoms**	**Iron-deficient (N = 22)**		**Iron-replete (N = 29)**		**P-value**
		N	%	N	%	
MADRS	Depressed	13	59.10 %	11	37.80 %	0.164
	Non-depressed	9	40.90 %	18	62.10 %	
BDI	Depressed	13	59.10 %	9	33.30 %	0.090
	Non-depressed	9	40.90 %	18	66.70 %	

37, 38, 39

[37] A Fisher's Exact test was used to generate the *p*-values of the iron status and depressive symptom categorical variables
[38] MADRS: Montgomery-Asberg Depression Rating Scale
[39] BDI: Beck's Depression Inventory

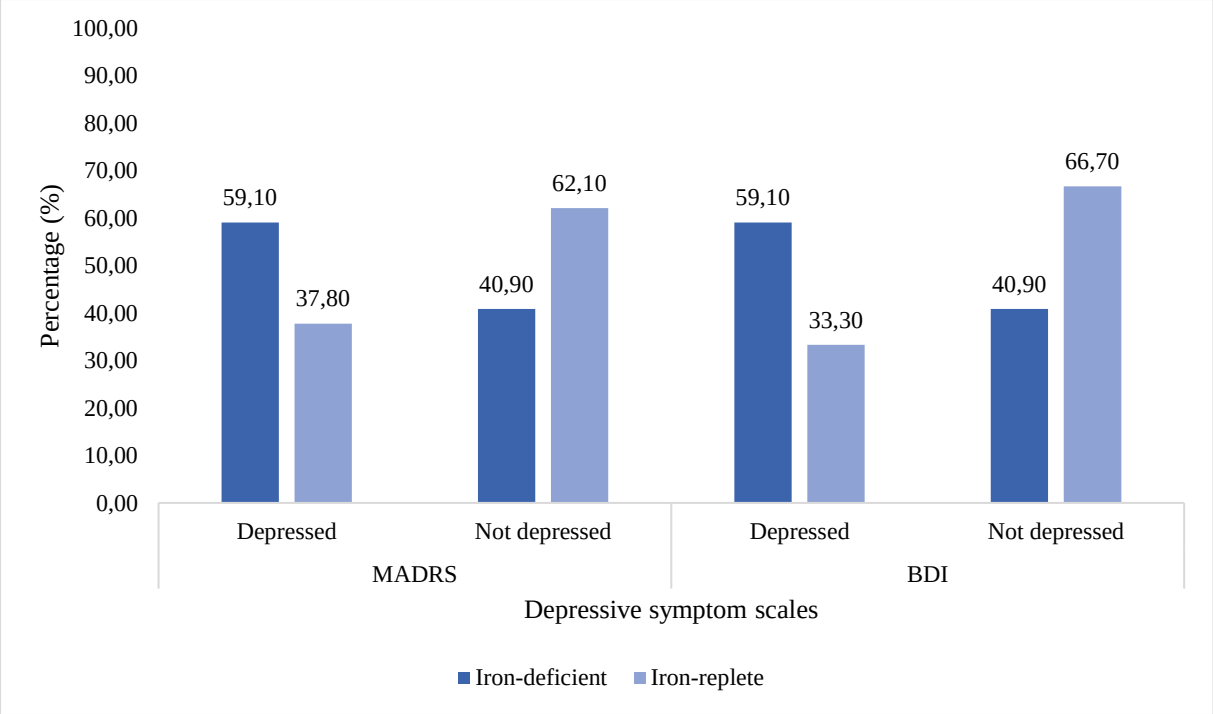


Figure 4.5.1.1: Bar graph representing the percentage distribution of the participants based on their iron status and depressive symptoms.

4.5.2 Iron deficiency versus obesity

Next, iron deficiency and obesity, BMI ⩾ 30.0 kg/m^2, were assessed in all 51 participants. As previously discussed, 12 (23.50 %) of the participants were considered obese, and 39 (76.50 %) had lower BMI values within typical ranges. The results are presented in Table 4.5.2.1 and Figure 4.5.2.1. Of those who were iron-deficient, 3 (13.60 %) participants were considered obese, while the remaining 19 (86.40 %) iron-deficient participants had lower BMI values. Regarding the iron-replete participants, 9 (31.00 %) fell within the obese range, and 20 (69.00 %) had lower BMI values. As assessed by Fisher's exact test, there was no statistically significant association between iron deficiency and obesity marked by BMI values, $p = 0.192$. The relationship between iron deficiency and obesity was also assessed using WC (cm) measurements in the definition of central obesity. When considering the iron-deficient participants, 7 (31.80 %) were centrally obese, while 15 (68.20 %) had typical WC measurements. In contrast, 11 (37.90 %) of the iron-replete participants were centrally obese, while the remaining 18 (62.10 %) participants were not. As assessed by Fisher's exact test, there was no association between iron deficiency and central obesity, $p = 0.770$.

Table 4.5.2.1: The association between iron status and obesity measures using Fisher's Exact tests.

Obesity measures	Obesity ranges	Iron-deficient (N = 22)		Iron-replete (N = 29)		P-value
		N	%	N	%	
BMI	Obese	3	13.69 %	9	31.00 %	0.192
	Absence of obesity	19	86.40 %	20	69.00 %	
WC	Central obesity	7	31.80 %	11	37.90 %	0.770
	Absence of central obesity	15	68.20 %	18	62.10 %	

40, 41, 42

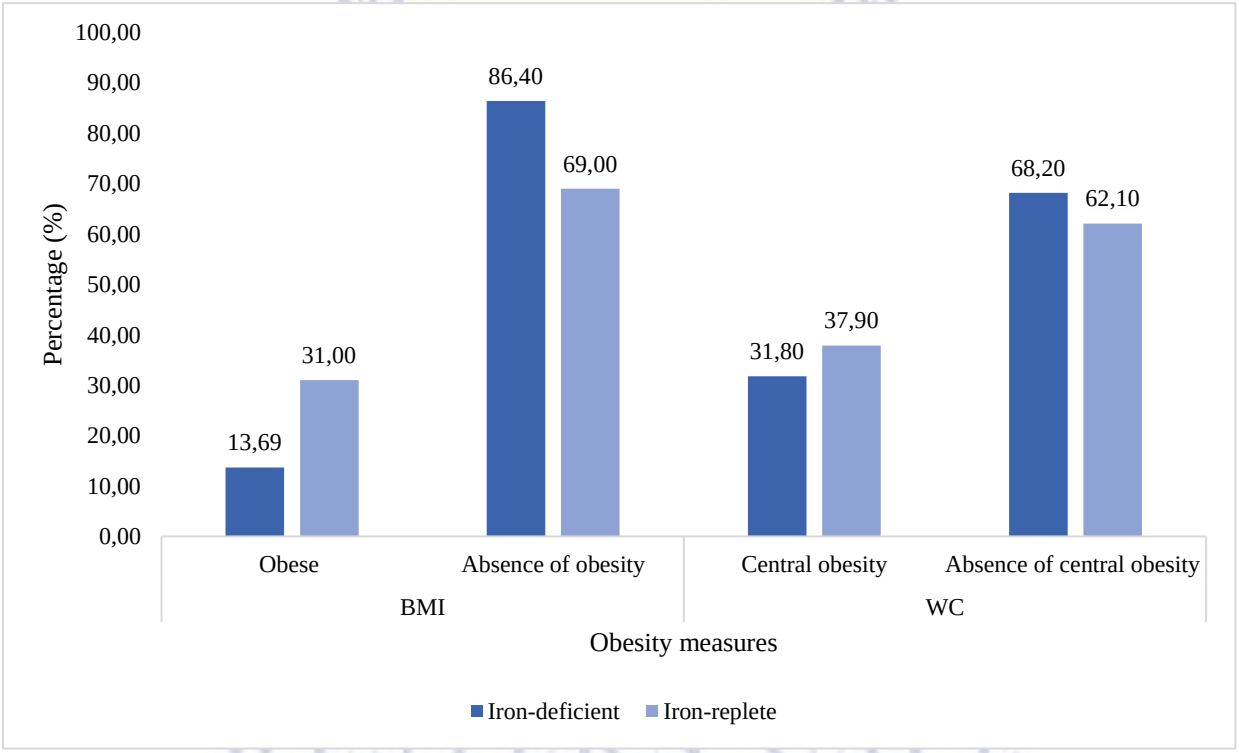


Figure 4.5.2.1: Bar graph representing the percentage distribution of the participants based on their iron status and obesity measures.

[40] A Fisher's Exact test was used to generate the p-values of the iron status and obesity measure categorical variables
[41] BMI: Body Mass Index
[42] WC: Waist Circumference

4.5.3 Obesity versus iron deficiency and depression

Subsequently, iron deficiency, depression, and obesity were assessed in all 51 participants to determine whether obesity was associated with iron deficiency and increased depressive symptoms. These results can be observed in Table 4.5.3.1. For the first analysis, obesity was defined according to the BMI values of the participants, and depression was determined using the MADRS scores. Of the 12 participants deemed obese, 2 (16.70 %) were both iron-deficient and depressed, and the remaining 10 (83.30 %) participants were iron-replete and had mild to minimal depressive symptoms. When assessing the participants with average BMI values, 11 (28.20 %) were both iron-deficient and depressed, while the remaining 28 (71.80 %) participants were iron-replete and had mild to minimal depressive symptoms. As assessed by Fisher's exact test, there was no statistically significant association between obesity and iron deficiency in depression, $p = 0.706$.

Furthermore, the relationship between obesity and iron deficiency in depression was analysed using the BDI scores of the participants as the depressive symptom assessor. Forty-nine participants were included in this analysis, and the results are presented in Table 4.5.3.1. Of the 11 participants who were deemed obese, 2 (18.20 %) were both iron-deficient and depressed, and 9 (81.80 %) were iron-replete with mild to minimal depressive symptoms. The 38 participants that did not fall within the obesity category comprised 11 (28.90 %) of those with iron deficiency and depression and 27 (71.10 %) of those iron-replete with typical depressive symptoms. As assessed by Fisher's exact test, there was no statistically significant association between obesity and iron deficiency in depression, $p = 0.703$.

For the second analysis of obesity and iron deficiency in depression, the WC measurements of the participants were utilised in the definition of central obesity and their MADRS scores in the assessment of depressive symptoms. All 51 participants were included in this analysis and are presented in Table 4.5.3.1. Eighteen participants were considered centrally obese altogether, with 4 (22.20 %) of these participants being iron-deficient and depressed, while 14 (77.80 %) were iron-replete with mild to minimal depressive symptoms. Of the 33 participants with healthy WC measurements, 9 (27.30 %) were iron-deficient and depressed, compared to the 24 (72.70 %) participants who were not. As assessed by Fisher's exact test, there was no statistically significant association between obesity and iron deficiency in depression, $p = 0.750$.

The relationship was then considered using the BDI scores as the assessor of depressive symptoms, and 49 participants were included in this analysis which is presented in Table 4.5.3.1. Of the 17 participants categorised as having central obesity, 4 (23.50 %) were iron-deficient and depressed, compared to the 13 (76.50 %) iron-replete participants with mild to minimal depressive symptoms. Regarding the 32 participants with low-risk WC measurements, 9 (28.10 %) were iron deficient and depressed, while 23 (71.90 %) were iron-replete and had mild to minimal depressive symptoms. As assessed by Fisher's exact test, there was no statistically significant association between obesity and iron deficiency in depression, p = 1.000. Based on the research question of whether an association exists between obesity and iron-deficient students experiencing moderate to severe depressive symptoms, no association exists between the two variables. As a result, we cannot reject the null hypothesis and cannot accept the alternative hypothesis.

Table 4.5.3.1: The association between iron status, depressive symptoms and obesity measures using Fisher's Exact tests.

		Depressive scales								
		MADRS					BDI				
Obesity measures	Obesity categories	Iron-deficient and depressed		Iron-replete and no depression		P-value	Iron-deficient and depressed		Iron-replete and no depression		P-value
		N	%	N	%		N	%	N	%	
BMI	Obesity	2	16.70 %	10	83.30 %	0.706	2	18.20 %	9	81.80 %	0.703
	Absence of obesity	11	28.20 %	28	71.80 %		11	28.90 %	27	71.10 %	
WC	Central obesity	4	22.20 %	14	77.80 %	0.750	4	23.50 %	13	76.50 %	1.000
	Absence of central obesity	9	27.30 %	24	72.70 %		9	28.10 %	23	71.90	

43,44,45,46

[43] MADRS: Montgomery-Asberg Depression Rating Scale
[44] BDI: Beck's Depression Inventory
[45] BMI: Body Mass Index
[46] WC: Waist Circumference

4.5.4 Sleep quality versus depression

Lastly, sleep quality and depression were assessed categorically among the 51 participants. Table 4.5.4.1 presents the *p*-values from the Fishers Exact test showing the association among the sleep quality questionnaire results between the depressed and non-depressed participants according to the MADRS questionnaire. The results of the sleep quality questionnaire are also visually represented in Figure 4.5.4.1, by way of a stacked bar graph that shows the distributions of the questionnaires categorical results between the depressed and non-depressed participants. Much like the Spearman's rho correlation analysis, no association was reported among the time it takes to fall asleep ($p = 0.098$), number of nights of problematic sleep ($p = 0.146$), daytime sleep ($p = 0.814$), and poor sleep affecting their mood, energy, or relationships ($p = 0.309$), and concentration or productivity ($p = 0.147$) results. Conversely, associations were observed among the sleep quality ($p = < 0.001$) and feeling drained after waking ($p = 0.009$) results between the two groups. Therefore, based on the research question of whether sleep quality is associated with depressive symptoms, an association exists, and we cannot reject the null hypothesis and cannot accept the alternative hypothesis.

Table 4.5.4.1: The association between sleep quality and depressive symptoms using Fisher's Exact tests.

Sleep quality questions	P-values
How long does it take you to fall asleep?	0.098
How many nights a week do you have a problem with your sleep?	0.146
Rate your sleep quality from very good to very poor.	< 0.001
Do you sleep during the day?	0.814
Do you feel drained after waking up?	0.009
Has poor sleep affected your mood, energy, or relationships?	0.309
Has poor sleep affected your concentration or productivity?	0.147

47

[47] Fisher's Exact tests were used to generate the *p*-values of the sleep quality questions and depression categorical data.

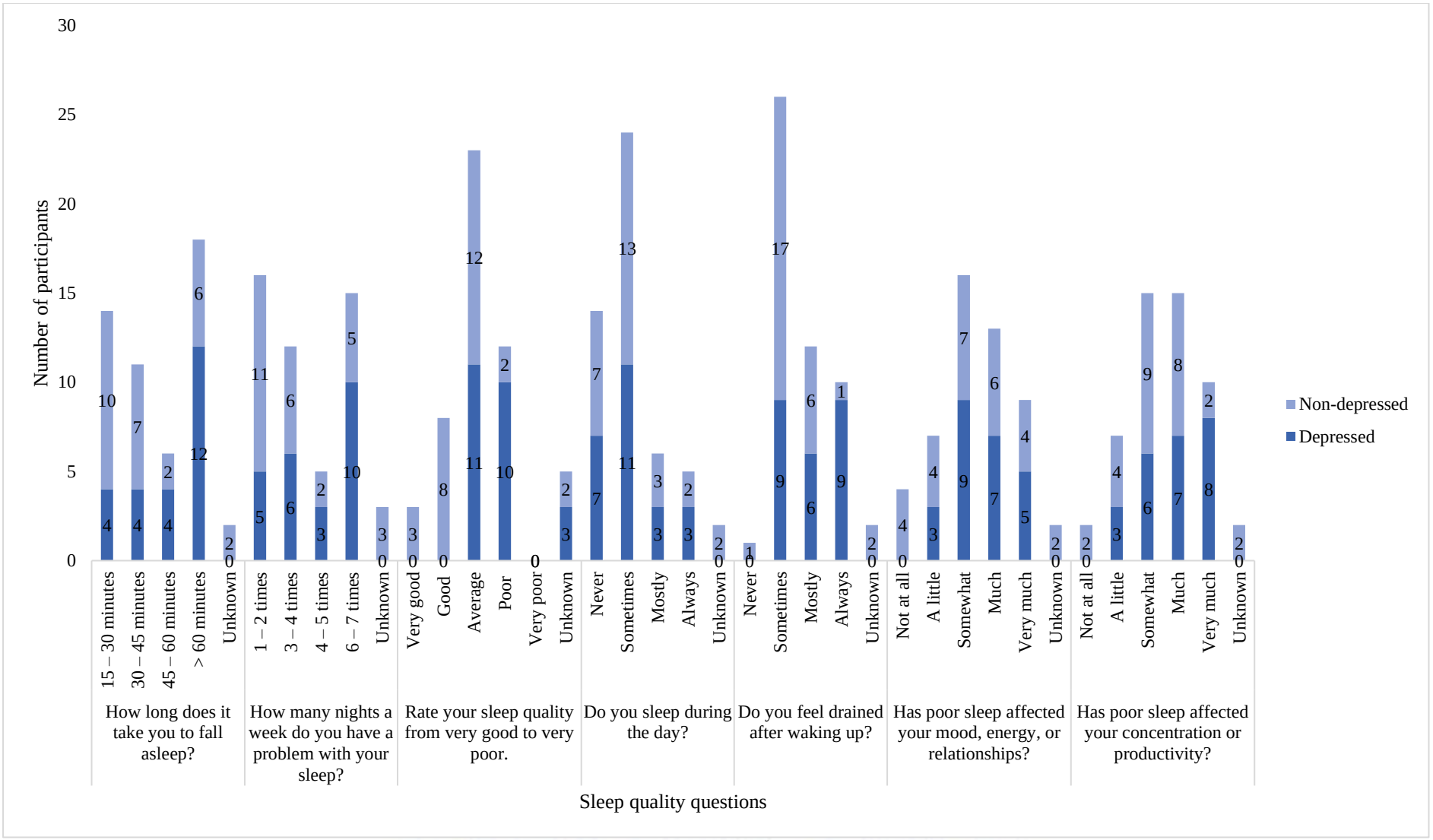


Figure 4.5.4.1: Stacked bar graph showing the results of the sleep quality questionnaire between the depressed and non-depressed participants.

4.6 Chapter summary

Chapter Four presented the results of the study. The characteristics of the study population were demonstrated, and iron deficiency, depression and obesity were assessed according to the iron status of the participants. Significant differences were observed between the iron status of the participants and their gender, and the iron status of the participants and the five iron status indicators. When assessing the relationships between the continuous variables, significant correlations were observed. The MADRS and BDI scores correlated with one another, and each of the four-body composition measurements correlated with one another. These results were to be expected. Additionally, significant correlations were observed between the age of the participants and their WC and WHtR measurements. Also, a correlation was observed between ferritin concentration and WHR measurements. Moreover, multiple sleep quality questionnaire results significantly correlated with both the MADRS and BDI scores. When evaluating the categorical data, it was revealed that no relationship existed between iron deficiency and depressive symptoms and iron deficiency and obesity. Furthermore, no significant association was observed between obesity and iron deficiency in depression. Lastly, an association was reported between the sleep quality and feeling drained after waking and depressive symptoms.

Chapter Five

Discussion

Chapter Five will discuss the demographic variables of the sample population in terms of iron status, race, inflammatory conditions, depression, and obesity. Thereafter, the results of the study will be discussed in terms of the research questions by utilising current literature to explain deviations from the hypotheses. Based on these results, no correlation existed between iron deficiency and moderate to severe depressive symptoms. Additionally, no association was observed between obesity and iron-deficient students experiencing moderate to severe depressive symptoms. Lastly, an association was observed between elements of sleep quality and depressive symptoms.

Regarding iron status, this study reported iron deficiency in 43.10 % (22 of 51) of the total sample population based on the definition provided in Chapter Three (TSAT < 20.00 % and ferritin < 30.00 μg/L). Moreover, of these iron-deficient participants, 100.0% were females. In 2012, the SANHANES-1 reported that 2.60 % of females living in the Western Cape aged between 16 and 35 were iron-depleted, i.e., iron-deficient. Moreover, 5.70 % of the females were considered iron-deficient anaemic, and 81.50 % were iron-replete (Shisana, et al., 2013). The higher prevalence of iron deficiency observed within this research study can be attributed to the differences in the definitions utilised by the SANHANES-1 and this study. The SANHANES-1 defined iron deficiency as a haemoglobin result greater than or equal to 12.00 g/dL and a ferritin concentration of less than or equal to 15.00 μg/L. Had this study used the benchmark of 15.00 μg/L for the ferritin concentration, the prevalence of iron deficiency would have been reduced. More recently, Phatlhane et al. assessed the iron status of a healthy South African adult population and reported that 39.80 % of the study population were iron deficient (ferritin < 30.0 μg/L). Additionally, 56.60 % of these iron-deficient participants were females (Phatlhane, et al., 2016). The total prevalence of iron deficiency within this sample population aligns with what was observed by Phatlhane. However, the increased prevalence of the condition in only the female participants could be attributed to the elevated male to female ratio (1:4) as more females responded to the online advertisements than males. Additionally, females are more susceptible to anaemia and thus iron deficiency than males, which could also account for the substantial increase (Turawa, et al., 2021).

Phatlhane et al. also observed that iron deficiency was specifically prevalent in 50.70 % of Black Africans (Phatlhane, et al., 2016). This trend was also reported by the SANHANES-1,

wherein most females with iron deficiency comprised of 6.70 % of Black Africans. Iron deficiency was also observed in 1.30 % of Coloured females, and there were too few observations of White and Asian/Indian females to record reliably (Shisana, et al., 2013). This study observed iron deficiency to be predominantly prevalent in 45.50 % of Black Africans and 45.50 % of Coloured participants.

Additionally, this research study reported that 59.10 % and 58.60 % of the iron-deficient and iron-replete participants presented with no inflammatory conditions, respectively. These results were self-reported by each participant. The most prevalent conditions were allergies (19.60 %) and asthma (5.90 %), which is less than the 20.00 – 30.00 % prevalence of allergies in Africa as reported by Mbugi and Chilongola (Mbugi & Chilongola, 2010). More recently, Thomas et al. investigated the relationship between inflammatory levels in first-year South African students and academic performance. Their investigation established that the inflammatory biomarkers for all students fell within normal ranges. (Thomas, et al., 2020).

Regarding depressive within the sample population, the prevalence of moderate to severe depressive symptoms according to the MADRS and BDI scores within this sample population was 47.06 % and 43.14 %, respectively. Moreover, 36.40 % and 50.00 % of the male and female participants had moderate to severe depressive symptoms according to the MADRS questionnaire, respectively. Similarly, these symptoms were observed in 27.30 % and 47.50 % of the male and female participants based on the BDI questionnaire. Tomlinson et al., reported the prevalence of lifetime major depression in an adult South African study population to be 9.70 %, and a high prevalence was observed in females (Tomlinson, et al., 2009). Similarly, Cuadros et al. stated that approximately 9.80 % of the adult population in South Africa were estimated to experience major depression at some point in their life (Cuadros, et al., 2019). When assessing depressive symptoms among South African university students, Banjes et al. reported that 11.20 % of the study population experienced moderate to severe depressive symptoms (Banjes, et al., 2016). This research study observed a higher prevalence in the severity of depressive symptoms, which can potentially be attributed to the change in circumstances that the COVID-19 pandemic brought about. When assessing South African university students' mental health during the pandemic, Visser and Law-van Wyk observed that 45.60 % and 35.00 % of students reported subjective experiences of anxiety and depression, respectively (Visser & Law-van Wyk, 2021). They observed a higher prevalence of depressive symptoms than previous studies, much like the present study results.

Lastly, the prevalence of obesity was high within this sample population. Initially, the South African Demographic Health Survey of 1998 and 2003 revealed that overweight and obesity have not significantly changed and remained prevalent in the female population. (Department of Health, Medical Research Council, OrcMacro, 2007). While the obesity trends in South Africa did not substantially change from 1998 to 2003, the SANHANES-1 reported considerable changes among the various anthropometric measures used to assess obesity in a population from 2003 to 2012. These changes consisted of increases in the BMI, WC and WHR values across all age categories, race groups and provinces (Shisana, et al., 2013).

This study observed that obesity, assessed through BMI values, was present in 27.30 % of the male participants, much like the 22.50 % observed in the female participants. These results contradict what was reported by the SANHANES-1, as they described 16.10 % and 37.90 % of males and females living in the Western Cape aged 15 years and older to be obese, respectively. This contradiction could be due to the decreased sample number, and it is not a true reflection of the sample population. Furthermore, the male participants only comprised of 21.57 % (11 of 51) of the total sample population. Therefore, reliable conclusions cannot be made. Additionally, Ter Goon et al. reported the prevalence of obesity to be 6.20 % in male students at the University of Venda and 9.00 % of the female students at the University (Ter Goon, et al., 2013). These statistics are much lower than what was observed by this research study and the SANHANES-1. The discrepancy between the increased BMI observed in this study compared to what was observed in these previous studies could be due to the COVID-19 pandemic. In 2020, Yang et al. aimed to investigate the changes in obesity and activity patterns among Chinese youths during the COVID-19 lockdown and observed that the mean BMI of all participants significantly increased. Furthermore, the team reported a decrease in the frequency of engaging in active transport, housework, and physical activity. While an increased frequency of sleeping and screen time was observed (Yang, et al., 2020). This could explain the higher frequency of obesity observed in this research study.

Furthermore, central obesity, assessed through WC measurements, was reported in 27.30 % and 37.50 % of the male and female participants, respectively. The SANHANES-1 reported higher frequencies of increased WC measurements in the Western Cape, as 29.80 % and 12.80 % of the male participants had WC measurements ⩾ 94.00 cm and ⩾ 102.00 cm, respectively. In contrast, 68.80 % and 50.40 % of the female participants had WC measurements of ⩾ 80.00 cm and ⩾ 88.00 cm, respectively (Shisana, et al., 2013). Interestingly, when Nkeh-Chungag et al. assessed the relationship between anthropometric measurements and blood pressure in

South African students attending the Walter Sisulu University, they observed that both the male (76.10 ± 1.10 cm) and female (77.90 ± 0.90 cm) participants had mean WC measurements that fell within the normal category (Nkeh-Chungag, et al., 2015). Pengpid and Peltzer later matched these results. They reported that the mean WC measurements of male (78.40 cm) and female (73.80 cm) university students across 26 countries were also in the normal category (Pengpid & Karl, 2018). The WC measurements of this research study are below what was observed by the SANHANES-1. However, above what was noted by Nkeh-Chungag et al. and Pengpid and Peltzer.

The WHR was assessed next, and it represented the metabolic risk of the participants in this study. An increased risk was observed in 27.30 % and 12.50 % of male (WHR ⩾ 0.90 cm) participants and female (WHR ⩾ 0.85 cm) participants, respectively. This risk was somewhat reflected by Hattingh et al., who observed that 16.50 % of South African females living in the Free State, aged between 24 - 34 years had WHR values equal to or greater than 0.80 cm (Hattingh, et al., 2008). This slight decrease could be attributed to province-specific differences. The SANHANES-1 reported that 43.10 % of females living in the Free State had WHR values equal to or greater than 0.85 cm, whereas 51.50 % of females living in the Western Cape had WHR values in this range (Shisana, et al., 2013). The provincial differences of the WHR values observed by the SANHANES-1 are reflected by this research study and Hattingh et al. at a smaller percentage. Regarding the males, the SANHANES-1 reported that 8.20 % of males living in the Western Cape have WHR values equal to or greater than 1.00 cm (Shisana, et al., 2013). This research study employed a cut-off value of 0.90 cm to measure increased metabolic risk. Had we used 1.00 cm as a measure, the percentage of males with increased metabolic risk may have been reduced, as observed by the SANHANES-1.

This next section of Chapter Five will discuss the results in terms of the research questions of this study. The first research question assessed whether a correlation existed between iron deficiency and moderate to severe depressive symptoms in students residing in the Western Cape. As previously discussed, the severity of the depressive symptoms reported by the study population tended to be increased. Table 4.3.2.1 presented the results of the depressive scores of the participants according to their iron status. This research study found that the MADRS scores were similar regardless of the iron status (p = 0.103). When assessing whether a significant difference existed between the BDI scores of the iron-deficient and iron-replete groups, a p-value of 0.056 was reported. This p-value result bordered on statistical significance, and had the sample size been larger, we might have observed statistically significant

differences between the BDI scores of both groups. Furthermore, Spearman's rho correlation analyses showed no significant correlations between the MADRS and BDI scores and any of the five iron status indicators. Lastly, Fisher's exact test analysis demonstrated no significant correlation between iron deficiency and depression (p = 0.164). As such, iron deficiency did not correlate with increased depressive symptoms in this sample population.

Many studies have assessed the relationship between iron deficiency and depression (Shariatpanaahi, et al., 2007), (Noorazar, et al., 2015), (Shafi, et al., 2018), (Yi, et al., 2011) & (Vulser, et al., 2016). They have often produced conflicting results, and the results of this study are in line with a few of these studies. The first studies analysed, investigated the relationship between ferritin concentrations and depression. As a decreased ferritin concentration formed part of this study's iron deficiency definition, it was essential to assess these studies. Shariatpanaahi et al. observed ferritin concentrations within a normal range in both the depressed and healthy groups of female medical students living in Tehran. However, the depressed group had a mean ferritin concentration of approximately 11.00 μg/L lower than the healthy group (Shariatpanaahi, et al., 2007). While this study grouped the participants according to their iron status in its analysis, a similar trend was observed when the participants were grouped according to their depressive symptoms which are presented in Appendices G and H. The ferritin concentrations were within a normal range in both the depressed and control group participants; however, the mean ferritin concentration of the depressed group was 22.18 μg/L less than the mean observed in the control group. While the difference in mean ferritin concentrations was not statistically significant, it is interesting to note the difference in concentrations between the two groups. Iron is vital for many metabolic processes, including monoamine metabolism, which synthesises dopamine and serotonin (Hare, et al., 2013). It is also an essential cofactor in the homeostasis of glutamate and GABA, neurotransmitters responsible for preserving the equilibrium between excitation and inhibition in the body (Kim & Wessling-Resnick, 2014) & (Sarawagi, et al., 2021). Deficiencies of both these neurotransmitters have been observed in individuals in depressive states (Richardson, et al., 2015), (Boku, et al., 2018), (Sarawagi, et al., 2021) & (Duman, et al., 2020). By way of reduced iron stores, these deficiencies could be attributed to the substantial difference in mean ferritin concentrations between the depressed and control group participants.

Contrastingly, Yi et al. reported no significant association between ferritin concentrations and depressive symptoms in Japanese females. However, a significant association existed between depressive symptoms experienced by Japanese males and reduced ferritin concentrations (Yi,

et al., 2011). This study observed a statistically significant difference (p = 0.001) in the prevalence of iron deficiency between the male (0.00%) and female (55.00%) participants. Furthermore, moderate to severe depressive symptoms according to the MADRS questionnaire were observed in 36.40 % of male and 50.00 % of female participants. Due to the lack of iron-deficient male participants, as well as a higher prevalence of depression in the female participants, the results of this study did not align with that of Yi et al. This increased prevalence of depression in South African adult females was in line with what was a current trend at the time. For example, Mutyambizi et al. reported depressive symptoms to be more pronounced in South African females (28.46 %) than males (24.38 %) when assessing the subjective social status and inequalities in depressive symptoms according to a gender-specific decomposition analysis (Mutyambizi, et al., 2019). However, a recent report by Oyenubi and Kollamparambil observed that depressive symptoms in a South African population have increased during the COVID-19 era. Moreover, the prevalence of increased depressive symptoms in females relative to males is no longer observed as there is no significant difference between positively identifying depressive symptoms across gender (Oyenubi & Kollamparambil, 2020). Had this study had more male participants, this trend might have been observed within the study population.

Furthermore, Su et al. reported no association between ferritin concentration and depressive symptoms among Chinese adults (Su, et al., 2016). Similarly, Okan et al. observed that ferritin levels did not correlate with anxiety, depressive symptoms, and sleep quality in non-anaemic patients with and without fibromyalgia syndrome (Okan, et al., 2019). The results of this study are in line with what was observed by Su et al. and Okan et al., as no association between iron deficiency and depressive symptoms in the study population was regarded. However, the iron-deficient group had a mean MADRS score (21.230), slightly higher than the iron-replete group (17.790). These mean scores reflect moderate and mild depressive symptoms, respectively. This trend was also observed with the BDI scores, whereby the mean BDI score for the iron-deficient group (23.090) was more significant than the mean score of the iron-replete group (18.180). It is interesting to note the differences in mean depressive scale scores between these two groups. The prevalence of depression was relatively high in this study compared to what was generally observed in a South African student population. As mentioned, Visser and Law-van Wyk assessed the mental health of South African university students during the peak of the COVID-19 pandemic. They reported increased feelings of anxiety and depression among the students. Furthermore, most students felt that the pandemic had: a negative effect on their

health and fitness (50.80 %), increased feelings of loneliness and isolation (56.70 %), a negative impact on their academics (58.90 %), increased financial strain (56.90 %) (Visser & Law-van Wyk, 2021). These negative attributes could have contributed to the increased mean depressive scale scores, thus creating similar mean scores between the iron-deficient and iron-replete groups.

The last set of studies investigating ferritin concentration and depressive symptoms observed that an increase in this iron status indicator was associated with depressive symptoms (Huang & Lee, 2007) & (Bozacı & Tatar, 2020). However, these studies measured depression in patients with chronic kidney disease. Chronic kidney disease is considered persistent a low-grade inflammatory condition, and due to ferritin being a positive acute-phase response protein, it will be increased in instances of inflammation (Akchurin & Kaskel, 2015) & (World Health Organization, 2011).

This section of Chapter Five assessed the relationship between iron deficiency and obesity. While this does not directly pertain to the second research question, it does offer a prelude to the question. As previously mentioned, the frequency of obesity in this study population tended to be high. Table 4.3.3.1 presented the ranges of the body composition measurements of the participants according to their iron status. As assessed by Mann-Whitney U tests, this research study found no statistically significant differences among the BMI ($p = 0.278$), WC ($p = 0.332$), WHR ($p = 0.156$) and WHtR ($p = 0.304$) measurements between the iron-deficient and iron-replete groups. However, Spearman's rho correlation analyses revealed positive correlations between the following: age and WC measurements (r_s (49) = 0.277, $p = 0.049$); age and WHtR values; (r_s (49) = 0.287, $p = 0.041$); and WHR values and ferritin concentrations (r_s (49) = 0.350, $p = 0.012$). Lastly, Fisher's exact test analyses revealed no correlation between iron deficiency and obesity marked by BMI values (p = 0.192) and between iron deficiency and central obesity marked by WC measurements ($p = 0.770$).

Many studies investigating the relationship between iron status and obesity have often produced conflicting results. Some studies have found no significant difference between the iron status of overweight and obese individuals (Pérez, et al., 2020), (Ghadiri-Anari, et al., 2014) & (Mchiza, et al., 2018). Mchiza et al. used results from the SANHANES-1 and reported that no significant difference existed between the BMI ranges of South African female adolescents and adults and their respective iron statuses. However, the prevalence of anaemia and IDA tended to decrease with increased BMI values of the adult participants (Mchiza, et al.,

2018). This present research study aligned with Mchiza et al., as no significant difference was observed between the four body composition measurements among the iron-deficient and iron-replete participants. Furthermore, the iron-deficient group participants tended to have slightly decreased BMI (24.012 kg/m^2), WC (75.367 cm), WHR (0.753 cm), WHtR (0.467 cm) mean values when compared to the BMI (26.040 kg/m^2), WC (81.867 cm), WHR (0.804 cm), and WHtR (0.503 cm) mean values of the iron-replete group. While these differences were not significant, the values coincide with the trend observed by Mchiza et al., whereby a higher BMI associates with lower prevalence of iron deficiency and anaemia. This trend was also observed by Kordas et al. and Qin et al., whereby overweight and obese Colombian women of reproductive age and adult Chinese women had a lower likelihood of anaemia, respectively (Kordas, et al., 2013) & (Qin, et al., 2013). This trend could be explained by individuals with increased body composition measurements having diets high in iron (Ghadiri-Anari, et al., 2014). Additionally, Qin et al. monitored the dietary consumption of the Chinese participants and observed that the women consumed adequate amounts of Vitamin C (Qin, et al., 2013). Vitamin C is a powerful enhancer of non-heme iron absorption and is the only known dietary component other than animal tissue to support iron absorption (Qin, et al., 2013) & (Li, et al., 2020). Diets high in Vitamin C could promote sufficient iron absorption in overweight and obese individuals.

Conversely, many studies have observed an inverse correlation between iron deficiency and obesity. Choma et al. observed that BMI values were inversely correlated with plasma iron, TSAT, and ferritin concentrations in South African women. In contrast, WC measurements were positively correlated with these iron status indicators (Choma, et al., 2015). Similarly, Jordaan et al. reported statistically significant inverse correlations between the BMI status and MCV, MCH and TSAT values of a South African female population residing in rural Free State. However, these values remained within a normal range. In contrast, this inverse correlation was also observed between the WC measurements and the three mentioned iron status indicators, unlike what was noted by Choma et al. (Jordaan, et al., 2020). Additionally, Stoffel et al. reported a significant positive correlation between the ratio of android fat to total body fat and CRP, AGP, hepcidin and TIBC in young women. An inverse correlation was reported between the ratio and TSAT (Stoffel, et al., 2020). As previously mentioned, hepcidin regulates iron metabolism by causing the degradation of ferroportin and inhibiting iron absorption (Nemeth & Ganz, 2021). Hepcidin, together with CRP and AGP, is synthesised and secreted in inflammatory states, including obesity. The results obtained by Stoffel et al. align

with this concept. The results of this research study did not align with the results obtained by these studies. This trend is potentially due to a dietary factor whereby the participants with higher BMI and WC values may have consumed foods high in iron, as observed by Qin et al.

When assessing the iron status indicators in isolation, it was observed that WHR values positively correlated with ferritin concentrations. Similarly, Gillum reported that the WHR values of Mexican American men positively correlated with their ferritin concentrations (Gillum, 2001). In a South African context, Aderibigbe et al. observed that increased ferritin concentrations were positively correlated with increased WHR and WC in a South African adult women population (Aderibigbe, et al., 2011). These results support what is known about ferritin and obesity. The persistent low-grade inflammation associated with obesity results in the overproduction of ferritin. This overproduction is due to ferritin being a positive acute response protein that increases in instances of inflammation (Alshwaiyat, et al., 2021) & (World Health Organization, 2011). As such, ferritin will be increased in individuals presenting with elevated body composition measures, such as the WHR. However, the BMI, WC and WHtR values should have also been positively correlated with ferritin concentrations in this context. Future studies need to assess this correlation in a larger sample population.

This section of Chapter Five assessed the results of the second research question, which investigated whether a correlation existed between obesity and iron-deficient students who experienced moderate to severe depressive symptoms. Fisher's exact test analyses revealed there to be no significant correlation between obese students with iron deficiency and increased depressive symptoms against obese students without the two conditions (BMI and MADRS: $p = 0.706$; BMI and BDI: $p = 0.703$; WC and MADRS: $p = 0.750$; WC and BDI: $p = 1.000$). As previously mentioned, very few studies have assessed iron deficiency, depression, and obesity concurrently in a sample population. Lee and Park were the closest to investigating these three variables by examining the relationship between the serum ferritin concentration and depressive symptoms in adult Korean males concerning their BMI values. Lee and Park observed that the BMI values were significantly positively correlated with the depressive scores of the participants as well as their serum ferritin concentrations. Furthermore, depressive symptoms were also significantly positively correlated with the serum ferritin concentrations (Lee & Park, 2019). These results were contradictory to what was observed in this research study.

When the participants were grouped according to obesity measures, which can be observed in Appendices I and J, twelve participants with BMI values of 30.0 kg/m^2 or greater (obese) presented with mean MADRS and BDI scores of 16.25 and 18.18, respectively. These scores reflect mild depressive symptoms. However, the 39 participants with BMI values less than 30.00 kg/m^2 (normal to overweight) presented with mean MADRS and BDI scores of 20.21 and 21.03, reflecting moderate depressive symptoms, respectively. Regarding the participants' iron status, the mean values of all five iron status indicators remained within normal ranges. However, the serum iron (12.342 µmol/L), transferrin (3.057 g/L), and TSAT (16.583 %) values were slightly reduced in the obese group compared to the serum iron (13.087 µmol/L), transferrin (3.182 g/L), and TSAT (17.308 %), values of the normal to overweight group. Contrastingly, the TIBC values of the obese group (69.508 µmol/L) were slightly elevated when compared to the values of the normal to overweight group (72.385 µmol/L). Also, the ferritin concentration of the obese group (102.917 µg/L) was substantially higher than the normal to overweight group (46.949 µg/L). However, this drastic difference could be due to ferritin being a positive acute-phase response protein, as previously mentioned (World Health Organization, 2011). It is important to note that none of these differences were significant.

While no significant results were obtained, the observed trends could be attributed to diet. When investigating iron deficiency and obesity, a tendency was observed whereby increased body mass decreased the likelihood of anaemia or iron-deficiency anaemia (Mchiza, et al., 2018), (Kordas, et al., 2013) & (Qin, et al., 2013). This research study observed that the iron-deficient sample group had lower mean BMI (24.012 kg/m^2) and WC (75.367 cm) values when compared to the iron-replete participants' mean BMI (26.040 kg/m^2) and WC (81.867 cm) values. This could be attributed to a diet rich in iron and vitamin C (Ghadiri-Anari, et al., 2014) & (Qin, et al., 2013). Péneau et al. aimed to assess the contribution of fruits and vegetables, based on their vitamin C and fibre content, to the iron status of a population. They determined a positive correlation between serum ferritin and fibre-poor fruits and vegetables in premenopausal women. However, these foods did not provide an enhancing effect in participants with high iron stores (Péneau, et al., 2008).

One diet that consists of a higher consumption of fruits and vegetables is the Mediterranean diet (Firth, et al., 2020). This diet may be a low-iron diet whereby the consumption of iron absorption inhibitors such as dairy products overpower the consumption of iron enhancers such as vitamin C and red meat. Thus, prolonged adherence to a Mediterranean style diet can reduce iron stores (Mascitelli, et al., 2015) & (Dwyer, et al., 2010). Additionally, Mediterranean diets

have been observed to reduce the risk of depression (Firth, et al., 2020), (Ventriglio, et al., 2020) & (Yin, et al., 2021). Various studies have assessed whether this diet also reduces depressive symptoms in university students. Corezzi et al. observed that students with a higher adherence to a Mediterranean diet had the lowest risk of depression (Corezzi, et al., 2020). Recently, Antonopoulou et al. analysed the current research regarding university students and their adherence to Mediterranean-style diets. The researchers observed trends whereby poor health statuses of students correlated with reduced adherence to the diet. Furthermore, increased adherence correlated with a lower risk of depression (Antonopoulou, et al., 2020). Interestingly, Metin and Okan Bakir reported no significant correlation between Mediterranean diet adherence in Turkish university students and their BDI scores. However, a correlation was observed between the students' diet and emotional states, including stress, anger, energy, and fatigue (Metin & Okan Bakir, 2021).

The results of this study align with what was documented by the various studies that evaluated the Mediterranean diet. However, due to the lack of information, we cannot be confident of the role of diet within this sample group, and further studies need to be conducted to assess this. Furthermore, depressive symptoms among university students have significantly increased during the COVID-19 pandemic and have potentially created a confounding effect when investigating the association between iron deficiency and depressive symptoms. Further studies need to assess the baseline depressive scores and iron statuses of South African university students pre-COVID-19 pandemic and compare it against the results obtained during the pandemic to better gauge the association.

The last set of studies discussed pertains to the secondary objective of this research study which sought to assess the association between sleep quality and depression (Reid & Baker, 2008), (Lemma, et al., 2012) & (Li, et al., 2020). This research study observed several correlations between many of the sleep quality questionnaire results and the MADRS and BDI scores of the participants. Current literature suggests a significant association between sleep disturbance and major depressive disorder, as it is one of the most consistent symptoms reported by individuals with depressive symptoms (Nutt, et al., 2008) & (Murphy & Peterson, 2015). These disturbances are not secondary to the disorder but often precede the depressive episode and can be characterised by the interruption of wake periods into the sleep process; the altered proportion and pattern of the sleep stages; and the enhancement of phasic phenomena within the REM stage of sleep (Murphy & Peterson, 2015) & (Borbely & Wirz-Justice, 1982). More recently, Fang et al. reviewed the relationship between depression in sleep disturbance and

concluded that sleep disturbance is both a comorbidity of depression and an early-onset symptom. Subsequently, the disturbance can predict the incidence and result of the disorder (Fang, et al., 2019).

Many young adults are at risk for sleep disorders (Gaultney, 2010) & (McArdle, et al., 2020). In 2008, Reid and Baker assessed the sleep habits of South African students and observed that 18.00 % of students reported having poor sleep quality (Reid & Baker, 2008). Poor sleep quality can substantially affect students' psychobiological well-being as they transition from high school to tertiary education (Dinis & Bragança, 2018). This research study observed a statistically significant, strongly positive correlation between the length of time it takes a participant to fall asleep and their BDI score ($\tau_b = 0.316$, $p = 0.004$). Also, a correlation was noted between the number of nights of problematic sleep and the participants' BDI score ($\tau_b = 0.320$, $p = 0.004$). Additionally, statistically significant, strongly positive correlations were observed between the participants' sleep quality and both their MADRS, ($\tau_b = 0.399$, $p = < 0.0005$) and BDI scores ($\tau_b = 0.595$, $p = < 0.0005$).

These results concur with what was observed by various studies. Lemma et al. reported that stress levels, anxiety and depressive symptoms were significantly associated with sleep quality in Ethiopian university students. Furthermore, female compared to male students, as well as second-and third-year students compared to students in fourth year or above had increased odds of poor sleep quality (Lemma, et al., 2012). Much like this research study, Li et al. observed that poor sleep quality and short sleep duration were significantly associated with the prevalence of increased depressive symptoms among Chinese university students (Li, et al., 2020). When assessing the physiological observations in depression, Nutt et al. reported that depressed patients presented with impaired sleep continuity, increased wakefulness, and reduced sleep efficiency. This inadequate sleep was potentially due to abnormalities observed in the circadian and homeostatic processes (Nutt, et al., 2008).

Researchers have recently investigated the relationship between depression and sleep quality concerning the COVID-19 pandemic. Carpi et al. aimed to assess the sleep quality among Italian university students during the late phase of the COVID-19 pandemic. They observed that many Sapienza University students presented with either poor sleep quality (65.00 %) or insomnia symptoms (55.00 %). Increased stress scores and decreased physical and mental health-related quality of life scores were detected (Carpi, et al., 2022). Lewis et al. aimed to assess the impact of sleep and other variables on depressive and anxiety-related symptoms

during the COVID-19 pandemic in a South African adult population. The team observed that regardless of the lockdown, an increase in the severity of insomnia symptoms predicted an increase in depressive symptoms. Furthermore, reduced physical activity during the lockdown period indicated increased insomnia symptoms, subsequently predicting an increase in depressive and anxiety-related symptoms (Lewis, et al., 2021). Coakley et al. supported this observation as they reported that 49.0 % of American university students did not meet the moderate to vigorous physical activity guidelines during the COVID-19 pandemic lockdown. Furthermore, a significant positive association existed between the hours spent sitting in a day and depressive and anxiety symptom severity (Coakley, et al., 2021). While the physical activity engagement of the participants was not noted in this research study, it would have been an interesting variable to consider. This research study observed a higher prevalence of depressive symptoms among the female participants when compared to the male participants in this research study. Furthermore, according to Bloemhoff, South African female university students tended to be significantly less active when compared to male students (Bloemhoff, 2010). Therefore, the higher prevalence of depressive symptoms in females could be attributed to reduced physical activity. Further studies need to be performed to assess physical engagement and depressive symptoms of university students pre-COVID 19 pandemic and during the pandemic.

A significant limitation of this research study is the relatively small sample size (n = 51), which could have attributed to the lack of significant results. Various studies investigating the relationship between iron deficiency and depressive symptoms included numerous participants in their respective studies. For example, Shariatpanaahi et al. had a sample population of 205 female medical students when assessing the association between depression and serum ferritin concentration (Shariatpanaahi, et al., 2007). Next, Yi et al. included 312 males and 216 females in their study to investigate the relationship between serum ferritin concentrations and depressive symptoms among Japanese municipal employees (Yi, et al., 2011). More recently, Noorazar et al. assessed the relationship between the severity of depressive symptoms and IDA in 100 women with MDD (Noorazar, et al., 2015). Interestingly, Lee and Park, whose study was most similar to this current research study, had a sample size of 55 participants (Lee & Park, 2019). However, this was only one of the few studies found with a smaller sample number. This research study found that many potential participants were no longer interested in participating when they were made aware that the measurements would be conducted at the University. Several of these students studied remotely in different provinces, as the country

was in Adjusted Alert Level 1 Lockdown in November 2021. Other potential participants could have disengaged out of fear of being on campus due to the COVID-19 pandemic. Furthermore, this research study had a time constraint challenge, as the data collection stage fell within the potential participants' final exam period, and many did not have time to participate. Ultimately, various factors affected the recruitment process of this study. Further studies need to be conducted with larger sample sizes to evaluate iron deficiency, depression, and obesity more accurately within a South African student sample population.

Additional limitations of this study were observed in the participant handling. Participants that consumed iron supplements during the data collection period of this study were initially excluded. However, due to the previously mentioned laboratory difficulties, these participants were included not further to compromise the already reduced sample number. Further studies need to monitor iron supplementation consumption within their sample population. Moreover, the sleep quality questionnaire was subjective. Each participant completed the questionnaire based on their individual experiences, not through standardised testing. Many sleep quality questions correlated with both the MADRS and BDI scores of the participants, and further studies should be conducted to assess this correlation more accurately.

Despite the various limitations, this study also has its strengths. To our knowledge, this was the first research study to investigate the relationship between iron deficiency and depressive symptoms in relation to body composition measurements in a South African university student population. This study had the participants' blood drawn by the same medical professional and the five selected iron status indicators were analysed through the same laboratory, PathCare. This reduced the variability between measurements based on methodology. This method was also applied to the anthropometric measurements, whereby one researcher was tasked to carry out these measurements on all 51 participants. While very few significant results were observed, this study provides the basis for future studies to assess iron deficiency, depression, and obesity within a larger South African student population.

Conclusion

It can be concluded that no correlation exists between iron deficiency and moderate to severe depressive symptoms in students residing in the Western Cape. Additionally, there is no correlation between obesity and iron-deficient students that experience moderate to severe depressive symptoms. This study was limited by a small sample size, the inclusion of participants who consumed iron supplements, and a subjective sleep quality questionnaire. Therefore, future studies should recruit participants during a low-stress period and strictly adhere to inclusion and exclusion factors. Furthermore, due to the significant correlations observed between the sleep quality questionnaire and depressive symptom scoring, it is recommended that standardised sleep quality instruments should be employed to assess the relationship between sleep quality and depressive symptoms in a South African university student population. It is also recommended that the participants' diet be analysed thoroughly and in relation to depressive symptoms.

References

Abbaspour, N., Hurrell, R. & Kelishadi, R., 2014. Review on iron and its importance for human health. *Journal of Research in Medical Sciences,* 19(2), pp. 164-174.

Abbott, 2018. *Alinity c: Iron Reagent Kit ,* Chicago : Abbott Laboratories .

Abbott, 2018. *Alinity c: Transferrin Reagent Kit,* Chicago: Abbott Laboratories.

Abbott, 2018. *Alinity i: Ferritin Reagent Kit,* Chicago : Abbott Laboratories .

Abo Zeid, A. A., El Saka, M. H., Abdalfattah, A. A. & Zineldeen, D. H., 2014. Potential factors contributing to poor iron status with obesity. *Alexandria Journal of Medicine,* 50(1), pp. 45-48.

Aderibigbe, O. R. et al., 2011. The relationship between indices of iron status and selected anthropometric cardiovascular disease risk markers in an African population: the THUSA study: cardiovascular topics. *Cardiovascular Journal of Africa,* 22(5).

Aigner, E., Feldman, A. & Datz, C., 2014. Obesity as an Emerging Risk Factor for Iron Deficiency. *Nutrients,* 6(9), pp. 3587-3600.

Akchurin, O. M. & Kaskel, F., 2015. Update on inflammation in chronic kidney disease. *Blood Purification ,* 39(1-3), pp. 84-92.

Al-Alimi, A. A., Bashanfer, S. & Morish, M. A., 2018. Prevalence of Iron Deficiency Anemia among University Students in Hodeida Province, Yemen. *Anemia,* Volume 2018, pp. 1-7.

Al-Jamea, L. et al., 2019. Prevalence of Iron-deficiency anemia and its associated risk factors in female undergraduate students at prince sultan military college of health sciences. *Journal of Applied Hematology,* 10(4), pp. 126-133.

Alkindi, S. et al., 2018. Iron deficiency and iron deficiency anemia in the adult omani population. *Journal of Applied Hematology,* 9(1), pp. 11-15.

Alshwaiyat, N. M., Ahmad, A., Hassan, W. M. R. W. & Al-Jamal, H. A. N., 2021. Association between obesity and iron deficiency (review). *Experimental and Therapeutic Medicine,* 22(5), pp. 1-7.

Antonopoulou, M. et al., 2020. Evaluating Mediterranean diet adherence in university student populations: Does this dietary pattern affect students' academic performance and mental health?. *The International Journal of Health Planning and Management ,* 35(1), pp. 5-21.

Apovian, C. M., 2016. Obesity: Definition, Comorbidities, Causes, and Burden. *The American Journal of Managed Care,* 22(7), pp. 176-185.

Ashwell, M., Cole, T. J. & Dixon, A. K., 1985. Obesity: new insight into the anthropometric classification of fat distribution shown by computed tomography. *British Medical Journal (Clinical Research Ed.),* 290(6483), pp. 1692-1694.

Ashwell, M., Cole, T. J. & Dixon, A. K., 1996. Ratio of waist circumference to height is strong predictor of intra-abdominal fat. *BMJ,* 313(7056), pp. 559-560.

Ashwell, M. & Gibson, S., 2016. Waist-to-height ratio as an indictaor of 'early health risk': simpler and more predictive than using a 'matrix' based on BMI and waist circumference. *BMJ Open,* 6(e010159), pp. 1-7.

Bailey, R. L., West Jr, K. P. & Black, R. E., 2015. The Epidemiology of Global Micronutrient Deficiencies. *Annals of Nutrition and Metabolism,* 66(2), pp. 22-33.

Banjes, J. R., Kagee, A., McGowan, T. & Steel, H., 2016. Symptoms of posttraumatic stress, depression, and anxiety as predictors of suicidal ideation among South African university students. *Journal of American College Health ,* 64(6), pp. 429-437.

Barton, J. C. & Acton, R. T., 2019. Chapter Eleven - Hepcidin, iron, and bacterial infection. In: G. Litwack, ed. *Vitamins and Hormones.* s.l.:Academic Press, pp. 223-242.

Beck, A. T. et al., 1961. An Inventory for Measuring Depression. *Archives of General Psychiatry,* 4(6), pp. 561-571.

Beck, K. L., Conlon, C. A., Kruger, R. & Coad, J., 2014. Dietary Detereminants of and Possible Solutions to Iron Deficiency for Young Women Living in Industrialised Countries: A Review. *Nutrients,* 6(9), pp. 3747-3776.

Behuet, S. et al., 2019. Developmental Changes of Glutamate and GABA Receptor Densities in Wistar Rats. *Frontiers in Neuroanatomy ,* 13(100), pp. 1-14.

Benca, R. M. & Peterson, M. J., 2008. Insomnia and depression. *Sleep Medicine,* 9(1), pp. 3-9.

Bhandari, P. M. et al., 2017. Sleep quality, internet addiction and depressive symptoms among undergraduate students in Nepal. *BMC Psychiatry* , 17(106).

Blank, P. R., Tomonaga, Y., Szucs, T. D. & Schwenkglenks, M., 2019. Economic burden of symptomatic iron deficiency - a survey among Swiss women. *BMC Women's Health*, 19(39), pp. 1-9.

Bloemhoff, H. J., 2010. Gender- and race-related physical activity levels of South African university students. *African Journal for Physical Activity and Health Sciences,* 16(4).

Boku, S., Nakagawa, S., Toda, H. & Hishimoto, A., 2018. Neural basis of major depressive disorder: Beyond monoamine hypothesis. *Psychiatry and Clinical Neurosciences* , 72(1), pp. 3-12.

Borbely, A. A. & Wirz-Justice, A., 1982. Sleep, Sleep Deprivation and Depression. *Human Neurobiology,* Volume 1, pp. 205-210.

Boukrim, M., Obtel, M., Lahlou, L. & Razine, R., 2021. University students' perceptions and factors contributing to obesity. *African Health Sciences*, 21(2), pp. 942-950.

Bouri, S. & Martin, J., 2018. Investigation of iron deficiency anaemia. *Clinical Medicine,* 18(3), pp. 242-244.

Bozacı, İ. & Tatar, E., 2020. Comparison of the Frequency and Severity of Depression Between Patients with Stage 4 and 5 Chronic Kidney Disease with and Without Kidney Transplantation. *The Medical Bulletin of Haseki,* Volume 58, pp. 376-381.

Bradl, M. & Lassmann, H., 2010. Oligodendrocytes: biology and pathology. *Acta Neuropathologica* , 119(1), pp. 37-53.

Bray, G. A., 2004. Medical Consequences of Obesity. *The Journal of Clinical Endocrinology & Metabolism,* 89(6), pp. 2583-2589.

Brock, J. M., Billeter, A., Muller-Stich, B. P. & Herth, F., 2020. Obesity and the Lung: What We Know Today. *Respiration,* Volume 99, pp. 856-866.

Bruce-Keller, A. J. et al., 2015. Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biological Psychiatry,* 77(7), pp. 607-615.

Camaschella, C., 2019. Iron Deficiency. *Blood,* 133(1), pp. 30-39.

Caminha, T. C. et al., 2017. Waist-to-height ratio is the best anthropometric predictor of hypertension. *Medicine,* 96(2), pp. 1-8.

Carpi, M., Cianfarani, C. & Vestri, A., 2022. Sleep Quality and Its Associations with Physical and Mental Health-Related Quality of Life among University Students: A Cross-Sectional Study. *International Journal of Environmental Research and Public Health,* 19(2874), pp. 1-13.

Castro, A. M., Macedode la Concha, L. E. & Pantoja-Meléndez, C. A., 2017. Low-grade inflammation and its relation to obesity and chronic degenerative diseases. *Revista Médica del Hospital General de México,* 80(2), pp. 101-105.

Çelik, N., Ceylan, B., Ünsal, A. & Çağan, Ö., 2018. Depression in health college students: relationship factors and sleep quality. *Psychology, Health and Medicine* , pp. 1-7.

Centers for Disease Control and Prevention , 2008. *National Report on Biochemical indicators of Diet and Nutrition in the U.S. Population 1999-2002,* Atlanta: Centers for Disease Control and Prevention .

Centers for Disease Control and Prevention , 2021. *Medical Conditions ,* Atlanta: Centers for Disease Control and Prevention .

Centers for Disease Control and Prevention, 2021. *Defining Adult Overweight & Obesity.* [Online]
Available at:

https://www.cdc.gov/obesity/adult/defining.html
[Accessed 26 May 2021].

Cepeda-Lopez, A. C., Aeberli, I. & Zimmermann, M. B., 2010. Does obesity increase risk for iron deficiency? A review of literature and the potential mechanisms. *International Journal for Vitamin and Nutrition Research,* 80(4-5), pp. 263-270.

Cepeda-Lopez, A. C., Melse-Boonstra, A., Zimmermann, M. B. & Herter-Aeberli, I., 2015. In overweight and obese women, dietary iron absorption is reduced and the enhancement of iron absorption by ascorbic acid is one-half that in normal-weight women. *The American Journal of Clinical Nutrition ,* 102(6), pp. 1389-1397.

Cerato, C. & Fonseca, F. A., 2019. Cardiovascular risk and obesity. *Diabetology & Metabolic Syndrome ,* 11(74), pp. 1-15.

Chen, M.-H.et al., 2013. Association between psychiatric disorders and iron deficiency anemia among children and adolescents: a nationwide population-based study. *BMC Psychiatry*, 13(161), pp. 1-8.

Choma, S. S. R., Alberts, M. & Modjadji, S. E. P., 2015. Conflicting effects of BMI and waist circumference on iron status. *Journal of Trace Elements in Medicine and Biology*, Volume 32, pp. 73-78.

Chukwudi, A. G., 2016. The Detereminants of Obesity among Students of the University of Venda, Limpopo Province of South Africa. *Journal of Obesity and Weight Loss Therapy*, 6(6), pp. 1-7.

Clark, S. F., 2008. Iron Deficiency Anemia. *Nutrition in Clinical Practice*, 23(2), pp. 128-141.

Coakley, K. E. et al., 2021. Physical Activity Behavior and Mental Health Among University Students During COVID-19 Lockdown. *Frontiers in Sports and Active Living*, 3(682175), pp. 1-8.

Cohen, J., 1988. *Statistical power analysis for the behavioural sciences.* 2nd ed. New York: Psychology Press.

Collaborative Laboratory Services, 2007. *Laboratory Procedure Manual - Total Iron Binding Capacity (TIBC),* Ottumwa: Collaborative Laboratory Services.

Corezzi, M. et al., 2020. Mediterranean diet and mental health in university students: an Italian cross-sectional study. *The European Journal of Public Health*, 30(5), p. v603.

Csige, I. et al., 2018. The Impact of Obesity on the Cardiovascular System. *Journal of Diabetes Research* , Volume 2018, pp. 1-12.

Cuadros, D. F. et al., 2019. Spatial structure of depression in South Africa: A longitudinal panel survey of a nationally representative sample of households. *Scientific Reports*, 9(979), pp. 1-10.

D'Angelo, G., 2013. Role of hepcidin in the pathophysiology and diagnosis of anemia. *Blood Research* , 48(1), pp. 10-15.

Daru, J. et al., 2017. Serum ferritin as an indicator of iron status: what do we need to know?. *The American Journal of Clinical Nutrition* , 106(6), pp. 1634-1639.

De Pergola, G. & Silvestris, F., 2013. Obesity as a Major Risk Factor for Cancer. *Journal of Obesity* , 2013(291546), pp. 1-11.

DeMaman, A. S. et al., 2010. Effectiveness of iron repletion in the diet for the optic nerve development of anaemic rats. *Eye,* Volume 24, pp. 901-908.

Department of Health, Medical Research Council, OrcMacro, 2007. *South Africa Demographic and Health Survey 2003,* Pretoria: Department of Health.

Department of Health: Republic of South Africa, 2015. *Strategy for the Prevention and Control of Obesity in South Africa 2015-2020,* Pretoria: Department of Health: Republic of South Africa.

Dinis, J. & Bragança, M., 2018. Quality of Sleep and Depression in College Students: A Systematic Review. *Sleep Science,* 11(4), pp. 290-301.

Dolatian, A., Arzaghi, S. M., Qorbani, M. & Pishva, H., 2017. The Relationship between Body Mass Index (BMI) and Depression According to the rs16139NPY Gene. *Iranian Journal of Psychiatry* , 12(3), pp. 201-205.

Duman, R. S., Sanacora, G. & Krystal, J. H., 2020. Altered connectivity in depression: GABA and glutamate neurotransmitter deficits and reversal by novel treatments. *Neuron,* 102(1), pp. 75-90.

Dwyer, B. E., Zacharski, L. R. & Balestra, D. J., 2010. Potential Role of Iron in a Mediterranean-style Diet. *Archives of Neurology* , 67(10), pp. 1286-1288.

Eknoyan, G., 2008. Adolphe Quetelet (1796-1874) - the average man and indices of obesity. *Nephrology Dialysis Transplantation* , 23(1), pp. 47-51.

Ellulu, M. S. et al., 2017. Obesity and inflammation: the linking mechanism and the complications. *Archives of Medical Science,* 13(4), pp. 851-863.

Elsayed, M. E., Sharif, M. U. & Stack, A. G., 2016. Chapter four - Transferrin Saturation: A Body Iron Biomarker. In: G. S. Makowski, ed. *Advances in Clinical Chemistry.* San Diego: Elsevier Science Publishing Co Inc, pp. 71-97.

Elsayed, M. E., Sharif, M. U. & Stack, A. G., 2016. Chapter Four - Transferrin Saturation: A Body Iron Biomarker. In: G. S. Makowski, ed. *Advances in Clinical Chemistry.* s.l.:Academic Press, pp. 71-97.

Espie, C. A. et al., 2014. The Sleep Condition Indicator: a clinical screening tool to evaluate insomnia disorder. *BMJ Open*, Volume 4, pp. 1-5.

Evans, K. et al., 2013. Chapter 9.11 - Hematology. In: D. Wild, ed. *The Immunoassay Handbook: Theory and Applications of Ligand Binding, ELISA and Related Techniques.* London: Newnes, pp. 795-815.

Fang, H., Tu, S., Sheng, J. & Shao, A., 2019. Depression in sleep disturbance: A review on a bidirectional relationship, mechanisms and treatment. *Journal of Cellular and Molecular Medicine,* 23(4), pp. 2324-2332.

Faruqi, A. & Mukkamalla, S. K. R., 2021. *Iron Binding Capacity,* Treasure Island: StatPearls Publishing.

Faul, F., Erdfelder, E., Lang, A.-G. & Buchner, A., 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioural, and biomedical sciences. *Behavior Research Methods,* Volume 39, pp. 175-191.

Firth, J. et al., 2020. Food and mood: how do diet and nutrition affect mental wellbeing?. *BMJ,* Volume 369, pp. 1-37.

Frater, J. L., 2021. The Top 100 Cited Papers in the Field of Iron Deficiency in Humans: a Bibliometric Study. *BioMed Research International* , 2021(5573790), pp. 1-9.

Fruh, S. M., 2017. Obesity: Risk factors, complications, and stratergies for sustainable long-term weight management. *Journal of the American Association of Nurse Practitioners,* 29(1), pp. S3-S14.

Gaultney, J. F., 2010. The Prevalence of Sleep Disorders in College Students: Impact on Academic Performance. *Journal of American College Health,* 59(2), pp. 91-97.

Georgieff, M. K., 2011. Long-term brain and behavioral consequences of early iron deficiency. *Nutritional Reviews,* 69(1), pp. 43-48.

Ghadiri-Anari, A., Nazemian, N. & Vahedian-Ardakani, H.-A., 2014. Association of Body Mass Index with Hemoglobin Concentration and Iron Parameters in Iranian Population. *ISRN Hematology,* Volume 2014, pp. 1-3.

Gillum, R. F., 2001. Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men --the Third National Health and Nutrition Examination. *Internation Journal of Obesity and Related Metabolic Disorders,* 25(5), pp. 639-645.

Ginzburg, Y. Z. & Shaz, B. H., 2013. Chapter 70 - Iron Overload. In: B. H. Shaz, C. D. Hillyer, M. Roshal & C. S. Abrams, eds. *Transfusion Medicine and Hemostasis (Second Edition).* London: Elsevier, pp. 449-451.

Girelli, D., Nemeth, E. & Swinkels, D. W., 2016. Hepcidin in the diagnosis of iron disorders. *Blood,* 127(23), pp. 2809-2813.

Guindi, M., 2018. 11 - Liver Disease in Iron Overload. In: K. D. Leslie & M. R. Wick, eds. *Practical Hepatic Pathology: a Diagnostic Approach (Second Edition).* Philadelphia: Elsevier, pp. 151-165.

Hare, D., Ayton, S., Bush, A. & Lei, P., 2013. A delicate balance: iron metabolism and diseases of the brain. *Frontiers in Aging Neuroscience* , 5(34), pp. 1-19.

Haregu, T. N., Lee, J. T., Oldenburg, B. & Armstrong, G., 2020. Comorbid Depression and Obesity: Correlates and Synergistic Association With Noncommunicable Diseases Among Australian Men. *Preventing Chronic Disease,* Volume 17.

Hattingh, Z. et al., 2008. Anthropometric and Biochemical Profiles of Black South African Women. *African Journal of Biomedical Research,* Volume 11, pp. 161-172.

Hruby, A. & Hu, F. B., 2015. The Epidemiology of Obesity: A Big Picture. *Pharmacoeconomics,* 33(7), pp. 673-689.

Huang, T.-L. & Lee, C.-T., 2007. Low serum albumin and high ferritin levels in chronic hemodialysis patients with major depression. *Psychiatry Research,* 152(2-3), pp. 277-280.

Huffington, A., 2017. Appendix A: Sleep-Quality Questionnaire. In: *The Sleep Revolution: Transforming Your Life, One Night at a Time.* London: W. H. Allen, pp. 287-289.

Human Sciences Research Council, 2014. *The South African National Health and Nutrition Examination Survey, 2012.* Cape Town: HSRC Press.

Hunt, J. M., 2002. Reversing Productivity Losses from Iron Deficiency: The Economic Case. *The Journal of Nutrition,* 132(4), pp. 794S-801S.

Jain, S., Gautam, V. & Naseem, S., 2011. Acute-phase proteins: A diagnostic tool. *Journal of Pharmacy and Bioallied Sciences,* 3(1), pp. 118-127.

Jankowich, M. et al., 2016. Relationship of Iron Deficiency and Serum Ferritin Levels with Pulmonary Hypertension: The Jackson Heart Study. *PLoS ONE,* 11(12), pp. 1-12.

Janse van Vuuren, C., Bodenstein, K. & Oberholzer, M., 2021. Exploring the psychological well-being of postgraduate accounting students at a South African university. *South African Journal of Accounting Research* , 35(3), pp. 219-238.

Jauregui-Lobera, I., 2014. Iron deficiency and cognitive functions. *Neuropsychiatric Disease and Treatment,* Volume 10, pp. 2087-2095.

Jordaan, E. M., Van den Berg, V. L., Van Rooyen, F. C. & Walsh, C. M., 2020. Obesity is associated with anaemia and iron deficiency indicators among women in the rural Free State, South Africa. *South African Journal of Clinical Nutrition* , 33(3), pp. 72-78.

Kaya, F., Daştan, N. B. & Durar, E., 2021. Smart phone usage, sleep quality and depression in university students. *The International Journal of Social Psychiatry,* 67(5), pp. 407-414.

Kerkadi, A. et al., 2021. Association between central obesity indices and iron status indicators among Qatari adults. *PLoS ONE,* 16(4), pp. 1-16.

Kim, J. & Wessling-Resnick, M., 2014. Iron and Mechanisms of Emotional Behavior. *The Journal of Nutritional Biochemistry,* 25(11), pp. 1101-1107.

Kim, M. K. et al., 2012. Increased serum ferritin predicts the development of hypertension among middle-aged men. *American Journal of Hypertension* , 25(4), pp. 492-497.

Knapskog, A. -B., Barca, M. L. & Engedal, K., 2011. A Comparison of the Validity of the Cornell Scale and the MADRS in Detecting Depression among Memory Clinic Patients. *Dementia and Geriatric Cognitive Disorders,* 32(4), pp. 287-294.

Kordas, K., Centeno, Z. Y. F., Pachón, H. & Soto, A. Z. J., 2013. Being Overweight or Obese Is Associated with Lower Prevalence of Anemia among Colombian Women of Reproductive Age. *The Journal of Nutrition* , 143(2), pp. 175-181.

Korkmaz, S. et al., 2015. Frequency of anemia in chronic psychiatric patients. *Neuropsychiatric Disease and Treatment* , 2015(11), pp. 2737-2741.

Kundrapu, S. & Noguez, J., 2018. Chapter six - Laboratory Assessment of Anemia. In: G. S. Makowski, ed. *Advances in Clinical Chemistry*. San Diego: Elsevier Science Publishing Co Inc, pp. 197-225.

Lee, H.-S.et al., 2020. Psychiatric disorders risk in patients with iron deficiency anemia and association with iron supplementation medications: a nationwide database analysis. *BMC Psychiatry* , 20(216), pp. 1-9.

Lee, H. S. & Park, E., 2019. Association of serum ferritin level and depression with respect to the body mass index in Korean male adults. *Nutrition Research and Practice,* 13(3), pp. 263-267.

Lemma, S. et al., 2012. Sleep quality and its psychological correlates among university students in Ethiopia: a cross-sectional study. *BMC Psychiatry,* 12(237), pp. 1-7.

Lewis, R. et al., 2021. The impact of sleep, physical activity and sedentary behaviour on symptoms of depression and anxiety before and during the COVID-19 pandemic in a sample of South African participants. *Scientific Reports,* 11(1).

Lin, C.-Y.et al., 2020. Advanced psychometric testing on a clinical screening tool to evaluate insomnia: sleep condition indicator in patients with advanced cancer. *Sleep and Biological Rhythms,* Volume 18, pp. 343-349.

Li, N. et al., 2020. The Efficacy and Safety of Vitamin C for Iron Supplementation in Adult Patients With Iron Deficiency Anemia: A Randomized Clinical Trial. *JAMA Network Open,* 3(11), pp. 1-9.

Litchford, M. D., 2008. Chapter 8 - Nutritional issues in the patient with diabetes and foot ulcers. In: J. H. Bowker & M. A. Pfeifer, eds. *Levin and O'Neal's The Diabetic Foot (Seventh Edition).* s.l.:Mosby, pp. 199-217.

Li, W. et al., 2020. Association between sleep duration and quality and depressive symptoms among university students: A cross-sectional study. *PLoS ONE,* 15(9).

Lohoff, F. W., 2010. Overview of the Genetics of Major Depressive Disorder. *Current Psychiatry Reports,* 12(6), pp. 539-546.

Luppino, F. S. et al., 2010. Overweight, Obesity, and Depression. A Systematic Review and Meta-analysis of Longitudinal Studies. *Archives of General Psychiatry ,* 67(3), pp. 220-229.

Mannan, M., Mamum, A., Doi, S. & Clavarino, A., 2016. Is there a bi-directional relationship between depression and obesity among adult men and women? Systematic review and bias-adjusted meta analysis. *Asian Journal of Psychiatry ,* Volume 21, pp. 51-66.

Mascitelli, L., Goldstein, M. R. & Zacharski, L. R., 2015. Chapter 24 - The Mediterranean Diet and Body Iron Stores. In: V. R. Preedy & R. R. Watson, eds. *The Mediterranean Diet.* s.l.:Academic Press, pp. 259-269.

Matlala, M., Maponya, M. L., Chigome, A. K. & Meyer, J. C., 2018. Overview of mental health: A public health priority. *South African Pharmaceutical Journal,* 85(6), pp. 46-53.

Mbugi, E. V. & Chilongola, J. O., 2010. Allergic Disorders in Africa and Africans: Is It Primarily a Priority. *World Allergy Organization Journal,* 3(5), pp. 175-181.

McArdle, N. et al., 2020. The prevalence of common sleep disorders in young adults: a descriptive population-based study. *Sleep,* 43(10).

McDermid, J. M. & Lonnerdal, B., 2012. Iron. *Advances in Nutrition ,* 3(4), pp. 532-533.

Mchiza, Z. J. et al., 2018. Understanding the determinants of hemoglbin and iron status: adolescent-adult women comparisons in SANHANES-1. *Annals of the New York Academy of Sciences,* pp. 1-17.

Means Jr, R. T., 2014. Iron Metabolism and Related Disorders. *Elsevier.*

Metin, D. & Okan Bakir, B., 2021. The Association Between Mediterranean Diet and Emotional Status Among University Students. *ESTÜDAM Halk Sağlığı Dergisi,* 6(2), pp. 159-168.

Milaneschi, Y., Simmons , W. K., van Rossum, E. F. C. & Penninx, B. W., 2019. Depression and obesity: evidence of shared biological mechanisms. *Molecular Psychiatry ,* Volume 24, pp. 18-33.

Milano, W. et al., 2020. Depression and Obesity: Analysis of Common Biomarkers. *Diseases,* 8(2), pp. 1-19.

Miller, J. L., 2013. Iron Deficiency Anemia: A Common and Curable Disease. *Cold Spring Harbor Perspectives in Medicine,* 3(7), pp. 1-13.

Monteiro, A. M. et al., 2018. Iron Deficiency and Obesity - Are we Diagnosing with Appropriate Indicators?. *Acta Medica Portuguesa,* 31(9), pp. 478-482.

Montgomery, S. A. & Asberg, M., 1979. A New Depression Scale Designed to be Sensitive to Change. *The British Journal of Pyschiatry*, Volume 134, pp. 382-389.

Motonishi, S., Tanaka, K. & Ozawa, T., 2018. Iron deficiency associates with depression in several symptoms independently from hemoglobin level among chronic hemodialysis patients. *PLoS ONE*, 13(8), pp. 1-14.

Murat, S. et al., 2015. Assessment of subjective sleep quality in iron deficiency anaemia. *African Health Sciences*, 15(2), pp. 621-627.

Murphy, M. & Peterson, M. J., 2015. Sleep Disturbances in Depression. *Sleep Medicine Clinics*, 10(1), pp. 17-23.

Mutyambizi, C., Booysen, F., Stornes, P. & Eikemo, T. A., 2019. Subjective social status and inequalities in depressive symptoms: a gender-specifc decomposition analysis for South Africa. *International Journal for Equality in Health* , 18(87), pp. 1-13.

Nabeshima, T. & Kim, H.-C., 2013. Involvement of Genetic and Environmental Factors in the Onset of Depression. *Experimental Neurobiology* , 22(4), pp. 235-243.

Nemeth, E., 2010. Targeting the Hepcidin-Ferroportin Axis in the Diagnosis and Treatment of Anemias. *Advances in Hematology*, 2010(750643), pp. 1-9.

Nemeth, E. & Ganz, T., 2021. Hepcidin-Ferroportin Interaction Controls Systemic Iron Homeostasis. *International Journal of Molecular Sciences*, 22(6493), pp. 1-13.

Nguse, S. & Wassenaar, D., 2021. Mental health and COVID-19 in South Africa. *South African Journal of Pyschology*, 51(2), pp. 304-313.

Ning, F. et al., 2020. Synergistic effects of depression and obesity on type 2 diabetes incidence in Chinese adults. *Journal of Diabetes*, 12(2), pp. 142-150.

Nkeh-Chungag, B. N., Mxhosa, T. H. & Mgoduka, P. N., 2015. Association of waist and hip circumeferences with the presence of hypertension and pre-hypertension in young South African adults. *African Health Sciences*, 15(3), pp. 908-916.

Noorazar, S. G. et al., 2015. Relationship between severity of depression symptoms and iron deficiency anemia in women with major depressive disorder. *Journal of Research in Clinical Medicine*, 3(4), pp. 219-224.

Ntini, I. et al., 2020. The Montgomery and Åsberg Depression Rating Scale – self-assessment for use in adolescents: an evaluation of psychometric and diagnostic accuracy. *Nordic Journal of Psychiatry*, 74(6), pp. 415-422.

Nunes, A. R. & Tata, M., 2017. The impact of anaemia and iron deficiency in chronic obstructive pulmonary disease: A clinical overview. *Revista Portuguesa de Pneumologia (English Edition)*, 23(3), pp. 146-155.

Nuttall, F. Q., 2015. Body Mass Index. *Nutrition Today* , 50(3), pp. 117-128.

Nutt, D., Wilson, S. & Paterson, L., 2008. Sleep disorders as core symptoms of depression. *Dialogues in Clinical Neuroscience*, 10(3), pp. 329-336.

Nutt, D., Wilson, S. & Paterson, L., 2008. Sleep disoreders as core symptoms of depression. *Dialogues in Clinical Neuroscience*, 10(3), pp. 329-336.

Ogden, C. L., Yanovski, S. Z., Carroll, M. D. & Flegal, K. M., 2007. Epidemiology of Obesity. *Gastroenterology*, Volume 132, pp. 2087-2102.

Ogun, A. S. & Adeyinka, A., 2021. *Biochemistry, Transferrin*, Treasure Island: StatPearls Publishing.

Oh, Y., Kang, B. J., Yoo, S. & Lopez, A., 2016. Overweight and Obese College Students' Perceived. *European Journal of Educational Sciences*, 3(4), pp. 1-10.

Okan, S. et al., 2019. Association of ferritin levels with depression, anxiety, sleep quality, and physical functioning in patients with fibromyalgia syndrome: a cross-sectional study. *Croatian Medical Journal*, 60(6), pp. 515-520.

Okan, S. et al., 2019. Association of ferritin levels with depression, anxiety, sleep quality, and physical functioning in patients with fibromyalgia syndrome: a cross-sectional study. *Croatian Medical Journal*, 60(6), pp. 515-520.

Ormel, J., Hartman, C. A. & Snieder, H., 2019. The genetics of depression: successful genome-wide association studies introduce new challenges. *Translational Psychiatry*, 9(114), pp. 1-10.

Oyenubi, A. & Kollamparambil, U., 2020. *COVID-19 and Depressive symptoms in South Africa*, Johannesburg: National Income Dynamics Study (NIDS) - Coronavirus Rapid Mobile Survey (CRAM).

Pagani, A., Nai, A., Silvestri, L. & Camaschella, C., 2019. Hepcidin and Anemia: A Tight Relationship. *Frontiers in Physiology* , 10(1294), pp. 1-7.

Pan, A. et al., 2012. Bidirectional Association between Depression and Obesity in Middle-aged and Older Women. *Interational Journal of Obesity* , Volume 36, pp. 595-602.

Pantalone, K. M. et al., 2017. Prevalence and recognition of obesity and its associated comorbidities: cross-sectional analysis of electronic health record data from a large US integrated health system. *BMJ Open*, Volume 7, pp. 1-9.

Parekh, A., Smeeth, D., Milner, Y. & Thuret, S., 2017. The Role of Lipid Biomarkers in Major Depression. *Healthcare,* 5(5), pp. 1-17.

Park, K. et al., 2020. The Montgomery and Åsberg Depression Rating Scale – self-assessment for use in adolescents: an evaluation of psychometric and diagnostic accuracy. *Frontiers in Psychology,* 10(2934), pp. 1-10.

Pasricha, S.-R., Tye-Din, J., Muckenthaler, M. U. & Swinkels, D. W., 2020. Iron Deficiency. *The Lancet,* 397(10270), pp. 233-248.

PathCare Reference Laboratory, 2021. *Final Report,* Cape Town: PathCare.

Pedersen , S. D., 2013. Metabolic complications of obesity. *Best Practice and Research. Clinical Endocrinology and Metabolism,* 27(2), pp. 179-193.

Peltzer, K. & Pengpid, S., 2012. Body weight and body image among a sample of female and male South African university students. *Gender and Behaviour,* 10(1).

Peltzer, K. et al., 2014. Prevalence of Overweight/Obesity and Its Associated Factors among University Students from 22 Countries. *International Journal of Environmental Research and Public Health,* 11(7), pp. 7425-7441.

Péneau, S. et al., 2008. Relationship between iron status and dietary fruit and vegetables based on their vitamin C and fiber content. *The American Journal of Clinical Nutrition,* 87(5), pp. 1298-1305.

Pengpid, S. & Karl, P., 2018. Religiosity and body mass index and waist circumference among male and female university students from 26 countries in Africa, Asia, Latin America and the Caribbean. *Gender and Behaviour,* 16(3).

Pengpid, S. & Peltzer, K., 2014. Prevalence of overweight/obesity and central obesity and its associated factors among a sample of university students in India. *Obesity Research and Clinical Practice,* Volume 8, pp. e558-e570.

Peng, Y. Y. & Uprichard, J., 2017. Ferritin and iron studies in anaemia and chronic disease. *Annals of Clinical Biochemistry ,* 54(1), pp. 43-48.

Pérez, M. O. et al., 2020. Relationship between Obesity and Iron Deficiency in Health Adolescents. *Childhood Obesity,* 16(6), pp. 440-447.

Peters, U. & Dixon, A. E., 2019. The effect of obesity on lung funtion. *Expert Review of Respiratory Medicine ,* 12(9), pp. 755-767.

Pfeiffer, C. M. & Looker, A. C., 2017. Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. *The American Journal of Clinical Nutrition ,* Volume 106, pp. 1606S-1614S.

Phatlhane, D. V. et al., 2016. The iron status of a healthy South African adult population. *Clinica Chimica Acta,* Volume 460, pp. 240-245.

Pietrangelo, A., 2018. 60 - Hemochromatosis. In: A. J. Sanyal, T. D. Boyer, K. D. Lindor & N. A. Terrault, eds. *Zakim and Boyer's Hepatology (Seventh Edition).* s.l.:Elsevier, pp. 941-959.

Pillay, A. L., Thwala, J. D. & Pillay, I., 2020. Depressive symptoms in first year students at a rural South African University. *Journal of Affective Disorders,* 15(265), pp. 579-582.

Pisa, P. T. & Pisa, N. M., 2017. Economic growth and obesity in South African adults: an ecological analysis between 1994 and 2014. *European Journal of Public Health,* 27(3), pp. 404-409.

Pi-Sunyer, X., 2009. The Medical Risks of Obesity. *Postgraduate Medicine,* 121(6), pp. 21-23.

Pizzini, A. et al., 2020. The Significance of iron deficiency and anemia ina real-life COPD cohort. *International Journal of Medical Sciences,* 17(14), pp. 2232-2239.

Podmore, C. et al., 2016. The Association of Multiple Biomarkers of Iron Metabolism and Type 2 Diabetes - the EPIC-InterAct Study. *Diabetes Care,* 39(4), pp. 572-581.

Portugal-Nunes, C. et al., 2020. Iron Status is Associated with Mood, Cognition, and Functional Ability in Older Adults: A Cross-Sectional Study. *Nutrients,* 12(3594), pp. 1-16.

Powell-Wiley, T. M. et al., 2021. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation* , 143(21), pp. e984-e1010.

Qin, Y. et al., 2013. Anemia in relation to body mass index and waist circumference among Chinese women. *Nutrition Journal,* 12(10), pp. 1-3.

Rad, H. A. et al., 2019. Obesity and iron-deficiency anemia in women of reproductive age in northern Iran. *Journal of Education and Health Promotion* , 8(115), pp. 1-16.

Ramakrishnan, L. et al., 2018. Pulmonary Arterial Hypertension: Iron Matters. *Frontiers in Physiology,* 9(641), pp. 1-11.

Regenold, W. T. et al., 2007. Myein staining of deep white matter in the dorsolateral prefronal cortex in schizophrenia, bipolar disorder, and unipolar major depression. *Psychiatry Research,* 151(3), pp. 179-188.

Reid, A. & Baker, F. C., 2008. Perceived Sleep Quality and Sleepiness in South African University Students. *South African Journal of Psycholog,* 38(2), pp. 287-303.

Rezende, A. C. et al., 2018. Is waist-to-height ratio the best predictive indicator of hypertension incidence? A cohort study. *BMC Public Health,* 18(281), pp. 1-11.

Richardson, A. C. et al., 2015. Higher Body Iron Is Associated with Greater Depression Symptoms among Young Adult Men but not Women: Observational Data from the Daily Life Study. *Nutrients* , 7(8), pp. 6055-6072.

Rodriguez-Garcia, C. et al., 2021. Transferrin-mediated iron sequestration suggests a novel therapeutic strategy for controlling Nosema disease in the honey bee, Apis mellifera. *PLoS Pathogens,* 17(2), pp. 1-30.

Ross, J. & Horton, S., 1998. *Economic Consequences of Iron Deficiency* , Ottawa: Micronutrient Initiative .

Ross, R. et al., 2020. Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. *Nature Reviews Endocrinology,* Volume 16, pp. 177-189.

Ruiter, G. et al., 2011. Iron deficiency is common in idiopathic pulmonary arterial hypertension. *European Respiratory Journal* , 37(6), pp. 1386-1391.

Ruiter, G. et al., 2015. Intravenous Iron Therpay in Patients with Idiopathic Pulmonary Arterial Hypertension and Iron Deficiency. *Pulmonary Circulation,* 5(3), pp. 466-472.

Sacchet, M. D. & Gotlib, I. H., 2017. Myelination of the brain in Major Depressive Disorder: An in vivo quantitative magnetic resonance imaging study. *Scientific Reports,* 7(2200), pp. 1-14.

Saito, H., 2014. Metabolism of Iron Stores. *Nagoya Journal of Medical Science* , 76(3), pp. 235-254.

Sanad, M., Osman, M. & Gharib, A., 2011. Obesity modulate serum hepcidin and treatment outcome of iron deficiency anemia in children: A case control study. *Italian Journal of Pediatrics,* 37(34), pp. 1-6.

Saonatse, L., De Witt, P. A. & van Niekerk, M., 2019. Experiences and perceptions of Return to Work (RTW) by clients with major depressive disorder in an extended sick leave period. *South African Journal of Occupational Therapy,* 49(1), pp. 36-42.

Sarawagi, A., Soni, N. D. & Patel, A. B., 2021. Glutamate and GABA Homeostasis and Neurometabolism in Major Depressive Disorder. *Frontiers in Psychiatry* , 12(637863), pp. 1-16.

Sato, M. et al., 2019. Impact of Transferrin Saturation on All-Cause Mortality in Patients on Maintenance Hemodialysis. *Blood Purification,* 48(2), pp. 158-166.

Saveanu, R. V. & Nemeroff, C. B., 2012. Etiology of Depression: Genetic and Environmental Factors. *Psychiatric Clinics,* 35(1), pp. 51-71.

Schachter, J. et al., 2018. Effects of obesity on depression: A role for inflammation and the gut microbiota. *Brain, Behavior, and Immunity* , Volume 69, pp. 1-8.

Schmidt, C. W., 2007. Environmental Connections: A Deeper Look into Mental Illness. *Environmental Health Perspectives,* 115(8), pp. 4-10.

Sciacqua, A. et al., 2020. Ferritin modifies the relationship between inflammation and arterial stiffness in hypertensive patients with different glucose tolerance. *Cardiovascular Diabetology,* 19(123), pp. 1-10.

Shadrina, M., Bondarenko, E. A. & Slominsky, P. A., 2018. Genetics Factors in Major Depression Disease. *Frontiers in Psychiatry* , 9(334), pp. 1-18.

Shafi, M. et al., 2018. Relation between Depressive Disorder and Iron deficiency Anemia among Adults Reporting to a Secondary Healthcare Facility: A Hospital-Based Case Control Study. *Journal of the College of Physicians and Surgeons Pakistan,* 28(6), pp. 456-459.

Shariatpanaahi, M. V. et al., 2007. The relationship between depression and serum ferritin. *European Journal of Clinical Nutrition ,* 61(4), pp. 532-535.

Shisana, O. et al., 2013. *The South African National Health and Nutrition Examination Survey (SANHNES-1),* Cape Town : HSRC Press.

Stack, A. G. et al., 2014. Transferrin saturation ratio and risk of total and cardiovascular mortality in the general population. *QJM: An International Journal of Medicine ,* 107(8), pp. 623-633.

Stander, M. P. et al., 2016. Depression in the South African workplace. *South African Journal of Psychiatry ,* 22(1), p. a814.

Statistics South Africa, 2016. *South Africa Demographic and Health Survey ,* Pretoria: Statistics South Africa.

Stoffel, N. U. et al., 2020. The effect of central obesity on inflammation, hepcidin, and iron metabolism in young women. *International Jouranl of Obesity ,* Volume 44, pp. 1291-1300.

Stone, T. W., McPherson, M. & Darlington, L. G., 2018. Obesity and Cancer: Existing and New Hypotheses for a Causal Connection. *EBioMedicine,* Volume 30, pp. 14-28.

Su, Q. et al., 2016. Association between Serum Ferritin Concentrations and Depressive Symptoms among Chinese Adults: A Population Study from the Tianjin Chronic Low-Grade Systemic Inflammation and Health (TCLSIHealth) Cohort Study. *PLoS ONE,* 11(9), pp. 1-12.

Su, Q. et al., 2016. Association between Serum Ferritin Concentrations and Depressive Symptoms among Chinese Adults: A Population Study from the Tianjin Chronic Low-Grade Systemic Inflammation nad Health (TCLSIHealth) Cohort Study. *PLoS ONE,* 11(9), pp. 1-12.

Svanborg, P. & Asberg, M., 2001. A comparison between the Beck Depression Inventory (BDI) and self-rating version of the Montgomery Asberg Depression Rating Scale (MADRS). *Journal of Affective Disorders,* Volume 64, pp. 203-216.

Taylor, C. L. & Brannon, P. M., 2017. Introduction to workshop on iron screening and supplementation in iron-replete pregnant women and young children. *The American Journal of Clinical Nutrition* , Volume 106, pp. 1547S-1554S.

Telleria-Aramburu, N. & Arroyo-Izaga, M., 2021. Risk factors of overweight/obesity-related lifestyles in university students: Results from the EHU12/24 study. *British Journal of Nutrition* , 127(6), pp. 914-926.

Ter Goon, D., Libelela, M., Amusa, L. O. & Muluvhu, T. C., 2013. Screening for total and abdominal obesity among University of Venda Students. *International Journal of Physical Medicine and Rehabilitation* , 1(6), p. 67.

Thomas, B. L., Bipath, P. & Viljoen, M., 2020. Inflammatory activity and academic performance in university students. *Journal of Psychology in Africa*, 30(1), pp. 53-57.

Tilea, I. et al., 2021. Short-term Impact of Iron Deficiency in Different Subsets of Patients with Precapillary Pulmonary Hypertension Referral Center. *International Journal of General Medicine* , 2021(14), pp. 3355-3366.

Tomlinson, M. et al., 2009. The epidemiology of major depression in South Africa: Results from the South African Stress and Health study. *South African Medical Journal*, 99(5), pp. 376-373.

Turawa, E. et al., 2021. prevalence of Anaemia, Iron Deficiency, and Iron Deficiency Anaemia in Women of Reproductive Age and Children under 5 Years of Age in South Africa (1997-2021): A Systematic Review. *International Journal of Environmental Research and Public Health*, 18(12799), pp. 1-17.

Ueda, T. et al., 2021. Serum iron: a new predicitor of adverse outcomes independently from serum hemoglobin levels in patients with acute decompensated heart failure. *Scientific Reports* , 11(2395), pp. 1-9.

Ugochukwu, E. F. et al., 2020. Waist Circumeference, Waist-to-Height Ratio and Body Mass Index as Parameters of Obesity Among Public Secondary School Students. *European Journal of Medical and Health Sciences*, 2(4), pp. 1-8.

van den Bosch, M. & Meyer-Lindenberg, A., 2019. Environmental Exposures and Depression: Biological Mechanisms and Epidemiological Evidence. *Annual Review of Public Health*, Volume 40, pp. 239-259.

van der Walt, S., Mabaso, W. S., Davids, E. L. & de Vries, P. J., 2020. The burden of depression and anxiety among medical students in South Africa: A cross-sectional survey at the University of Cape Town. *South African Medical Journal,* 110(1), pp. 69-76.

van Empel, V. P. M., Lee, J., Williams, T. J. & Kaye, D. M., 2014. Iron Deficiency in Patients With Idiopathic Pulmonary Arterial Hypertension. *Heart, Lung and Circulation ,* 23(3), pp. 287-292.

Ventriglio, A. et al., 2020. Mediterranean Diet and its Benefits on Health and Mental Health: A Literature Review. *Clinical Practice and Epidemiology in Mental Health ,* Volume 16, pp. 156-164.

Visser, M. & Law-van Wyk, E., 2021. University students' mental health and emotional wellbeing during the COVID-19 pandemic and ensuing lockdown. *South African Journal of Psychology ,* 51(2), pp. 229-243.

Vuik, S. et al., 2019. *The economic burden of obesity,* Paris: Organisation for Economic Co-operation and Development.

Vulser, H. et al., 2016. Association between depression and anemia in otherwise healthy adults. *Acta Psychiatrica Scandinavica,* 134(2), p. 1.

Wang, W. et al., 2010. Serum Ferritin: Past, Present and Future. *Biochimica et Biophysica Acta,* 1800(8), pp. 760-769.

Wells, J. C. K., 2014. Commentary: The paradox of body mass index in obesity assessment: not a good index of adiposity, but not a bad index of cardio-metabolic risk. *International Journal of Epidemiology,* 43(3), pp. 672-674.

Wiencek, J. R., Duh, S.-H. & Christenson, R. H., 2020. Chapter 22 - Proteins: analysis and interpretation in serum, urin, and cerebrospinal fluid. In: W. Clarke & M. A. Marzinke, eds. *Contemporary Practice in Clinical Chemistry (Fourth Edition).* s.l.:Academic Press, pp. 365-390.

Williams, M. R. et al., 2019. Axonal myelin decrease in the splenium in major depressive disorder. *European Archives of Psychiatry and Clinical Neuroscience ,* Volume 269, pp. 387-395.

World Health Organisation, 2001. *Iron Deficiency Anaemia: Assessment, Prevention and Control. A guide for programme managers,* Geneva: World Health Organisation .

World Health Organization , 2010. *WHO guidelines on drawing blood: best pratices in phlebotomy,* Geneva: World Health Organization.

World Health Organization , 2018. *Country Cooperation Strategy at a Glance ,* Geneva: World Health Organization .

World Health Organization, 1998. *Obesity: Preventing and managing the global epidemic,* Geneva: World Health Organization.

World Health Organization, 2007. *Assessing the iron status of populations: including literature reviews. Second edition,* Geneva: World Health Organization.

World Health Organization, 2008. *Worldwide prevalence of anaemia 1993-2005,* Geneva: World Health Organization.

World Health Organization, 2011. *Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations,* Geneva: World Health Organization.

World Health Organization, 2011. *Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations,* Geneva: World Health Organization.

World Health Organization, 2011. *Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations,* Geneva: World Health Organization.

World Health Organization, 2011. *Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation,* Geneva: World Health Organization.

World Health Organization, 2017. *Depression and Other Common Mental Disorders Global Health Estimates,* Geneva: World Health Organization.

World Health Organization, 2017. *WHO STEPS Surveillance Manual: The WHO STEPwise approach to noncommunicable disease risk factor surveillance,* Geneva: World Health Organization.

World Health Organization, 2019. *A heavy burden: the indirect cost of illness in Africa,* Brazzaville: WHO Regional Office for Africa.

World Health Organization, 2021. *Obesity and Overweight,* Geneva: World Health Organization .

Yang, S. et al., 2020. Obesity and activity patterns before and during COVID-19 lockdown among youths in China. *Clinical Obesity ,* Volume 10, pp. 1-7.

Yiannikourides, A. & Latunde-Dada, G. O., 2019. A Short Review of Iron Metabolism and Pathophysiology of Iron Disorders. *Medicines* , 6(85), pp. 1-15.

Yin, W. et al., 2021. Mediterranean diet and depression: a population-based cohort study. *International Journal of Behavioral Nutrition and Physical Activity* , 18(153), pp. 1-10.

Yi, S. et al., 2011. Association between serum ferritin concentrations and depressive symptoms in Japanese municipal employees. *Psychiatry Research,* 189(3), pp. 368-372.

Zhao, L. et al., 2015. Obesity and iron deficiency: a quantitative meta-analysis. *Obesity Reviews,* 16(12), pp. 1081-1093.

Appendices

Appendix A: Demographic questions

The following questions were obtained from the Google Form that the participants were sent prior to their scheduled appointments. This form contained the MADRS questionnaire. The participants were required to either provide short answers or select a response from the lists provided.

1) Name and student number:

_____

2) Gender:

Female	
Male	
Other	

3) Race:

Black	
White	
Coloured	
Indian	
Asian	
Other	

4) Age:

18 – 20	
21 – 23	
24 – 26	
27 – 29	
30 – 32	
33 – 35	
36 and over	

5) Faculty and department:

_____

6) Year of study: first, second, third, fourth, honours, masters, PhD

First	
Second	
Third	
Fourth	
Honours	
Masters	
PhD	

7) BEFORE WE GET STARTED: Did your depressive symptoms begin before or after the COVID-19 pandemic? AND did the pandemic better or worsen your symptoms?

	Better	Worsen
Before		
After		

8) Have you been professionally diagnosed with depression? AND are you currently on medication?

	Yes, I am on medication	No, I am not on medication
Yes, I have been diagnosed.		
No, I have not been diagnosed.		

Appendix B: Montgomery-Asberg Depression Rating Scale

1 Apparent sadness. Representing despondency, gloom, and despair (more than just ordinary transient low spirits), reflected in speech, facial expression, and posture. Rate by depth and inability to brighten up.

0	No sadness
2	Looks dispirited but does brighten up without difficult
4	Appears sad and unhappy most of the time
6	Looks miserable all the time. Extremely despondent

2 Reported sadness. Representing reports of depressed mood, regardless of whether it is reflected in appearance or not. Includes low spirits, despondency, or the feeling of being beyond help and without hope.

0	Occasional sadness in keeping with the circumstances
2	Sad or low but brightens up without difficulty
4	Pervasive feelings of sadness or gloominess. The mood is still influenced by external circumstances
6	Continuous or unvarying sadness, misery or despondency

3 Inner tension. Representing feelings of ill-defined discomfort, edginess, inner turmoil, mental tension mounting to either panic, dread, or anguish. Rate according to intensity, frequency, duration, and the extent of reassurance called for.

0	Placid. Only fleeting inner tension
2	Occasional feelings of edginess and ill-defined discomfort
4	Continuous feelings of inner tension of intermittent panic which the patient can only master with some difficulty
6	Unrelenting dread or anguish. Overwhelming panic

4 Reduced sleep. Representing the experience of reduced duration or depth of sleep compared to the subject's own normal pattern when well.

0	Sleeps as normal
2	Slightly difficult dropping off to sleep or slightly reduced, light or fitful sleep
4	Moderate stiffness and resistance
6	Sleep reduced or broken by at least 2 hours

5 Reduced appetite. Representing the feeling of a loss of appetite compared with when-well. Rate by loss of desire for food or the need to force oneself to eat.

0	Normal or increased appetite
2	Slightly reduced appetite
4	No appetite. Food is tasteless
6	Needs persuasion to eat at all

6 Concentration difficulties. Representing difficulties in collecting one's thoughts mounting to an incapacitating lack of concentration. Rate according to intensity, frequency, and degree of incapacity produced.

0	No difficulties in concentrating
2	Occasional difficulties in collecting one's thoughts
4	Difficulties in concentrating and sustaining thought which reduced ability to read or hold a conversation
6	Unable to read or converse without great difficulty

7 Lassitude. Representing difficulty in getting started or slowness in initiating and performing everyday activities.

0	Hardly any difficulty in getting started. No sluggishness
2	Difficulties in starting activities
4	Difficulties in starting simple routine activities which are carried out with effort
6	Complete lassitude. Unable to do anything without help

8 Inability to feel. Representing the subjective experience of reduced interest in the surroundings, or activities that normally give pleasure. The ability to react with adequate emotion to circumstances or people is reduced.

0	Normal interest in the surroundings and in other people
2	Reduced ability to enjoy usual interests
4	Loss of interest in the surroundings. Loss of feelings for friends and acquaintances
6	The experience of being emotionally paralysed, inability to feel anger, grief, or pleasure and a complete or even painful failure to feel for close relatives and friends

9 Pessimistic. thoughts Representing thoughts of guilt, inferiority, self-reproach, sinfulness, remorse, and ruin.

0	No pessimistic thoughts
2	Fluctuating ideas of failure, self-reproach, or self- depreciation
4	Persistent self-accusations, or definite but still rational ideas of guilt or sin. Increasingly pessimistic about the future
6	Delusions of ruin, remorse, or irredeemable sin. Self- accusations which are absurd and unshakable

10 Suicidal thoughts Representing the feeling that life is not worth living, that a natural death would be welcome, suicidal thoughts, and preparations for suicide. Suicide attempts should not in themselves influence the rating.

0	Enjoys life or takes it as it comes
2	Weary of life. Only fleeting suicidal thoughts
4	Probably better off dead. Suicidal thoughts are common, and suicide is considered as a possible solution, but without specific plans or intentions
6	Explicit plans for suicide when there is an opportunity. Active preparations for suicide

Appendix C: Beck's Depression Inventory

1) Sadness

0	I do not feel sad
1	I feel sad
2	I am sad all the time and can't snap out of it
3	I am so sad and unhappy I can't stand it

2) Pessimism

0	I am not particularly discouraged by the future
1	I feel discouraged about the future
2	I feel I have nothing to look forward to
3	I feel the future is hopeless and that things cannot improve

3) Sense of failure

0	I do not feel like a failure
1	I feel that I have failed more than the average person
2	As I look back on my life, all I can see are failures
3	I feel I am a complete failure as a person

4) Dissatisfaction

0	I get as much satisfaction out of things as I used to
1	I don't enjoy things the way I used to
2	I don't get real satisfaction out of anything anymore
3	I am dissatisfied or bored with everything

5) Guilt

0	I do not feel particularly guilty
1	I feel guilty a good part of the time
2	I feel quite guilty most of the time
3	I feel guilty all the time

6) Expectation of punishment

0	I do not feel I am being punished
1	I feel I may be punished
2	I expect to be punished
3	I feel I am being punished

7) Self-dislike

0	I do not feel disappointed in myself
1	I am disappointed with myself
2	I am disgusted with myself
3	I hate myself

8) Self-blame

0	I do not feel that I am any worse than anybody else
1	I am critical of myself for my weaknesses or mistakes
2	I blame myself all the time for my faults
3	I blame myself for everything bad that happens

9) Suicidal thoughts

0	I do not have any thoughts of killing myself
1	I have thoughts of killing myself, but I would not carry them out
2	I would like to kill myself
3	I would kill myself if I had the chance

10) Episodes of crying

0	I do not cry more than usual
1	I cry more than I used to
2	I cry all the time now
3	I used to be able to cry, now I can't cry even though I want to

11) Irritability

0	I am no more irritated by things than I ever was
1	I am slightly more irritated now than usual
2	I am quite annoyed/irritated a good deal of the time
3	I feel irritated all the time

12) Social withdrawal

0	I have not lost interest in other people
1	I am less interested in other people than I used to be
2	I have lost most interest in other people
3	I have lost all my interest in other people

13) Indecisiveness

0	I makes decisions about as well as I ever could
1	I put off making decisions more than I used to
2	I have greater difficulty in making decisions more than I used to
3	I can't make decisions at all anymore

14) Worthlessness

0	I do not feel that I look any worse than I used to
1	I am worried that I am looking old or unattractive
2	I feel that there are permanent changes in my appearance that make me look unattractive
3	I believe that I look ugly

15) Retardation

0	I can work about as well as before
1	It takes extra effort to get started at doing something
2	I have to push myself very hard to do anything
3	I can't do any work at all

16) Insomnia

0	I can sleep as well as usual
1	I don't sleep as well as I used to
2	I wake up 1-2 hours earlier than I used to and find it hard to get back to sleep
3	I wake up several hours earlier than I used to and cannot get back to sleep

17) Fatigability

0	I do not get more tired than usual
1	I get tired more easily than I used to

2	I get tired from doing almost nothing
3	I am too tired to do anything

18) Loss of appetite

0	My appetite is no worse than usual
1	My appetite is not as good as it used to be
2	My appetite is much worse now
3	I have no appetite at all anymore

19) Loss of weight

0	I haven't lost much weight, if any, lately
1	I have lost more than 5 pounds (approx. 2.25kg)
2	I have lost more than 10 pounds (approx. 4.5kg)
3	I have lost more than 15 pounds (approx. 6.8kg)

20) Somatic preoccupation

0	I am no more worried about my health than usual
1	I am worried about my physical problems – aches, pains, upset stomach or constipation
2	I am very worried about my physical problems and it's hard to think of much else
3	I am so worried about my physical problems that I cannot think of anything else

21) Lack of interest in sex

0	I have not noticed any recent change in my interest in sex
1	I am less interested in sex than I used to be
2	I have almost no interest in sex
3	I have lost interest in sex completely

Appendix D : Sleep quality questionnaire

1 How long does it take you to fall asleep?

15-30 minutes	30-45 minutes	45-60 minutes	60 minutes >

2 How many nights a week do you have a problem with your sleep?

1-2 times	3-4 times	4-5 times	6-7 times

3 How long have you had a problem with your sleep?

3 months <	3-6 months	6-12 months	12 months >

4 How would you rate your sleep quality?

Very good	Good	Average	Poor	Very poor

5 Do you take any medications/aids to help you fall asleep? (Ranging from sedatives to chamomile tea).

Never	Sometimes	Mostly	Always

6 Do you ever sleep during the day?

Never	Sometimes	Mostly	Always

7 Do you feel drained after you have woken up?

Never	Sometimes	Mostly	Always

Thinking about the past month, to what extent has poor sleep… (applicable to questions 8 and 9).

8 Affected your mood, energy, or relationship?

Not at all	A little	Somewhat	Much	Very much

9 Affected your concentration, productivity, or ability to stay awake?

Not at all	A little	Somewhat	Much	Very much

Appendix E: Informed consent and information sheets

University of the Western Cape Ethics Committee (Reference Code: BM21/9/15)

Informed Consent Form for Clinical Studies

ROBERT SOBUKWE RD, BELLVILLE, CAPE TOWN, 7535

This informed consent form is for students residing in the Western Cape who wish to participate in a research study titled "Investigating the relationship between iron deficiency and depressive symptoms in relation to the body compositions of students residing in the Western Cape".

Name of Principal Investigator: Kiyara Govender
Name of Organization: University of the Western Cape

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you).
- Certificate of Consent (for signatures if you agree to partake).

You will be given a copy of the complete Informed Consent Form.

PART I: Information Sheet

Introduction

I am Kiyara Govender, a master's student majoring in Medical Bioscience at the University of the Western Cape. I am researching the relationship between iron deficiency and depressive

symptoms as a possible means to combat depression, which unfortunately remains rife throughout South Africa. Additionally, I will be investigating whether a relationship exists between obesity and iron-deficient individuals experiencing moderate to severe depressive symptoms. I will give you further information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the study. Before you choose, you can talk to anyone you feel comfortable with about the research. This consent form may contain some words that you do not understand. Please ask me to stop as we go through the information, and I will take the time to explain. If you have questions later, you can ask them of my supervisors or me.

Purpose of the research

Iron deficiency is the consequence of a prolonged diminished iron balance, whereby iron stores are no longer sufficient to meet the requirements of regular iron turnover. It is the most ubiquitous single nutrient deficiency, accounting for most cases of anaemia globally. Iron deficiency can cause symptoms of fatigue, issues regulating body temperature and inadequate physical endurance. Additionally, it has been associated with cognitive impairments, including attention span, intelligence, emotions, and behaviour. Subsequently, the association between iron deficiency and depression was proposed. Depression is one of the world's most common mental illnesses, the consequences of which place a heavy burden on the individual with the condition and their family and the economy. Depression is also associated with poor diets and obesity. It has been hypothesized that individuals alter both their food choices and the quantities of food they consume as a means to regulate their mood and emotions, potentially creating a detrimental cycle. Therefore, all aetiologies of depression must be explored to develop novel ways of treating the condition, thus relieving the burden. This research study investigates the role of iron deficiency in the experience of depressive symptoms concerning the body composition of a university student population.

Type of Research Intervention

This research study comprises of fifty-one (n = 51) participants that will answer standardized questionnaires, namely the Beck Depression Inventory (BDI) and the Montgomery-Asberg Depression Rating Scale (MADRS), as well as a sleep quality questionnaire, of which you have already completed. The standardized questionnaires allow for quantifying your depressive symptoms and the sleep quality questionnaire to assess your sleep health. Upon arrival, you will have your body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR),

and waist-to-height ratios (WHtR) measured to determine your weight range. Thereafter, you will have 5.0 ml of whole venous blood drawn from their median cubital vein located on their forearm by a registered medical doctor, Dr Juley De Smidt. The blood samples will be transported to PathCare, N1 City, Goodwood, where they will be analysed. The following five iron status indicators will be evaluated: ferritin, serum iron, transferrin, transferrin saturation and total iron-binding capacity. Based on these results, you will either be characterized as being iron-deficient or iron-replete.

Participant selection

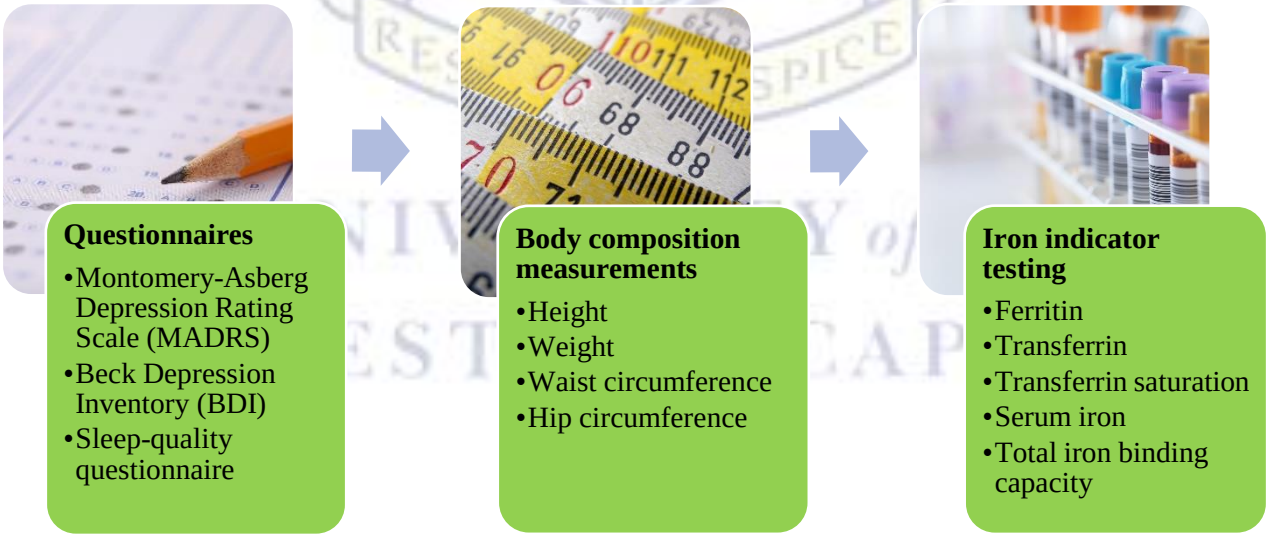
You have been invited to participate in this research study based on your depressive scores that were captured after completing the two standardized depression questionnaires.

Voluntary Participation

Your participation in this research is entirely voluntary. You may change your mind during this process and stop participating even if you had agreed initially.

Procedures and Protocol

Description of the Process:



1) Questionnaires

Two standardised questionnaires will be used to measure your depressive scores. These consist of the Montgomery-Asberg Rating Scale (MADRS) and the Beck Depression Inventory (BDI). Furthermore, a sleep-quality questionnaire and a food frequency questionnaire will also be used to gather additional data on your eating and sleeping habits. These questionnaires have already been completed online.

2) Body composition measurements

The body composition measurements that will be included in this research study are anthropometric measurements that will aid the assessment of different weight classifications. They will comprise of height, weight, waist circumference and hip circumference. Height will be measured using a portable stadiometer, the weight will be measured using a scale, and waist and hip circumferences will be measured using a stretch-resistant measuring tape. These measurements will allow us to calculate your body mass index (BMI), waist to hip ratio (WHR), and waist to height ratio (WHtR), which will allow us to quantify your weight ranges and nutritional statuses. These measurements will be performed at the University of the Western Cape.

3) Iron indicator testing

This research study will focus on serum iron, ferritin, transferrin, transferrin saturation, and total iron-binding capacity concentrations to measure and assess iron deficiency. The measuring of these iron status indicators will be done at PathCare, N1 City, Goodwood. The 5.0 ml of whole venous blood will be drawn during your allocated time slot by a registered medical doctor, Dr Juley De Smidt. Plasters will be on hand should there be residual bleeding.

Duration

You, as the participant, will be required to get to the University of the Western Cape, where your body composition measurements will be taken, and your blood will be drawn. This process altogether will take approximately 30 minutes of your time.

Side Effects/risks

Other than the initial discomfort of having your blood drawn, there are no long-lasting side effects or risks involved in this study.

Incentives

You will be given a snack bag for your participation in this study, comprising one of the following items: water, juice, lollipop, biscuits, chips, peanuts and raisins, an energy bar, and a cereal snack.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no one, but the researchers will be able to see it. Any information about you will have a number on it instead of your name, and only the researchers will know what your number is. It will not be shared with or given to anyone except your clinicians and my supervisors.

Sharing the Results

The knowledge we get from doing this research will be shared with you through online meetings before it is made widely available. Confidential information, such as personal information, will not be shared.

Right to Refuse or Withdraw

You do not have to participate in this research if you do not wish to do so. You may also stop participating in the study at any time you choose. It is your choice, and all your rights will still be respected.

Who to Contact?

If you have any questions, you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

- Kiyara Govender – Principal investigator
    - Email address: 3981498@myuwc.ac.za
- Ammaarah Gamieldien – Co-investigator
    - Email address: 3568307@myuwc.ac.za
- Dr. Juley De Smidt – Supervisor
    - Email address: jdesmidt@uwc.ac.za

This proposal has been reviewed and approved by the ethics review committee of the University of the Western Cape (ref. code: BM21/9/15), a committee whose task is to make sure that research participants are protected from harm. If you wish to find out more about the ethical clearance, please contact the Biomedical Research Ethics Committee (BMREC), Department of Research and Development, at the University of the Western Cape (Tel: 0219594111; email: research-ethics@uwc.ac.za).

You can ask me any more questions about any part of the research study if you wish to. Do you have any questions?

Part two consists of the certificate of consent and requires the signatures of three individuals. As the participant, the first would be to ensure that you have read and understood all the information provided to you and have had all your questions or concerns addressed. The second signature is that of a witness. A witness verifies that all information has been provided and that all questions and concerns have been addressed accurately. Furthermore, the witness ensures that you have given consent freely while also verifying your identity. The last signature is that of the researcher. This is to confirm that all relevant information was provided to you and that you understand what will happen to both you and your information throughout the study while addressing all your questions and concerns.

Part II: Certificate of Consent

I have read the previous information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____

Day/month/year

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant and, to the best of my ability, made sure that the participant understands that the following will be done:

1. Questionnaire participation
2. Body composition measurements
3. Iron indicator testing

I confirm that the participant was allowed to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

An e-copy of this ICF will be provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Appendix F: Data collection sheet

Study participant ID: _____

Age: _____

Gender: _____

Date: _____

1) Anthropometric data

Anthropometric measurement	**Measurements**			
	Attempt 1	Attempt 2	Attempt 3	Average
Weight (kg)				
Height (cm)				
Waist circumference (cm)				
Hip circumference (cm)				

Index/Ratio	**How it is calculated**	**Result**
Body mass index (BMI)	Weight/height	
Waist-to-hip ratio (WHR)	Waist circumference/hip circumference	
Waist-to-height ratio (WHtR)	Waist circumference/height	

2) Clinical data

a. Blood pressure (BP)

Clinical measurement	Measurements			
	Attempt 1	Attempt 2	Attempt 3	Average
Systolic BP				
Diastolic BP				
Mean arterial pressure (MAP)				
Heart rate (HR)				

b. Iron status indicators

Iron status indicators	Result
Haemoglobin (g/dL)	
Haematocrit (%)	
Ferritin (µg/L)	
Transferrin (g/L)	
Iron (µmol/L)	
Unsaturated iron binding capacity (UIBC) (µmol/L)	

Iron status indicators	How it is calculated	Result
Total iron binding capacity (TIBC) (µmol/L)	Serum iron + UIBC	
Transferrin saturation (%)	Serum iron / transferrin	

c. Lipid profile

Lipid profile	Result
Total cholesterol	
LDL	
HDL	
Triglycerides	
Total chol/HDL	

3) Depressive Scoring

Standardised questionnaire	Score	Severity
Montgomery-Asberg Depression Rating Scale (MADRS)		
Beck Depression Inventory (BDI)		

4) Inflammatory conditions

Inflammatory condition	Yes/No	Comment
Seasonal/chronic allergies		
Chronic urinary tract disease (UTI)		
Chronic cough		
Asthma		
Gout		
Type 1 diabetes		
Gastro-duodenal ulcer		
Rheumatoid arthritis		
Other		

Appendix G: Descriptive statistics of participants with minimal to mild depressive symptoms according to their MADRS scores.

Study variables	Variables	Sample number	Minimum	Maximum	Mean	Standard error
Age	Age	27	18.000	52.000	23.481	1.563
Iron status indicators	Serum iron	27	3.700	25.700	12.907	1.144
	Transferrin	27	2.320	4.200	3.100	0.092
	TSAT	27	3.000	34.000	17.074	1.677
	Ferritin	27	4.000	400.000	70.556	17.592
	TIBC	27	52.800	95.600	70.522	2.092
Depressive scales	MADRS	27	2.000	19.000	12.148	1.066
	BDI	25	2.000	22.000	12.600	1.183
Body composition measurements	BMI	27	17.303	41.070	26.077	1.182
	WC	27	56.800	140.330	81.631	3.722
	WHR	27	0.662	1.047	0.795	0.019
	WHtR	27	0.352	0.793	0.500	0.022

Appendix H: Descriptive statistics of participants with moderate to severe depressive symptoms according to their MADRS scores.

Study variables	Variables	Sample number	Minimum	Maximum	Mean	Standard error
Age	Age	24	18.000	46.000	21.833	1.214
Iron status indicators	Serum iron	24	0.900	35.400	12.917	1.760
	Transferrin	24	2.230	4.320	3.211	0.115
	TSAT	24	1.000	48.000	17.208	2.642
	Ferritin	24	2.000	186.000	48.375	11.373
	TIBC	24	50.700	98.300	73.042	2.613
Depressive scales	MADRS	24	20.000	53.000	27.292	1.439
	BDI	24	10.000	50.000	28.500	2.217
Body composition measurements	BMI	24	16.467	37.038	24.140	1.050
	WC	24	59.833	115.467	76.174	2.937
	WHR	24	0.630	1.064	0.768	0.018
	WHtR	24	0.364	0.684	0.473	0.017

Appendix I: Descriptive statistics of obese participants according to their BMI values.

Study variables	Variables	Sample number	Minimum	Maximum	Mean	Standard error
Age	Age	12	18.000	52.000	27.917	3.661
Iron status indicators	Serum iron	12	4.000	25.700	12.342	1.870
	Transferrin	12	2.510	3.770	3.057	0.118
	TSAT	12	4.000	34.000	16.583	2.734
	Ferritin	12	6.000	400.000	102.917	32.199
	TIBC	12	57.100	85.800	69.508	2.694
Depressive scales	MADRS	12	2.000	30.000	16.250	2.658
	BDI	11	3.000	50.000	18.182	4.022
Body composition measurements	BMI	12	30.003	41.070	33.882	0.930
	WC	12	81.500	140.330	104.116	4.445
	WHR	12	0.738	1.064	0.900	0.031
	WHtR	12	0.529	0.793	0.634	0.020

Appendix J: Descriptive statistics of normal-overweight participants according to their BMI values.

Study variables	Variables	Sample number	Minimum	Maximum	Mean	Standard error
Age	Age	39	18.000	30.000	21.103	0.498
Iron status indicators	Serum iron	39	0.900	35.400	13.087	1.208
	Transferrin	39	2.230	4.320	3.182	0.088
	TSAT	39	1.000	48.000	17.308	1.806
	Ferritin	39	2.000	255.000	46.949	9.341
	TIBC	39	50.700	98.300	72.385	1.993
Depressive scales	MADRS	39	2.000	53.000	20.205	1.597
	BDI	38	2.000	47.000	21.026	1.848
Body composition measurements	BMI	39	16.467	28.888	22.483	0.472
	WC	39	56.800	91.733	71.354	1.274
	WHR	39	0.630	0.892	0.746	0.008
	WHtR	39	0.352	0.591	0.442	0.009