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The effect of probiotic yoghurt on C-reactive protein and related gut microbiome in females with obesity

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ANNEXURES

Abstract

Background: Obesity is an escalating health issue worldwide, and in South Africa, it particularly impacts women, significantly increasing the prevalence of non-communicable diseases. Recent findings suggest a strong correlation between obesity and an imbalance in the gut microbiota, which may contribute to chronic, low-level inflammation, as indicated by elevated levels of C-reactive protein (CRP). This inflammatory condition is, in essence, linked to the metabolic issues commonly associated with obesity. Our study aimed to describe the baseline characteristics and explore the relationships between gut microbiota composition, weight status, CRP levels, and dietary intake, and to assess the effect of a 12-week probiotic yoghurt intervention on changes in weight status and CRP levels.

Methods: The study utilised an observational descriptive design, followed by a single-blind, randomised, placebo-controlled trial. The study sample consisted of 30 South African women aged 25-55 years who had obesity. Participants were randomly allocated to either consume a probiotic yoghurt that includes *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis* BB-12, or a control yoghurt, daily for 12 weeks. Weight status measurements and CRP levels were measured at the beginning and after the intervention. The composition of gut microbiota was evaluated only at baseline.

Results: Our participants had a mean baseline CRP level of 4.18 mg/L (SD = 2.19). The most abundant phyla: Bacillota (46.19%), Bacteroidota (23.95%), Firmicutes (11.72%) and Bacteroidetes (7.19%). The mean F/B ratio was 2.28 (SD = 1.88). No statistically significant relationships were found between weight status measurements and any bacterial phyla or F/B ratio. A statistically significant negative correlation was found between *Bacteroides* and WHR ($r = -0.479$, $p = 0.044$) and between *Akkermansia* and WHR ($r = -0.488$, $p = 0.04$). A statistically significant negative correlation was found between F/B ratio and CRP levels ($r = -0.409$, $p = 0.013$). No notable alterations were detected in weight status or CRP levels in the intervention group ($n = 13$) after the 12-week intervention.

Conclusion: Our study revealed mixed results on the relationships among gut microbiome composition and the other variables. The significant negative correlation between the F/B ratio and CRP levels offers preliminary support for the gut microbiota's role in inflammation in this population. However, the observed lack of significant changes in weight status or CRP levels following a 12-week probiotic intervention, potentially due to the study's limited sample size and duration, highlights the need for larger, longer-term trials. This study enhances our understanding of gut microbiota, weight status, dietary patterns, and inflammation in South African women with obesity, emphasising the importance of population-specific considerations.

ABBREVIATIONS

Abbreviation	Meaning
BIA	Bioelectrical Impedance Analyser
BMI	Body Mass Index
CRP	C-reactive Protein
CVD	Cardiovascular diseases
EDTA	Ethylendiaminetetra acetic acid
F/B Ratio	Firmicutes/Bacteroidetes Ratio
FMI	Fat Mass Index
GPCRs	G protein-coupled receptors
HDAC	Histone deacetylase
IFN- gamma	Interferon-gamma
IL-1β	Interluekin-1 beta
IL - 6	Interleukin-6
LDL	Low-density lipoprotein

LMIC	Low- and middle-income
mCRP	Monomeric CRP
NCDs	Noncommunicable diseases
NK	Natural Killer
pCRP	Pentameric C-reactive protein
RCT-SB	Randomised controlled trial -single blind
Th1-dependent	T helper-dependent
TNF-α	Tumour necrosis factor-alpha
SADHS	South African Demographic and Health Survey
SCFAs	Short-chain fatty acids
SDG	Sustainable Development Goals
WHO	World Health Organisation

1 INTRODUCTION

1.1 BACKGROUND

South Africa possesses the highest obesity prevalence in Sub-Saharan Africa (Jayawardena et al., 2020). According to the 2016 South African Demographic and Health Survey (SADHS), 68% of women were classified as overweight or obese, while 31% of men also fell into the overweight or obese categories (National Department of Health, 2016). A comparison with the 1998 SADHS indicates that the prevalence of overweight or obesity among women has risen from 56% to 68%. For men, the prevalence increased from 29% to 31% (National Department of Health, 2016). It is projected that the prevalence of obesity will increase by 47.7% in females and 23.3% in males (Van Vollenstee et al., 2021). These findings highlight the escalating health concern posed by obesity within the South African population, specifically among females.

Besides serving as a metabolic risk factor for non-communicable diseases (NCDS) like diabetes, cancer, neurological conditions, chronic respiratory ailments, digestive disorders, and cardiovascular diseases (CVDS), obesity is recognised as a state of chronic, low-grade systemic inflammation (Gomes et al., 2017; WHO, 2021). The primary factor contributing to mortality globally is CVD (WHO, 2021). Three-quarters of the 17.9 million deaths from CVD in 2019 occurred in low- and middle-income countries, according to World Health Organisation (WHO) reports (WHO, 2021). Systemic inflammation linked to obesity contributes to the development and progression of these diseases by releasing a variety of inflammatory markers (BabyChitra et al., 2019; Uludag et al., 2023).

An example of a widely used and well-known inflammatory marker is C-reactive protein (CRP), a protein produced by the liver that helps the immune system recognise patterns and respond to threats (Mouliou, 2023; Rizo-Téllez et al., 2023). Practitioners typically use CRP as a key test to detect inflammation in the body (Mouliou, 2023). The strong coonection between systemic inflammation, obesity, and cardiovascular risk is clearly shown by the consistent relationship between high CRP levels and both higher body mass index (BMI) and the presence of CVDs (Uludag et al., 2023). Two examples of

inflammatory markers released from adipose tissue found in obese individuals are interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), which stimulate hepatic CRP production, leading to elevated systemic CRP levels (BabyChitra et al., 2019; Uludag et al., 2023). Together with these systemic factors, the gut microbiome has a significant influence on the growth and advancement of obesity (BabyChitra et al., 2019; Uludag et al., 2023).

There is increasing proof that the gut microbial diversity in obese people differs from that of those who have a healthy weight, often leading to dysbiosis (Hruby et al., 2015; Gomes et al., 2017; Cao et al., 2020). Dysbiosis is characterised by altered ratios of dominant phyla, such as a higher Firmicutes to Bacteroidetes (F/B) ratio, and reduced microbial diversity (Hruby et al., 2015; Gomes et al., 2017; Cao et al., 2020). Dysbiosis can worsen inflammation through various mechanisms; for example, a compromised gut barrier (also known as a leaky gut) permits bacterial components, such as lipopolysaccharide (LPS), to enter the bloodstream, which initiates an inflammatory response and increases CRP levels (Gomes et al., 2017). Additionally, the metabolic functions of a dysregulated microbiome can affect the host's energy extraction and fat accumulation, which can further contribute to obesity and inflammation (Cao et al., 2020).The administration of probiotics is believed to help reduce low-grade inflammation in obese individuals. This is accomplished by ingesting particular bacterial species, like *Bifidobacterium* and *Lactobacillus*, which have been demonstrated to reduce inflammation and enhance body composition in overweight or obese individuals (Cao et al., 2020; Wicinski et al., 2020). The passage of toxic substances through the intestinal wall and a reduction in inflammatory markers may be the cause of these benefits (Gomes et al., 2017).

Previous research has several limitations and knowledge gaps, particularly regarding the gut microbiome profiling of South Africans and the impact of probiotics on this population. Since many of these studies have used multiple strains, it is challenging to determine the effectiveness of specific bacterial strains. Furthermore, a few studies have combined probiotic supplementation with diet and exercise programs, which can lead to conflicting findings about the direct correlation between probiotic supplementation and outcomes pertaining to CRP levels, anthropometric and body composition measurements (Madjd et al., 2016; Gomes et al., 2017; Hassan et al., 2022). Moreover, although there is a

considerable interest, a clear and consistent connection between the Firmicutes/Bacteroidetes ratio (F/B ratio) and obesity has not been universally confirmed across various populations and study methods, highlighting the intricate nature of gut microbiome interactions with host metabolism. Exploring the role that gut microbial composition has in obesity in the South African context and the potential of probiotics to improve metabolic health offers promising strategies to address this growing global health issue, especially among South African women.

1.2 PROBLEM STATEMENT

Even though there is mounting proof that gut microbiota and obesity are related, more research on African populations and worldwide is still required. Comprehending the profiles of the gut microbiome of obese South African women is crucial to understanding how these profiles relate to CRP levels and how obesity develops. Improving obese women's well-being and standard of living aligns with the third goal of the Sustainable Development Goals (SDGs) to "ensure healthy lives and promote well-being for all at all ages" (United Nations, Department of Economic and Social Affairs, 2022). Therefore, this pilot study aims to address this important knowledge gap by providing key insights into the complex connections among gut microbiome, CRP levels, and obesity specifically within the South Africa context. By focusing on South African women, this research seeks to shed light on the population-specific nuances of the obesity pandemic that disproportionately impacts them. . As illustrated in Figure 1, which outlines our contextual framework, This research examines the connection between the baseline gut microbiome composition and established markers of inflammation (CRP) and obesity (anthropometric and body composition measurements) within this under-represented population, while also assessing the effects of a specific probiotic intervention. This approach contributes to essential baseline data and explores potential intervention strategies relevant to the South African health landscape.

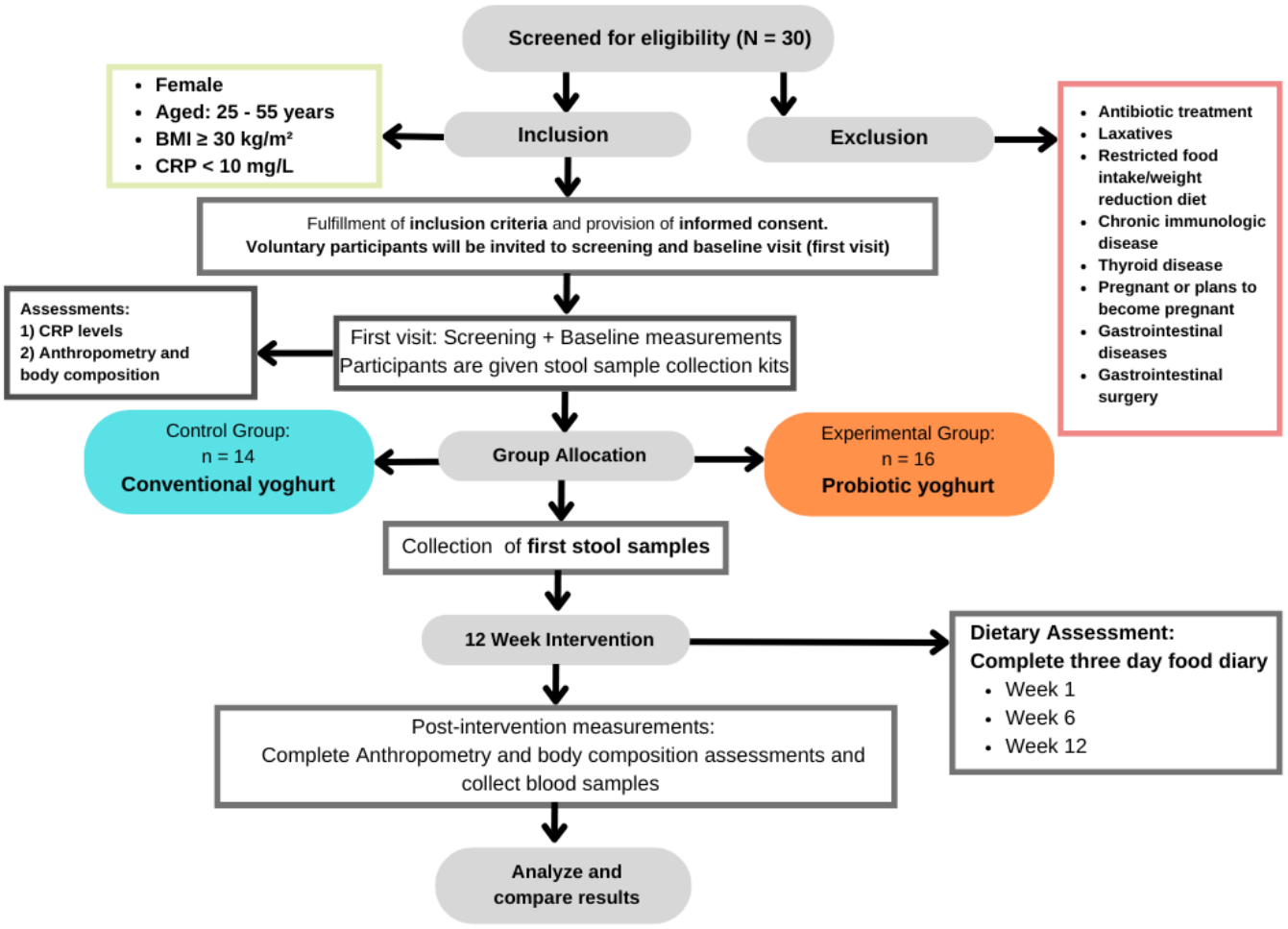

Figure 1: Conceptual Framework

1.3 AIM

The study aims to describe the baseline characteristics and explore the relationships between gut microbiota composition, weight status, CRP levels, and dietary intake in South African females with obesity (25-55 years), and to assess the effect of a 12-week probiotic yoghurt intervention on changes in weight status and CRP levels.

1.4 OBJECTIVES

1.4.1 Primary Objectives:

1. To evaluate and describe the characteristics of South African females aged 25-55 years with obesity:
   1.1. Weight status including anthropometry (weight (kg), BMI (kg/m²) and Waist - Hip Ratio (WHR)) and body composition (fat mass index (FMI) (kg/m²) and fat mass (FM) (kg))
   1.2. C-reactive protein levels (mg/L)
   1.3. Gut microbiota composition including gut bacterial counts (cfu/g) at phylum (including Firmicutes/Bacteroidetes ratio (F/B ratio)) and genus levels
2. To monitor and describe dietary intake, including energy (kJ), protein (g), lipids (g), carbohydrates (g) and dietary fibre (g).
3. To explore the relationships between gut microbiota composition (at phylum and genus levels) and weight status, CRP levels, and dietary intake respectively.

1.4.2 Secondary Objectives

To determine the effect of a 12-week probiotic yoghurt intervention on changes in weight status and CRP levels.

2 LITERATURE REVIEW

2.1 INTRODUCTION

One in eight people worldwide suffers from obesity (WHO, 2024), making it a global concern (Alvarez-Arrano et al., 2021). According to the WHO, in 2022, 44% of the 2.5 billion adults aged 18 and over who were overweight were female. This indicates that overweight and obesity are becoming more widespread worldwide, with obesity rates increasing (WHO, 2024). The WHO characterises overweight and obesity as “abnormal or excessive fat accumulation that presents a risk to health” (WHO, 2021). The nutrition transition has resulted in a transition away from conventional diets to a Westernised diet characterised by the intake of calorie-rich foods that are high in saturated fats and sugars, while being low in dietary fibre, often accompanied by decreased physical activity (Mbogori et al., 2020; Alvarez-Arrano et al., 2021). Factors that have contributed to the nutrition transition include economic status, mass media, and urbanisation (Mbogori et al., 2020). This dietary shift has had a profound impact on gut health.

Food consumption significantly impacts the diversity of microorganisms in the gut microbiome (Lee et al., 2021). Individuals living with obesity have a reduced bacterial diversity characterised by having lower microbial counts of Bacteroides and higher Firmicutes counts (Alvarez-Arrnao et al., 2021). Obesity and other chronic conditions, such as diabetes and atherosclerosis, have also been linked to enhanced uptake across the gut’s epithelial barrier (Szulinska et al., 2018).

The literature review that follows provides a broad overview of obesity in both South Africa and globally. It will cover the prevalence and impact of obesity in South Africa, the impact of gut microbiota on wellness and illness, the connection between diet, probiotics, the gut microbiome, inflammation, and obesity, along with the existing research gaps and the reasoning behind this study, are covered.

2.2 THE PREVALENCE AND IMPACT OF OBESITY

2.2.1 The Economic Impact of Obesity

Despite the pervasive issues of undernutrition and food insecurity, obesity is particularly evident in Africa (Tzioumis et al., 2016; WHO, 2024). Obesity places substantial financial burdens due to healthcare expenses and reduced productivity (Boachie et al., 2022). According to the WHO, without intervention, it is projected that the worldwide expenses associated with being overweight and obese will increase to US$ three trillion annually by 2030 (WHO , 2024). This presents a significant challenge for countries that previously did not have high obesity rates (WHO , 2024).

2.2.2 Obesity in South Africa: specific challenges and disparities

The prevalence of obesity in South Africa is shaped by distinct social, economic, and cultural influences. Approximately nine percent of total health expenditure in Africa and 13.2% globally can be attributed to overweight and obesity (Boachie et al., 2022). In 2020, a study investigating the healthcare costs in the South African public sector linked to treating weight-related conditions estimated that the total cost of overweight and obesity amounted to ZAR33,194 million (Boachie et al., 2022). This demonstrates the significant financial toll that obesity and overweight have on South Africa's public healthcare system.

The prevalence of overweight and obesity in Africa is the result of various cultural beliefs and values (Manafe et al., 2022). For example, being overweight is seen as a sign of wealth in some African societies because it is thought that having money enables one to buy a lot of food (Manafe et al., 2022). The 2016 SADHS, which discovered that severe obesity increases with wealth in both men and women, supports this. As an additional illustration, mothers are urged to eat more than they need to after giving birth in order to promote both their own and their baby's health, which results in weight gain (Manafe et al., 2022). Additionally, being thin carries a stigma that results in the unjust labelling of thin people as HIV-positive, which encourages people to put on weight (Manafe et al., 2022). The rising rates of overweight and obesity in these societies are a result of these misconceptions.

Additionally, individuals struggle to achieve a weight that is healthy for the body because of the availability and low cost of unhealthy food in their surroundings (Manafe et al., 2022). Certain food items, including meat, oils and fats, sauces, dressings, condiments, as well as soft drinks, have experienced a 30% rise in consumption in South Africa since 1994 (Ronquest-Ross et al., 2015). A high intake of these foods has been linked to NCDS, increased weight, and a greater prevalence of obesity among both adults and children (Ronquest-Ross et al., 2015). These factors, when combined with South Africa's largely passive lifestyle and reported physical immobility, contribute to the problem of obesity (Manafe et al., 2022).

The 2016 SADHS found significant differences in obesity prevalence across different demographic groups, including gender, age, race, and socioeconomic status (National Department of Health, 2016). The survey indicates the frequency of obesity in South Africa is significantly greater among women compared to men, as it was found that 41% of women and 11% of men aged 15 and older were obese (Nglazi and Ataguba, 2022). Furthermore, the greatest rates of overweight or obesity were observed in women between the ages of 45 and 64 (81%–82%), with men aged 65 and older following at 54% (National Department of Health, 2016). There was minimal variation in the proportion of overweight or obese women across population groups (67%–70%) (National Department of Health, 2016). These findings demonstrate the significant impact that obesity has on South African women.

2.3 Physiology of C-Reactive Protein

C- reactive protein is a key acute-phase reactant and an important element of the innate immune system. It is recognised as a molecule for pattern recognition and is part of the pentraxin family. C-reactive protein consists of five identical sub-units linked non-covalently, forming a planar ring structure, and is mainly produced by hepatocytes in the liver (Moulioue, 2023; Rizo-Téllez et al., 2023). The production of CRP is rapidly and markedly elevated in reaction to inflammation stimuli, mainly influenced by IL-6 , along with input from interluekin-1 beta (IL-1β) and TNF-α, in addition to stress signals associated

with tissue damage (Mouliou, 2023; Rizo-Téllez et al., 2023). C-reactive protein acts as a sensitive inflammation biomarker and helps modulate host defense mechanisms by interacting with various ligands, such as components from bacteria and fungi, phosphocholine, oxidised low-density lipoprotein (LDL), and apoptotic cells (Mouliou, 2023; Rizo-Téllez et al., 2023). There are primarily two distinct conformational states of CRP: the native pentameric CRP (pCRP) and the monomeric CRP (mCRP). While pCRP is present in the bloodstream and is typically associated with opsonisation, complement activation, and the regulation of immune responses, it can dissociate into mCRP in inflammatory regions. Monomeric CRP is believed to have strong proinflammatory effects, increasing local inflammatory reactions, promoting monocyte chemotaxis, and enhancing endothelial activation (Mouliou, 2023; Rizo-Téllez et al., 2023).

2.3.1 C-Reactive Protein in the South African Context

C-reactive protein assays allows for the measurement of lower concentrations associated wih chronic low-grade inflammation, with levels above 10 mg/L indicating acute inflammation (George et al., 2018). Typically, CRP levels are classified as: a) low risk: below 1mg/L; b) moderate risk: 1-3 mg/L; and c) high risk: above 3 mg/L (George et al., 2018; Myburgh et al., 2020). In South Africa, Black individuals tend to have higehr CRP levels compared to other ethnicities, indicating increased systemic inflammation (Myburgh et al., 2020). For instance, George et al (2018) found that the median CRP for Black South African women was 2.3 µg/ml, slightly higher than for White women after adjustments. Myburgh et al. (2020) noted a significant portion of Black South Africans had CRP levels exceeding 3 mg/L, emphasizing a heightened inflammatory burden. Research also highlights sex differences in CRP levels. Myburgh et al. (2020) reported that South African women, especially post-menopausal, had higher CRP concentrations, with a median of 4.31 mg/L for post-menopausal women compared to 2.42 mg/L for men. Adjusting for waist circumference diminished the disparities, suggesting abdominal obesity plays a key role in the observed inflammation levels (George et al., 2018; Myburgh et al., 2020).

2.4 INFLAMMATION AND OBESITY

C-reactive protein levels ranging from 3 to 10 mg/L have been linked to obesity (Nehring et al., 2023). Low-grade inflammation refers to the release of various substances, including

chemokines, which are responsible for attracting immune cells, anti-inflammatory and pro-inflammatory proteins, and pro-inflammatory signalling molecules such as chemokines and cytokines (Khanna et al., 2022). When an individual is obese and has low-grade inflammation, it greatly impacts their insulin sensitivity, which affects blood sugar regulation. This is because the fat tissue is inflamed, which causes further inflammation and changes the immunity of the individual (Khanna et al., 2022). Excess fat produces substances that attract immune cells, leading to further inflammation and the release of pro-inflammatory substances. (Khanna et al., 2022; Su et al., 2024). This sequence of events impacts glucose processing and promotes fat buildup in the liver, muscles, and fat tissue (Khanna et al., 2022). It can cause cellular damage, increased levels of pro-inflammatory agents and oxidative stress, resulting in a chronic low-grade inflammatory response when harmful substances are not neutralised. (Khanna et al., 2022).

Uludağ et al. (2023) aimed to assess the link between CRP levels and females' BMI. The findings demonstrated a notable variation in CRP levels across BMI classifications. They particularly noted a notable distinction between the healthy weight group and those categorised as obese (P = 0.001) and morbidly obese (P = 0.0001). Additionally, the mean CRP level increased significantly with increasing BMI category, with values of 0.07 ± 0.05 mg/L for normal weight (n = 22), 0.87 ± 1.36 mg/L for overweight (n = 25), 1.44 ± 1.3 mg/L for obese (n = 38), and 2.12 ± 1.22 mg/L for morbidly obese (n = 46) individuals. Comparable findings were observed in a systematic review and meta-analysis investigating the influence of obesity and overweight on CRP levels and rheumatoid arthritis (RA). The study found that CRP levels are generally higher in female RA patients with an elevated BMI (Flores-Alvarado et al., 2023).

Inflammatory cytokines, including resistin, visfatin, interleukins (IL-6, IL-18), TNF-α , and CRP, are found in varying amounts in the blood of obese individuals (Khanna et al., 2022; Su et al., 2024). A cross-sectional study that looked at inflammatory markers and BMI categories in 7526 men and 3219 women found that those with higher BMI had significantly higher levels of CRP than those with normal BMI ($p<0.0001$). The

inflammatory marker CRP increased most noticeably, especially in women (Cohen et al., 2021).

A systematic review by Su et al. (2024), which encompassed 91 studies and 435,007 adult participants, observed similar findings. This research compared the levels of CRP, IL-6, and TNF-α in different groups based on metabolic health and weight status. The review showed that CRP levels were higher in obese participants who were metabolically healthy (MHO) compared to non-obese, metabolically healthy (MHNO) participants and non-obese, metabolically unhealthy (MUNO) participants. In contrast, participants who were metabolically unhealthy and obese (MUO) had even higher CRP levels than those who were MHO (Su et al., 2024). Inflammation and obesity status may be related even in the absence of other metabolic factors, as the study found that MHO participants had greater levels of TNF-α, IL-6, and CRP than MHNO participants (Su et al., 2024). These results emphasise the intricate connection between inflammation and obesity, emphasising how critical it is to treat obesity in order to lower systemic health risks.

2.5 THE ROLE OF GUT MICROBIOME IN HEALTH AND DISEASE

2.5.1 Overview of the gut microbiome

The microorganisms that make up the gut microbiome are diverse and interact with both the host and one another (Hutchinson et al., 2021). There are 500 distinct types of microorganisms in the human gut, including bacteria, viruses, fungi, protozoa, and archaea (Chenhuichen et al., 2022). It is well-established that the gut microbiome has a vital function in maintaining overall health by regulating nutritional, metabolic, and immune functions (Hutchinson et al., 2021; Shen et al., 2018). It is important to acknowledge the recent changes in bacterial classification, where the names of phyla now consistently end with the suffix "-ota" (Oren, 2024). The main phyla include:

A) Bacillota (formerly known as Firmicutes): This large group primarily consists of Gram-positive bacteria, which are often considered metabolically efficient. Many genera within this phylum are involved in fermenting food components and producing SCFAs.

These processes may be linked to higher intakes of calories, protein, fat, and sugar (Karačić et al., 2024; Koliada et al., 2017).

B) Bacteroidota (formerly known as Bacteroidetes): This phylum is composed of Gram-negative bacteria and is generally associated with higher fibre consumption and is understood to be less efficient in energy extraction compared to Bacillota (Karačić et al., 2024; Koliada et al., 2017).

C) Pseudomonadota (formerly known as Proteobacteria): An increased presence of this phylum is often linked to dysbiosis and inflammation. Many Pseudomonadota produce lipopolysaccharides (LPS), which are molecules recognised for eliciting strong pro-inflammatory responses in the host (Karačić et al., 2024).

D) Actinomycetota (previously Actinobacteria):Although less abundant than Bacillota and Bacteroidota, this phylum contains beneficial genera such as *Bifidobacterium*, which contribute to promoting gut health (Koliada et al., 2017; Mazloom et al., 2019).

The ratio of Bacillota to Bacteroidota, referred to as the F/B ratio, has historically been considered a significant indicator of gut microbial composition and health, particularly in relation to obesity (Oren, 2024; Ragonnaud & Biragyn, 2018). Previous research often linked a higher F/B ratio with obesity, suggesting that an increased presence of Bacillota might facilitate greater energy extraction from food, leading to weight gain and fat storage (Koliada et al., 2017; Mazloom et al., 2019). Conversely, a lower F/B ratio was generally associated with a leaner body type and a varied, stable gut microbiome (Oren, 2024). However, more recent and detailed studies have cast doubt on the F/B ratio's reliability as a solitary biomarker for obesity or dysbiosis. Research indicates that this relationship can frequently differ among various populations and is greatly impacted by lifestyle choices, individual characteristics, and the research methods used (Karačić et al., 2024; Koliada et al., 2017; Mazloom et al., 2019). Therefore, while changes in this ratio can signal more extensive shifts in microbial communities, replying solely on the F/B ratio may oversimplify the complex metabolic and immunological interactions occurring in the gut (Ragonnaud & Biragyn, 2018).

A more detailed and insightful understanding of the gut microbiome' impact on health can be gained by examining it at the genus level (Karačić et al., 2024; Oren, 2024). A genus is made up of closely related bacterial species that share specific characteristics and

functional roles, allowing for clearer insights into how certain bacteria impact host metabolism and immune responses (Karačić et al., 2024). Several genera within the predominant phyla have been extensively researched for their roles in obesity, inflammation and overall gut health:

A) *Bifidobacterium* (within Actinomycetota): Generally considered beneficial, species of *Bifidobacterium* are associated with leanness, gut acidity, immune regulation, and improve metabolic profiles (Mazloom et al., 2019; Ragonnaud & Biragyn, 2018).

B) *Lactobacillus* (within Bacillota): Although some species may be present in greater numbers in obese individuals, *Lactobacillus* is widely recognised as a probiotic genus known for its positive effects on gut health, including lactic acid production and maintaining the integrity of the gut barrier (Mazloom et al., 2019; Oren, 2024).

C) *Faecalibacterium* (within Bacillota): Specifically, *Faecalibacterium prausnitzii* is predominant in the gut and is noted for its anti-inflammatory properties and production of SCFAs (Ragonnaud & Biragyn, 2018).

D) *Bacteroides* (within Bacteroidota): Species in this genus are crucial for breaking down complex polysaccharides and modulating immune responses (Oren, 2024). However, some species have been linked to inflammation and metabolic changes associated with obesity (Mazloom et al., 2019).

E) *Akkermansia* (specifically *Akkermansia muciniphila*): Known for its ability to degrade mucin, this bacterium helps maintain gut barrier integrity and promotes the growth of benefical SCFA-prodcuing bacteria (Ragonnaud & Biragyn, 2018).

By understanding the presence and relative abundance of these specific genera, we gain a more nuanced view compared to phylum-level analyses, allowing for a better evaluation of specific microbial functions and their impacts on host health.

2.5.2 The gut microbiome of the African population

The gut microbiome of African populations, including those in South Africa, displays unique traits influenced by specific dietary habits, lifestyles, environmental factors, and ongoing changes in disease patterns (Allali et al., 2021; Ecklu-Mensah et al., 2023). These attributes often set them apart form Western populations, while also exhibiting variation within different regions of the continent. In terms of overall diversity and composition,

African groups typically show higher microbial diversity compared to individuals from more urbanised or wealthier nations, though this is not uniform. For example, South Africans generally exhibit lower diveristy than Ghanaians (Ecklu-Mensah et al., 2023). A distinctive feature of the gut microbiota in South Africa is the high prevalence of the *Prevotella* enterotype (P-type), found in about 62% of individuals, which is closely linked to traditional, plant-based, fiber-rich diets (Ecklu-Mensah et al., 2023; Syromyatnikov et al., 2022). This stand in contrast to Western diets, where Bacteroides are more commonly seen (Syromyatnikov et al., 2022).

In addition to *Prevotella*, the most commonly found phlya in South African adults usually incldue Firmicutes, Bacteroidetes, and Proteobacteria, with their proportions often indicating a transitional microbiome that lies taxonomically between traditional non-Western and heavily Westernized groups (Oduaran et al., 2020; Tamburini et al., 2022). Frequently identified key genera include *Bacteroides* and *Faecalibacterium* alongside *Prevotella* (Tamburini et al., 2022). Notably, South African microbiomes may also contain taxa that are rare in Western microbiomes, such as members of VANISH taxa (for example, *Treponema* and *Succinatimonas*), especially in rural areas (Tamburini et al., 2022). Other significant genera that have been observed include *Clostridium*, *Olsenella*, *Bacilli*, and *Mogibacterium* (Ecklu-Mensah et al., 2023).

2.5.3 The gut microbiome and immunity

The intestinal barrier is a multilayered physical barrier that acts as the initial line of defence against toxins and pathogens (Martin et al., 2019). A healthy intestinal barrier is necessary for regulating water and solute fluxes, allowing nutrients to enter selectively, and preventing the infiltration of toxins and pathogens (Martin et al., 2019). Inflammation is triggered when pathogens interact with the epithelial cells in the intestine (Martin et al., 2019; Zhao et al., 2021). The epithelial barrier comprises Peyer's patches, which facilitate the interaction of antigens with immunoreactive cells. The lamina propria is situated within the gastrointestinal tract and is abundant in cytokine-producing cells that shield the intestinal tissue from interactions with pathogens (Nyangale et al., 2015).

The gut microbiota is essential for the synthesis of SCFAs by breaking down indigestible fibres (Ragonnaud and Biragyn, 2021). These SCFAs are essential for maintaining the gut microbiome because they provide energy to microorganisms and colonocytes and support immune function by fighting pathogens, protecting the intestinal barrier, and regulating immune cells (Ragonnaud and Biragyn, 2021). Additionally, SCFAs promote mucus production, which fortifies the intestinal barrier and aids in increasing healthy gut bacteria. Short-chain fatty acids directly stimulate immune cells and are primarily produced by Firmicutes (Ragonnaud and Biragyn., 2021). Figure 2 illustrates the relationship between the metabolic processes derived from microbiota and their effects on the gut epithelium (Youn Yoo et al., 2020).

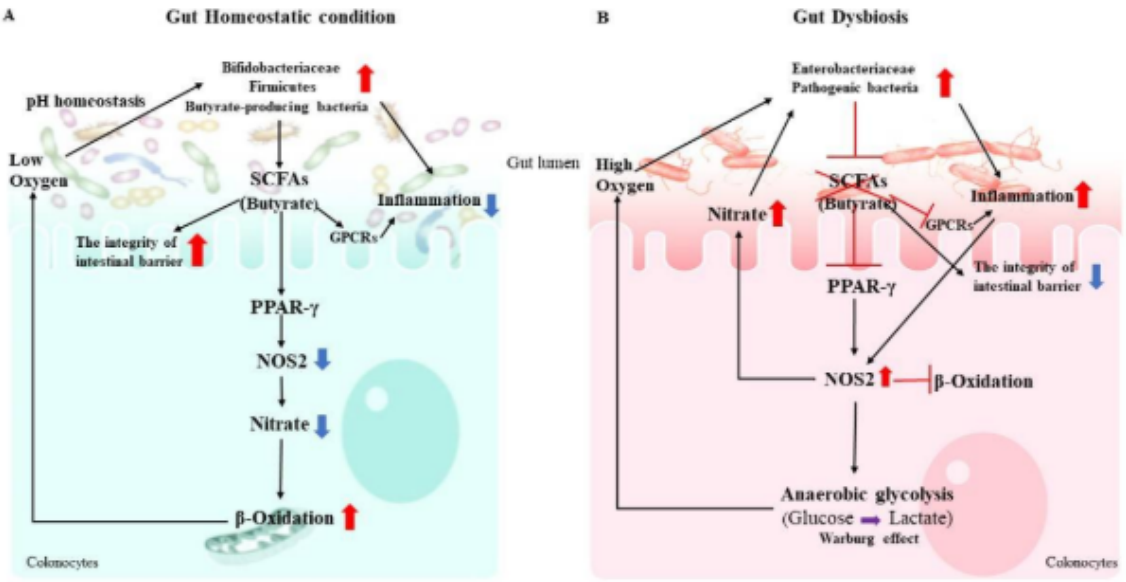

Figure 2: The relationship between the metabolic processes derived from microbiota and their effects on the gut epithelium (Youn Yoo et al., 2020).

Figure 2 A) illustrates a healthy gut condition (Youn Yoo et al., 2020). In a healthy gut (gut homeostatic condition), butyrate-producing bacteria convert dietary fibre into SCFAs. These SCFAs promote PPAR-γ-dependent mitochondrial oxidation in colonocytes, resulting in a hypoxic environment that supports anaerobic SCFA-producing bacteria while inhibiting facultative pathogens. Short-chain fatty acids also bind to G protein-coupled receptors (GPCRs) on epithelial and immune cells, reducing inflammation. They enter host cells, where they are metabolised an may inhibit histone deacetylase (HDAC) activity, contributing to immune regulation (Youn Yoo et al., 2020). On the other hand, Figure 2 B)

illustrates the gut in an unhealthy or imbalanced condition (Youn Yoo et al., 2020). When the gut bacteria become imbalanced (gut dysbiosis), harmful Enterbacteriscese ca cuase neutrophils, a type of white blood cell, to move across the gut lining. This reduces SCFA-producing bacteria, leading to lower levels of SCFAs like butyrate (Youn Yoo et al., 2020). As a result, oxygen and lactate levels rise, which helps harmful bacteria grow in low-oxygen conditions. The figure uses red arrows for increases and blue arrows for decreases in bacteria, metabolites, and effects seen in healthy and unhealthy guts (Youn Yoo et al., 2020).

2.5.4 The gut microbiome and obesity

Research has shown that the gut microbiota plays a crucial role in the development of obesity in various studies (Turnbaugh et al., 2009; Chen et al., 2021; Zsálig et al., 2023). Even though they ate less food, mice raised in a typical environment had 40% more body fat and 47% more fat surrounding their reproductive organs than mice that were brought up in sterile surroundings (Bäckhed et al., 2004). Without affecting their food intake or energy expenditure, germ-free mice's body fat increased by 60% when their gut microbiome from regular mice was transferred into them (Bäckhed et al., 2004). The study discovered that the altered gut microbiota affected the host's genes linked to fat cell energy storage in addition to increasing the energy availability from plant-based diets (Bäckhed et al., 2004).

The microbial diversity in obese individuals differs from that in healthy weight individuals (Hruby et al., 2015). Dysbiosis is characterised by the reduction of healthy microbiota, the increase of bacteria that could be harmful, and a decline in microbial diversity (Alvarez-Arrnao et al., 2021; Zsálig et al., 2023). A metagenomic study of the gut microbiome in lean and obese twins found that individuals who are lean exhibited higher proportions of Actinobacteria, while showing lower proportions of Bacteroidetes and less bacterial diversity compared to their obese counterparts (Turnbaugh et al., 2009). Firmicutes ratios did not differ significantly (Turnbaugh et al., 2009). However, subsequent studies have questioned whether obesity and the F/B ratio are related. This indicates that the microbial community in the gut regulates obesity in ways other than a straightforward imbalance of these phyla (Zsálig et al., 2023).

The composition of the gut microbiome in South African adults is increasingly associated with metabolic health factors, including obesity. While the *Prevotella* enter-type is prevalent in South Africa and is often linked to plant-based diets, its impact on obesity seems complex and reliant on context. Oduaran et al. (2020) found a correlation between the abundance of *Prevotella* and obesity, particularly in the rural cohort from Bushbuckridge. This indicates that although *Prevotella* flourishes on fibre-rich diets, specific species or their interactions in a changing microbiome may contribute to obesity in specific situations. Other microorganisms associated with obesity in South African populations include *Intestinimonas* and *Oscillibacter* (Oduaran et al., 2020). On the other hand, certain bacteria that produce SCFAs, like *Oscillospira* and *Christensenella*, which are linked to higher SCFA levels and a leaner phenotype, are present in South African populations. These bacteria generate SCFAs such as butyrate, recognized for enhancing insulin sensitivity and decreasing inflammation, indicating a potential protective function (Ecklu-Mensah et al., 2023). Additionally, the South African cohort has displayed a higher relative abundance of predicted genes responsible for enzymes involved in butyrate production pathways, particularly enriched in individuals without obesity, thus reinforcing the possible metabolic advantages of certain microbial compositions (Ecklu-Mensah et al., 2023).

2.5.5 The gut microbiome, human health and diet

The significance of the gut microbiome in human health was originally proposed by Elie Metchnikoff (Ragonnaud and Biragyn, 2021). He suggested that the build-up of "putrefactive bacterial autotoxins" leaking from the colon could harm healthy tissues (Ragonnaud and Biragyn, 2021). The gut microbiome is vital in energy metabolism and maintaining the body's homeostasis, and numerous studies have shown that interactions between gut microbiome, host genotypes, and diet may affect health (Ghosh and Pramanik, 2021; Ragonnaud and Biragyn, 2021;Song et al., 2021). An imbalance in gut microbiome has been linked to various health issues, such as obesity and related chronic diseases, fatty liver disease, heart disease, irritable bowel syndrome, and allergies (Ghosh and Pramanik, 2021; Song et al., 2021).

The composition of the gut microbiome and its function are influenced by diet and nutrition (Sonneburg and Backhed, 2016; Lee et al., 2020; Spencer et al., 2019). Short-term dietary modifications can quickly alter the composition of gut microbes, even though long-term dietary patterns are linked to specific microbial traits (Spencer et al., 2019). Diets rich in refined sugar promote *Bacteroides*, whereas diets rich in high-fibre foods support Firmicutes (Lee et al., 2020). Additionally, a diet low in dietary fibre decreases the diversity and richness of gut microbial diversity (Lee et al., 2020). Consuming more high-fibre carbohydrates and less fat results in an elevated F/B ratio and the generation of SCFAs (Lee et al., 2020; Ragonnaud and Biragyn, 2021). Firmicutes ferment dietary fibre to produce SCFAs, including butyrate (Shen et al., 2018). Changes in the F/B ratio are linked to obesity, diabetes, and liver cirrhosis, and negatively affect carbohydrate metabolism and SCFA production (Komaroff, 2017; Zhang et al., 2021).

The notable impact of lifestyle and urbanization on the gut microbiome is clearly illustrated in South Africa. Research contrasting rural and urban groups reveals distinct variations (Oduaran et al., 2020; Tamburini et al., 2022). For example, the rural community of Bushbuckridge often shows greater gut microbiome alpha diversity (Shannon diversity) and a higher prevalence of traditional taxa such as *Prevotella, Treponema,* and *Succinatimonas,* reflecting a diet rich in plant-based foods and fibre (Oduaran et al., 2020; Tamburini et al., 2022; Ayeni et al., 2022, for a comparable rural study in Nigeria). Conversely, urban populations like those in Soweto typically present an increased prevalence of genera associated with Western diets, including *Bacteroides, Bifidobacterium,* and *Streptococcus* (Tamburini et al., 2022; Ayeni et al., 2022, for a comparable urban study in Nigeria). This shift underscores that as South African communities undergo urbanisation, their gut microbiomes start to resemble those of Western populations, likely influenced by dietary changes towards more processed food and reduced fibre intake (Allali et al., 2021; Ecklu-Mensah et al., 2023)).

Prebiotics are “substances that are used by the microorganisms that live in our bodies and that positively affect our health” (Akatsu et al., 2021; Gibson et al., 1994). A synbiotic refers to “a mixture comprising live microorganisms and substrate (s) selectively utilised by host microorganisms that confers a health benefit on the host” (Hill et al., 2014; Zhao et al., 2021). Prebiotics are mainly indigestible oligosaccharides, which are absorbed by the host and used as fuel for beneficial bacteria, which promotes their growth and production

(Zhao et al., 2021). Prebiotics encourage the growth of *Bifidobacterium* in the intestine, influence the B-cell response, and improve Th1-dependent immune reactions, natural killer (NK) cell function, and interferon-gamma (IFN-gamma) production (Akatsu et al., 2021). Probiotics consist of live microorganisms that offer health advantages to the host ((FAO/WHO, 2002, p. 12)).

Fermented and probiotic foods have a notable impact on gut health and general well-being primarily through their direct and indirect effects on the composition of the gut microbiome. These foods introduce live microorganisms, mainly lactic acid bacteria (LAB) like *Lactobacillus* and *Bifidobacterium* species, along with various yeasts and bioactive compounds that can change the existing microbial populations (Dahiya and Nigam, 2022; Leeuwendaal et al., 2022; Roselli et al., 2021). Although many of these microbes from food may only temporarily colonise the gut, regular consumption can lead to beneficial changes, such as increases in helpful bacteria like *Bacteroides, Faecalibacterium prausnitzii, Lactobacilli,* and *Bifidobacteria*, and occasionally a decrease in harmful bacteria (Roselli et al., 2021; Shah et al., 2023). Additionally, the fermentation process improves the bioavailablity of nutrients and generates metabolites like SCFAs, particularly butyrate, acetate, and propionate, which are essential energy sources for gut cells and help maintain a lower gut pH, fostering a healthy environment (Dahiya and Nigam, 2022; Leeuwendaal et al., 2022; Shah et al., 2023).

Health-promoting foods come in a variety of forms, including fermented dairy products like yoghurt, kefir, and different cheeses; fermented vegetables such as sauerkraut and kimichi; fermented legumes like tempeh, miso and natto; and fermented drinks such as kombucha (Dahiya and Nigam, 2022; Leeuwendaal et al., 2022; Shah et al., 2023). The benefits of these foods each beyond digestive health, positively affecting overall well-being. They improve gut barrier integrity, influence immune responses by activating immune cells and boosting cytokine levels, and can help ease gastrointestinal issues such as lactose intolerance, antibiotic-related diarrhea, and irritable bowel syndrome (Dahiya and Nigam, 2022; Roselli et al., 2021; Shah et al., 2023). Additionally, the bioactive compounds and SCFAs generated during fermentation provide antioxidant, antihypertensive,a nd anti-diabetic effects, potentially lowering the risk of chronic conditions such as CVD, type 2 diabetes, and obesity (Leeuwendaal et al., 2022; Shah et

al., 2023). The influence of fermented and probiotic foods on the gut microbiome also affects the gut-brain axis, impacting mental health, mood, and even neurological disorders (Dahiya and Nigam, 2022; Leeuwendaal et al., 2022).

2.6 PROBIOTICS

According to the World Gastroenterology Organisation, the effectiveness of probiotics depends on their strain and dosage (Garcia-Burgos et al., 2020). Probiotics have the greatest effect when there is a high concentration of living bacteria, with at least 10^7 colony-forming units (CFU/mL), remaining at the conclusion of the food's shelf life. (Ghosh et al., 2019; Baruah et al., 2022). It was found that probiotics have the ability to activate the immune system and protect against pathogens (Ghosh et al., 2019). Probiotic bacteria consist of various surface markers which are responsible for the interactions with the host's intestinal epithelial cells. The interactions between the surface markers and the host's epithelial cells are essential, as this impacts the synthesis of chemokines and cytokines released by the enterocytic cells (Ghosh et al., 2019). Research has shown (Cao et al., 2020; Wicinski et al., 2020) that consuming certain types of bacterial strains, such as *Lactobacillus* and *Bifidobacterium*, can reduce body composition measurements and reduce inflammation in overweight and obese adults. For this reason, the following research report will focus on reporting findings on these two types of probiotic bacteria.

2.6.1 Lactobacillus

The genus *Lactobacillus* contains a wide variety of Gram-positive bacteria (Martin et al., 2019). They are present in various settings, such as fermented dairy products and the human body (Martin et al., 2019). Because of its capacity to promote gut homeostasis and mitigate illnesses linked to dysbiosis, *Lactobacillus. rhamnosus* is recognised as a probiotic (Martin et al., 2019). It has specific characteristics which allow the bacterium a better chance of survival, such as the adaptability to acidic and basic conditions within the body, which allows it to adhere to and colonise the intestinal walls (Toscano et al., 2017). The probiotic strain *L. rhamnosus* has been found to enhance mucosal integrity and reduce intestinal permeability, helping to prevent a "leaky gut" (Martin et al., 2019) and has

the potential to change the microbial diversity of the gut microbiome in healthy individuals (Toscano et al., 2017).

2.6.2 Bifidobacterium

Bifidobacterium are commonly found in the gastrointestinal tract and are a key microorganism in gut colonisation (Yao et al., 2021). Individuals with gastrointestinal conditions tend to have lower levels of B. longum compared to healthy individuals (Yao et al., 2021). Moreover, *Bifidobacterium* have been demonstrated to positively impact weight status in obese individuals aged 18 to 65 (Pedret et al., 2018). Pedret et al (2018) conducted a study that explored the impact of 12 weeks of daily *B. animalis subsp. lactis* CECT 814 on body composition in obese adults and revealed a decrease in weight status measurements compared to the placebo group ($P < 0.05$) (Pedret et al., 2018).

2.7 PROBIOTICS AND OBESITY: CLINICAL TRIALS INVESTIGATING PROBIOTICS FOR OBESITY MANAGEMENT

A review of eight studies suggests that probiotic interventions may reduce inflammation in overweight and obese adults by lowering CRP levels and pro-inflammatory markers (Rajkumar et al., 2014; Sabico et al., 2018; Tanaka et al., 2019; Tenorio-Jimenez et al., 2019; Majewska et al., 2020; Toejing et al., 2021). However, one study found no significant effects on inflammatory markers, concluding that the lack of inflammatory processes, such as high-fat consumption, was the reason. (Gomes et al., 2017). A compilation of the particular details from the research is shown in Table 1.

Table 1: Summary Of Previous Studies Investigating The Effects Of Probiotic Consumption On Inflammatory Markers In Obese Adults

Author Year	Population	Study design	Number of strains and dosage	Intervention duration	Results
Rajkumar 2014	N = 60 (n = 15 per group)	RCT	*8 x bacterial strains* .112.5 × 10^9 CFU/capsule	6 weeks	↓CRP in probiotic group (p < 0 .01)
Gomes 2017 Brazil	N = 43 Probiotic Group = 21 Control Group = 22	RCT-DB	5 x bacterial strains 2 × 10^{10} CFU/day	8 weeks	No significant changes
Sabico 2018	N = 61 Probiotic Group = 31 Control Group = 30	RCT -DB	8 x bacterial strains Twice daily placebo or 2.5 10^9 cfu/g	24 weeks	↓ TNF-α (67%), ↓ IL-6 (77%) ↓ CRP (53%)
Tanaka 2019	N = 96 Probiotic Group = 47 Control Group = 49	RCT -DB	1 x Heat-killed Bacterial strain . One tablet per day containing 50 mg	12 weeks	↓ CRP

Tenorio- Jimenez 2019	N = 53	RCT	*1 x bacterial strain* (5 × 10^9 CFU) or a placebo once daily	12 weeks	↓ IL-6 (p > 0.05)
Toejing 2021	N = 36 Probiotic Group = 18 Control Group =18	RCT-DB	*1 x bacterial strain* (50 × 10^9 CFU/day) or a placebo (corn starch 10 mg/day)	12 weeks	↓ TNF-α (p > 0.05) ↓ IL-6 (p > 0.05) ↓ CRP (p > 0.05)

*Abbreviations: RCT = Randomised control trial; DB = Double-blind; CFU = colony-forming units; TNF-α = tumor necrosis factor - alpha; IL-6 = interlukin 6; CRP = C-reative protein.

The impact of probiotics consumption on body composition was examined in seven studies (Jung et al., 2015; Gomes et al., 2016; Madjd et al., 2016; Kim et al., 2017; Pedret et al., 2018; Szulinska et al., 2018; Hassan et al., 2022), with a focus on BMI, body weight, and WC. The majority of the studies demonstrated decreases in these metrics following the interventions (Pedret et al. 2018; Hassan et al. 2022; Jung et al. 2015; Gomes et al. 2016; Kim et al. 2017). In one study, participants in both groups followed a specific dietary program, which resulted in a reduction of over 5% in their respective body weight. Nevertheless, the probiotic group and the control group did not differ significantly, according to the study. The researcher hypothesised that the short intervention period might have contributed to this lack of difference and suggested that a more extended intervention period might be more successful (Madjd et al., 2016). Table 2 provides a condensed overview of the particular details from the research studies.

Table 2: Summary Of Previous Studies Investigating The Effects Of Probiotic Consumption On Anthropometry and Body Composition In Obese Adults

Author Year	Population	Study design	Number of strains and dosage	Intervention duration	Results
Jung 2015 South Korea	N = 95 Probiotic Group = 49 Control Group = 46	RCT-DB	2 x bacterial strains 5 × 10^9 CFU/day, twice a day	12 weeks	↓ body weight (p = 0.008); ↓ BMI (p = 0.006); and ↓WC (P = 0.015).
Kim 2018 South Korea	N = 90 Probiotic Group (BNR17 - L) = 30 Probiotic Group (BNR17 - H) = 30 Control Group =30	RCT-DB	1 x bacterial strain Low-dose 10^9 CFU/day High-dose 10^{10} CFU/day	12 weeks	Within Group Comparisons BNR17 - L Group: ↓ WC (p = .045); and HC (p = 0.033) BNR17 - H Group: ↓ WC (p = 0.012)
Pedret 2018	N = 126 Probiotic Group (Ba8145) = 42 Probiotic Group (h-k Ba8145) = 44 Control Group =40	RCT-DB	1 x bacterial strain Heat-killed capsule/day 10^{10} CFU/day	12 weeks	↓ WC; ↓ W/H ratio; and ↓ BMI compared with placebo group (P < 0.05)

Madjd 2016 Iran	N = 89 Probiotic Group = 44 Control Group =45	RCT-SB	2 x bacterial strains 1 × 107 CFU	12 weeks	No significant differences.
Gomes 2017 Brazil	n = 43	RCT-DB	5 x bacterial strains 2 × 10^{10} CFU/day	8 weeks	↓ WC(p = 0.03), and W/H ratio (p = 0.02) in comparison with the dietary intervention
Szulinska 2018 Poland	N = 71 Probiotic Group (high dose) = 23 Probiotic Group (low dose) = 24 Control Group =24	RCT - DB	*9 x bacterial strains*	12 weeks	High dose: ↓ WC (p=0.0199); and FM (p=0.03974) Low dose: ↓ WC (p=0.0001); FM(p=0.0099); and F percentage (p = 0.0103.
Hassan 2022 Egypt	n = 58	Longitudinal study	*2 x bacterial* strains	12 weeks	↓ body weight (p < 0.05); ↓BMI (p < 0.05); ↓WC (p < 0.05); ↓HC (p < 0.05); ↓ fat percentage (p < 0.05); ↓ FM (p < 0.05); and ↓ FFM (p < 0.05)

*Abbreviations: BNR17 - L = low-dose group of Lactobacillus gasseri BNR17; BNR17 - H = high-dose group of Lactobacillus gasseri BNR17 ; Ba8145 = Bifidobacterium animalis subsp. lactis CECT 8145; h-k Ba8145 = heat-killed form of the probiotic strain Bifidobacterium animalis subsp. lactis CECT 8145; RCT = Randomised control trial; DB = Double-blind; Sb = Single-blind; CFU = colony-forming units; WC = waist circumference; BMI = body mass index; FM = fat mass; FFm = fat free mass.

Inflammation has been demonstrated to be reduced by single-strain probiotic treatments, especially those involving Lactobacillus species. For instance, lower levels of TNF-α, IL-6, and CRP have been linked to *L. plantarum, L. reuteri,* and *L. paracasei* (Tanaka et al., 2019; Tenorio-Jimenez et al., 2019; Toejing et al., 2021). Improvements in body composition have been associated with specific strains of *Lactobacillus*. Reductions in WC, body weight, and BMI have been shown in studies employing *L. gasseri* (Kim et al., 2017) and a mixture of *L. curvatus* and *L. plantarum* (Jung et al., 2015). Probiotic treatments lasted anywhere from six to twenty-four weeks. While longer interventions (24 weeks) resulted in significant reductions in TNF-α, IL-6, and CRP (Sabico et al., 2018), shorter interventions (6 weeks) produced lower CRP levels and modest decreases in pro-inflammatory markers (Rajkumar et al., 2014). Jung et al. (2015), Madjd et al. (2016), Kim et al. (2017), Pedret et al. (2018), Szulinska et al. (2018), Tanaka et al. (2019), Tenorio-Jimenez et al. (2019), Majewska et al. (2020), Toejing et al. (2021), and Hassan et al. (2022) were among the majority of studies that used 12-week interventions.

Many studies incorporated additional components into their interventions, such as weight loss plans and exercise programs (Madjd et al., 2016; Gomes et al., 2017; Hassan et al., 2022), and omega-3 fatty acid supplementation (Rajkumar et al., 2014). Probiotic strains varied widely, ranging from single-strain to multi-strain formulations. Dosages and delivery methods also differed across studies. A review conducted by Telle-Hansen et al (2018) found “the heterogeneity among the intervention studies makes it difficult to conclude whether diets or dietary components affect gut microbiota homeostasis and inflammation”. The varying results of probiotic studies make it challenging to understand how probiotics affect gut bacteria and inflammation. This issue reflects the broader challenges identified by Telle-Hansen et al. (2018) in dietary intervention studies.

2.8 CONCLUSION

The high prevalence of obesity among South African women was brought to light by this literature review, which also highlighted several important findings about the complex relationships between dietary intake, gut microbial composition, CRP levels, and obesity. The gut microbiota is crucial in the onset of obesity as it influences the maintenance of

overall health by regulating various bodily functions, including immune, metabolic, and nutritional processes. According to the literature, probiotics may help obese adults who have low-grade inflammation. Nevertheless, prior studies have some limitations and research gaps.

Numerous studies have aimed to understand the apparent dysbiosis observed in obesity globally. However, studies on African populations still need to be investigated. It is essential to gain insight into the current gut microbiome profiling of South African women living with obesity to learn about its associations with CRP levels and its effect on the development of obesity. By addressing this knowledge gap, we seek to contribute to developing effective strategies for obesity management, specifically for South African women. Identifying a correlation between CRP levels and the gut microbiome is essential. Such a correlation could lead to a cost-effective strategy for gaining insights into an individual's gut microbiome and health. Stool sample analysis is currently quite costly, but if a correlation between CRP levels and gut microbiome profile exists, we could identify issues with the gut microbiome by analysing CRP levels.

Numerous limitations and knowledge gaps exist in earlier research, and further research is required to look at the direct effects of specific probiotic strains. For instance, previous studies have used multiple probiotic strains, which limits insight into the effectiveness of specific probiotic strains. Probiotic treatments were administered alongside particular diet and exercise plans in several studies, which could have influenced the outcomes. To fill in these knowledge gaps and gain a deeper understanding of the complex relationship among gut microbiota, CRP levels, and obesity, the following research study will be conducted.

3 METHODS

3.1 STUDY DESIGN

The study used a two-phase design. The initial phase utilised an observational descriptive design to characterise the baseline characteristics of the participants and explore the relationships between key variables. This was followed by a single-blind, randomised, placebo-controlled trial to assess the impact of the intervention on changes in CRP (representing chronic inflammation) and weight status over time.

3.2 STUDY SETTING

For logistical reasons and to allow easy access for participants, the study was conducted at three different locations. The Pathology building in the Immunological Department at Prinshof Campus, the Consumer and Food Science Department at Hillcrest Campus, both at the University of Pretoria, and an egg packaging station in Delmas, Mpumalanga. All assessment visits, including screening and data collection, were conducted at these venues.

3.3 STUDY POPULATION AND SAMPLING

3.3.1 Study population

The study population consists of a diverse group of South African females aged 25 to 55 years with obesity. Although not an inclusion criterion, these individuals were expected to have obesity-related chronic lower-grade inflammation associated with NCDs, such as Diabetes Mellitus, cancer, CVD, and chronic lung disease. The inclusion criteria are summarised in Table 3.

TABLE 3: INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA	EXCLUSION CRITERIA
Female Aged: 25 - 55 years BMI ≥ 30 kg/m² CRP levels < 10 mg/L	Antibiotic treatment Laxatives Restricted food intake/weight reduction diet Chronic immunologic disease Thyroid disease Pregnant or plans to become pregnant Gastrointestinal diseases Gastrointestinal surgery

3.3.2 Recruitment and sampling

The study participants were recruited using a non-probability convenience sampling approach. Recruitment occurred from February to May 2024. A poster (Annexure B), inviting people to take part in the study, was distributed through various channels, including social media platforms such as WhatsApp and Instagram, pharmacies, malls, and office blocks. Additionally, the Department of Consumer and Food Science, University of Pretoria, sent out the poster via an existing consumer database. Individuals who wish to take part were requested to fill out a Google form (Annexure C). The Google form served as a pre-screening assessment to identify individuals meeting essential inclusion criteria such as BMI, age, and gender and to identify those who did not meet the criteria. Participants who voluntarily consented to take part and met the primary inclusion criteria were then invited to be screened for eligibility. During the screening, weight status (BMI in kg/m²), medical history, and CRP levels (mg/L) were assessed as part of the process to determine eligibility. Subsequently, participants were randomly assigned to either a Control Group or an Intervention Group. Randomisation was completed using a Randomiser App.

3.3.3 Sample size

A power analysis for two-sample t-tests was conducted using RStudio software (R Version 4.4.2) to determine the required sample size. Based on a previous study (Toejing et al., 2021) reporting a mean reduction in CRP levels of 0.0019 mg/L with a standard deviation of 0.0024 mg/L, an effect size of - 0.792 was estimated. To achieve a desired power of 0.80 and a significance level of 0.05, the analysis revealed that 26 participants are needed in each group, leading to an overall sample size of 52.

However, this research formed part of a pilot study; the sample size depended on the available funds for stool samples and blood analysis, and allowed for the assessment of 30 participants, pre- and post-intervention. Recruitment continued to reach the required number, with 15 participants allocated to each group: control and intervention. Guided by other studies (Sabico et al., 2018; Toejing et al., 2021), a sample of 30 was deemed sufficient for an initial pilot. Sabico et al (2018) included a total of 31 participants and found reductions in chronic inflammatory markers, including TNF- α (67%), IL-6 (77%), and CRP (53%). Toejing et al (2021) had similar findings with 36 participants. The study found a notable decrease in the plasma levels of CRP when compared to the baseline ($p < 0.05$) within the Intervention Group.

3.4 DATA COLLECTION

3.4.1 Measurement tools

3.4.1.1 Anthropometric Assessments

The measurement tools used to measure anthropometric measurements include a Seca digital scale (Model 813, SECA GmbH & Co. KG, Hamburg, Germany), a Seca non-stretchable measuring tape (Model 201, SECA GmbH & Co. KG, Hamburg, Germany), a Seca stadiometer (Model 213, SECA GmbH & Co. KG, Hamburg, Germany) and a Seca body composition analyser (Bioelectrical Impedance) (BIA) (seca mBCA 525 SECA GmbH & Co. KG, Hamburg, Germany).

3.4.1.2 Blood Sample Collection and CRP Measurement Kit

Ethylendiaminetetra acetic acid (EDTA)--containing blood collection tubes were used for blood collection. A CardioPhase® high-sensitivity CRP kit (Siemens, MU, DE) was used to determine the levels of CRP in the samples.

3.4.1.3 Dietary Assessment

Two dietary assessment tools were used to assess the participants' dietary intake and monitor changes to dietary patterns during the intervention. A Three-Day Food Diary (Lee and Nieman, 2013) (Annexure D), where participants had to track all food and drinks consumed over the course of three days, consisting of two weekdays and one weekend day. They were urged to include as much information as they could, such as food brands, serving sizes, and preparation techniques. For participants with lower literacy levels, a 24-hour recall was used (Annexure E).

3.4.1.4 Gut Microbiome

Stool sample collection kits were given to participants, along with detailed written instructions on how to securely gather and preserve stool samples at home (refer to Annexure G). DNA/RNA shield faecal collection tubes (Catalog # R1101, Zymo Research, Irvine, CA, USA) were included in the kits. These tubes were made especially for the extraction and storage of nucleic acids from stool samples. Each fecal collection tube has a prefilled 9mL DNA/RNA shield and comes with a spoon attached to the screw cap. Additionally, the kit includes a faeces catcher (Catalog # R1101-1-10, Zymo Research, Irvine, CA, USA), which is a strip of faecal collection paper that can be fitted onto any toilet seat.

3.4.2 Measurement methods

3.4.2.1 Anthropometric Assessments

Anthropometric data were recorded during the initial visit (screening/baseline) and then again at the second visit (post-intervention). All measurements were recorded on the screening and baseline measurements assessment sheet (Annexure H). Participants were instructed to don light and non-restrictive attire on the days when anthropometric

measurements were taken. All measurement procedures were conducted in accordance with the Anthropometry Procedures Manual issued by the National Center for Health Statistics (NCHS), as reflected in the context regarding the National Health and Nutrition Examination Survey (NHANES) (National Center for Health Statistics, 2021) and were thoroughly explained to the participants prior to any measurements being taken. The calculation of body mass index (BMI) was performed by dividing weight (kg) by the square of height (m) (WHO, BMI).

Weight was measured using the following procedures (NCHS, 2021). Participants were measured for weight in kilograms utilising a calibrated Seca digital scale, with precision to the nearest 100 grams (0.1 kg). Participants were instructed to stand barefoot in the middle of the scale platform. They were to face the researcher or a BSc Nutrition student, keeping their hands at their sides and looking straight ahead. After ensuring the participant was properly positioned, the researcher recorded the measurement shown on the digital scale.

Height was measured according to the NCHS manual procedures (NCHS, 2021). Height was recorded in meters (m), rounded to the nearest 0.5m with a stadiometer. Participants were instructed to take off any hair accessories, jewellery, or styles such as buns or braids from the top of their heads. They were directed to stand upright on the stadiometer platform with their backs aligned against the backboard. Their weight should be evenly balanced, and their feet must be flat on the platform. The participants were told to position their heels together while allowing their toes to point outward at a 60-degree angle. The researcher made sure that the participants' backs of the head, shoulder blades, buttocks, and heels made contact with the backboard. The head of the participant was aligned in the Frankfort horizontal plane. The stadiometer headpiece was then lowered so that it rested firmly on top of the participant's head. Participants were asked to stand as upright as they could, take a deep breath, and maintain that posture. The measurement was recorded, and then the participant was instructed to exhale.

Waist circumference was measured using the following procedures (NCHS, 2021). Participants were instructed to gather their shirts above their waists, cross their arms, and place their hands on their opposite shoulders. The researcher found the ilium, which is part

of the pelvis, on the right side of each participant and drew a horizontal line just above the upper edge of the right ilium. Next, the researcher made a cross mark at the midaxillary line, which runs from the armpit down the side of the body. The waist circumference was measured to the nearest 0.1 cm using a non-stretchable measuring tape. The researcher wrapped the measuring tape around the waist at the level of the measurement mark. The tape should be level, parallel to the floor, and fit snugly without pressing on the skin (NCHS, 2021).

Body composition measurements were conducted using a BIA, an electronic instrument that passes an alternating current through the body using four electrodes. Participants were instructed to take off their shoes, socks, and jewellery. Following this, the researcher cleaned their hands and feet using sanitising wipes. The electrodes were placed on the hands and feet, and the patient's cables were attached to the participant (Figure 3). The participant was asked to avoid moving while the analyser took the measurements. After the measurements had been completed, the researcher documented the reactance (Xc) and resistance (R) (Lee and Nieman, 2013). To determine fat-free mass and fat mass, the formula for females (Sun et al., 2003) was applied.

FFM = −9.53 + 0.69 × (stature²/ resistance) + 0.17 × body weight + 0.02 × resistance (Sun et al., 2003)

FM = body weight - FFM

Where:

- FFM and FM are in kilograms,
- Stature is in centimetres,
- Resistance is in ohms (Ω),
- Body weight is in kilograms.

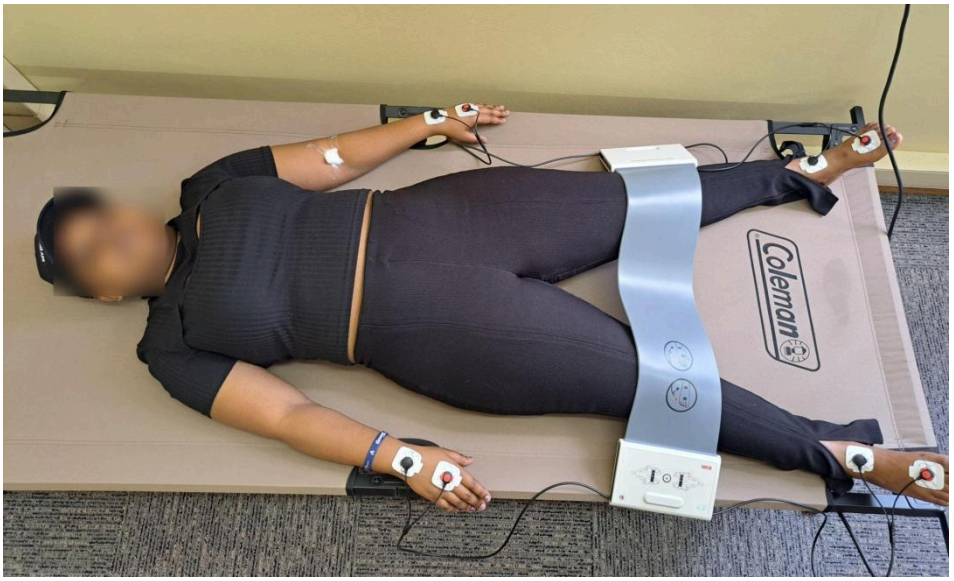

Figure 3: Bioelectrical Impedance Analysis (BIA) for Body Composition.

3.4.2.1.1 Quality Control of Anthropometry and Body Composition Measurements

To ensure the accuracy and consistency of anthropometric and body composition measurements, the following quality control procedures were implemented:

Standardised Training: Before data collection, all participating fourth-year BSc Nutrition students underwent a standardised training session conducted by the researchers. This training covered: 1) Proper measurement techniques for height, weight, waist circumference, hip circumference, and body composition; and 2) Standardised positioning of participants for each measurement. These control measures reduced intra-observer variability as they minimised variations in technique by a single person over time and reduced inter-observer variability as the exact same techniques were used by each person. The standardised training also ensured accuracy of measurements, which improved the validity. This was achieved by minimising systematic error as the standardised training emphasises correct techniques that aim to capture the true value of the measurement. When measurements are taken using standardised techniques, the data collected is more comparable across different participants and even with data from other studies that have used similar standardised methods. This allowed for data comparability, which improved the validity of the data.

Calibration of Equipment: All equipment used for anthropometry and body composition measurements was calibrated regularly, both prior to the commencement of the study and at regular intervals throughout the data collection phase. This ensures that repeated measurements under the same conditions will yield more consistent results, which reduces systematic error and bias contributing to the consistency of measurements. Additionally, it minimised random error as the calibration of equipment ensured greater consistency across measurements taken at different times. Calibration directly addresses accuracy by ensuring that the equipment's readings are traceable to a recognised standard, thereby improving the validity of the data.

Duplicate Measurements: To minimise intra-observer variability, duplicate measurements were taken for each anthropometric parameter by the same student. If the variation between the two measurements surpassed a set threshold (for instance, 0.5 cm for waist circumference and 0.1 kg for weight), a third measurement was conducted, and the average of the two measurements that were closest was noted. Duplicate measurements and regular checks for consistency among measurers minimised intra-observer variability and contributed to more accurate measurements.

3.4.2.2 Blood Sample Collection and Measurements of CRP levels

Blood sample collection:

A phlebotomist from the Immunology department took 10ml blood samples from each participant (Figure 4). After collection, blood samples were taken to the Immunology Department, located in the Pathology Building, Prinshof 349-Jr, Pretoria, 0084, to be processed and then analysed for CRP levels.

Figure 4: Blood Sample Collection

Blood Sample Processing:

Whole blood (10mL) collected in EDTA- containing blood collection tubes were centrifuged at 1800xg at room temperature (24 ºC) for 10 minutes. The plasma will be carefully aspirated from the sample and stored in 250µL aliquotes at -80 ºC until use.

C-reactive protein measurement procedures:

The high sensitivity (hs) CRP concentration was measured using an assay comprising human-specific antibodies bound to polystyrene particles that bind to the CRP present in the sample. Light scatters and passes through the sample, resulting in different recorded intensities proportional to the CRP concentration. A CardioPhase® high sensitivity CRP kit (Siemens, MU, DE) was used to determine the levels of CRP in the samples. The assay was performed according to the manufacturer's specifications using the Attelica 630N nephelometer (Siemens, MU, DE). A reference curve was generated by multi-point calibration, and N Rheumatology Standard SL serial dilutions were automatically prepared by the Attelica 630N 45 nephelometer (Siemens) using N diluent. Samples were inserted into the instrument and diluted (1:20). The CRP concentrations were presented as milligrams/Litre. Table 4 displays the interpretation of CRP levels. For the purpose of this

study, a CRP level ≥ 10 mg/L was considered to be systemic inflammation (Nehring et al., 2023).

Table 4: Interpretation of CRP levels

CRP Ranges	Interpretation
Less than 3 mg/L	Normal/Healthy
3 - 10 mg/L	Normal or minor elevation (Seen in obesity)
10 - 100 mg/L	Moderate elevation (Systemic inflammation)

***(Nehring et al., 2023)**

3.4.2.3 Dietary Assessment

Dietary assessments were conducted in the first week, the sixth/seventh week and the 12th week of the intervention. Participants received reminders to complete their food diaries and were asked to email their three-day food diaries to the researcher. The 24-hour recall interviews were conducted in person by the researcher and trained fourth-year BSc Nutrition students (Figure 5). Participants were required to name all of the foods and drinks they had consumed the day before the interview. Following this first recollection, the interviewer probed further to find out if any particular foods had been eaten. A portion size guide was used to determine the estimated portions of foods consumed for all participants including those completing the three day food diaries and the 24-Hour recall dietary assessments (Annexure F).The food diaries and 24-hour recalls were analysed by 4th-year BSc Nutrition students using FoodFinder (v2023-05-11, 2024), a web-based dietary analysis software developed by the South African Medical Research Council (SAMRC) and maintained by the South African Food Data System (SAFOODS) (SAFOODS, 2024).

Figure 5: Dietary Assessment via 24-Hour Recall

3.4.2.3.1 Quality Control of Dietary Assessments and Analysis

To maintain the integrity of the dietary data, the subsequent measures were put in place:

Standardised Training: All participating students received standardised training on conducting dietary assessments, including: 1) Proper use of three-day food diaries; 2) Techniques for probing and clarifying food descriptions and portion sizes with participants; and 3) Methods for minimising participant recall bias. The standardised training improved the reliability in several ways. It minimised variability in how instructions were given, how probing questions were asked, and how portion sizes were estimated, which led to more consistent data across participants and across different data collectors. The standardised probing questions reduced the likelihood of interviewers unintentionally leading participants or influencing their responses, which reduced interviewer bias.

Standardised Food Diary and 24-Hour Recall Instructions: Participants were provided with clear and comprehensive written instructions on how to complete the three-day food diaries, including examples of typical portion sizes and food items. The 24-hour recalls were performed in person, and the participants were given clear instructions before the interview took place. The Researcher and students made use of a food groups visual aid to help the participants describe their portion sizes. The standardised instructions for the dietary assessments improved reliability and validity by providing clear written and verbal instructions. This ensured all participants understood the requirements for completing the food diaries and participating in the recalls in the same way, and minimised

misunderstandings and errors in recording food intake, which reduced participant error. The use of the food groups visual aid provided a standardised reference for participants to estimate portion sizes, improving the consistency of portion size estimation.

Review of Food Diaries and 24-Hour Recalls: Completed food diaries were reviewed by the researcher. If any information was missing or unclear, participants were contacted to provide clarification. This improved reliability by data cleaning, which ensured a more complete and consistent dataset for analysis and reduced ambiguities in food descriptions or portion size, led to more consistent interpretation during data entry. Identifying and correcting missing information or unclear entries improved the accuracy of the reported dietary intake. Contacting participants for clarification helped retrieve potentially valuable dietary information that might have been lost otherwise.

Standardised Food Analysis Software/Database: Dietary data from the food diaries were analysed using FoodFinder (v2023-05-11, 2024). All students were trained on the proper use of the software/database to ensure consistency in data entry and analysis. Using the same software and database ensured that nutrient calculations were performed consistently for all dietary assessments, eliminating variability due to different analysis methods or databases. Utilising a validated and up-to-date food composition database ensured that the nutrient values used in the analysis are as accurate as possible and also minimised calculation errors, as the software automated the nutrient calculation process, reducing the risk of manual calculation errors.

Quality Checks of Dietary Analysis: The researcher reviewed the analysed food diaries to ensure the accuracy of the nutrient calculations and to identify any potential errors. Reviewing the analysed data helped to identify and correct any inconsistencies or errors that might have occurred during data entry or analysis. Ensuring the accuracy of nutrient calculations confirmed that the dietary intake data was being interpreted correctly. Quality checks helped to identify potential outliers or unusual dietary patterns that might warrant further investigation and ensured the overall validity of the findings.

3.4.1.4 Gut Microbiome

3.4.1.4.1 Collection of Stool Samples

Participants received their stool sample collection kits during their screening visit. They used these kits to collect their stool samples at home. The participants were given detailed written instructions on how to securely gather and preserve stool samples at home (refer to Annexure G). Participants were instructed to collect their stool samples either the night before or the morning of their drop-off. Once the samples are collected, they should be stored in the refrigerator. After collection, the participants dropped off their samples at the Agriculture Building in the Consumer and Food Science Department at the University of Pretoria. Each stool sample was labelled with a permanent marker using its ID code and stored in a specialised freezer purchased explicitly for this purpose. The samples were then transported to Mr. DNA Molecular Research LP (Shallowater, TX 79363, USA) within three days while maintaining low temperatures.

3.4.1.4.2 Analysis of Stool Samples

All procedures for analyzing stool samples were carried out by Mr. DNA Molecular Research LP located in Shallowater, TX 79363, USA. The stool samples underwent analysis at both the phylum and genus levels. The assessment of gut microbiota was performed on 18 distinct phyla. Additionally, the F/B ratio was determined by examining the abundance of Firmicutes and Bacteroidetes. At the phylum level, several bacterial groups were identified, including Bacillota, Firmicutes, Bacteroidota, Bacteroidetes, Pseudomonadota, Proteobacteria, Actinomycetota, and Actinobacteria.

The results for Bacillota and Firmicutes, Bacteroidota and Bacteroidetes, Pseudomonadota and Proteobacteria, and Actinomycetota and Actinobacteria are reported separately throughout this study. While this approach may introduce some redundancy in the data presentation, it is maintained for clarity and to address specific considerations relevant to this research project. Separating these terms made it easier to compare our findings with existing research that uses the old phylum names. For instance, the term "Firmicutes" is particularly important when discussing the Firmicutes/Bacteroidetes ratio, which is a widely studied metric. Additionally, different

microbiome analysis pipelines and databases may produce results using both naming conventions. Therefore, this study presents the findings in a way that reflects the raw output of the analysis based on the database utilised.

The assessment of gut microbiota was performed on 238 different genera. However, since this study's main focus is on genera with known associations with obesity (Companys et al., 2021; Politi et al., 2023), the report was limited to the following seven primary genera: *Faecalibacterium, Bacteroides, Eubacterium, Bifidobacterium, Clostridium, Akkermansia,* and *Lactobacillus.*

The protocol utilised for laboratory analysis involved the following steps: PCR primers 515/806 targeting the V4 variable region of the 16S rRNA gene were employed in a PCR process that varied between 30 to 35 cycles based on the specific primers and DNA. The PCR was conducted using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: an initial denaturation step lasting 5 minutes at 95°C, followed by 30-35 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C for 40 seconds, and extension at 72°C for 1 minute. A final elongation step was performed for ten minutes at 72°C.

Following amplification, the PCR products were analysed with a 2% agarose gel to determine the effectiveness of the amplification and the relative strength of the bands. The samples were multiplexed with distinct dual indices and combined in equal amounts according to their molecular weight and DNA concentrations. The combined samples underwent purification using calibrated Ampure XP beads.

The pooled and purified PCR product was subsequently utilised to create an Illumina DNA library. Sequencing took place at MR DNA (Shallowater, TX, USA) on a MiSeq, adhering to the manufacturer's instructions. The sequence data were analysed through the MR DNA analysis pipeline. In brief, sequences were concatenated, and those shorter than 150 bp were discarded, as well as sequences with ambiguous base calls. Quality filtering was conducted using a maximum expected error threshold of 1.0, and dereplication was carried out to obtain unique sequences. Denoising was utilised to detect and eliminate

distinct sequences that contained sequencing and/or PCR errors, followed by the removal of chimeras. This led to the generation of denoised sequences or zero-radius Operational Taxonomic Units (zOTUs). Ultimately, the zOTUs underwent taxonomic classification using BLASTn with a curated database sourced from NCBI. The results, including phylum and genus classifications, were transferred to an Excel spreadsheet and shared with the researcher.

3.4.1.5 Production of the yoghurt products

The yoghurt was made by honours Food Science students under the guidance of a postgraduate PhD student in the food science laboratories of the Department of Food and Consumer Science at the University of Pretoria.

3.4.1.5.1 Probiotic and Yoghurt Start Culture

A commercial source of freeze-dried yoghurt start cultures (Sacco Lyofast Y259A) containing a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus* was used to prepare the probiotic yoghurt. Additionally, a commercial freeze-dried culture of *Bifidobacterium animalis subspecies lactis* (BB-12) (Chr.Hansen), as well as a commercial freeze-dried culture of *Lacticaseibacillus rhamnosus GG (LGG)* (Chr.Hansen), was used. For confocal microscopy and flow cytometry, single strains of *S. thermophilus* and *L. delbrueckii subspecies bulgaricus*, which had been cryopreserved in glycerol and stored in a - 80℃ freezer, were utilised.

3.4.1.5.2 Yoghurt Production

The Experimental Dairy Farm at the Hillcrest Campus of the University of Pretoria supplied 25 liters of chilled raw milk. A batch pasteuriser was filled with the raw milk and heated to 25°C. After the milk reached 25°C, about 600 millilitres were taken out of the batch pasteuriser. To create a smooth mixture, 600g of commercial yoghurt stabiliser (Bianka Dairy Supplies IFATECH DD 25) was whisked into 600ml of milk. The batch pasteuriser was then filled with the smooth mixture using the remaining milk. The milk was then pasteurised for 30 minutes at 63°C.

Twelve 2 L glass bottles were filled with the milk after it had been pasteurised and cooled to 50°C. Furthermore, 3g of the commercial freeze-dried yoghurt starter cultures were added to 300 ml of pasteurised milk in a 500 ml glass bottle. The commercial freeze-dried probiotic cultures were inoculated with 0.6g each in 70 ml of pasteurised milk in a different 100 ml glass bottle. For at least three minutes, these two glass bottles were shaken thoroughly.

A pipette was used to transfer 20 ml of the inoculated milk containing starter cultures into each of the twelve 2 L bottles. Six of the bottles, designated as plain yoghurt, were set aside specifically for the addition of starter cultures. The two probiotic bacterial strains were pipetted into 10 ml of the inoculated milk in the remaining six bottles, which were labelled as probiotic yoghurt. Furthermore, two bottles were made in order to track the pH levels of the probiotic and plain yoghurts throughout the fermentation process.

Every bottle was thoroughly shaken before being submerged in a water bath set at 42°C. The milk's pH was checked every hour until it reached 4.6. When the pH hit 4.6, the bottles were removed from the water bath, shaken to break the coagulation, and then submerged in a cold water bath to halt the fermentation. The yoghurt was poured into 100g tubes after it had cooled and refrigerated at 4°C to solidify. The production of both probiotic and regular yoghurt was carried out weekly throughout the 12-week intervention period.

3.4.1.5.*3* Physicochemical Tests, pH and Titratable Acidity

During the 12-week intervention phase, physicochemical assessments were performed on day 0 and day 7 of each production cycle for both the plain yoghurt and the probiotic yoghurt. These tests included pH, degree of syneresis, and titratable acidity.

A meter (HANNA Instruments Inc., USA) was used to measure the pH of both plain and probiotic yoghurt. Three separate measurements of titratable acidity were made for the probiotic and plain yoghurt. To make a 10^{-1} dilution, 2g of yoghurt was mixed with 18g of distilled water. Each yoghurt sample that was diluted was treated with four drops of phenolphthalein indicator, followed by titration with 0.1N sodium hydroxide (NaOH) until a

pale pink color persisted. The percentage of lactic acid in the yoghurt samples was calculated using the following formula:

% Lactic acid = [(titre x N x 90) ÷ (M_X x 10)]

In this context, N represents the normality of the NaOH utilised in the titration, which was determined to be 0.1 N; M_X denotes the mass of yogurt used during the titration, which was 2 grams; and titre refers to the volume of 0.1 N NaOH employed to achieve the endpoint, characterised by a faint pink color, in the titration.

3.4.1.5.4 . Determination of Total Plate Counts, Total Lactic Acid Bacteria Counts, and Viability of Probiotics During Storage of Probiotic Yoghurt

During the 12-week intervention, the viability of *B. animalis subspecies lactis* BB-12 and *L. rhamnosus* GG, along with the total plate count and overall lactic acid bacterial count, were evaluated for the probiotic yogurt using the plate count method on days 0 and 7 of each production cycle.

A dilution factor of 10^7 was achieved by serially diluting the probiotic yoghurt samples. A duplicate of 100 µL of dilutions 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} were spread plated onto the various media types. The first type of media used to count all lactic acid bacteria was MRS agar (de Man, Rogosa, Sharpe 1960). Blood heart infusion (BHI) agar (Millipore), an enriched non-selective medium, was the second type of media used to count a broad range of microorganisms, such as molds, yeasts, and bacteria. To quantify L. rhamnosus GG, the third type of medium used was MRS agar enriched with vancomycin (13 mg/L) (MRS-Vancomycin agar).

The final media type utilised for the enumeration of B.animalis subspecies lactis BB-12 was MRS agar supplemented with lithium chloride (3g/L), L-cysteine (5mg/L), nalidixic acid (15 mg/L), neomycin (100mg/L), and paramomycin (200mg/L) (MRS-NNLP agar) (Moriya, Fachin, Gandara & Viotto, 2006). The MRS, MRS-Vancomycin, and MRS-NNLP agar plates were kept in an anaerobic environment at 37°C for a duration of 48 to 120 hours. In particular, the MRS-NNLP agar plates were incubated for a full 120 hours. The BHI agar plates were incubated for 48 hours at 37°C in an aerobic environment.

Physiochemical Properties of Plain and Probiotic Yoghurt

The amount of lactic acid in both plain and probiotic yoghurt was the same on day 0 and day 7.

Table 5: Analysis of variance of lactic acid (%) in plain and probiotic yoghurt during shelf life of 7 days at 4℃

Factor	Degrees of Freedom	p-value
Day (0,7)	1	0.1162
Type of yogurt (Plain, probiotic)	1	0.1016
Interaction between day and type of yogurt	1	0.5800

3.4.1.4.4 Quality control of yoghurt products

3.4.1.4.4.1 Quality Assurance of yoghurt products

The following quality and safety procedures ensure that all yoghurt products are safe for human consumption. Quality and safety control protocols were conducted by postgraduate students in Food Science within the laboratories of the Department of Food and Consumer Science at the University of Pretoria.

3.4.1.4.4.2 Viability assessment and Microbial safety assessments

The viability of probiotics and yoghurt starter cultures was determined using the standard protocols of the International Dairy Federation (IDF). Viability was determined immediately after fermentation and on days 14 and 28 during cold storage.

i. For *Bifidobacterium* BB-12. viability was determined by enumeration on MRS-NNLP agar.

ii. For LGG, viability was determined by enumeration on MRS agar with vancomycin.

iii. For *Streptococcus thermophilus*, viability was determined by enumeration on M17 agar with lactose.

iv. For *Lactobacillus delbrueckii subsp. bulgaricus*, viability was determined by enumeration on MRS agar adjusted to pH5.4.

All batches of yoghurt were tested for *Escherichia coli, Salmonella spp*., and *Staphylococcus aureus* in accordance with the Dairy Standards Agency (DSA) standard methods.

3.4.1.4.5 Delivery of the yoghurt products and monitoring of yoghurt consumption

The participants received a week's supply (7 yoghurt products, 100g each) every week for 12 weeks. Figure 6 shows an example of yoghurt containers labelled with participant name, intervention week, and day of the week, ready for distribution. The researcher and 4th-year BSc students in Nutrition and Food Science delivered the yoghurt products. The majority of participants collected their yoghurts from the Department of Food and Consumers Science building of the University of Pretoria. Participants were instructed to keep all of the yoghurt containers and return them each week. This procedure enabled the researcher to monitor their consumption by counting the containers to ensure that all were submitted. Additionally, each week, participants were asked if they had consumed all of their yoghurt to verify their consumption. This information was then recorded in a spreadsheet (Annexure I).

Figure 6: Yoghurt Intervention Products

3.4.3 Intervention

The intervention group consumed the "treated" product, probiotic yoghurt, containing *L. rhamnosus* GG and *B. animalis* subsp. *lactis* BB-12, during the intervention period, while the control group consumed a conventional product, specifically non-probiotic yoghurt. Participants were instructed to eat the yoghurt once daily for the duration of the 12-week intervention. Participants could eat the yoghurt at any time during the day and incorporate it into their daily dietary routine as they wished. This period was selected based on earlier research evaluating the impact of probiotic supplementation in adults with obesity (A. Gomes et al., 2017; Toejing et al., 2021). Participants were instructed to steer clear of heavily fermented foods like kombucha and kimchi, along with other probiotic sources, while maintaining their regular eating habits. The researcher monitored yoghurt consumption compliance once a week via WhatsApp messages or emails. Additionally, participants' dietary intake was monitored using dietary assessments completed at the beginning, during the sixth week, and upon conclusion of the intervention.

3.4.4 Variables

Independent variables:

- The bacterial content of the yoghurt (10^{10}cfu/g of *B. animalis subspecies lactis* (BB-12) (Chr.Hanse) and 10^{10}cfu/g of *L. rhamnosus GG* (LGG) (Chr.Hansen))

Dependent variables:

- Gut bacterial counts (cfu/g) at phylum and genus levels.
- The CRP levels (mg/L)
- BMI (kg/m²), weight (kg), height (cm), WC (cm), HC(cm), W/H Ratio, fat mass (kg), fat-free mass (kg), and FMI (kg/m²).

Control and monitoring:

- Dietary intake: Total energy (kj/day), protein (g/day), lipids (g/day), carbohydrates (g/day), and dietary fibre (g/day).

The study investigated several variables to assess the effects of a 12-week probiotic yoghurt intervention in obese South African females. These variables were categorised into anthropometric, body composition, biochemical, dietary, and gut microbiome composition outcomes. Each variable was measured using validated tools and instruments at specific time points, primarily at baseline and post-intervention. A detailed overview of the variables, along with the measurement units, instruments utilised, and the timing for data gathering, is displayed in Table 6.

Table 6: Summary of Study Variables, measurement unit and Instruments, and Time points

Variable	Category	Unit of Measurement	Instrument/Tool	Time points
Weight	Anthropometric	Kilograms (kg)	Seca digital scale (Model 813)	Baseline, Post-intervention
Height	Anthropometric	Centimetres (cm)	Seca stadiometer (Model 213)	Baseline only
Body Mass Index (BMI)	Anthropometric	kg/m²	Calculated: weight (kg)/ height (m²)	Baseline, Post-intervention
Waist Circumference	Anthropometric	Centimetres (cm)	Seca non-stretchable tape (Model 201)	Baseline, Post-intervention
Waist-Hip Ratio	Anthropometric	Unitless (ratio)	Calculated from waist and hip circumference	Baseline, Post-intervention
Fat Mass Index (FMI)	Body Composition	kg/m²	Seca mBCA Bioelectrical Impedance Analyser	Baseline, Post-intervention
Fat Mass (FM)	Body Composition	Kilograms (kg)	Seca mBCA Bioelectrical Impedance Analyser	Baseline, Post-intervention
CRP	Biochemical	mg/L	Siemens CardioPhase® hs-CRP kit with Attelica 630N	Baseline, Post-intervention
Energy Intake	Dietary	kJ/day	FoodFinder software v2023-05-11	Week 1, Week 6, Week 12
Macronutrient Intake	Dietary	% of total intake / grams	3-Day Food Diary and 24-Hour Recall (FoodFinder)	Week 1, Week 6, Week 12
Dietary Fibre Intake	Dietary	Grams/day	3-Day Food Diary and 24-Hour Recall (FoodFinder)	Week 1, Week 6, Week 12
Gut Microbiota at Phylum Level	Microbiological	CFU/g	16S rRNA sequencing (MR DNA)	Baseline only
Gut Microbiota at Genus Level	Microbiological	CFU/g	16S rRNA sequencing (MR DNA)	Baseline only
Firmicutes/Bacteroidetes Ratio (F/B Ratio)	Microbiological	Ratio	Calculated from sequencing output	Baseline only

3.5 DATA MANAGEMENT AND STATISTICAL ANALYSIS

This section outlines the steps involved in statistical analysis, data management, and cleaning. All relevant data, including anthropometric measurements, body composition information, and participant demographics, were recorded using data collection forms. Using 24-hour recalls and three-day food diaries, dietary intake was collected and recorded. After the data was gathered, Microsoft Excel was used to move all of the data into a secure electronic database (Annexure J). Anthropometric measurements, body composition information, dietary intake, CRP levels, gut microbial composition, and participant demographics were all included.

After data entry, data cleaning procedures were performed to identify and correct any errors or inconsistencies. This included checking for missing data and implementing appropriate strategies for handling missing values, defining the criteria used for outlier identification and checking for data consistency and logical errors. All information was saved safely on the researcher's computer, which is protected by a password. Access to the data was restricted to authorised personnel only. Data were anonymised by assigning unique participant identification numbers to protect participant confidentiality.

Statistical analyses were conducted using R Version 4.4.2 and RStudio. Descriptive statistics summarised the characteristics of participants and the study variables. This included calculating means and standard deviations (SD) for continuous variables such as age, BMI, and CRP levels. Inferential statistical methods were selected based on data distribution and the nature of comparisons, as summarised in Table 7.

Table 7: Summary of Statistical Analyses Used

Analysis Objective	Statistical Test	Purpose
Compare baseline differences between the control and intervention groups	Independent t-test	To assess group comparability
Within-group comparisons (pre- and post-intervention)	Paired t-test	To assess the effect of the intervention
Assess correlations between weight status, CRP, gut microbiota, and dietary intake	Pearson (parametric) or Spearman (non-parametric) correlation	To explore linear and monotonic associations

Every statistical test was performed with a confidence level of 95%, and the significance limit was established at $p < 0.05$. Data were stratified where applicable to allow comparisons by group and time point.

The following statistical tests were employed:

Baseline Comparisons: Independent samples t-tests were used to compare baseline characteristics between the intervention and control groups, as well as between urban participants and rural participants. The characteristics assessed included age, weight (kg), height (cm), BMI (kg/m²), WC (cm), HC (cm), W/H ratio, FM (kg), FFM (kg) and FMI (kg/m²), CRP levels (mg/L), dietary intake including energy (kJ), protein (g/day), lipids (g/day), carbohydrates (g/day), and dietary fibre (g/day) and gut bacterial composition (cfu/g) both at phylum (including F/B ratio) and genus level. At the genus level, the study focused on *Faecalibacterium, Bacteroides, Eubacterium, Bifidobacterium, Clostridium, Akkermansia*, and *Lactobacillus.*

Group Comparisons: T-tests for independent samples were conducted to analyze differences in outcomes between the intervention and control groups at both baseline and post-intervention. The evaluated outcomes comprised weight (kg), BMI (kg/m²), WC (cm), HC (cm), W/H ratio, FM (kg), FFM (kg) and FMI (kg/m²) and CRP levels (mg/L).

Within Group Comparisons: A paired t-test was utilized to evaluate the means of participants before and after the intervention. The assessed outcomes included weight (kg), BMI (kg/m²), WC (cm), HC (cm), W/H ratio, FM (kg), FFM (kg) and FMI (kg/m²), CRP levels (mg/L), dietary intake including energy (kJ), protein (g/day), lipids (g/day), carbohydrates (g/day) and dietary fibre (g/day).

Correlations: Pearson correlation coefficients were utilised to evaluate the connections between:

1. CRP levels (mg/L) and weight status measurements, including BMI (kg/m²), FMI (kg/m²), FM (kg), WC (cm), and WHR.
2. Gut bacterial composition (cfu/g) both at phylum (including F/B ratio) and genus level and a) weight status measurements including BMI (kg/m²), FMI (kg/m²), FM (kg), WC (cm), and WHR; b) CRP levels (mg/L); and c) Dietary intake including energy (kJ), protein (g/day), lipids (g/day), carbohydrates (g/day), and dietary fibre (g/day).

4 ETHICAL AND LEGAL CONSIDERATIONS

Every participant was fully informed about the study and its methods, and written consent (Annexure K) was obtained before any data was collected. After being given all relevant information about the study, participants were encouraged to discuss any aspect of it and ask questions. All participants were required to provide written informed consent prior to being screened and taking part in the study. The researcher assisted Participants with lower literacy in completing consent forms and questionnaires. All participants gave consent. All information and data samples collected were handled carefully to protect participants' privacy. The evaluation excluded any personal details, and every participant was assigned a distinct study code number for sample identification. English was used for all questionnaires. Before the study began, ethical clearance was obtained from the Faculty of Natural and Agricultural Sciences and Health Sciences (Ethical reference number: NAS/142/2023).The letter of ethical approval is located in Annexure L.

4 RESULTS

This chapter outlines the results of a 12-week randomised controlled trial designed to characterise baseline traits and explore the connections between gut microbiota, weight status, CRP levels, and dietary habits in South African women with obesity, in addition to evaluating the effects of a 12-week probiotic yoghurt intervention on weight status and CRP. The findings are presented in alignment with the study's objectives: (1) baseline characteristics , (2) dietary intake , (3) correlation between baseline variables, and (4) the effect of the 12-week probiotic yoghurt intervention on changes in weight status and CRP Levels. Due to logistical constraints, gut bacterial analysis was conducted only at baseline. Therefore, the presented gut bacterial data describe the baseline composition of the study participants and explore its relationship with other baseline variables.

4.1 DESCRIPTION OF THE PARTICIPANTS

At baseline, the study included 30 participants who were randomly assigned to either the intervention group (n = 16) or the control group (n = 14). Baseline assessments included weight status measurements (n = 30), CRP levels (n =25) and gut bacterial analysis (n = 18). Five participants were excluded from the CRP and gut bacterial results as they did not meet the required CRP criteria at baseline. An additional seven participants had their stool samples randomly selected for metagenomic analysis; consequently, they were excluded from the gut bacterial analysis performed at baseline. Therefore, the baseline CRP analysis included 25 participants, while the baseline gut bacterial analysis included 18 participants. Throughout the intervention, two participants, one from each group, chose to exit the study. Furthermore, two participants were omitted from the CRP results due to their CRP levels being above 10 mg/L, which could have affected the overall CRP findings. After the intervention, weight status measurements were performed for 28 participants, and CRP levels were assessed for 21 participants. The progression of participants throughout the study is outlined in Figure 7.

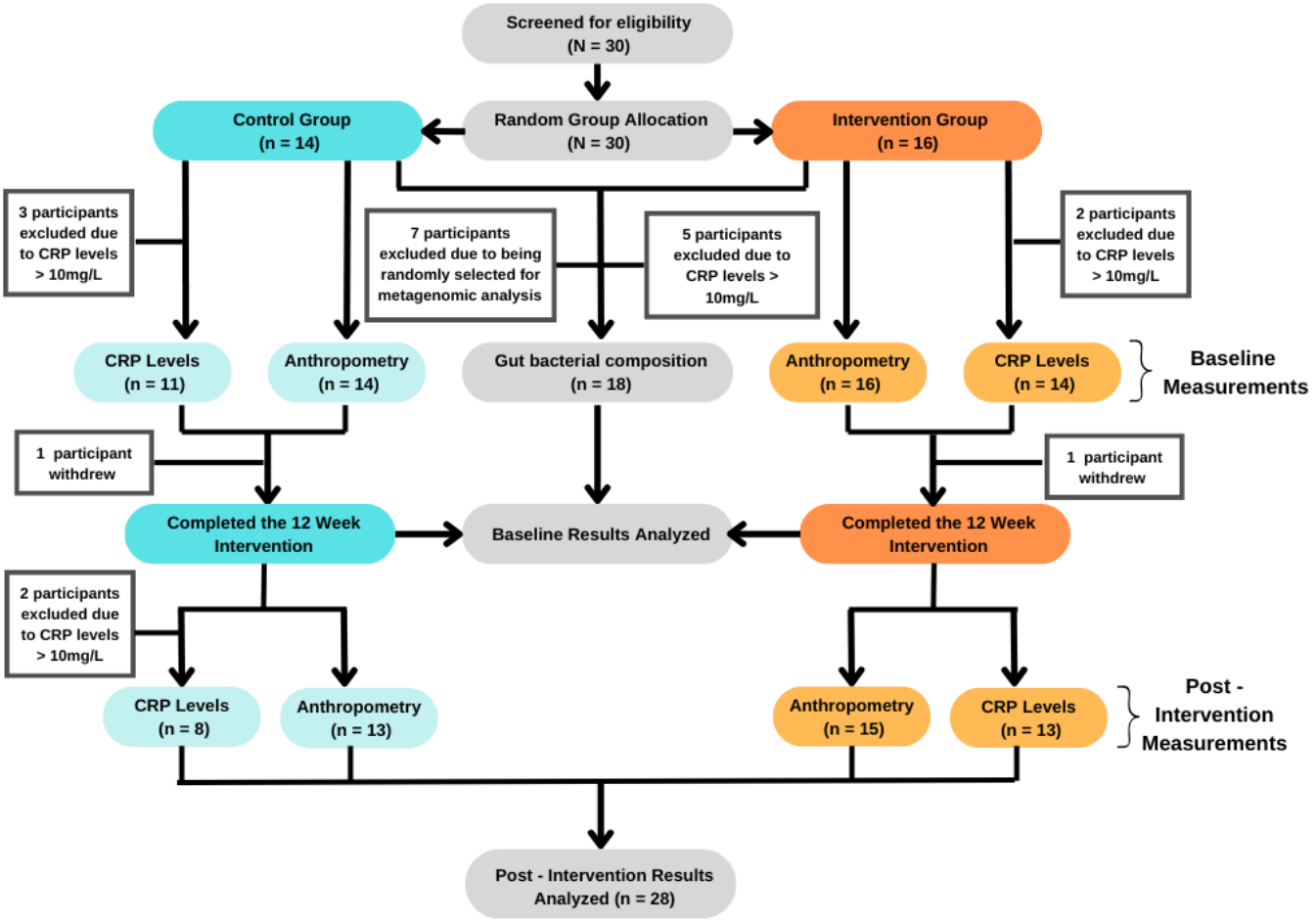

Figure 7: Flow chart illustrating the participant flow through the study, including inclusion/exclusion criteria, randomisation, intervention/control groups, withdrawals and final analysis.

4.1.1 Sociodemographic description of the participants

The study included 25 black African participants (83%) and five white participants (17%). The mean age of the participants was 37.63 (SD = 10.13) years. Two participants were 56 years old. These two participants were not excluded from the study, as it was expected that the one year above the upper limit set for this study would not affect the outcomes. There were no statistically significant differences between the control and intervention groups regarding age (p = 0.2086). Participants resided in various areas, with 63.3% of participants residing in urban areas and 36.6% residing in rural areas.

4.1.2 Participants characteristics and results at baseline

4.1.2.1 Weight Status Characteristics of the Participants

The Table 8 illustrates the baseline weight status characteristics of the total sample, including mean values for weight (kg), height (cm), BMI (kg/m²), WC (cm), HC (cm), WHR, FFM (kg), FM (kg), and FMI (kg/m²). Additionally, Table 8 presents the weight status characteristics of the control group (n = 14) vs the intervention group (n = 16) at baseline. No statistically significant differences ($p > 0.05$) were observed between the two groups at baseline for any of the variables.

Table 8: Weight Status Characteristics of the participants and the Control vs Intervention Groups

Weight Status Characteristics	Total Sample (N = 30)		Control Group (n = 14)		Intervention Group (n = 16)		value
	Mean	SD	Mean	SD	Mean	SD	
Weight (kg)	94.9	14.66	96.28	16.80	93.96	12.42	0.733
Height (cm)	160.36	6.24	159.84	6.41	161.43	6.55	0.674
BMI (kg/m²)	36.98	5.89	37.60	5.61	36.32	6.32	0.680
Waist circumference (cm)	100.6	11.25	103.7	12.03	97.91	10.25	0.273
Hip circumference (cm)	124.42	11.63	126.26	12.27	122.93	10.78	0.537
Waist-Hip Ratio	0.81	0.06	0.82	0.057	0.80	0.07	0.441
Fat-free mass (kg)	49.79	5.26	50.80	6.70	49.72	3.36	0.561

Fat mass (kg)	45.10	10.34	45.48	11.11	44.24	10.22	0.855
FMI (kg/m²)	17.613	4.28	17.77	4.05	17.18	4.78	0.851

**Values are presented as mean ± SD.

*Independent t-test was used to compare the mean values between the two groups.

*p < 0.05 was considered statistically significant.

Abbreviations: BMI= Body Mass Index; FMI = Fat Mass Index

*Healthy ranges BMI: 18 - 24.9 kg/m²; Waist - Hip Ratio: < 0.85; FMI: 5 - 9 kg/m²

The comparison of weight status characteristics between the urban (n = 19) and rural participants (n = 11), including mean values for weight (kg), height (cm), BMI (kg/m²), WC (cm), HC (cm), WHR, FFM (kg), FM (kg), and FMI (kg/m²) was assessed. No statistically significant differences (p > 0.05) were observed between the two groups for any of the variables.

4.1.2.2 CRP levels of the Participants

At baseline, the participants had a mean CRP level of 4.18 mg/L (SD = 2.19). Table 10 shows CRP levels for both groups at baseline. There was no statistically significant difference (p = 0.097) in CRP levels between the control group (n = 11) and the intervention group (n = 14) at baseline.

Table 9 : CRP Levels (mg/L) of the Control vs Intervention Groups

Parameters	**Control Group (n = 11)**		**Intervention Group (n = 14)**		**P-value**
	Mean	SD	Mean	SD	
CRP (mg/L)	3.50	4.36	3.94	2.20	0.097

*Values are presented as mean ± SD.

*Independent t-test was used to compare the mean CRP levels between the two groups.

*p < 0.05 was considered statistically significant.

Abbreviations: CRP = C-reactive Proteins

*Normal CRP levels: 3 - 10 mg/L

The mean CRP level for the urban (n = 14) and rural (n = 11) participants was 4.43 mg/L (SD = 2.3) and 3.8 mg/L (SD = 2.07), respectively. The analysis found no statistically significant difference in CRP levels between the urban and rural participants (p = 0.483).

4.1.2.3 The Gut microbiome profile of the Participants

4.1.2.3.1 Gut bacterial counts at the phylum level

The examination of gut microbiota was performed on 18 distinct phyla. Table 10 displays the mean, standard deviation and mean percentage of the total gut bacterial counts (CFU/g) for the 18 identified phyla, highlighting the most abundant phyla: Bacillota (13857cfu/g; 46.19%), Bacteroidota (7186cfu/g; 23.95%), Firmicutes (3515 cfu/g; 11.72%), Bacteroidetes (2158cfu/g; 7.19%), Pseudomonadota (1678 cfu/g; 5.59%), and Actinomycetota (624cfu/g; 2.08%). The following are taxonomically synonymous but were reported separately: Bacillota and Firmicutes, Bacteroidota and Bacteroidetes, Pseudomonadota and Proteobacteria, and Actinomycetota and Actinobacteria. All other phyla had gut bacterial counts of less than 1.22%. The mean F/B ratio was 2.28 (SD = 1.88) and was calculated using Firmicutes and Bacteroidetes.

Table 10: Bacterial Counts (CFU/g) at Phylum level, expressed in mean, standard deviation and percentage in descending order

Phlyum	Bacterial Counts (CFU/g)		
	Mean	SD	Mean Percentage
Bacillota	13857	2001	46.19%
Bacteroidota	7186	1977	23.95%
Firmicutes	3515	1179	11.72%
Bacteroidetes	2158	1305	7.19%
Pseudomonadota	1678	1998	5.59%
Actinomycetota	624	448	2.08%
Verrucomicrobiota	367	1138	1.22%
Euryarchaeota	247	416	0.82%
Candidatus Melainabacteria	91.4	173	0.30%
Thermodesulfobacteriota	71.6	104	0.24%
Lentisphaerota	65.1	120	0.22%
Mycoplasmatota	50.4	83	0.17%
Proteobacteria	45	68	0.15%
Eukaryota	22.9	70.7	0.08%
Actinobacteria	8.11	10.4	0.03%
Cyanobacteriota	7.83	33.2	0.03%
Verrucomicrobia	4.56	16	0.02%
Elusimicrobiota	1.39	5.89	0.00%

*Values are presented as mean ± SD.

4.1.2.3.3 Gut Bacterial counts at the genus level

The analysis of gut microbiota was performed on 238 different genera. However, since this study's main focus is on genera that have known associations with obesity and inflammation, the in-depth analysis was limited to seven primary genera: *Faecalibacterium* (10.27%), *Bacteroides* (5.23%), *Eubacterium* (2.92%), *Bifidobacterium* (1.71%), *Clostridium* (1.65%), *Akkermansia* (1.09%), and *Lactobacillus* (0.4%).

Table 11: Gut Bacterial Counts (cfu/g) for Seven Key Genera at Baseline

Genus	Bacterial Count (CFU/g)		
	Mean	SD	Mean Percentage
Faecalibacterium	2881	1586	10.27 %
Bacteroides	1809	1904	5.23%
Eubacterium	752	303	2.92%
Bifidobacterium	377	414	1.71%
Clostridium	482	648	1.65%
Akkermansia	351	1141	1.09%
Lactobacillus	117	162	0.4%

Group comparisons were performed to compare gut bacterial counts between urban (n = 9) and rural (n = 9) participants. The analysis was performed on all 18 phyla, the F/B ratio and the seven genera. The Welch Two-Sample t-test revealed a statistically significant difference for Proteobacteria (p = 0.013). The mean gut bacterial counts for Proteobacteria for the urban and rural participants were 3.89 (cfu/g) and 86.11 (cfu/g), respectively. We found no statistically significant differences for any other phyla and the F/B ratio (p > 0.05). The Welch Two-Sample t-test revealed a statistically significant difference for Bacteroides (p = 0.043). The mean gut bacterial counts for *Bacteroides* for the urban and rural participants were 2709.11 (cfu/g) and 909.11 (cfu/g), respectively.

4.2 DIETARY INTAKE OF THE PARTICIPANTS

The dietary assessments were collected at weeks 1, 6, and 12. While dietary data were collected at week 1, insufficient information was provided regarding the food consumed, which prevented an accurate macronutrient analysis. Therefore, nutritional data from week 1 were excluded from further analysis. Only one participant failed to submit their dietary intake assessments, and hence, the dietary intake analysis was performed on 29 participants. Furthermore, dietary intake analysis assessed macronutrients including energy (kj), total protein (g), total lipids (g), total carbohydrates (g) and total dietary fibre

(g). Table 12 presents the mean and standard deviation of macronutrient intake at weeks 6 and 12. Paired t-tests revealed no statistically significant differences in any macronutrients between week 6 and week 12 (all p values > 0.05). The average intake of the sample for each dietary variable was assessed by averaging the results from week 6 and week 12. Table 12 displays the means and standard deviations of the average intake of the sample for energy (kj/day), protein (g/day), lipids (g/day), carbohydrates (g/day), and dietary intake (g/day). Table 12 also displays the average macronutrient distribution for total protein (g/day), lipids (g/day), and carbohydrates (g/day).

Table 12 Comparison of dietary intake at week 6 and week 12 and the average intake and macronutrient distribution of the participants

Nutrient	Week 6		Week 12		Average Intake		Macro-nutrient Distribut-ion (%)	P - value
	Mean	SD	Mean	SD	Mean	SD		
Energy (kj)	8895.11	4896.34	8480.70	4844.18	8687.91	4366.02	-	0.622
Total Protein (g)	73.44	52.44	67.34	34.63	70.39	33.78	13.77%	0.588
Total Lipids (g)	95.17	72.72	78.95	47.58	87.06	52.48	37.08%	0.199
Total Carbo-hydrates (g)	242.22	135.89	256.49	174.76	249.36	146.28	48.79%	0.512
Total Dietary Fibre (g)	17.31	11.87	16.79	9.14	17.09	8.28	-	0.840

*Values are presented as mean ± SD.

*Paired t-test was used to compare the mean values between the two groups.

*p < 0.05 was considered statistically significant

*Average intake refers to the average dietary intake between week 6 and week 12

*The Acceptable Macronutrient Distribution Ranges (AMDRs) for adults are: 10 - 35% from protein; 20 - 35% from lipids; 45 - 65% from carbohydrates.

*Recommended Total Dietary Fibre (g/day) = 25 - 30 g/day

The dietary intake was compared between urban (n = 18) and rural participants (n = 11). The dietary variables assessed include energy (kj/day), protein (g/day), lipids (g/day), carbohydrates (g/day), and dietary fibre (g/day). The analysis (n = 29) found statistically significant differences in energy (p = 0.006), lipids (p = 0.027), carbohydrates (p = 0.012), and dietary fibre (p = 0.034) between the urban and rural participants. Although not initially planned, we noticed some interesting differences when we looked at the baseline data based on where participants lived. We found that rural participants had higher energy and carbohydrate intake compared to urban participants. We also saw variations in gut bacteria, specifically with more Proteobacteria in rural areas. These unexpected findings give us useful insights into how where people live may affect their diets and gut bacteria in this South African population. This calls for more research to explore these differences further.

4.3 CORRELATION ANALYSIS

4.3.1 The Correlations Between Gut Bacterial Counts and Weight Status

Table 13 shows the relationship between gut bacterial counts (cfu/g) at the phylum level and weight status characteristics, including BMI (kg/m²), WC (cm), WHR, FMI (kg/m²), and FM (kg), respectively. The analysis found a statistically significant positive correlation between WHR and Eukaryota (r = 0.556, p = 0.017) and a negative correlation between WHR and Verrucomicrobiota (r = -0.474, p = 0.047). The analysis found no other correlations between any weight status characteristics and any bacterial phyla, including the F/B ratio.

Table 13 : Relationship between gut bacterial counts (cfu/g) at the phylum level and weight status characteristics, respectively

Phylum	BMI (kg/m²)		Waist Circumference (cm)		Waist-Hip Ratio		Fat Mass (kg)		FMI (kg/m²)	
	r	p-value	**r**	p-value	**r**	p-value	**r**	p-value	**r**	p-value
Bacillota	0.267	0.283	0.344	0.163	0.266	0.285	0.198	0.431	0.192	0.444
Bacteroidota	0.14	0.58	0.024	0.925	-0.189	0.453	0.207	0.41	0.156	0.537
Firmicutes	-0.149	0.555	-0.319	0.197	-0.253	0.31	-0.142	0.575	-0.168	0.504
Bacteroidetes	-0.092	0.717	0.009	0.971	-0.056	0.825	0.023	0.928	-0.045	0.858
Pseudomonadota	-0.217	0.388	-0.143	0.57	0.088	0.73	-0.273	0.273	-0.186	0.46
Actinomycetota	-0.322	0.193	-0.252	0.313	-0.078	0.759	-0.253	0.311	-0.296	0.232
Verrucomicrobiota	-0.284	0.254	-0.387	0.113	-0.474	0.047	-0.209	0.405	-0.266	0.286
Euryarchaeota	0.153	0.545	0.17	0.501	0.27	0.278	0.076	0.764	0.193	0.443
Candidatus Melainabacteria	0.191	0.447	0.191	0.448	0.415	0.087	0.086	0.735	0.165	0.513
Thermodesulfo-bacteriota	-0.324	0.19	-0.268	0.283	-0.212	0.399	-0.25	0.316	-0.309	0.212
Lentisphaerota	0.117	0.645	0.105	0.679	0.231	0.355	0.029	0.91	0.128	0.612
Mycoplasmatota	0.206	0.413	0.291	0.241	0.327	0.185	0.153	0.544	0.215	0.391
Proteobacteria	-0.092	0.716	-0.031	0.901	0.211	0.4	-0.136	0.59	-0.06	0.813
Eukaryota	-0.04	0.874	0.061	0.809	0.556	0.017	-0.135	0.592	-0.057	0.821
Actinobacteria	0.182	0.471	0.195	0.439	0.323	0.191	0.034	0.894	0.111	0.662
Cyanobacteriota	-0.284	0.254	-0.299	0.228	-0.246	0.325	-0.317	0.2	-0.268	0.283
Elusimicrobiota	0.062	0.808	0.101	0.69	0.315	0.203	-0.024	0.923	0.048	0.85
Verrucomicrobia	0.268	0.282	0.211	0.401	0.049	0.846	0.237	0.343	0.308	0.213
F/B Ratio	-0.049	0.779	-0.202	0.237	-0.157	0.36	-0.087	0.732	-0.114	0.506

*Correlation coefficients (r) and p-values were calculated using Pearson's correlation.
*$p < 0.05$ was considered statistically significant.
* Abbreviations: BMI = Body Mass index, FMI = Fat Mass Index and Ratio F/B Ratio = Firmicutes/ Bacteroidetes Ratio

Table 14 shows the relationship between gut bacterial counts (cfu/g) at the genus level and weight status characteristics, including BMI (kg/m²), WC (cm), WHR, FMI (kg/m²), and FM (kg), respectively. A statistically significant negative correlation was found between *Bacteroides* and WHR ($r = -0.479$, $p = 0.044$) and between *Akkermansia* and WHR ($r = -0.488$, $p = 0.04$). The analysis found no other statistically significant correlations between any weight status characteristics and any bacterial genera.

Table 14: Relationship between gut bacterial counts (cfu/g) at genus level and weight status characteristics, respectively

Genus	Weight Status Characteristic	Correlation Coefficient (r)	p-value
Faecalibacterium	BMI (kg/m²)	-0.081	0.75
	Waist Circumference (cm)	-0.165	0.513
	Waist-Hip Ratio	-0.286	0.249
	FMI (kg/m²)	-0.084	0.742
	Fat mass (Kg)	**0.005**	0.985
Bacteroides	BMI (kg/m²)	-0.331	0.179
	Waist Circumference (cm)	-0.366	0.136
	Waist-Hip Ratio	-0.479	**0.044**
	FMI (kg/m²)	-0.355	0.149
	Fat mass (Kg)	-0.241	0.336
Eubacterium	BMI (kg/m²)	-0.021	0.936
	Waist Circumference (cm)	0.039	0.879
	Waist-Hip Ratio	0.199	0.428
	FMI (kg/m²)	-0.002	0.995
	Fat mass (Kg)	-0.013	0.960
Bifidobacterium	BMI (kg/m²)	-0.246	0.325
	Waist Circumference (cm)	-0.224	0.371
	Waist-Hip Ratio	-0.069	0.787
	FMI (kg/m²)	-0.241	0.336
	Fat mass (Kg)	-0.198	0.431
Clostridium	BMI (kg/m²)	-0.127	0.615
	Waist Circumference (cm)	-0.089	0.727
	Waist-Hip Ratio	-0.02	0.938
	FMI (kg/m²)	-0.107	0.671
	Fat mass (Kg)	-0.096	0.706
Akkermansia	BMI (kg/m²)	-0.284	0.253
	Waist Circumference (cm)	-0.39	0.11
	Waist-Hip Ratio	-0.488	**0.04**
	FMI (kg/m²)	-0.266	0.286
	Fat mass (Kg)	-0.206	0.413
Lactobacillus	BMI (kg/m²)	0.261	0.295
	Waist Circumference (cm)	0.188	0.454
	Waist-Hip Ratio	0.034	0.895
	FMI (kg/m²)	0.224	0.372
	Fat mass (Kg)	0.267	0.284

*Correlation coefficients (r) and p-values were calculated using Pearson's correlation.
*$p < 0.05$ was considered statistically significant.
* Abbreviations: BMI = Body Mass index, FMI = Fat Mass Index, WC = Waist Circumference, and WHR = Waist-Hip Ratio

4.3.2 The *Correlations Between Gut Bacterial Counts and CRP Levels*

The relationship between gut bacterial counts (cfu/g) at the phylum level and CRP levels among the participants is displayed in Table 15 . The bacterial phyla examined include all 18 identified phyla and the F/B ratio. A statistically significant negative correlation was found between the F/B ratio and CRP levels (r = -0.409, p = 0.013). No statistically significant correlations were found between CRP levels and any other phylum.

Table 15 : Relationship between gut bacterial counts (cfu/g) at phylum level and CRP levels respectively

Phylum	Correlation Coefficient (r)	p-value
Bacillota	0.016	0.951
Bacteroidota	0.063	0.804
Firmicutes	-0.1	0.693
Bacteroidetes	0.288	0.246
Pseudomonadota	-0.44	0.068
Actinomycetota	0.042	0.869
Verrucomicrobiota	0.314	0.204
Euryarchaeota	-0.042	0.867
Candidatus Melainabacteria	0.394	0.106
Thermodesulfo- bacteriota	0.43	0.075
Lentisphaerota	-0.304	0.22
Mycoplasmatota	0.238	0.342
Proteobacteria	-0.283	0.255
Eukaryota	0.256	0.306
Actinobacteria	0.169	0.503
Cyanobacteriota	-0.364	0.138
Elusimicrobiota	-0.249	0.32
Verrucomicrobia	0.061	0.811
F/B Ratio	-0.409	0.013

*Correlation coefficients (r) and p-values were calculated using Pearson's correlation. *p < 0.05 was considered statistically significant. *Abbreviations: F/B Ratio = Firmicutes/ Bacteroidetes Ratio; CRP = C-Reactive Proteins

The relationship between gut bacterial counts (cfu/g) at the genus level and CRP levels among the participants is displayed in Table 16. The bacterial genera examined include *Faecalibacterium, Bacteroides, Eubacterium, Bifidobacterium, Clostridium, Akkermansia* and *Lactobacillus.* The analysis (n = 18) revealed no statistically significant correlations between CRP levels and any of the bacterial genera.

Table 16 : Relationship between gut bacterial counts (cfu/g) at genus level and CRP levels respectively

Genus	Correlation Coefficient (r)	p-value
Faecalibacterium	-0.265	0.288
Bacteroides	0.023	0.928
Eubacterium	0.157	0.533
Bifidobacterium	-0.064	0.8
Clostridium	0.067	0.792
Akkermansia	0.327	0.186
Lactobacillus	-0.02	0.936

*Correlation coefficients (r) and p-values were calculated using Pearson's correlation.
*$p < 0.05$ was considered statistically significant.

4.3.3 The Correlation Between CRP Levels and Weight Status

Table 17 displays the relationship between CRP levels (mg/L) and various weight status characteristics, including BMI (kg/m²), WC (cm), WHR, FMI (kg/m²), and FM (kg), in the study sample (n = 25), Control Group (n = 11) and the Intervention Group (n = 14). A statistically significant positive correlation was found between the CRP levels (mg/L) and FM (kg) ($r = 0.632$, $p = 0.037$) in the Control Group. The analysis showed no other statistically significant correlations between CRP levels and any of the examined weight status characteristics in the control and intervention groups.

Table 17: Relationships between CRP levels (mg/L) and weight status characteristics in the participants, control group and intervention group

Group	Weight Status Characteristic	Correlation Coefficient (r)	p - value
Study sample (n =25)	BMI (kg/m²)	0.311	0.131
	Waist Circumference (cm)	0.292	0.157
	Waist-Hip Ratio	0.066	0.753
	FMI (kg/m²)	0.397	0.049
	Fat mass (Kg)	0.315	0.125
Control (n = 11)	BMI (kg/m²)	0.496	0.120
	Waist Circumference (cm)	0.354	0.285
	Waist-Hip Ratio	-0.189	0.579
	FMI (kg/m²)	0.568	0.068
	Fat mass (Kg)	0.632	0.037
Intervention (n = 14)	BMI (kg/m²)	0.226	0.436
	Waist Circumference (cm)	0.329	0.251
	Waist-Hip Ratio	0.336	0.240
	FMI (kg/m²)	0.175	0.550
	Fat mass (Kg)	0.181	0.535

*Correlation coefficients (r) and p-values were calculated using Pearson's correlation.
*p < 0.05 was considered statistically significant.
* Abbreviations: BMI = Body Mass index, FMI = Fat Mass Index.

4.3.4 The Correlation Between Dietary Intake and Gut Bacterial Counts

The relationship between mean daily dietary intake and gut bacterial counts at the phylum level, including F/B Ratio, of the participants is summarised in Table 21. The analysis revealed statistically significant positive correlation between Mycoplasmatota and energy (r = 0.484, p = 0.042) and carbohydrates (r = 0.592, p = 0.01), and dietary fibre (r = 0.52, p = 0.027); Proteobacteria and energy (r = 0.687, p = 0.002), lipids (r = 0.515, p = 0.029) carbohydrates (r = 0.701, p = 0.001) and dietary fibre (r = 0.58, p = 0.012). No statistically significant correlations were found between any of the dietary variables and the F/B Ratio and any other phylum.

Table 18 : The Relationship between Average Daily Dietary Intake and Gut Bacterial Counts at the phylum level

Phylum	Energy (kJ/day)		Protein (g/day)		Lipids (g/day)		Carbohydrates (g/day)		Dietary Fibre (g/day)	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Bacillota	0.157	0.534	-0.003	0.991	0.012	0.964	-0.267	0.285	0.104	0.682
Bacteroidota	0.272	0.274	-0.238	0.342	0.231	0.357	-0.241	0.336	0.447	0.063
Firmicutes	0.029	0.908	0.164	0.515	0.147	0.56	-0.195	0.438	0.226	0.367
Bacteroidetes	0.084	0.74	0.321	0.193	0.261	0.296	-0.118	0.641	0.231	0.356
Pseudomonadota	0.297	0.231	-0.069	0.787	0.088	0.727	0.457	0.056	0.337	0.171
Actinomycetota	0.333	0.178	0.42	0.083	0.195	0.439	0.336	0.173	0.422	0.081
Verrucomicrobiota	0.191	0.447	-0.176	0.485	0.285	0.252	-0.071	0.779	0.018	0.944
Euryarchaeota	0.321	0.194	0.119	0.638	0.116	0.647	0.439	0.068	0.271	0.277
Candidatus Melainabacteria	0.14	0.578	-0.008	0.976	0.228	0.364	0.071	0.78	-0.16	0.526
Thermodesulfo-bacteriota	0.224	0.371	-0.197	0.433	0.338	0.17	-0.081	0.749	0.087	0.732
Lentisphaerota	0.183	0.466	-0.073	0.774	0.012	0.963	0.323	0.192	0.34	0.168
Mycoplasmatota	0.484	0.042	0.246	0.325	0.246	0.326	0.592	0.01	0.52	0.027
Proteobacteria	0.687	0.002	0.398	0.102	0.515	0.029	0.701	0.001	0.58	0.012
Eukaryota	0.043	0.864	-0.118	0.642	0.052	0.838	-0.008	0.974	0.062	0.806
Actinobacteria	0.305	0.218	-0.292	0.239	0.321	0.194	-0.226	0.368	0.125	0.62
Cyanobacteriota	0.054	0.832	-0.191	0.449	0.109	0.668	0.032	0.899	0.1	0.692
Elusimicrobiota	0.215	0.391	0.051	0.842	0.108	0.671	0.277	0.267	0.287	0.248
Verrucomicrobia	-0.05	0.843	-0.017	0.947	0.113	0.656	0.005	0.986	-0.11	0.663
F/B Ratio	0.119	0.639	-0.177	0.482	0.088	0.728	-0.103	0.685	0.342	0.165

*Correlation coefficients (r) and p-values were calculated using Pearson's correlation.
*$p < 0.05$ was considered statistically significant.
*F/B Ratio = Firmicutes/Bacteroidetes Ratio

The relationship between mean daily dietary intake and gut bacterial counts at the genus level of the participants is summarised in Table 19 . The analysis revealed a statistically significant positive correlation between Lactobacillus and dietary fibre ($r = -0.49$, $p = 0.039$), Clostridium and energy ($r = 0.52$, $p = 0.027$), protein ($r = 0.827$, $p < 0.001$) and lipids ($r = 0.778$, $p < 0.001$). No statistically significant correlations were found between any of the dietary variables and any other genus.

Table 19: The Relationship between average daily dietary intake and gut bacterial counts at the genus level

Genus	Energy (kJ/day)		Protein (g/day)		Lipids (g/day)		Carbohydrates (g/day)		Dietary Fibre (g/day)	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Faecalibacterium	-0.26	0.297	-0.061	0.808	-0.15	0.552	-0.319	0.197	0.201	0.424
Bacteroides	0.451	0.06	-0.307	0.215	0.448	0.062	-0.368	0.134	0.355	0.148
Eubacterium	0.045	0.861	0.062	0.808	0.015	0.954	0.056	0.826	0.206	0.412
Bifidobacterium	0.296	0.232	0.424	0.079	0.218	0.384	0.253	0.31	0.333	0.176
Clostridium	0.52	0.027	0.827	0.0001	0.778	0.0001	0.125	0.62	0.312	0.208
Akkermansia	0.202	0.421	-0.178	0.48	-0.29	0.243	-0.086	0.734	0.035	0.89
Lactobacillus	0.222	0.376	-0.075	0.767	0.063	0.804	-0.321	0.194	-0.49	0.039

*Correlation coefficients (r) and p-values were calculated using Pearson's correlation.
*$p < 0.05$ was considered statistically significant.

4.4 THE EFFECT OF A INTERVENTION ON CHANGES IN WEIGHT STATUS AND CRP LEVELS

4.4.1 Post Intervention Weight Status

An analysis of weight status characteristics of both groups after the intervention showed no statistically significant differences between the Control Group (n = 13) and the Intervention Group (n = 15) for any of the measured variables, however, all meaurements for intervention groups was lower than the control. Table 20 presents the weight status characteristics of both groups at baseline and after the intervention, along with a comparison of changes within each group. The only statistically significant change observed was in the WHR for the control group (p = 0.009). However, the change is not clinically significant. No other significant changes in weight status measurements were found in either the control group (n = 13) or the intervention group (n = 15) following the 12-week intervention.

Table 20 : The Within-Group Differences Between Baseline and Post-Intervention Anthropometric and Body Composition Characteristics in the Control and Intervention

Parameters	Groups	Baseline		Post - Intervention		p-value (Within-Group)
		Mean	SD	Mean	SD	
Weight (kg)	Control	96.92	16.75	96.86	16.72	0.498
	Intervention	94.83	13.34	94.69	13.94	0.769
BMI (kg/m²)	Control	37.99	5.38	37.96	5.32	0.505
	Intervention	37.02	6.28	37.30	6.42	0.881
Waist circumference (cm)	Control	103.31	12.50	104.08	11.37	0.206
	Intervention	99.33	10.12	98.32	9.98	0.098
Hip circumference (cm)	Control	126.48	12.78	124.37	12.81	0.291
	Intervention	124.06	10.81	123.78	12.46	0.596
Waist/Hip ratio	Control	0.82	0.06	0.84	0.07	0.009
	Intervention	0.80	0.07	0.80	0.08	0.287
Fat-free mass (kg)	Control	50.70	6.97	50.91	7.07	0.108
	Intervention	49.32	3.73	49.46	4.07	0.057
Fat mass (kg)	Control	46.22	10.52	45.96	10.36	0.636
	Intervention	45.51	10.41	45.2	10.52	0.202
FMI (kg/m²)	Control	18.12	3.78	18.02	3.72	0.612
	Intervention	17.82	5.32	17.87	4.68	0.257

*Values are presented as mean ± SD.

*Paired t-test was used to compare the means at baseline and post-intervention.

*$p < 0.05$ was considered statistically significant.

*Abbreviations: BMI = Body Mass Index; FMI = Fat Mass Index

4.4.2) Post Intervention CRP Levels

An analysis of these CRP levels (mg/L) showed statistically significant differences ($p = 0.004$) between the Control Group ($n = 8$; mean =1.52 mg/L; SD = 1.18 mg/L) and the Intervention Group ($n = 13$; mean = 4.5 mg/L; SD = 2.92mg/L). Table 21 shows the CRP levels (mg/L) for both groups at baseline and after the intervention, along with a comparison of the changes within each group. No significant changes were observed in the intervention group ($n = 13$) following the 12-week intervention. However, a significant change ($p = 0.033$) was observed in the control group ($n = 8$).

Table 21 : The Difference Between Baseline and Post-Intervention CRP (mg/L) Levels Between Control and Intervention Groups.

Parameter	Groups	Baseline		Post - Intervention		P-value
		Mean	SD	Mean	SD	
CRP (mg/L)	Control	2.27	1.21	1.52	1.18	0.033
	Intervention	4.99	1.71	4.50	2.91	0.429

*Values are presented as mean ± SD.

*Paired t-test was used to compare the mean CRP levels between the two groups.

*$p < 0.05$ was considered statistically significant.

5 DISCUSSION AND CONCLUSION

Our pilot study employed a two-phase study design, consisting of an initial observational descriptive phase followed by a randomised controlled trial. The research investigated the connections among the gut microbiome, CRP levels, and weight status in the context of South Africa to better understand the obesity epidemic that disproportionately impacts women in South Africa. Our study aimed to describe baseline characteristics and explore the relationships between gut microbiota composition, weight status, CRP levels, and dietary intake in South African females with obesity (25-55 years old), and to assess the effect of a 12-week probiotic yoghurt intervention on changes in weight status and CRP levels. As far as we know, this is the first study of its kind to be conducted in South Africa and serves as a pilot study to guide future research in the South African context.

Importantly, none of the measured variables, including weight status characteristics and CRP levels, showed statistically significant differences ($p > 0.05$) between the intervention and control groups at baseline, which is a strong indication that the randomisation process was effective in creating two comparable groups. We also found no statistically significant differences in the mean intake of energy, protein, lipids, carbohydrates, or dietary fibre between week six and twelve. This implies that, at least in terms of the macronutrient composition and fibre content, the participants' overall dietary intake was largely consistent during the intervention phase, thereby minimising the effect of dietary changes on CRP and weight status. The following section will delve deeper into the study's key findings.

5.1 BASELINE CHARACTERISTICS OF THE PARTICIPANTS

5.1.1 Sociodemographic Characteristics

The sociodemographic profile of the study participants , comprising of 25 black African participants (83%) and five white participants (17%), accurately depicts the proportion of black African and white women in South Africa with 87% of women self-reported as black African, compared to three percent who self-reported as white in the 2016 SADHS poll (National Department of Health, 2016). It was also found that obesity is more common among black African women (40.9%) than in white women (30.6%). It is important to consider this ethnic makeup when evaluating the generalizability of our study's findings to other ethnic groups in South Africa and around the world, as lifestyle, cultural, and genetic factors may influence the gut microbiota and the prevalence of obesity (Oduaran et al., 2021).

Our participants ranged in age from 25 to 56 years, with an average age of 37.63 years (SD = 10.13), which, based on the findings from the 2016 SADHS poll, indicates the greatest levels of obesity in South Africa (National Department of Health, 2016). The 2016 SADHS indicated that the occurrence of obesity increases with age, reaching its peak in the 45–54 age bracket before decreasing in subsequent older age groups (National Department of Health, 2016). Given the established chronic nature of obesity and the relatively short duration of the intervention, it was reasoned that a one-year deviation was unlikely to significantly impact the primary outcomes related to weight status and CRP levels. Therefore, the study included the two participants who were 56 years old, which was slightly older than our initial upper limit of 55 years. The results did not reveal any clear patterns or outliers associated with these two participants that would suggest their inclusion influenced the overall findings. Our participants' geographic distribution showed that 36.6% of participants lived in rural areas, while the majority (63.3%) lived in urban areas. This urban majority is likely a result of the recruitment strategy used, which mostly focused on populations that were closest to the study setting.

5.1.2 Weight Status Characteristics

The baseline weight status characteristics of the study group (N = 30) indicated a high prevalence of obesity, with a mean BMI of 36.98 kg/m², which is notably above the clinical threshold of ≥30 kg/m² (WHO, 2000). The study participant's mean waist circumference of 100.6 cm suggests that the participants may have WC above the increased risk threshold (> 80cm), which heightens the likelihood of metabolic complications, including type 2 diabetes, hypertension, and CVD (WHO, 2011). Interestingly, the mean WHR of the study participants was 0.81, which falls below the threshold linked to increased health risks associated with excessive abdominal fat. The mean FMI of 17.613 kg/m² falls in the Obese class II range (Kelly et al., 2009) which highlights the high levels of fat within this group, aligning with the widespread issue of obesity in South African women (National Department of Health, 2016) and highlighting the importance of researching this population.

5.1.3 CRP Levels

The mean CRP level for our participants (n = 25) was 4.18 mg/L (SD = 2.1), which is within the normal or slightly elevated range (3 - 10 mg/L) (Nehring et al., 2023) and corresponds with the type of inflammation commonly observed in obese adults (Nehring et al., 2023). The mean CRP level in our study was greater than the levels reported in a Turkish study by Uludağ et al. (2023). They observed CRP levels of 1.44 mg/L for obese women (n = 38) and 2.12 mg/L for morbidly obese women (n = 46) (Uludağ et al., 2023). However, in our study, participants were not categorised based on BMI categories, whereas in Uludağ et al. (2023), their participants were divided into obese and morbidly obese groups. Since our study didn't separate the obese participants from the morbidly obese participants, the average CRP levels might be higher as a result. These findings underscore how specific factors such as ethnicity, genetics, and degree of adiposity and BMI, within a population, can influence inflammation levels (Uludağ et al., 2023).

Moreover, the study conducted by Myburgh et al. (2020) offers important insights into CRP levels among the South African population. In their analysis of 1,569 black South Africans

aged over 30, the median CRP level for obese individuals was noted to be 8.24 mg/L, with an interquartile range of [3.74; 15.9]. The distribution of CRP values among a broader cohort of obese South Africans, reaching up to 15.9 mg/L within the interquartile range, suggests a potentially greater inflammatory burden compared to the range found in our study, which excluded participants with baseline CRP levels above 10 mg/L. Their study notes that a CRP level above 10 mg/L usually indicates an acute infection (Myburgh et al., 2020). However, many of their participants had high CRP levels without signs of disease, as their body temperatures were normal (≤ 38.0 °C). Specifically, 23.1% had elevated CRP levels but no evidence of acute infection. The authors caution that excluding people with CRP >10 mg/L could skew the results by omitting certain gene types linked to higher CRP levels in this black South African population, leading to a misrepresentation of the true causes of increased CRP levels in this group (Myburgh et al., 2020). Their evaluation further emphasised the significant impact of central adiposity, as indicated by waist circumference, as a major factor contributing to increased CRP levels, especially in women, reinforcing the recognised connection between abdominal obesity and systemic inflammation (Myburgh et al., 2020).

Additionally, George et al. (2018) conducted research on 194 black African and 153 white South African women, aged 18 to 45 years, who appeared to be healthy. Participants with CRP levels exceeding 10 mg/L were not included in the study. Their findings indicated that black women had higher CRP levels (2.5 mg/L) compared to white women (2 mg/L), even when controlling for age and BMI (George et al., 2018). Their finding may explain why our participant's CRP was higher, as we had predominantly black participants in our study. George et al. (2018) also highlighted that race and ethnic backgrounds influence the link between CRP and metabolic risk factors, which are affected by body fat distribution. For example, they found that black women generally had less visceral adipose tissue (VAT) but greater subcutaneous adipose tissue (SAT) compared to white women. This may partly explain the differences in how inflammation relates to metabolic risk factors, such as lipids. Our average CRP levels reflect low-grade inflammation associated with obesity. Still, they are lower than the median levels identified in the larger black South African participants investigated by Myburgh et al (2023). This discrepancy is likely due to our exclusion of higher CRP readings. Both studies demonstrate that variables such as body fat, education,

ethnicity, and potentially other lifestyle and genetic aspects significantly influence inflammation levels (George et al., 2018; Myburgh et al., 2023).

In the control group, we observed a statistically significant positive correlation between CRP levels (mg/L) and FM (kg), indicating that those with greater body fat generally had higher CRP levels in our study. However, no other statistically significant correlations were observed between CRP levels and any of the examined weight status characteristics in either the control or the intervention group. Our finding differs from BabyChitra et al. (2019) and Uludağ et al. (2023). For obese participants, BabyChitra et al. (2019) observed statistically significant positive relationships between CRP and WHR ($r = 0.368$, $p = 0.019$) and BMI ($r = 0.592$, $p < 0.001$) and Uludağ et al. (2023) discovered that CRP levels increased considerably as BMI increased ($r = 0.480$, $p < 0.001$). While our study only found a significant link with FM in the control group, BabyChitra et al. (2019) discovered stronger correlations between general measures of obesity in obese individuals and systemic inflammation. In our control group, our results identify FM as a significant correlate of CRP, while BabyChitra et al. (2019) and Uludağ et al. (2023) revealed a more widespread association between BMI and CRP across all participants. Both studies emphasise the connection between inflammation and adiposity (BabyBabyChitra et al., 2019; Uludağ et al., 2023).

5.2 AVERAGE DIETARY INTAKE

It is important to note that we did experience challenges when collecting dietary intake data from our participants. We made use of food diaries and 24-hour recalls; however, the reported dietary intakes may not be a true reflection of our participants' diets, as participants might have withheld some information. Nevertheless, this information still provides valuable insights into the dietary patterns of our participants, which can be used in future studies conducted in South Africa.

Based on the Acceptable Macronutrient Distribution Ranges (AMDRS), it is recommended that 45–65% of daily energy/calories come from carbohydrates, 20–35% from fats, and 10–35% from proteins (Institute of Medicine, 2002/2005). Our participants had their

protein intake around the lower end of its recommended range, suggesting a relatively lower consumption compared to other macronutrients, while the participants reported carbohydrate intake was in line with the AMDR's midpoint. However, our participants had a high-fat eating behaviour since its lipid intake exceeded the AMDR upper limit. Furthermore, dietary fibre intake in the participants was lower than the WHO's recommended daily intake of 25–30g (World Health Organization, 2003). A different study conducted in South Africa on middle-aged black women living in Soweto, Johannesburg, found a similar distribution of macronutrients to our participants (Makura-Kankwende et al., 2021). The average macronutrient distribution for the obese participants in their study indicates that around 53.5% of total energy intake came from carbohydrates, 11.6% from protein, and 30.2% from fat (Makura-Kankwende et al., 2021). When compared to recommended levels, these values generally indicate a nutritional profile that is heavy in fats and low in dietary fibre.

South Africa has been experiencing a nutrition transition, which has impacted the dietary intake of the population as more South Africans are consuming a Westernised diet (Makura-Kankwende et al., 2021; Frank et al., 2024). The eating habits observed in our participants, which contain a lot of fats and have a low amount of dietary fibre, indicate a shift towards a typical Western diet. This type of diet is marked by high levels of total fat, saturated fats and sugars, along with a reduced intake of plant-based foods such as whole grains, legumes, and vegetables (Makura-Kankwende et al., 2021; Frank et al., 2024). This shift in dietary patterns is leading to an increase in obesity and related NCDs within the South African population. (Makura-Kankwende et al., 2021; Frank et al., 2024).

Although examining urban versus rural differences was not a primary objective of this study, our baseline data unexpectedly highlighted notable distinctions in dietary intake and some related gut microbiome variables between these two geographical groups. These observed differences, which are consistent with findings from other diverse populations, emphasise the need for more focused research in future studies to fully understand the impact of geographical context on metabolic health and the gut microbiome in South African women with obesity.

A comparison of dietary intake between rural and urban participants showed significant differences, with rural individuals consuming more energy, lipids, carbohydrates, and dietary fibre. This aligns with Mukoma et al. (2023), who noted that rural diets are higher in animal protein, fat, and added sugar, while urban diets feature more plant protein and carbohydrates. Overall, rural areas tend to have diets richer in energy-dense, animal-derived foods.The finding underscores the impact of the nutrition transition on rural low-income individuals, who often rely on ultra-processed foods that are high in energy and fat but lack nutritional value (Frank et al., 2024). This dietary pattern increases their susceptibility to non-communicable diseases (NCDs) linked to high intake of fat, sodium, and sugar (Frank et al., 2024). Thus, the nutrition transition worsens dietary quality and raises the risk of obesity and diet-related NCDs among low-income South Africans.

5.3 GUT MICROBIOME PROFILE

5.3.1 Phylum Level

The assessment of the baseline gut microbial composition revealed that the four most abundant phyla in the study of South African women with obesity were Bacillota (46.19%), Bacteroidota (23.95%), Firmicutes (11.72%), and Bacteroidetes (7.19%), collectively representing approximately 90% of the total bacterial counts. The prevalence of Bacillota, also referred to as Firmicutes, and Bacteroidota, also referred to as Bacteroidetes, in the participants aligns with the conclusions of a systematic review and meta-analysis conducted by Pinart et al. (2022). This review consistently identified Firmicutes and Bacteroidetes as the dominant phyla in the gut microbiota of both groups (Pinart et al., 2022). Notably, some studies mentioned in Pinart et al. (2022) observed greater levels of Firmicutes and reduced levels of Bacteroidetes in obese individuals, similar to our results. However, Pinart et al (2022) noted that their findings were not consistent across all studies, with some studies finding no difference between the gut microbiome of obese and lean individuals.

While a greater F/B ratio has often been linked to obesity due to Firmicutes' perceived metabolic efficiency in energy harvest (Koliada et al., 2021; Turnbaugh et al., 2009), the

relationship appears to be more complex and controversial than initially proposed, varying in populations and methodologies. Our study observed a mean F/B ratio of 2.28 (SD = 1.88), a value within the range reported for overweight and obese individuals in other studies. For instance, Karačić et al. (2024) found a higher median F/B ratio of 3.11 [IQR: 2.35–4.47] in a Croatian overweight adult population. Conversely, Koliada et al. (2021) reported lower median F/B ratios in Ukrainian adults with overweight (1.3 [IQR: 0.7–2.0]) and obesity (1.6 [IQR: 1.1–2.2]). Politi et al. (2023) reported a mean F/B ratio of 0.83 ± 0.36 in an Italian population (BMI ≥ 25 kg/m²). Contrary to the typical association, Politi et al. (2023) also found a significant inverse correlation between the F/B ratio and BMI, with a greater F/B ratio being linked to 76% reduced odds of overweight/obesity per unit increase. Our mean F/B ratio is considerably higher than the mean of 0.83 reported by Politi et al. (2023), further emphasising the complex and sometimes contradictory, and likely non-linear connection between the F/B ratio and weight status across diverse populations.

Furthermore, our analysis found no statistically significant correlation between the F/B ratio and any weight status characteristics, including BMI or WHR. This finding aligns with other studies such as Companys et al. (2021) and Karaci et al (2024). Both studies found no correlation between the F/B ratio and any weight status characteristics, including BMI. These findings add to the growing doubt about the effectiveness of the F/B ratio as a reliable biomarker for obesity. The discrepancies observed among various studies, as suggested by Karačić et al. (2024), can likely be attributed to many confounding factors, such as lifestyle choices (such as dietary habits, levels of physical activity), environmental factors, and significant variability in methodologies concerning sample processing, DNA extraction, sequencing methods, and bioinformatic evaluations. Indeed, meta-analyses indicate that the composition of gut microbiota typically shows stronger clustering based on study methodologies rather than BMI categories, suggesting that methodological variations may overshadow true biological differences (Karačić et al., 2024). Although the suggested mechanism for an elevated F/B ratio in obesity points to more efficient energy extraction (Turnbaugh et al., 2009; Koliada et al., 2017), the lack of a consistent correlation in our study and others, along with the c difficulties in identifying small yet significant differences in this ratio, strongly implies that the F/B ratio by itself may not be a reliable or

universal biomarker for obesity. Instead, a more comprehensive understanding of specific microbial taxa and their functional roles, rather than generalized ratios at the phylum level, is likely to offer more insight in relation to host metabolism and body fat.

We discovered a positive correlation between WHR and the phylum Eukaryota. This suggests a potential link between this bacterial group and body fat distribution, but further research is necessary to completely understand its effects. Our study found no significant correlations between the F/B ratio and weight status, which is consistent with the general knowledge that the link between the F/B ratio and obesity is not consistently observed across all research (Pinart et al., 2022). Variations may be due to differing dietary habits, ethnicity, location, and methodologies.

The analysis of CRP levels and the F/B ratio revealed a negative connection, indicating that a increased F/B ratio is linked to lower CRP levels. This result is in opposition to the broad patterns highlighted in the evaluations by Mazloom et al. (2019) and Telle-Hansen et al. (2018). An elevated F/B ratio has been linked to metabolic dysregulation, including obesity and insulin resistance, according to Telle-Hansen et al. (2018)'s review. However, there is no conclusive proof linking this to CRP levels. For example, a study by Balfego et al. (2016), referenced by Telle-Hansen et al. (2018), revealed that Type II Diabetes (T2D) participants who received a nutritional intervention had a lower F/B ratio without any simultaneous changes in CRP. This suggests that the F/B ratio and inflammation may not be related.

Similarly, Mazloom et al. (2019) report a greater F/B ratio in obese individuals associated with inflammation and metabolic dysfunction, usually linking obesity-related inflammation with an elevated F/B ratio. They do, however, acknowledge that the F/B ratio is controversial and that different studies have produced conflicting results about its relationship to inflammation and obesity. Our findings add to this understanding by showing that, in this specific population, a higher F/B ratio may be linked to an anti-inflammatory profile. This is in contrast to the concept that modifications in the gut microbiota associated with obesity, which are frequently characterised by a higher F/B ratio, promote inflammation.

The results of our study were comparable to another South African cohort that examined the gut microbiome of adults living in both urban and rural regions (Oduaran et al., 2020). Although the proportions varied slightly, their study found that Proteobacteria, Bacteroidetes, and Firmicutes were the major phyla. In contrast to Oduaran et al. (2020), who reported Firmicutes at 43.7%, Bacteroidetes at 40%, and Proteobacteria at 12.5%, our study revealed similar percentages, but with slightly lower quantities of Proteobacteria and Bacteroidetes. Low amounts of Proteobacteria are linked to a stable gut microbiome, which reflects effective host regulation and symbiotic interactions (Shin et al., 2015). This contradicts the notion that dysbiosis, having high quantities of Proteobacteria, is linked to obesity (Shin et al., 2015).

Interestingly, our study found that rural participants had a greater abundance of Proteobacteria compared to urban participants. Higher prevalence of Proteobacteria is often associated with dysbiosis and may be caused by a range of dietary and environmental influences, such as soil and plants (Shin et al., 2015; Cohen et al., 2021). High prevalence of Proteobacteria in the gut is usually linked to diets that are rich in energy, containing a high amount of fat and a low level of dietary fibre (Shin et al., 2015), which is the type of dietary pattern we observed in our sample as previously mentioned. Additionally, rural participants are typically exposed to a greater range of bacteria through soil, plants, and other environmental factors, which may affect their gut microbiota (Shi et al., 2015).. Proteobacteria are known to thrive in a range of environments, including soil and plants, which are more prevalent in rural areas (Shi et al., 2015). These two elements could account for the increased occurrence of Proteobacteria among our rural participants; however, given our limited sample size, these results should be viewed as initial observations.

Moreover, our study investigated the relationships between mean daily dietary intake (energy, protein, lipids, carbohydrates, and dietary fibre) and gut bacterial counts at the phylum level, including the F/B ratio. Our results showed a complicated, sometimes contradicting picture, even while the literature emphasises the important influence of nutrition on the gut microbial diversity (Zsálig et al., 2023; Smidowicz and Regula, 2015). We found positive correlations between Proteobacteria and dietary fibre consumption, energy, lipids, and carbohydrates, as well as between Mycoplasmatota and these same

factors. These findings indicate a more comprehensive association with total energy and macronutrient consumption, whereas Zsálig et al. (2023) associated elevated Proteobacteria abundance with gut dysbiosis and high saturated fat diets. This may suggest that in our participants , a higher consumption of energy and macronutrients, regardless of their specific makeup, may promote the growth of Proteobacteria, a phylum frequently linked to pro-inflammatory conditions (Zsálig et al., 2023). The positive correlation with dietary fibre, which is generally considered beneficial and associated with taxa like Prevotella (Smidowicz & Regula, 2015; Tamburini et al., 2022), warrants further investigation in our specific population. It is possible that the source or type of fibre consumed plays a crucial role, or that other host factors influence this relationship (Telle-Hansen et al., 2018).

Interestingly, we found no correlations between any dietary variables and the F/B ratio, a widely discussed metric in the context of obesity (Koliada et al., 2017). This lack of association aligns with some studies that have reported inconsistent relationships between diet, F/B ratio, and metabolic health (Pinart et al., 2022). The dominant influence of overall dietary patterns (Western vs. non-Western) on phylum dominance, as described by Smidowicz and Regula (2015) and Tamburini et al. (2022), might suggest that examining broader dietary patterns rather than individual macronutrient intake could be more relevant for understanding the F/B ratio in our study.

5.3.2 Genus Level

The relative abundance of the seven analysed genera shows some consistency with other research on gut microbiota in obese and lean adults, although direct comparisons are limited due to variations in reported genera (Companys et al., 2021; Politi et al., 2023). Companys et al. (2021) found *Faecalibacterium* and *Bacteroides* to be the main genera in both weight groups, with comparable levels of Faecalibacterium (~ 12.7%) but a higher *Bacteroides* abundance in lean individuals (17.82%) compared to overweight/obese (14.45%). The relative abundance of both these genera in our study was lower than Companys et al. (2021). *Faecalibacterium* is thought to be preventive against obesity and is typically seen in increased abundance in lean individuals as it produces butyrate, which has anti-inflammatory properties (Companys et al., 2021). On the other hand, *Bacteroides*

is usually found to be more abundant in obese individuals as it produces acetate and propionate, which may be involved in the metabolic consequences associated with obesity (Companys et al., 2021).

Our study also found relatively higher abundances of *Bifidobacterium* (1.71%) and *Akkermansia* (1.09%) compared to the reduced median levels reported in overweight individuals by Politi et al. (2023) (*Bifidobacterium*: 0.21%; *Akkermansia*: 0.03%). Recognised for its properties that reduce inflammation and ability to regulate mucin production, which maintains the integrity of the intestinal barrier, **Akkermansia**, and in particular *Akkermansia muciniphila*, are generally found in lower abundance in obese individuals compared to lean individuals (Companys et al., 2021; Politi et al., 2023). Likewise, Politi et al. (2023) discovered that overweight people had reduced levels of *Bifidobacterium*, which are essential for the synthesis of GLP-2, which maintains gut barrier function. Hence, decreased *Bifidobacterium* levels are connected to compromised gut barrier integrity, elevated LPS leakage, and consequent inflammation, which exacerbates insulin resistance and the metabolic problems linked to obesity. Therefore, the higher abundances of *Bifidobacterium* and *Akkermansia* observed in our participants suggest that our participants has a healthier gut microbiome than what is typically seen in overweight/obese individuals.

We observed a negative connection between *Akkermansia* and WHR and between *Bacteroides* and WHR in our study. Our results suggest a potential relationship between higher abundances of these genera and a lower WHR, which aligns with previous studies that link Bacteroides to dietary patterns high in fibre and lean body composition (Daniali et al., 2020; Karaci et al., 2024). Similarly, *A. muciniphila* is recognised as a beneficial gut bacterium that enhances gut health, controls metabolism, strengthens the gut barrier, and lowers inflammation in addition to preventing obesity (Daniali et al., 2020; Karaci et al., 2024). Nevertheless, the small sample size of our study restricts our ability to generalise these findings to the broader population.

Additionally, a negative correlation was observed between *Lactobacillus* abundance and dietary fibre intake. This finding contrasts with the general understanding that fibre serves as a prebiotic, promoting the production of healthy bacteria like *Lactobacillus* (Zsálig et al., 2023). This correlation suggests a potential interaction between the types of fibre consumed and the *Lactobacillus* species present in our small sample. The positive

correlations between *Clostridium* abundance and energy, protein, and lipid intake are also notable. While Zsálig et al. (2023) report a decrease in *Clostridium leptum* in obese individuals, our analysis of the broader *Clostridium* genus suggests a potential link with higher energy-rich macronutrient consumption. This could reflect the metabolic capabilities of certain *Clostridium* species to utilise these substrates. Nevertheless, given the limited sample size of our research, these results should be viewed as initial.

5.4 IMPACT OF THE YOGHURT INTERVENTION

5.4.1 Weight Status

Our study assessed the effect of a 12-week probiotic yoghurt intervention containing *L. rhamnosus* GG and *B. animalis* subsp. *lactis* BB-12 on weight status and CRP levels in South African females with obesity. Our findings revealed no statistically significant differences in weight status measurements between the intervention and control groups after the 12 weeks, although the intervention group consistently showed lower measurements. These results align with some existing literature but contrast with others, highlighting the complex and often inconsistent outcomes of probiotic interventions in managing obesity-related parameters.

This aligns with Madjd et al. (2016) who found no statistically significant differences in WC, BMI reduction, or weight loss between a group that consumed probiotic yoghurt (*L. acidophilus LA5* and *B. lactis BB12*) and a control group that consumed low-fat yoghurt over 12 weeks. The study involved obese women following an energy-restricted diet. This implies that even in the setting of an energy-restricted diet, the probiotic inclusion in Madjed et al.'s study did not result in greater weight reduction advantages when compared to a control yoghurt. Additionally, similar to our lack of significant weight status changes, Czajeczny et al. (2020) similarly found no notable differences in weight-related measurements after a 6-week supplementation with B. lactis BS01 and L. acidophilus LA02in healthy young females without specific dietary restrictions. This supports the idea that probiotics alone may not be very effective in causing noticeable modifications in weight status measurements in the absence of a focused dietary intervention (Madjd et al., 2016; Czajeczny et al., 2020).

In their 12-week trial, Pedret et al. (2018) found no significant differences in their groups' overall weight status biomarkers. But according to Pedret et al. (2018), visceral fat, WC, WHR, and conicity index all significantly decreased, specifically in women, while diastolic blood pressure and HOMA-IR improved as well. Their findings highlight the potential that various probiotic strains could influence particular metabolic and body fat markers differently. Additionally, a 12-week intervention utilising strains of *Bifidobacterium* and *L. acidophilus* together with a low-calorie, high-protein, high-fibre diet did not produce any significant changes in overall weight status, according to Hassen et al. (2023). Their research did, however, show notable alterations in the gut microbiota composition (increased *Lactobacillus* and *Bifidobacterium*, decreased F/B ratio) and obesity-related biomarkers (Leptin, ALT, AST). This suggests that probiotics, especially when paired with dietary changes, may influence specific markers and gut health without necessarily resulting in significant overall weight loss.

Although the intervention group displayed a trend toward lower measurements, our findings of no significant changes in weight status following a 12-week probiotic yoghurt intervention are in line with other research. This suggests that probiotics by themselves may not be sufficient to produce observable anthropometric changes when managing obesity without a more thorough dietary intervention that involves a significant caloric deficit. The inconsistent results from previous studies, which used different probiotic strains in combination with dietary changes and focused on specific adiposity and metabolic markers, highlight the importance of these factors. This also highlights the importance of considering strain-specificity, intervention design, and outcome measures in comprehending the possible impact of probiotics on managing obesity-related outcomes. In our research, participants adhered to their usual dietary habits, which probably reduced the likelihood of significant changes in anthropometric measurements.

5.4.2 CRP Levels

We observed a statistically significant decrease in CRP levels in the control group consuming conventional yoghurt (from a mean of 2.27 mg/L to 1.52 mg/L, $p = 0.033$). In comparison, the intervention group showed no significant change in CRP levels despite

having a higher baseline mean of 4.99 mg/L (decreasing non-significantly to 4.5 mg/L). These findings present a complex picture when compared to existing literature on probiotic interventions and inflammation.

Numerous studies agree that probiotics have an anti-inflammatory impact on CRP (Rajkumar et al., 2014; Tanaka et al., 2021; Toejing et al., 2021). Over six weeks, Rajkumar et al. (2014) observed that their VSL#3 probiotic intervention significantly decreased CRP levels. While Lactobacillus paracasei HII01 also significantly reduced hsCRP, according to Toejing et al. (2021). Lastly, heat-killed L. plantarum L-137 dramatically decreased CRP, especially in those with higher baseline levels, according to Tanaka et al. (2021). The difference between these findings and our results could be attributed to multiple factors, one of which may include the particular probiotic strains utilized. In contrast to the strains used in our study, the strains in VSL#3 (a multi-strain formulation), *L. paracasei* HII01, and heat-killed *L. plantarum L-1*37 may have distinct immunomodulatory characteristics and modes of action. The specific anti-inflammatory mechanisms that are triggered, or their effectiveness in influencing the overall inflammatory response, may differ greatly among probiotic strains, even within the same genera.

It may also be worthwhile to consider baseline CRP levels. The responsiveness to the probiotic intervention may have been affected by the intervention group's noticeably higher baseline CRP levels. Tanaka et al. (2021) found that probiotics had a more noticeable effect on those with higher baseline CRP, which is different from our findings. Although our intervention group's baseline inflammation was higher, there was no significant reduction. This unexpected finding in our intervention group, despite higher baseline CRP, may be due to several factors including insufficient dose or duration, inter-individual variability, compensatory mechanisms and sample size. The 12-week intervention might not have been long enough, or the strains and dosages used may have been inadequate for individuals with higher inflammation, who may need a stronger or longer intervention for a measurable effect. Moreover, responses to probiotics can vary based on factors such as gut microbiome composition, genetics, and lifestyle. It is possible that the baseline profiles in our group did not support the expected ant-inflammatory benefits of the strains used. Those with higher chronic inflammation mat have complex physiological responses that are resistant to probiotic interventions without broader lifestyle changes. Lastly the limited

sample size reduces statistical power to detect clinically relevant changes in CRP levels, meaning small fluctuations may not be statically significant.

The overall dietary context (participants in our study maintained their regular diets, unlike some studies with specific dietary treatments) and the particular delivery mechanism (yoghurt vs. capsules or sachets) may also have contributed to the inconsistent results. Our findings emphasise the importance of more investigation in finding specific probiotic strains and the most effective intervention strategies for the targeted decrease of inflammatory markers in this population. More thought needs to be given to the decline in CRP in our control group. Possible factors include the natural range of CRP levels, other unmeasured lifestyle changes within this group, or perhaps a slight influence of the conventional yoghurt itself (for example, the broad health benefits of diary products, or slight changes in gut microbiota resulting from daily intake of non-probiotic yoghurt), but this is less frequently documented. It might also represent a regression to the mean phenomenon, especially if the baseline CRP levels in the control group experienced natural variations.

5.5 STRENGTHS AND LIMITATIONS

This pilot study had several strengths. The first strength is that the randomised, placebo-controlled, single-blind design allowed us to attribute the observed effects to the probiotic intervention, thereby reducing bias and enhancing internal validity. Second, the study addressed a major research gap by focusing on a particular population, obese South African women, and provided valuable preliminary data, as well as the feasibility and potential efficacy of probiotic treatments in this high-risk group. Thirdly, the detailed evaluation of anthropometry, body composition, CRP levels, and gut microbiota composition provided a clear depiction of the intervention's effectiveness, and the use of a probiotic yoghurt matrix enhanced participants' adherence. Lastly, the 16S rRNA gene sequencing used allowed for examining the possible associations between variations in the gut microbiota and various outcomes.

However, several restrictions need to be mentioned. Our study's first significant limitation is that the limited sample size (n = 30) might have restricted statistical power, which could make it harder to generalise the findings. Secondly, the use of self-reported dietary intake data from three-day food diaries and 24-hour recalls may have introduced bias due to incomplete reporting and recall errors. Additionally, the current study only investigated CRP levels as a measure of inflammation in the participants. It did not include inflammatory markers associated with obesity, such as TNF-α, IL-6 and IL-12, which limited our understanding of the level of inflammation in our participants. Another limitation is the use gut microbiome analysis methods; compared to shotgun metagenomic sequencing, 16S rRNA gene sequencing yielded less precise information regarding the gut microbiota Additionally, our study only investigated seven key genera, therefore, only reporting on a limited portion of the whole diverse gut microbiome. Future research using metagenomic sequencing may provide a better understanding of the gut microbiota's function. Finally, by only analysing the baseline gut microbiome composition, we were unable to investigate whether the probiotic yoghurt had an influence on the gut microbiome composition after the 12-week intervention period. These strengths and limitations should all be taken into consideration when determining the next steps for future research.

5.6 CONCLUSIONS AND RECOMMENDATIONS

To our knowledge, this study was the first of its kind in a South African population and served as an investigative pilot study to explore the impact of a newly developed probiotic yoghurt compared with conventional yoghurt on CRP levels (mg/l) and anthropometric and body composition outcomes. It also evaluated gut bacterial profiles at the phylum and genus levels. Our findings indicated that CRP levels corresponded with low-grade inflammation and obesity (Nehring et al., 2023), being higher than in a European study (Uludağ et al., 2023) but similar to other South African research, suggesting a potential trend of elevated CRP levels in South Africans (George et al., 2018; Myburgh et al., 2023). Additionally, our analysis showed low protein intake and high fat intake exceeding AMDR limits (Institute of Medicine, 2002/2005), along with dietary fibre intake below the WHO recommendation of 25–30g (WHO, 2003). This underscores the impact of the nutrition transition on food choices and how it contributes to a higher likelihood of developing obesity and NCDS related to diet (Makura-Kankwende et al., 2021; Frank et al., 2024).

We identified the dominant phyla in our participants, aligning with previous obesity studies but showing lower levels of Proteobacteria compared to another South African study (Oduaran et al., 2020). Our study did not reveal a notable connection between the F/B ratio and weight status, which contrasts with other research results. Our mean F/B ratio was at the midpoint compared to other overweight or obese populations (Koliada et al., 2021; Politi et al., 2023; Karačić et al., 2024). Importantly, CRP levels exhibited a significant inverse correlation with the F/B ratio, which challenges earlier research (Telle-Hansen et al., 2018; Mazloom et al., 2019). We did not observe any meaningful relationships between dietary factors and the F/B ratio, but did observe positive correlations between Proteobacteria, Mycoplasmatota, and dietary fibre, energy, lipids, and carbohydrates, which opposes typical associations with dysbiosis and low-fibre diets.

In our study, lower abundances of *Faecalibacterium* and *Bacteroides* were linked to obesity, while higher levels of *Bifidobacterium* and *Akkermansia* indicated a healthier gut microbiome compared to overweight individuals (Politi et al., 2023). We found a notable negative relationship between *the abundance of Akkermansia* and *Bacteroides* and the WHR, indicating that increased levels are linked to improved metabolic health. Interestingly, *Lactobacillus* abundance negatively correlated with dietary fibre intake, which contradicts the idea that fibre boosts beneficial bacteria (Zsálig et al., 2023). Additionally, some *Clostridium* species appeared to thrive on high-energy diets. Following the intervention, there were no notable alterations in body composition or CRP levels, probably due to the limited sample size, which emphasises the necessity for additional research.

Our study suggests practical recommendations based on our findings. The F/B ratios of our participants differed from those of other obese adult populations, emphasising the need to consider factors like ethnicity, race, diet, and lifestyle when assessing the gut microbiome (Oduaran et al., 2020). Most research has focused on European or Asian populations, with limited information on African populations. This underscores the essential need for more research on the gut microbiome in Africa and a consideration of F/B ratio ranges that are specific to different populations. The analysis of gut microbiota within the sample population revealed important findings for public health and future interventions. Notably, The negative correlation between *Akkermansia* and *Bacteroides*, along with the

improved WHR, and the connection between the Eukaryota phylum and the WHR, underscore the potential for microbiota-targeted interventions to reduce abdominal fat. Future research should investigate how these microbial groups affect body fat distribution to inform intervention development.

Future research should focus on recruiting larger sample sizes to enhance statistical power and generalizability. To mitigate bias from self-reported dietary data, more objective methods, such as food frequency questionnaires or weighed food records, should be employed. Additionally, future studies should broaden the assessment of inflammation by including markers such as TNF-α, IL-6, and IL-12. To gain a better insight into the composition and functionality of the gut microbiome, shotgun metagenomic sequencing is preferred over 16S rRNA gene sequencing, concentrating on a broader spectrum of bacterial taxa. Lastly, longitudinal sampling of the gut microbiome during the 12-week probiotic intervention period is suggested to evaluate its impact.

To further examine the potential benefits of probiotics within the South African setting, future research should look into interventions that utilize specific strains, dosages, and duration's that have previously demonstrated effectiveness. For example a multi-strain probiotic blend as employed by Sabico et al (2019), which featured *B. bifidum W23, B. lactis W52, L. acidophilus W37, L. brevis W63, L. casei W56, L.salivarius W24, L. lactis W19*, and *L. lactis W58*, was given at a dosage of 2.5 x 10^9 CFU/g twice each day for 6 months and proved effective in lowering inflammation. In a similar case, for outcomes related to weight status, strains such as L.curvatus HY7601 and L. plantarum KY1032, provided at a dosage of 2.5 × 10^9 CFU of each strain twice daily for 12 weeks, similar to the approach of Jung et al (2015), could be specifically investigated. Future studies in South Africa could use these successful cases to create targeted interventions featuring optimized strain combinations, suitable dosages, and possibly extended duration's to more effectively determine their impact on reducing inflammation and enhancing weight status in obese women.

In conclusion, this research enhances our knowledge of the gut microbiota, weight status, dietary patterns, and inflammation in South African women with obesity, highlighting the need for population-specific considerations and further investigation into the complex interplay between these factors. Future studies, incorporating the recommendations

outlined, will be crucial for developing effective interventions to enhance metabolic health and alleviate the impact of obesity-related issues in this group.

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ANNEXURES

ANNEXURE A
- Declaration regarding plagiarism

DEPARTMENT OF HUMAN NUTRITION

University of Pretoria

Declaration regarding Plagiarism

The Department of Human Nutrition emphasises integrity and ethical behaviour with regard to the preparation of all written assignments.

Although the lecturer/course facilitator will provide you with information regarding reference techniques, as well as ways to avoid plagiarism, you also have a responsibility to fulfil in this regard. Should you at any time feel unsure about the requirements, you must consult the lecturer/course facilitator concerned before submitting an assignment.

You are guilty of plagiarism whenever you extract information from a book, article, web page or any other information source without acknowledging the source and pretend that it is your own work. This does not only apply to cases where you quote verbatim, but also when you present someone else's work in a somewhat amended (paraphrased) format or when you use someone else's arguments or ideas without the necessary acknowledgement. You are also guilty of plagiarism if you copy and paste information directly from an electronic source (eg a web site, e-mail message, electronic journal article, or CD ROM), even if you acknowledge the source.

You are not allowed to submit another student's previous work as your own. You are furthermore not allowed to let anyone copy or use your work with the intention of presenting it as his/her own.

Students who are guilty of plagiarism will forfeit all credits for the work concerned. In addition, the matter will be referred to the Committee for Discipline (Students) for a ruling. Plagiarism is considered a serious violation of the University's regulations and may lead to your suspension from the University. The University's policy regarding plagiarism is available on the Internet at http://upetd.up.ac.za/authors/create/plagiarism/students.htm.

For the period that you are a student at the Division of Human Nutrition, the following declaration must accompany **all** written work that is submitted for evaluation. No written work will be accepted unless the declaration has been completed and is included in the particular assignment.

I (full names and surname):	Gabriela Lourenco
Student number:	17099776
Module code: VDG 890	Research Proposal

Declare the following:

1. I understand what plagiarism entails and am aware of the University's policy in this regard.
2. I declare that this assignment is my own, original work. Where someone else's work was used (whether from a printed source, the Internet or any other source) due acknowledgement was given and reference was made according to departmental requirements.
3. I did not copy and paste any information directly from an electronic source (eg a web page, electronic journal article or CD ROM) into this document.
4. I did not make use of another student's previous work and submitted it as my own.
5. I did not allow and will not allow anyone to copy my work with the intention of presenting it as his/her own work.

Signature

30 April 2025
Date

I, Gabriela Maria Lourenco declare that the thesis/dissertation, which I hereby submit for the degree MSc Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: .....  .....
DATE: .....30 April 2025.....

ANNEXURE B
Participation Poster

CAN A PROBIOTIC YOGHURT IMPROVE WEIGHT STATUS AND RELATED INFLAMMATION?

Would you like to take part in a research study conducted by the Department of Consumer and Food Sciences at the University of Pretoria?

Aim: to determine the effect of a probiotic yoghurt on the gut microbiome and related effects on weight status and immune markers.

We are looking for:

- Females
- Age: 25 - 55 years
- BMI ≥ 30 kg/m²
- Willing to consume a provided yoghurt (100g) everyday for 12 weeks

WHAT DOES THIS STUDY ENTAIL?

- The **daily consumption** of either a probiotic or conventional yoghurt for **12 weeks.**
- **Refraining** from other **probiotic foods** and **beverages** such as kimchi, kombucha, etc.
- Two **stool sample collections.**
- Two **blood sample collections.**

PRACTICALITIES:

- **Date:** August - November 2023
- **Venue**: University of Pretoria, Prinshof campus, Dr Savage RD.
- **Transport:** transport costs will be reimbursed

BENEFITS OF PARTICIPATING IN THIS STUDY:

- You will receive insights into your **health status.**
- You may experience improvements in your **weight status, gut health** and **immunity**.
- You will be helping increase **information** and **knowledge** regarding **female health status** in South Africa.
- You will be the first to try a **newly developed yoghurt product.**
- This is the **first study** of its kind to ever be done in **South Africa**.

Are you eligible for the study?

Scan the QR code to find out!

Screening is based on **weight status** and the absence of **acute inflammation.**

Please note that should you not meet all the criteria that we require, you will not be able to participate in the study.

If you should be interested in participating in this study or would like to learn more about it, please contact the researcher:

- Gabriela Lourenco: 079 973 1274/ gabriela.lourenco6@gmail.com

Faculty of Natural and Agricultural Sciences
Department of Consumer and Food Sciences

ANNEXURE C

- Pre-screening Google Form

Screening Form - Dairy Project

The **purpose** of the following screening form is to identify **eligible participants** to participate the study intervention. The form includes various sections including; personal and contact details; medical history; and Body Mass Index (BMI) measurements.

All personal information is confidential and will not be shared externally. Eligible participants who are invited to participate in the intervention will be contacted by the researcher.

Disclaimer: Those who do not meet the requirements/inclusion criteria will not be invited to participate in the intervention.

* Indicates required question

1. Name *

2. Surname *

3. Age *

4. Contact number *

5. Email address *

6. Physical Address *
(Physical addresses are required to ensure participants live in the set radius (10km) to the study setting)

_____

7. Please check the boxes that apply to you. *

Tick all that apply.

- [ ] Antibiotic treatment
- [ ] Use of laxatives
- [ ] Restricted food intake and/or following a weight reduction diet or program
- [ ] Chronic immunologic disease (lupus, rheumatoid arthritis, Crohn's disease and ulcerative colitis)
- [ ] Thyroid disease
- [ ] Pregnant or plans to become pregnant
- [ ] Undergone gastrointestinal surgery
- [ ] Gastrointestinal diseases (diverticular disease, colon polyps, colon cancer and inflammatory bowel disease)

8. Are you willing to consume a yoghurt (100g) provided by the researcher, everyday for 12 weeks, without the consumption of any other highly fermented foods and beverages such as kombucha, kimchi, etc. *

Mark only one oval.

- ◯ Yes
- ◯ No

9. Body weight (kilograms) *

_____

10. Height (cm) *

_____

Thank you for taking the time to complete the screening form!

ANNEXURE D
Three-Day Food Diary

THREE DAY FOOD DIARY

Please complete the food diary to the best of your ability. The more specific and detailed your entries are, the better it is for the researchers to access and analyse your dietary intake.

Please save the title of your food diary document as such "Code number - Week1".

For example: 1 - Week1

Directions for using the food diary

1. Keep your food diary current. List foods immediately after they are eaten. Please print all entries.
2. Record only one food item per line in this record booklet.
3. Be as specific as possible when describing the food item eaten: the way it was cooked (if it was cooked) and the amount that was eaten.
4. Include brand names whenever possible.
5. Report only the food portion that was actually eaten — for example: T-bone steak, 4-oz broiled. (Do not include the bones.)
6. Record amounts in household measures — for example: ounces, tablespoons, cups, slices or units, as in 1 cup nonfat milk, two slices of wheat toast, or one raw apple.
7. Include methods that were used to prepare food items — for example: fresh, frozen, stewed, fried, baked, canned, broiled, raw, or braised.
8. For canned foods, include the liquid in which it was canned — for example: sliced peaches in heavy syrup, fruit cocktail in light syrup, or tuna in water.
9. Food items listed without specific amounts eaten will be analysed using portion sizes.
10. Do not alter your normal diet during the period you keep this diary.
11. Remember to record the amounts of visible fats (oils, butter, salad dressings, margarine, and so on) you eat or use in cooking.

EXAMPLE :

Time	Food Item and Method of preparation	Amount eaten
7am	Apple, raw, fresh	1 medium
12 am	Beef stew	10 oz portion
12 pm	Bread, wholewheat, fresh	2 slices
3pm	Cereal, corn flakes	2 cups
3pm	With sugar	2 Tbsp
3pm	With milk, non-fat	½ cup

Page 1

DAY 1 (Weekday)

DATE:_____

Time	Food Item and Method of preparation	Amount eaten

DAY 2 (Weekday)

DATE:_____

Time	Food Item and Method of preparation	Amount eaten

Page 2

DAY 3 (Weekend day)

DATE:_____

Time	Food Item and Method of preparation	Amount eaten

Page 3

ANNEXURE E
24-Hour Recall

24-Hour Recall

Interviewer Instructions:

1. Ask the respondent to recall **all the food and beverages** (meals and snacks) consumed yesterday, from morning to night. Ask them to provide as **much detail as possible**, including **portion sizes, preparation methods** (boiled, steamed, pan-fried, deep-fried, baked), and **brand names.**
2. Start with the first thing they consume in the morning.
3. List all items and their estimated quantities in the dietary assessment table.
4. If composite dishes (stews, salads) are mentioned, list all the ingredients.
5. Ask if any snacks were eaten between meals.
6. Ask about any added foods like sugar in tea, oil in mixed dishes, or fried foods.
7. Once they are done with the 24-hour recall, indicate which target foods they consumed by ticking the food item off. Identify which target foods were not consumed and ask if they consumed them.

Name/Code:_____
Date:_____

Breakfast	Snack	Lunch	Snack	Dinner	Snack
-Soft Pap 1 fist size Cooked with just water **-2 pieces of wors** Cooked with brown soup and water **-1cup of tea** black tea 2 teaspoons of sugar	**None**	**-Soft Pap** 1 fist size Cooked with just water **-2 pieces of wors** Cooked with brown soup and water **1 litre of tap water**	**None**	**-Soft Pap** 1 fist size Cooked with just water **-1 chicken piece** Breast chicken Cooked with water, added onion, beef stock, chill beef spice **1 cup of tap water**	**None**

ANNEXURE F
Food Portion Size Guide

ANNEXURE G
- Stool Sample Collection Instructions-

STOOL SAMPLE COLLECTION INSTRUCTIONS

Stool sample collection supplies:

- DNA/RNA Shield Fecal Collection Tube
- Faeces catcher
- Self-adhesive stickers (for labeling your stool sample tube)

Stool sample collection instructions:

Please read the following step-by-step instructions (with pictures) to successfully collect a stool sample. If you wish to watch the step-by-step demonstration of the stool collection process, click here.This link takes you to the Zymo Research website which will provide a product video for the DNA/RNA Shield Fecal Collection Tube.

Step-By-Step Instructions:

Step 1: Remove faeces catcher from packaging and peel adhesive from both sides.

Step 2: Place faeces catcher on the toilet seat. Firmly press adhesive to secure.

Step 3: Deposit sample onto faeces catcher.

Page 2

Step 4: Unscrew collection tube cap and use scoop to collect one spoonful of sample.
CAUTION: DO NOT INGEST LIQUID STABILISER.

Page 3

Step 5: Screw cap tightly. Shake vigorously to ensure complete mixing

Step 6: Labelling of the stool sample tube

Write your name and surname as well as the date on the tube

Example:
Jane Doe 16/05/2024

.

Step 7: Store stool sample tube in the fridge.

Step 8: Bring the stool sample tube with you to the next study visit.

ANNEXURE H
Data Collection Forms

Pre-Assessment Visit
Screening and data collection form

Name					
Surname					
Date of birth (dd/mm/yyyy)					
Age (years)					
Contact number					
Email address					
Race	**White**	**Black**	**Indian**	**Coloured**	**Other**

Medical History

1) Non-communicable diseases

Please check all the boxes that apply to you.

- Diabetes Mellitus (Type II)
- Cancer
- Cardiovascular Diseases (stroke, heart attack)
- Chronic Respiratory Diseases (chronic obstructed pulmonary disease and asthma)

2) Please list all medication you are currently administering

_____
_____
_____
_____
_____
_____
_____
_____

Dietary Assessment

How would you like to complete your dietary assessment?

I will complete my food diary via Word document	
I will send my food diary via WhatsApp message	
I would like the researcher to call me and I will give a 24-hour recall	

Anthropometric Measurements

Weight (kg)	1	2	3	Average
Height (cm)	1	2	3	Average
BMI (kg/m2)				
Waist circumference (cm)	1	2	3	Average
Hip circumference (cm)	1	2	3	Average
BIA measurements	Fat mass	Fat-free mass	Resistance	Reactance

Blood Sample

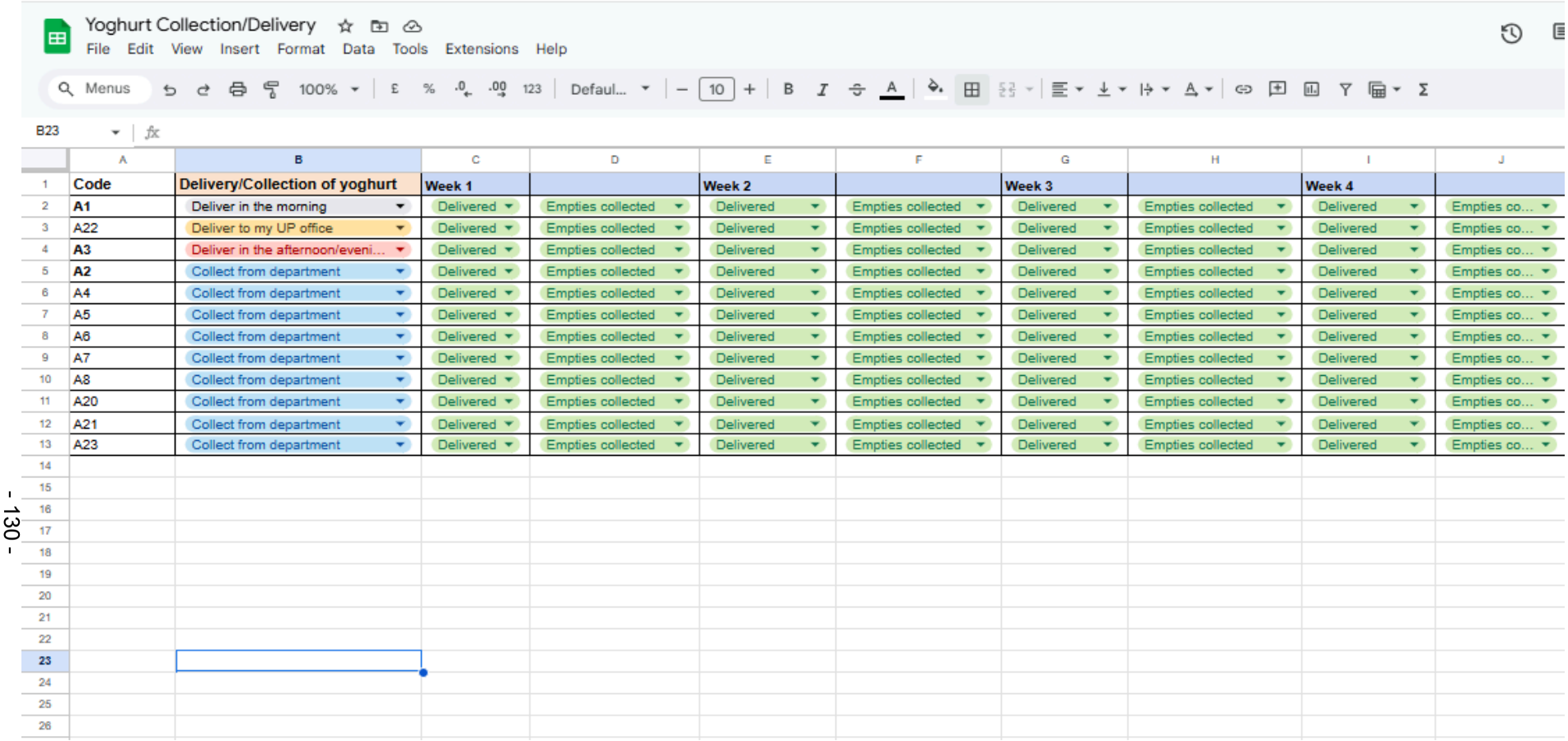
Blood sample completed	Yes	No

Stool Sample

A stool sample kit has been given to the participant	Yes	No
Stool samples have been collected from participant	Yes	No

ANNEXURE I

- Yoghurt Consumption Monitoring Spreadsheet -



	A	B	C	D	E	F	G	H	I	J
1	**Code**	**Delivery/Collection of yoghurt**	**Week 1**		**Week 2**		**Week 3**		**Week 4**	
2	**A1**	Deliver in the morning	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
3	A22	Deliver to my UP office	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
4	**A3**	Deliver in the afternoon/eveni...	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
5	**A2**	Collect from department	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
6	A4	Collect from department	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
7	A5	Collect from department	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
8	A6	Collect from department	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
9	A7	Collect from department	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
10	A8	Collect from department	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
11	A20	Collect from department	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
12	A21	Collect from department	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
13	A23	Collect from department	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties collected	Delivered	Empties co...
14										
15										
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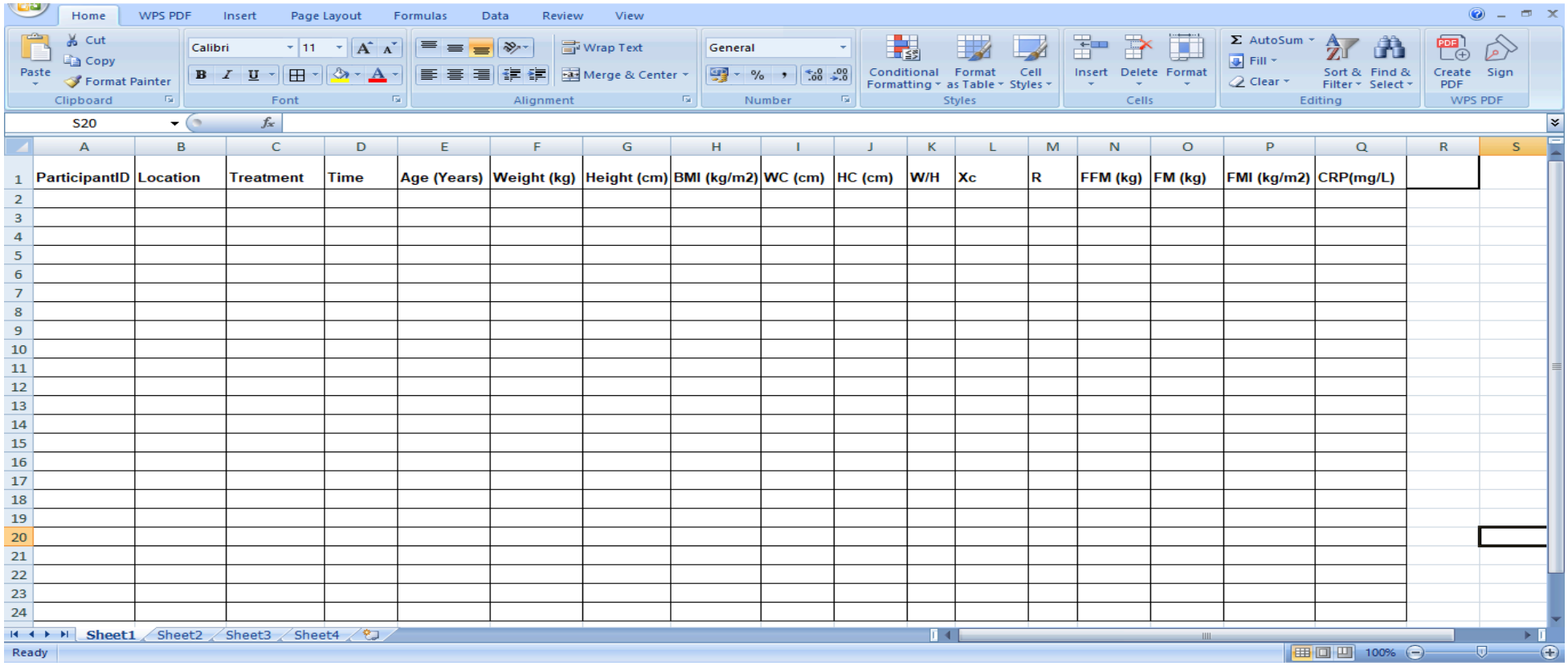
ANNEXURE J

- Data Collection Excel Spreadsheet -



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	ParticipantID	Location	Treatment	Time	Age (Years)	Weight (kg)	Height (cm)	BMI (kg/m2)	WC (cm)	HC (cm)	W/H	Xc	R	FFM (kg)	FM (kg)	FMI (kg/m2)	CRP(mg/L)		
2																			
3																			
4																			
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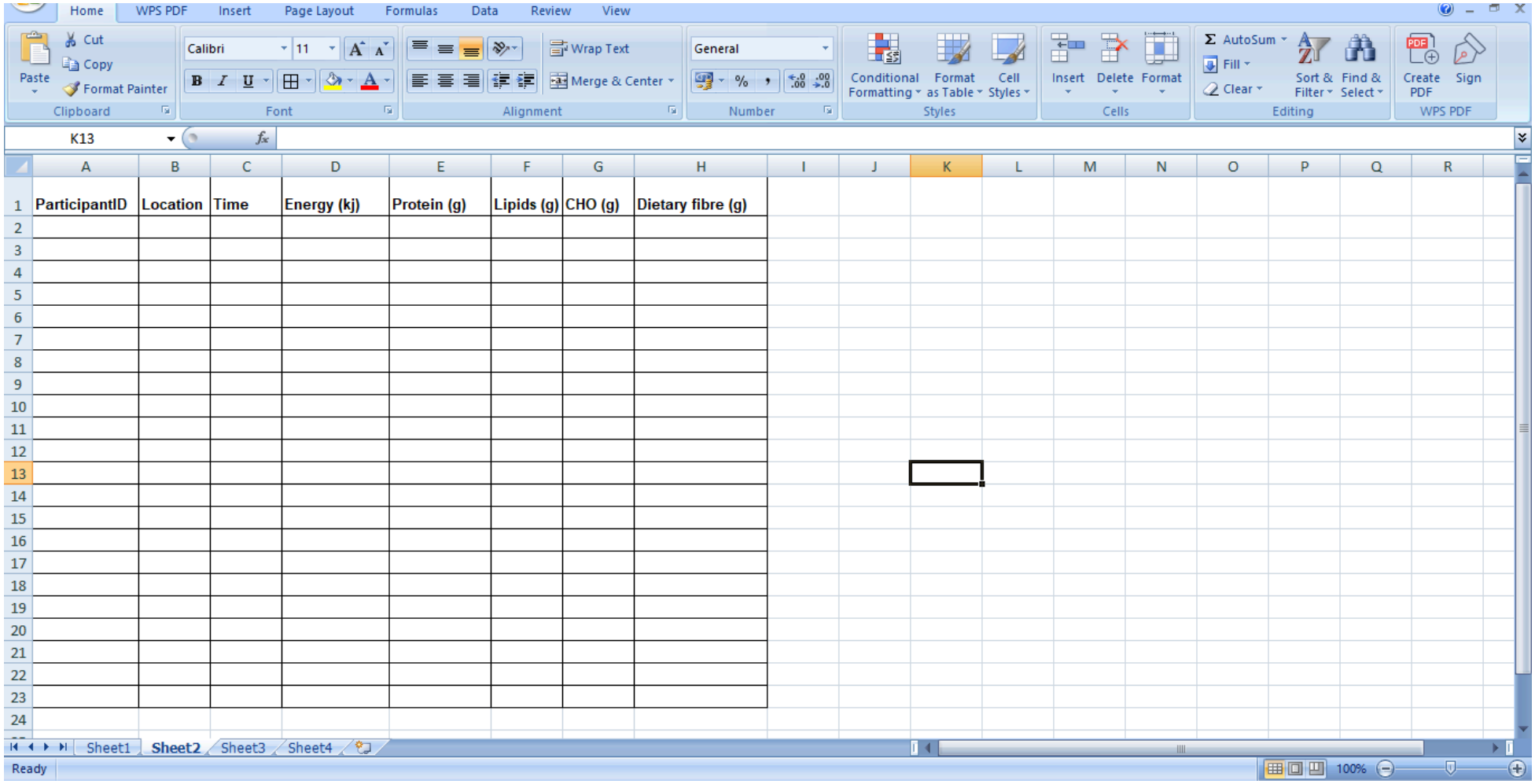
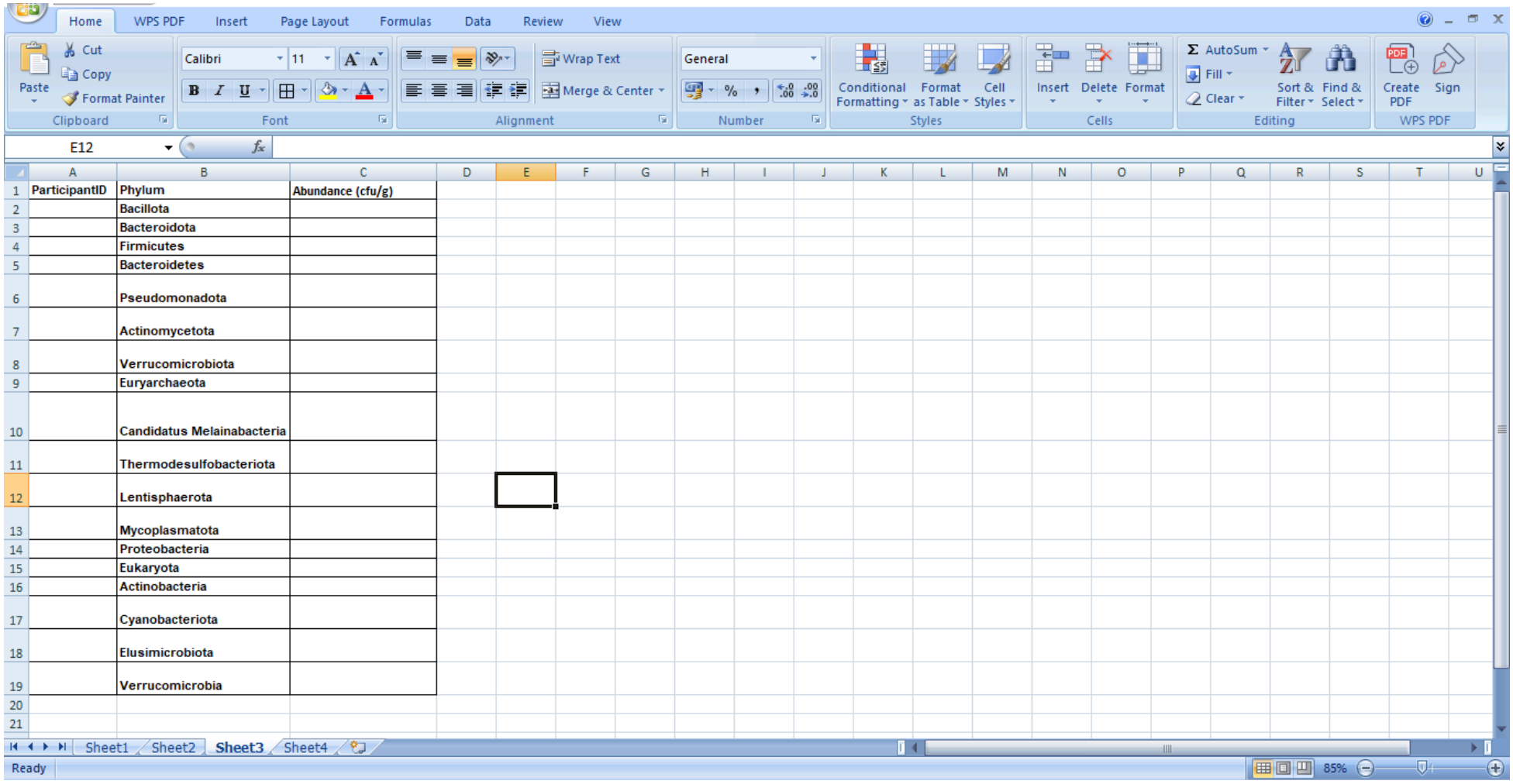
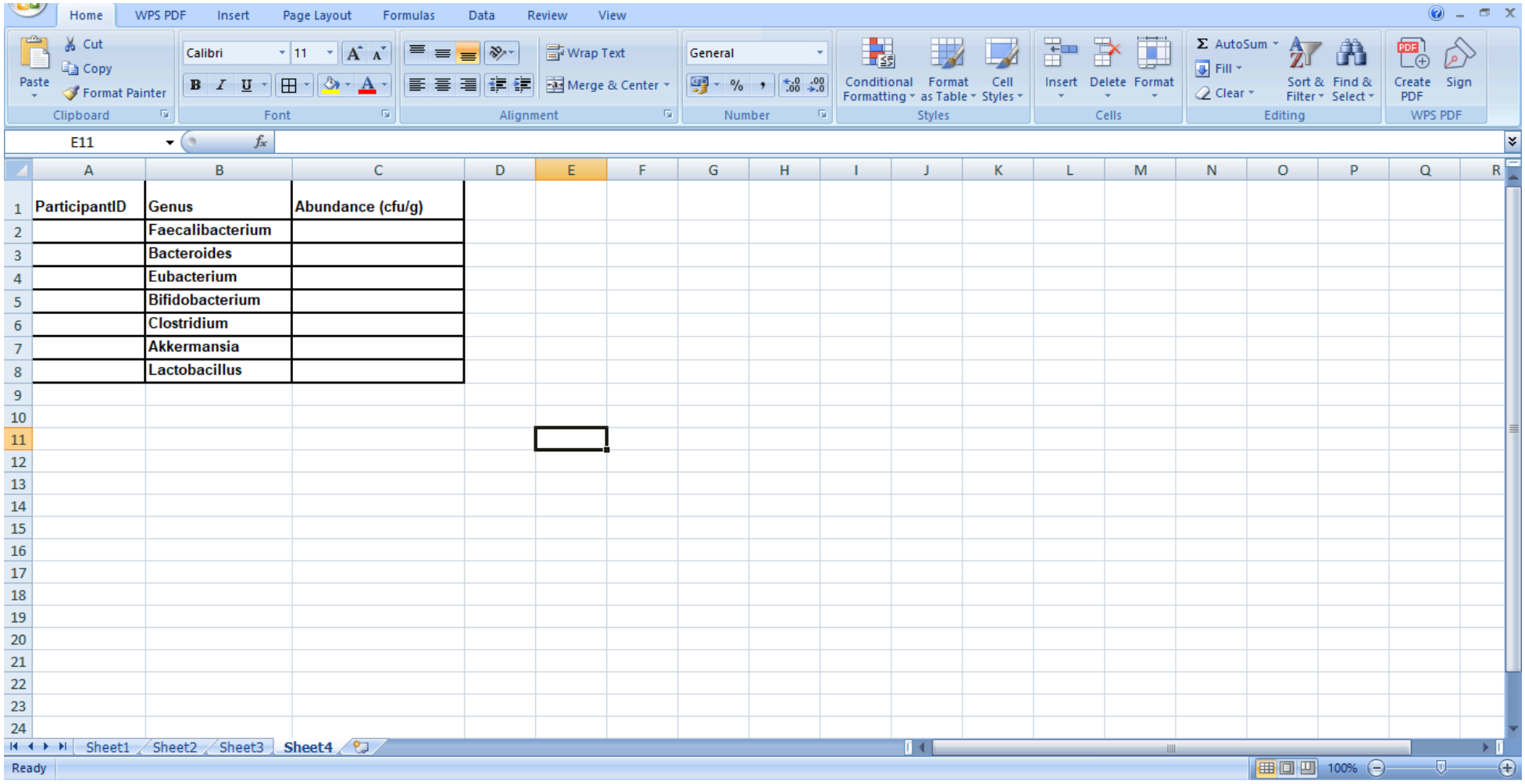


ParticipantID	Location	Time	Energy (kj)	Protein (g)	Lipids (g)	CHO (g)	Dietary fibre (g)



	A	B	C
1	ParticipantID	Phylum	Abundance (cfu/g)
2		Bacillota	
3		Bacteroidota	
4		Firmicutes	
5		Bacteroidetes	
6		Pseudomonadota	
7		Actinomycetota	
8		Verrucomicrobiota	
9		Euryarchaeota	
10		Candidatus Melainabacteria	
11		Thermodesulfobacteriota	
12		Lentisphaerota	
13		Mycoplasmatota	
14		Proteobacteria	
15		Eukaryota	
16		Actinobacteria	
17		Cyanobacteriota	
18		Elusimicrobiota	
19		Verrucomicrobia	
20			
21			



ParticipantID	Genus	Abundance (cfu/g)
	Faecalibacterium	
	Bacteroides	
	Eubacterium	
	Bifidobacterium	
	Clostridium	
	Akkermansia	
	Lactobacillus	

ANNEXURE K

- Participant Invitation and Informed Consent Form -

PARTICIPANT INVITATION AND INFORMED CONSENT FORM

Title of the study: ***THE EFFECT OF A PROBIOTIC YOGHURT ON GUT MICROBIOME, LOW GRADE INFLAMMATION AND WEIGHT STATUS OF OBESE SOUTH AFRICAN WOMEN***

Sponsor: Milk SA

Principal Investigator: Gabriela Lourenco

Institution: University of Pretoria, Department of Consumer and Food Sciences

DAYTIME AND AFTER HOURS TELEPHONE NUMBER(S):

Daytime number: 079 973 1274

After Hours number: 079 973 1274

DATE AND TIME OF FIRST INFORMED CONSENT DISCUSSION:

Date	**month**	**year**

:
Time

Dear Prospective Participant

You are invited to volunteer for a research study. I am doing research for a Master's of Science Nutrition Degree purpose at the University of Pretoria. This information in this document is to help you to decide if you would like to participate. Before you agree to take part in this study you should fully understand what is involved. If you have any questions, which are not fully explained in this document, do not hesitate to ask the researcher. You should not agree to take part unless you are completely happy about all the procedures involved.

NATURE AND PURPOSE OF THE STUDY

The increasing prevalence of overweight and obesity amongst South Africa adults, particularly women, is alarming. South Africa is regarded as one of the countries with the highest growing prevalence of obesity (Van Vollenstee et al., 2021). It is estimated that by the year 2025, there will be an increase in obesity by 47.7% in females and 23.3% in males (Van Vollenstee et al., 2021) This highlights the need for alternative interventions that target obesity related inflammation and weight status in obese South African women. A dietary intervention that targets the gut microbiome as well as immune functioning is a cost effective, safe and noninvasive strategy which could potentially be used to maintain microbiome homeostasis for obese individuals.

The aim of the study is to determine the effect of a 12- week administration of a probiotic yoghurt containing a culture of *Lactobacillus rhamnosus* GG (ATCC 53103) (LGG) and a commercial freeze-dried culture of *Bifidobacterium animalis* subspecies *lactis* BB-12, both at levels of 10^{10}cfu/g, on the gut microbial count and the related outcomes in CRP levels and weight status of females with a BMI >30 kg/m, between the ages of 25 - 55 years.

EXPLANATION OF PROCEDURES AND WHAT WILL BE EXPECTED FROM PARTICIPANTS

To participate in the study, you would need to be a female aged 25 - 55 years, with a BMI ≥ 30 kg/m². This study involves answering some questions regarding your medical history, weight status measurements, dietary assessments, blood and stool sample tests. The following tests will be done for the research specifically. Blood tests will be done to determine C-reactive protein levels to identify whether inflammation is present. Stool samples tests will be done to determine the bacterial counts of the two bacterial strains that are present in the gut microbiome. Weight status measurements include weight (kilograms), height (centimetres), waist circumferences (centimetres), fat mass and lean body mass (kilograms). To determine dietary intake, you will be required to complete a three day food diary which will be completed at three different points in time. All measurements, excluding the dietary assessment, will be completed twice with the first measurements being performed at baseline and the second at the end of the intervention.

Blood sample collection: A clinical nurse from the Immunology department, will collect a blood sample at the SKills Laboratory at HW Snyman, faculty of Health Sciences from you at a pre-arranged date and time. Please do not administer any anti-inflammatory medication on the day of blood sample collection.

Stool sample collection: Stool sample collection kits will be given to participants at the screening visit. The kits include everything that will be needed to safely collect your stool sample, including written instructions with pictures. A video link will also be made available.

Weight status measurements: You will be asked to wear light and non-restrictive clothing on the days of taking weight status measurements. You will also be required to remove your shoes when taking weight and height measurements.

Dietary Assessment: You will be asked to complete a three-day food diary whereby they will record every food and beverage consumed. The more detail you can provide, the better. For example, providing specific quantities consumed or the brand name of specific food and beverage products. The food diary should include two weekdays (Monday to Friday) and one weekend day (Saturday and Sunday). The food diary will be completed at three different points in time, namely: the first week, the sixth week (mid-intervention), and the last week of the intervention.

Intervention: During the intervention period, you will be expected to take 100g of a treated/conventional yoghurt product provided to you by the researcher. The intervention period will be 12 weeks and participants will be required to consume the product once a day. The product will be delivered to you once per week. You will be asked to continue with your usual dietary habits and to refrain from the consumption of any other probiotic or highly fermented products such as kombucha and kimchi. Compliance with the consumption of the yoghurt products will be monitored one a week by a phone call or message sent by the researcher.

RISK AND DISCOMFORT INVOLVED

There are no medical risks associated with the study. The only possible risk and discomfort involved is the taking of blood from a vein which can result in bruising and bleeding from the puncture site.

COMPENSATION

You will not be paid to take part in the study. However, any cost you do have as a result of taking part in the study, for example transport costs will be paid back to you (reimbursed). You will receive an R200 food voucher to compensate for all the traveling costs to and from the clinic.

YOUR RIGHTS AS A RESEARCH PARTICIPANT

Your participation in this trial is entirely voluntary and you can refuse to participate or stop at any time without stating any reason. Your withdrawal will not affect your access to other medical care.

ETHICS APPROVAL

This Protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, telephone numbers 012 356 3084 / 012 356 3085, and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2013), which deals with the recommendations guiding doctors in biomedical research involving humans/subjects. A copy of the Declaration may be obtained from the investigator should you wish to review it.

CONFIDENTIALITY

All information obtained during the course of this study will be regarded as confidential. Each participant who is taking part will be provided with an alphanumeric coded number e.g. A001. This will ensure the confidentiality of the information so collected. Only the researcher will be able to identify you as a participant. Results will be published or presented in such a fashion that patients remain unidentifiable. The hard copies of all your records will be kept in a locked facility at the Consumer and Food Sciences Department at the University of Pretoria.

CONTACT DETAILS:

Please do not hesitate to contact me, Gabriela Lourenco, MSc Nutrition student at the University of Pretoria (gabriela.lourenco6@gmail.com/ 079 973 1274), or the principal supervisor (Adeline Pretorius, Registered Dietician, Lecturer at the University of Pretoria (adeline.pretorius@up.ac.za; Tel 071 369 7793), should you require further information. Please note that should you not meet all the criteria that we require, you might not be able to participate in the study.

Consent to participate in the study:

I confirm that I have been informed about the nature, process, risks, discomforts, and benefits of the study. I have also received, read, and understood the above-written information (Participation Invitation And Informed Consent) regarding the study. I am aware that the results of the study, including personal details, will be anonymously processed into research reports. I will participate willingly. I have had time to ask questions and have no objections to participating in this study. I understand that there is no penalty should I wish to discontinue with the study and the withdrawal will not affect me in any way. If I decide to withdraw, I will be given the option that my information and data collected to be either destroyed or to be used for the results of the study. I have received a signed copy of this informed consent agreement.

Volunteer's Name (Please print):_____

Volunteer's contact number: _____

Volunteer's signature: _____ Date: _____

Emergency Contact Name (Please print): _____

Emergency contact number: _____ Date: _____

Researcher's Name (Please print): _____

Researcher's signature: _____ Date: _____

Witness' Name (Please print): _____

Witness' signature: _____ Date: _____

ANNEXURE L
- Ethical Approval Letter -

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through June 30, 2025 and Expires 07/28/2026.

Faculty of Health Sciences **Research Ethics Committee**

10 October 2023

Endorsement Notice

Dear Miss GM Lourenco

Ethics Reference No: NAS142/2023
Title: The effect of a probiotic yoghurt on gut microbiome, low-grade inflammation and weight status of obese South African women

The **New Application** as supported by documents received between 2023-08-30 and 2023-10-09 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-10-09 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Ethics Approval is valid for 1 year and needs to be renewed annually by 2024-10-10.
- Please remember to use your protocol number (NAS142/2023) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

Research Ethics Committee
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University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo