UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES

THE EFFECT OF A DIETARY SUPPLEMENT WITH ALPHA LIPOIC ACID AND OTHER NEUTRACEUTICALS ON WEIGHT CONTROL AND SERUM LIPID PROFILE IN SPRAGUE DAWLEY RATS

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DECLARATION

I, Albert Kyei-Kankam Poakwah, hereby declare that except for reference to other people’s work, which I have duly cited, this thesis is the result of an original research work and that the material has not been presented either in whole or in part elsewhere for another degree and all experimental works was performed by me under the supervision of Dr Charles Brown and Dr Kwame Benoit Nguessan Banga.

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ABSTRACT

Background: Obesity is a major health problem and a risk factor for morbid conditions such as type-2 diabetes, cardiovascular disorders, non-alcoholic fatty liver disease and metabolic syndrome. Dietary modifications and pharmacological agents, among others, are measures often used by individuals to decrease body fat.

Aim: The aim of this study was to determine the effect of a dietary supplement (MS01) on weight control and serum lipid profile in Sprague Dawley (SD) rats.

Methods: A total of twenty-four SD rats were randomly put into 4 groups of 6 rats each. Group 1 was fed on a normal diet (NCD) and groups 2 - 4 fed on a high-fat diet (HFD) for 30 days. The rats on the HFD were treated with the dietary supplement (MS01), at low (group 3, MS01/L) and high (group 4, MS01/H) doses. The other HFD-fed rats received distilled water and served as a control of the treatment (group 2). The doses of MS01/L and MS01/H administered orally to the SD rats were 4 mg/kg and 8 mg/kg body weight, respectively. The weights of the rats were measured at the end of every week to determine the weight gain. Blood samples from the animals were collected by cardiac puncture at the end of the experiment. Serum lipid profiles (total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (HDL-C)) were assayed using an automated blood chemistry analyser. Additionally, the heart, liver and kidney of rats in the various groups were harvested and weighed at the end of the 30-day period.

Results: Rats in group 2 (fed with HFD plus distilled water) showed a significant increase in weight (99.08% weight gain, p<0.001) compared to the rats fed with normal diet after the 30-day period. Rats administered MS01 showed a significant reduction in weight (84.87% and 84.83%, p<0.001 for MS01/L and MS01/H, respectively) compared to the rats fed with normal diet. However, the decrease in weight observed for rats administered with MS01 was not found to be dose-dependent (MS01/L vs MS01/H p>0.05). It is noteworthy, however, that there was no statistically significant difference (p>0.05) among groups with respect to feed intake over the period (14.74 ± 5.60, 13.52±1.59, 13.17±1.59 and 12.72±1.86 for NCD, control HFD, MS01/L and MS01/H, respectively). The serum total cholesterol (2.08 ± 0.47), triglyceride (1.56 ± 0.81) and HDL-cholesterol (0.98 ± 0.19) was found to be significantly higher (p<0.05) in control HFD rats compared to rats fed with the normal diet (1.51 ± 0.18; 0.75 ± 0.22; 0.59 ± 0.64 respectively). Also, serum concentration of triglyceride was significantly increased (p<0.01) in the rats treated...
with MS01/L (2.28 ± 1.09) compared to rats treated with MS01/H (0.84 ± 0.48) and control HFD rats (1.56 ± 0.81). The effect of the dietary supplement on treated groups was found not to be dose-dependent (MS01/L vs MS01/H p>0.05). The organs (heart, liver, kidneys) tissue weight of rats fed with the control high fat diet only, showed an increase (0.35±0.04; 2.72±0.4; 0.325±0.055) compared to the rats fed with the normal diet (0.30±0.02; 2.12 ±0.1; 0.275±0.035) respectively but was not statistically significant (p>0.05). Moreover, the organs (heart, liver, kidneys) tissue weight of rats treated with the high dose of the dietary supplement was noted significantly inferior (0.26±0.02; 1.88 ±0.1; 0.245±0.025) compared to the rats treated with a low dose of the dietary supplement (0.30±0.06; 1.93±0.3; (0.25±0.045) and the rats that fed the control high fat diet only (0.35 ±0.04; 2.72 ±0.4; 0.325±0.055) respectively. Despite the difference in means for tissue weight of rat organs (heart, liver and kidneys) both treated and untreated groups, the rat body weight to organ ratio showed no statistically significant difference (p>0.05) among all groups.

**Conclusion:** The dietary supplement MS01 (both low and high doses) was found to be effective in limiting weight gain in the SD rats fed on a high-fat diet after the 30-day period. However, the supplement did not significantly decrease the serum lipid profile of the SD rats. Further studies, probably over a longer period is recommended to ascertain the possible effect of this dietary supplement MS01 on serum lipids.
DEDICATION

This dissertation is dedicated to the memory of my late mother Miss Mavis Koduah and late Grandfather Mr Francis Koduah, for their encouragement and support, but who did not live to see the completion of this work.
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LIST OF ABBREVIATIONS

AMPK  Adenosine monophosphate-activated protein kinase
ALA   Alpha - lipoic acid
CHD   Coronary heart disease
CVD   Cardiovascular disease
FDA   Food and Drugs Authority
GROUP-3 MS01/L (rats that fed HFD then treated with a low dose of the MS01 dietary supplement)
GROUP-4 MS01/H (rats that fed HFD then treated with a high dose of the MS01 dietary supplement)
HDL   High-density lipoprotein
HFD   High-fat diet
LDL   Low-density lipoprotein
MS01  The dietary supplement
MS01/L Low dose of the dietary supplement
MS01/H High dose of the dietary supplement
NAFLD Non-alcoholic fatty liver disease
NCD   Normal control diet
TG    Triglycerides
TCHOL Total cholesterol
T2DM  Type-2 diabetes mellitus
VLDL  Very low-density lipoprotein
CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Obesity is a multifaceted condition of fat and carbohydrate breakdown occurring as a result of an extreme fat build-up in adipose tissue, liver and skeletal muscle, among others (WHO, 2011). In obesity, there is an unjustified upsurge in adipose tissue which is often as a result of nutrition and genetic influence (Ferranti & Mozaffarian, 2008). The American Medical Association classified obesity as an illness in 2013 (Weinstock, 2013).

Type-2 diabetes mellitus (T2DM), cardiovascular diseases (CVDs) and non-alcoholic fatty liver disease (NAFLD) are conditions that can affect an individual when obese (Saravanan et al., 2014). Obesity has been classified as an avoidable basis of death globally and has been categorised as a challenge to the well-being of humans in the 21st century (Barness et al., 2007). Individuals that are obese are stigmatized in most places (mainly in the Western world), although obesity was originally assumed to be a sign of being wealthy (Haslam & James, 2005). The stigmatization of obesity can lead to several negative effects including reduced confidence and self-image (Tillman et al., 2007; Puhl & Heuer, 2010), and hence likely to affect obese individuals especially in their childhood and adolescence periods (Pearce et al., 2002).

The worldwide prevalence of obesity is high across sex, age groups, race/ethnicity and socio-economic status (Cossrow & Falkner, 2004; Wang & Lim, 2012; Flegal et al., 2012; Dinsa et al., 2012). WHO estimated that, among African adults aged 20 years and over, 27% are overweight and 8% obese (WHO, 2010). WHO again estimated that there is a rise in number, among individuals who are overweight and obese in sub-Saharan Africa
(Abrahams, 2011). In a 2013 study conducted in sub-Sahara Africa, Equatorial Guinea recorded a 25% prevalence of obesity, the highest, and Uganda recorded the least, 1.7% (Fleming et al., 2014). A 2003 study done in Ghana by Amoah across cities and villages in the Greater Accra Region, among individuals over 25 years old, revealed the occurrence of 23.4% and 14.1% for overweight and obesity, respectively, hence prevalence appeared to be high then.

Relationships between obesity, high blood pressure and abnormal lipid profile occur in both sexes among ethnicities in Africa (Must et al., 1999). Dyslipidaemia results from excessive accumulation of fat that is stimulated by an unhealthy diet and a sedentary lifestyle which leads to the rise in levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and reduce high-density lipoprotein cholesterol (HDL-C). Other variations associated with the prevalence of dyslipidaemia includes ethnicity, nationality, genetics, socio-cultural and economic factors (Durrington, 2003; Han et al., 2015).

Dyslipidaemia is another modifiable risk factor for CVD (WHO, 2011). WHO in 2012 reported that 2.6 million (4.5%) deaths worldwide were related to dyslipidaemia and these deaths were generally due to lipid accumulation in large and medium-sized arteries (Mahmoud et al., 2010). Diabetes mellitus (DM), atherosclerosis (AS) and NAFLD have an association with dyslipidaemia (Ghouri et al., 2010; Popkin et al., 2001).

Currently, dyslipidaemia remains a health problem in Africa. In Nigeria, the occurrence of dyslipidaemia among T2DM persons from the rural and urban centres increased from 82.6% to 90.7% from 2008 to 2011 (Okafor et al., 2008; Ogbera et al., 2009; Jisieike-
Onuigbo et al., 2011). In South Africa, the prevalence is over 90% among Blacks with T2DM (Vezi & Naidoo, 2005). In Tanzania, the prevalence of diabetic dyslipidaemia was 95% in 2007 (Chattanda & Mgonda, 2008). The high levels of dyslipidaemia among middle-aged men and women are mainly due to the adopted western diet, sedentary lifestyle, and physical inactivity (Njeleka et al., 2002). A prospective study in Ghana among 708 persons with CVD at the National Cardiac Referral Centre revealed that 66.3% were hypertensive and 8.8% had hyperlipidaemia (Amoah, 2000).

The pharmacological approach in controlling weight gain along with lifestyle adjustments comprises, amongst others, the use of agents that reduce nutrient absorption, modulate lipogenesis-lipolysis, and regulate adipose signals and appetite centres (Colagiuri, 2010). For example, the possibility of myocardial infarction and stroke was significantly low in hypertensive subjects with dyslipidaemia when they were treated with lipid regulatory medications (Sever et al., 2003). Moreover, the risk of coronary heart disease (CHD) may be reduced when patients with uncontrolled hypertension have their lipid profiles normalized (Perreault et al., 1999).

Therapeutic agents like lipase inhibitors (orlistat) and monoamine reuptake inhibitors (sibutramine) are among the few approved by the Food and Drugs Authority (FDA) for managing obesity. Others like the statins (atorvastatin, fluvastatin, lovastatin, pravastatin, pitavastatin, and simvastatin) and fibrates (gemfibrozil and fenofibrate, and niacin) have been developed to control lipid profiles (Kobayashi et al., 2008; Wat et al., 2016). However, epidemiological studies have recommended the use of natural agents to decrease the possibility of adverse events related to the long-term use of these drugs (Kishino, 2006).
One such natural agent, with lipid-lowering and obesity-reducing potential, is alpha-lipoic acid (ALA). ALA is normally found in meats and vegetables (in lesser amounts) and has an effective antioxidant capacity (Liu et al., 2002). The management of diabetic neuropathy has been demonstrated to improve with the use ALA (Mihai et al., 2010), likewise the management of atherosclerosis (Ying et al., 2010), liver-related pathologies (Foo et al., 2011), and hypertriglyceridemia (Kandeil et al., 2011). ALA is also known to possess anti-ageing properties (Sena et al., 2008). The management of dyslipidaemia and hyperglycaemia with ALA has been proven to be effective in diabetic persons (Ying et al., 2010, Kandeil et al., 2011). Lee et al. (2005) reported that muscle cells sensitivity to insulin improved with the use of ALA. Additionally, weight loss characteristics of ALA have been reported to facilitate metabolism (Kim et al., 2004).

Other natural agents are ginseng, citrulline and leucine. Ginseng belongs to the genus Panax and family Araliaceae. Its benefits are usually obtained from the fleshy roots and berries. Ginseng has been established to be effective in controlling metabolism while enhancing weight loss, reducing blood glucose and controlling appetite (Attele, 2002). Citrulline is largely obtained from watermelon and has been shown to suppress appetite (Rimando & Perkins-Veazie, 2005). Leucine which is an essential amino acid and has been shown to aid in muscle protein synthesis. It slows down muscle degradation and helps in resolving dyslipidaemia, hyperglycaemia and maintaining weight loss (Halton & Hu 2004).

1.2 PROBLEM STATEMENT

The imbalance between energy expenditure and energy intake through overeating, stress and lack of exercise leads to metabolic syndrome (Susanti et al., 2014). However, proper
alteration of metabolic disorders, obesity and lipoproteins are necessary (Shin et al., 2013). Obesity and reduced lipid metabolism present an association for developing life-threatening circumstances that create major risk factors including microvascular disorders (Shin et al., 2013).

However, weight loss and balancing blood lipid levels can be a difficult endeavour even for the most motivated individuals. This is because, with determined lifestyle adjustments, obese participants in clinical trials can lose between 7% to 10% of their initial weight after a year (Carvajal et al., 2013). Irrespective of weight loss success, maintaining this new weight is often challenging (Middleton et al., 2012). Likewise, adapting to these lifestyle modifications with numerous biological and environmental stressors is also challenging. Thus, adjunctive therapies such as agents to help patients lose or sustain weight are needed (Yanovski & Yanovski, 2014).

1.3 STUDY JUSTIFICATION

While there is data of individual nutraceuticals and weight loss with lipids regulatory potential, there is a dearth of information on the MS01 dietary supplement, which is a combination of various nutraceuticals. The MS01 dietary supplement being studied has a proprietary blend of ALA, ginseng, citrulline (citrulline malate), leucine, riboceine (D-Ribose-L-Cysteine) and pantetheine; each of these individually affects metabolism. Also, the supplement has vitamin D2, thiamine, vitamin B6, vitamin B12, and potassium. The overall effect of this conglomerate of nutraceuticals on weight and hyperlipidaemia control needs validation, thus the relevance of this study.

The effectiveness of the MS01 dietary supplement in the rats may form the basis of a clinical study in humans. This may give the possibility of it being adopted to contribute
to achieving a healthier lifestyle and may help reduce the economic strain of managing as well as preventing obesity and dyslipidemia-related medical conditions among individuals and on the nation.

1.4 AIM

The aim of this study was to determine the effect of a dietary supplement on weight control and serum lipids in *Sprague Dawley* rats.

1.5 SPECIFIC OBJECTIVES

The specific objectives of the study were to:

1. Determine the degree of weight control in the *Sprague Dawley* rats fed on a high-fat diet and treated with the MS01 dietary supplement.

2. Determine the effect of the MS01 dietary supplement on the serum lipid profile of *Sprague Dawley* rats fed on a high-fat diet.
CHAPTER TWO

‘2.0 LITERATURE REVIEW

2.1 OBESITY AND DYSLIPIDAEMIA

2.1.1 Definitions

2.1.1.1 Obesity

Obesity is defined by the World Health Organisation as an extreme fat build-up that affects health. Body Mass Index (BMI) is commonly used to define an individual’s weight; calculated as a person’s weight in kilograms divided by the square of the height in meters (kg/m\(^2\)). Overweight and obesity are defined by the World Health Organization as BMI ≥ 25 kg/m\(^2\) and BMI ≥ 30 kg/m\(^2\), respectively, for adults (WHO, 2017). Obesity is a condition caused by multiple physiological systems (Rutter, 2018).

2.1.1.2 Dyslipidaemia

Dyslipidaemia is a disorder leading to higher plasma (TG) and free fatty acids (FFAs) levels and is characterized by lipid abnormalities that include alterations in both atherogenic as well as anti-atherogenic lipoproteins (Su et al., 2014; Soran et al., 2016). Dyslipidaemia is the accumulation of one or more lipids in the plasma and is a manifestation of abnormalities in lipid transport metabolism. Clinically, dyslipidaemia is expressed as hypertriglycerideremia, an increase in LDL-cholesterol and/or decreased HDL-cholesterol (Bjornson et al., 2017).

2.1.2 Descriptions

2.1.2.1 Obesity

Shakespeare said that a rose by any other name would smell as sweet. Obesity, currently defined as a BMI ≥ 30 kg/m\(^2\), remains basically the same thing whatever we name it. BMI
is calculated from just two basic anthropometric measurements, weight and height, and is somehow limited as a sole diagnostic standard for obesity. Though BMI does not measure adiposity in the true sense of the word, it is easy to use in health screenings and epidemiological surveys (Ortega et al., 2016). Obesity reflects an energy imbalance; related to dietary intake and energy expenditure (Hall et al., 2012). Physical inactivity is a major contributor to morbidity and mortality (Dietz et al., 2015). The global developments in obesity prevalence, along with high mortality attributed to obesity, have led to projections of decreased life expectancy (Gortmaker et al., 2011). In advanced countries, factors influencing obesity are closely related to lifestyle or dietary habits (Marcos et al., 2014). This latest development in obesity is mostly due to lifestyle factors including sedentary lifestyle, poor dietary choices and patterns (McHill & Wright, 2017). However, in developing countries, studies have shown obesity is influenced by a sedentary lifestyle, demographic changes, geographic patterns, and socio-economic and environment factors (Kandala & Stranges, 2014; Bhurosy & Jeewon, 2014). Also, there are wider system-level adaptations, such as the food industry altering pricing or increasing marketing and promotions in response to regulations on labelling. Moreover, obesity can be conceptualized as an emergent property of food, physical inactivity, and other systems, with the rising trend in the prevalence of excess weight, an unintended but inherent consequence of human behaviour within those systems (Rutter, 2018)

2.1.2.2 Dyslipidaemia

Dyslipidaemia is related to excess production of very low-density lipoprotein (VLDL), low-density lipoproteins (LDL) and a decreased synthesis of high-density lipoproteins (HDL) (Christian & Su, 2014). Postprandial lipemia (PPL) is as a result of the increase in plasma lipid levels after a meal (Stenger et al., 2010). This leads to an increase in the
VLDL quantity that is converted to LDL which is delivered to the arterial walls, a reduction of HDL particle number and function, and increased production of LDL. This adverse lipid profile increases the development of atherosclerosis (Matsumoto et al., 2014; Paloma et al., 2014).

Majority of people spend most of their time in the fed state with a recurrent variation in the lipemia levels throughout the day. According to the typical American eating pattern, “most people consume three or more meals per day and each of these meals is mostly consumed before the plasma triglyceride levels returned to baseline levels; this produces a lipemic condition resulting from the previous meal intake” (Ridker, 2008). The occurrence of high lipid levels after a meal and impaired postprandial lipoprotein clearance is related to atherosclerosis compared with levels in the fasting state (Parthasarathy, 2010). In other studies, 40% of all patients with premature coronary artery disease have normal fasting plasma lipids, while they suffer from impaired clearance of postprandial lipoproteins (Cox-York et al., 2013). Several factors affect the metabolism of postprandial lipoprotein. These include dietary pattern, food composition, other conditions associated with lifestyle, physiological factors (age, gender, genetic background and postmenopausal status) and cardiometabolic conditions (Alcala-Diaz et al., 2014).

2.1.3 Prevalence

2.1.3.1 Global

2.1.3.1.1 Obesity

Obesity is acknowledged as a public health issue and is of global health concern (Gaio et al., 2018; Lim & Park, 2018). Worldwide, obesity was estimated to occur in about 500
million adults in 2011 (Finucane et al., 2011). WHO in 2014 reported prevalence of obesity as 13% globally. It was again reported in 2016 by WHO that 11% of men and 15% of women of age 18 years and over were obese. The prevalence of obesity in the United Kingdom (UK) has nearly doubled between 1993 and 2011 from 13% to 24% (Jones, 2018). Also, from 2013 to 2014, approximately one-third of adults in the United States of America (USA) were both overweight and obese (Fryar et al., 2016). In a study conducted among Portuguese speaking people, the prevalence of obesity was recorded at 28.6% and obesity was higher in females than in males like other countries (Gaio et al., 2018; Jones, 2018).

2.1.3.1.2 Dyslipidaemia

Coronary heart disease and cerebrovascular disease are two major types of cardiovascular diseases (CVDs). They were ranked as the top two causes of early death and disability-adjusted life-years (DALYs) globally in 2015 (Murray et al., 2015; Mortality and Causes of Death Collaborators, 2015) and both contributed significantly to the increasing healthcare expenditures, especially, in low- and middle-income countries as well as in China (Stevens et al., 2016). Pathogenesis of metabolic syndrome (MetS) is complex and lipids contribute to the risks associated with this syndrome.

Regardless of the diagnostic criteria used, the prevalence of metabolic syndrome is associated with geographic and socio-demographic factors. A National Health and Nutrition Examination Survey data estimated that 35% of adults in the United States, and as much as 50% of the over-60-year-old population had a diagnosis of MetS (30.3% in men and 35.6% in women). This was based on the National Cholesterol Education Program Adult Treatment Panel III criteria with recent trends suggesting that the overall
prevalence was stable, and men had a lowered prevalence (Aguilar et al., 2015). The highest MetS prevalence has been reported in Mexican-American women (Beltrán-Sánchez et al., 2013). Using the International Diabetes Federation diagnostic criteria, MetS prevalence in Europe was estimated as 41% and 38% in men and women, respectively (Gao, 2008). A systematic review of epidemiologic data from the Middle East reports a prevalence of MetS in men of 20.7-37.2% and 32.1-42.7% in women (using Adult Treatment Panel III criteria) (Mabry et al., 2010). Data from China suggest a 58.1% prevalence in adults of 60 years and older age group (Liu et al., 2013).

2.1.3.2 Africa

2.1.3.2.1 Obesity

In Africa, the prevalence of overweight and obesity differs from country to country. A meta-analysis study of the prevalence of adult obesity in Africa reported a prevalence rate of 21.8%, with increasing rates over time, among individuals who are less educated, females and persons of poor economic status (Tulp et al., 2018). Obesity rates in African countries fall between a range of 7.6% to 26.8% with South Africa being the highest (Jones, 2018). A study done in Algeria by Dalichaouch-Benchaoui and Abadi in 2014 reported a prevalence of overweight and obesity among adults of 32.5% and 30.9%, respectively. The prevalence of overweight and/or obesity in Ethiopia was found to be 9.4% (Alemu et al., 2014). The crude prevalence of overweight and obesity among civil servants in Lagos, Nigeria, was 70.7% (Ajani et al., 2015). The prevalence of obesity among adults in Issele-uku, a community in Nigeria, reported a prevalence of 5.5% (Agofure, 2017). Among Sudanese individuals, the prevalence rate was reported at 21.2% (Ahmed et al., 2017). In Zambia, it was 24.7% (21.0% among males and 27.3% among females) (Zyaambo et al., 2012).
2.1.3.2 Dyslipidaemia

In the Black African populations, dyslipidaemia is to a large extent, similar to worldwide averages (Shisana et al., 2013). In Africa, dyslipidaemia prevalence rates of between 14% and 69% have been found using community-level assessments (Manning et al., 2016). In Nigeria, the prevalence of dyslipidaemia ranged from 60% among apparently healthy Nigerians to 89% among diabetic Nigerians (Oguejiofor et al., 2012). The prevalence of MetS in urbanized sub-Sahara African population ranges from 11% in Benin (Ntandou et al., 2009) to 34.6% in Kenya (Kaduka et al., 2012). A South African survey revealed that 28% of women and 19% of men older than 18 years had elevated total cholesterol; 52% and 44% of men and women, respectively, had low levels of HDL (Shisana et al., 2013).

2.1.3.3 Ghana

2.1.3.3.1 Obesity

A systematic review and meta-analysis study of obesity between 1998 and 2016 in Ghana reported a national prevalence rate of 17.1% with the majority being females and urban dwellers (Ofori-Asenso et al., 2016). In Ghana, the overall prevalence of overweight among women aged 15 - 49 years increased from 25.5% to 30.5% between 2003 and 2008 (Dake et al., 2010). Childhood obesity increased from 0.5% in 1988 to 1.9% in 1993 and to 5% in 2008. Among the youth aged 15 -19 years and 20 - 24 years, obesity increased from 7.2 - 9.0% and 15.1 - 16.6% respectively in the same period (Dake et al., 2010). Also, Agbeko et al. in 2013 reported a prevalence of overweight and obesity among women as 20.5% and 9.3%, respectively.
2.1.3.3.2 Dyslipidaemia

Little is known about the lipid profile of children and adolescents in Ghana. One study conducted in the Ga-East Municipality showed a low level of hypercholesterolemia (2.8%) among overweight and obese school children (Steiner-Asiedu et al., 2012).

2.1.4 Pathophysiology

2.1.4.1 Obesity

The pathophysiology of obesity involves two parallel viewpoints: dietary intake and energy expenditure (Mozaffarian & Ludwig, 2015; De Souza et al., 2012). All the same, the distinction between the causes and consequences of obesity must be given due consideration, as well as the importance of understanding obesity-independent and obesity-dependent pathophysiology comorbidities including CVDs. Majority of scientists agree that based on evidence that individual adult body weight barely changes during short-term experimental up or down distresses under constant environmental conditions, body weight or adiposity is dynamically controlled or protected (Schwartz et al., 2017). Latest developments point to the fact that increased body weight/adiposity in the majority of obese persons is defended just as it is in normal weight persons (Hall & Guo, 2017); backing the view that obesity is a disease, thus putting the blame on the physiology rather than the individual (Figure 2.1).

A genetic predisposition for obesity has been indicated following the identification of more than 140 genetic chromosomal regions related to obesity from data obtained from genome-wide association studies (Fall et al., 2017).
Figure 2.1: Schematic diagram shows the 3 heavily interconnected major brain areas constituting the core processor for the control of ingestive behaviour and its relation to the gastrointestinal tract and other peripheral organs involved in energy storage and utilization. The hindbrain is mainly concerned with meal size control because it possesses all the elements to detect sensory information mediated by vagal afferents and circulating factors and generate a motor output associated with the ingestion, digestion, and absorption of food. The corticolimbic system, consisting of large cortical areas, basal ganglia, hippocampus, and amygdala, is intimately connected to the hypothalamus and brainstem and provides the emotional, cognitive, and executive support for ingestive behaviour. The hypothalamus via its connections with the other areas is central for the drive to eat and can potently modulate peripheral organs by autonomic and endocrine outflow. (Source: Berthoud et al., 2017).
Though gene expression related to BMI and general adiposity is highly embedded in the central nervous system (Locke et al., 2015), some genes with effects on BMI have been identified. These include the genes that code for components of leptin and melanocortin signalling, in addition to paternally expressed genes along a specific region of chromosome 15 responsible for Prader-Willi syndrome (Angulo et al., 2015). The “thrifty” gene hypothesis argues that “the evolutionary selection pressure for genes keeping body weight or adiposity to a minimum relaxed when humans invented weapons and fire about 2 million years ago and thus were no longer threatened by predators, with the consequence of a random drift of genes allowing increased adiposity” (Speakman, 2008).

The early origins of adult disease hypothesis suggest that obesity can develop in offspring from mothers exposed to metabolic hardship such as under-nutrition, obesity, and diabetes (Barker, 2004). Early-life metabolic programming occurs through epigenetic gene modifications (Cordero et al., 2015). Importantly, such epigenetically determined increased risk for adult obesity can be passed on to future generations leading to a further increase in the obesity epidemic. Consequently, finding the tools and therapies to break the vicious circle of epigenetic programming is an important target of obesity research. Due to “the disproportionally high expression of obesity-associated genes and epigenetic modifications in the central nervous system, it is highly likely that obesity genes act, not only within the hypothalamic homeostatic regulator of energy balance but also within neural circuits that are involved in interactions with an obesogenic environment, including circuits underlying reward-based decision making, learning and memory, delayed discounting, and spatial orientation” (Kishore et al., 2018).
2.1.4.2 Dyslipidaemia

Though, there is an uncountable number of vascular insults to the vascular system and blood vessels, the vascular endothelium, vascular and cardiac smooth muscle can only respond in three specific ways (inflammation, oxidative stress and vascular immune-dysfunction) to these insults. Endothelial dysfunction (ED) in vascular smooth muscle and cardiac dysfunction are the results of these pathophysiologic processes. The vascular outcomes include cardiovascular diseases (CVDs), coronary heart disease (CHD), myocardial infarction (MI) and cerebrovascular accidents (CVA) (Houston, 2010; Tian et al., 2010; Ungvari et al., 2010).

According to Houston (2012), “Genetics, epigenetics, chronic inflammatory micro and macro-nutrient intake, obesity (visceral obesity), chronic infections, toxins and some specific pharmacological agents including some of the older β-blockers and the thiazide or thiazide-like diuretics, tobacco products, DM and lack of exercise contribute to dyslipidaemia”. Several genetic phenotypes, such as apolipoproteins-E (apoE), result in variable serum lipid responses to diet, as well as contributing to CHD and MI risks (Plourde et al., 2009). The sortilin-1 allele variants on chromosome 1p13 increase LDL and CHD risk by 29% (Calkin et al., 2010).

Insight into pathophysiologic steps that result in dyslipidemia-induced vascular damage is required in order to treat this condition in a logical and innovative way (Fig. 2.2). Recent studies suggest that increasing dietary cholesterol intake will not significantly alter serum total or LDL cholesterol levels or CHD risk; some saturated fats, depending on their carbon chain length, may have minimal influences on serum lipids and CHD risk.
Figure 2.2: The various steps in the uptake of LDL cholesterol, modification, macrophage ingestion with scavenger receptors, foam cell formation, oxidative stress, inflammation and autoimmune cytokines and chemokine production (Source: Houston, 2014).

whereas monounsaturated and polyunsaturated fats have a favourable influence on serum lipids and CHD risk. Increased refined carbohydrate intake may be more important in changing serum lipids and lipid sub-fractions than saturated fats and cholesterol. Refined carbohydrates have more adverse effects on insulin resistance, atherogenic LDL, small dense LDL, LDL particle number (LDL-P), VLDL, triglycerides (TG), total HDL, HDL sub-fractions and HDL particle number, thus contributing to CHD risk more than saturated fats (Houston et al., 2009; Djousse and Caziano, 2009; Siri-Tarino et al., 2010).
2.2 FACTORS INFLUENCING OBESITY AND DYSLIPIDAEMIA

2.2.1 Genetic

2.2.1.1 Obesity

Although the ultimate cause for obesity is as a result of energy intake compared to energy utilization, obesity is also the result of an interplay between genetic and environmental factors (Albuquerque et al., 2015). Epidemiological studies have repeatedly indicated that certain diets and lifestyles enhance the risk of obesity among adults genetically at high risk. For example, consistent evidence has shown that sugar-sweetened beverages (Brunkwall et al., 2016; Olsen et al., 2016), fried foods (Qi et al., 2014), physical activity and sedentary lifestyles (Tyrrell et al., 2017) are associated with genetic variants in the cause of obesity. Similar findings were observed in a study of Swedish adults; a stronger association was seen between sugar-sweetened beverages with BMI in people with a genetic predisposition to obesity (Brunkwall et al., 2016). Consumption of fried food interacts with a genetic background in relation to obesity emphasizing the need for lowering the consumption of fried food among individuals with a genetic predisposition to obesity (Qi et al., 2014). The FTO genotype shows the strongest interaction among all obesity predisposing variants (Qi et al., 2014).

The European ancestry investigated “a composite score representing healthy diet (which was calculated based on self-reported intakes of whole grains, fish, fruits, vegetables, nuts/seeds and red/processed meats, sweets, sugar-sweetened beverages and fried potatoes) and modified associations of their genetic variants associated with obesity using GRSs based on 32 BMI and 14 waist to hip ratio (WHR) associated single nucleotide polymorphisms (SNPs)” (Nettleton et al., 2015). Their results indicated that correlations between genetic predisposition and obesity traits were higher among individuals who had
healthier diet scores (Nettleton et al., 2015). A recent study of the United Kingdom (UK) Biobank also gave comparable outcomes and the effect of genetic risk of obesity on BMI was stronger for people who watched TV for at least four hours daily compared with those who watched TV for three hours or less (Tyrrell et al., 2017). The UK Biobank study also reported that “associations of genetic predisposition and measures of adiposity (such as BMI and waist circumference) were modified by a variety of sleep characteristics including sleep duration, chronotype, day napping, shift work, and night-shift work” (Celis-Morales et al., 2017). Their results indicated that the relation of genetic risk and adiposity was increased by adverse sleeping characteristics.

2.2.1.2 Dyslipidaemia

According to the Mendelian randomization studies, there is a causal link between lipid profile and CHD (Voight, 2012). The pleiotropic effects of genetic mutants or variants affecting dyslipidaemia and hyperglycaemia appear to play a role, hence the nature for the relationship is not well understood. Undeniably, a few rare genetic mutations involving ABCA1, LIPE, LPL, or LRP6 (Mani et al., 2007) form the basis of both dyslipidaemia and hyperglycaemia. A study of 4,052 “subjects who have personal or family history of stroke and coronary heart disease, or family history of dyslipidaemia had higher prevalence of dyslipidaemia than those without any history of these conditions and participants with family history of stroke were more likely to suffer from high low-density lipoprotein cholesterol compared to those without family history of stroke. Whereas, participants with a family history of coronary heart disease or dyslipidaemia had increased prevalence of elevated total cholesterol and triglyceride as against those without any history of these conditions (Zhang et al., 2017).
2.2.2 Socio-demographic and Environmental

2.2.2.1 Age

2.2.2.1.1 Obesity

Ageing of the population and the rise in obesity are among the major public health concerns in developed countries. Demographic trends of the older and obese population have increased at exceptional rates (Kaeberlein et al., 2015). Each of these trends (ageing and obesity) has important effects on body composition, functional disability, morbidity, and mortality (Fabbri et al., 2015). The body shape changes at the mid-section along with age and this is in relation to the deposition of fats in the abdomen. This is as a result of decreased height and loosening of the abdominal wall muscle strength (Mogre et al., 2012).

An ageing-dependent increase in fat mass (obesity) is associated with the onset of other diseases, such as metabolic syndrome. It represents a cluster of risk factors, including insulin resistance (IR), dyslipidaemia and hypertension that altogether result in higher risk of type 2 diabetes mellitus (T2DM), cardiovascular disorders, in addition to sleep apnoea, arthritis, and some cancer types (Allott & Hursting, 2015; O’Neill & O’Driscoll, 2015).

2.2.2.1.2 Dyslipidaemia

According to a Korean study, among males and females of an average age of 54.1 years, average TC, LDL-C, and HDL-C levels were higher in females than in males, whereas the average TG levels were higher in males than in females (Jongseok et al., 2017). Prevalence of dyslipidaemia was also higher among the female population of 60 years through to 70 years age groups (Jongseok et al., 2017).
Lipid-lowering medication used by many Americans is related to age (Gu et al., 2014). In a study by Zhang et al. (2017), dyslipidaemia first increased, and then decreased with age and the peak prevalence appeared in the 60 - 69 years age group and the same trend occurred in high TC measurements.

2.2.2.2 Diet

2.2.2.2.1 Obesity

Several studies conducted suggests that eating at the right or wrong time, restricting eating hours, time allocation for meals, timing of macronutrient consumption during the day and even variety of the diet may also have an important role in total energy intake and therefore in the regulation of adiposity and body weight (Johnston, 2014; Garaulet & Gómez-Abellán, 2014; Vadiveloo & Parekh, 2015). It has been observed that characteristics of dietary behaviour such as skipping breakfast (Watanabe et al., 2014), eating more of the day’s total energy intake during the evening (Wang et al., 2014), higher frequency of meals eaten away from home (Bes-Rastrollo et al., 2010), and, higher eating and snack frequency (Murakami & Livingstone, 2015) are associated with a higher risk of being overweight/obese or having adverse metabolic consequences (Hermengildo et al., 2016). In addition, the main contributor to obesity in older adults is not likely to be increased energy intake, but rather reduced energy expenditure (Mathus-Vliegen, 2012). Sodium intake stimulates thirst and appetite, and subsequently increases energy intake and extracellular volume (Libuda et al., 2012).

Prevention of obesity is most of the time wrongly depicted simply as a bookkeeping issue; intake of calories must be adjusted to the expenditure of the calories (Levine, 2017).
Based on this “calories in, calories out” model, treatment of obesity is reduced to simply advising people to just eat less and be more mobile, thereby shifting the calorie balance scale and thus leading to a steady weight loss that accrues according to the popular but flawed 3500 kcal per pound rule (Guth, 2014; Hall & Chow, 2013). Consequently, when an individual fails to experience a significant weight loss it implies that an individual does not possess strong determination to stick to a modest lifestyle intervention over a reasonable time period. This simplistic view, however, is wrong because it considers “energy intake and expenditure to be independent parameters that can be adjusted at will and thereafter remain static without being influenced by homeostatic signals related to weight loss” (Hall & Chow, 2013). Energy intake and expenditure are interdependent variables that are dynamically influenced by each other and body weight (Hall et al., 2012). Attempts to change energy balance through diet or exercise are opposed by physiological adaptations that resist weight loss (Ochner et al., 2015).

2.2.2.2 Dyslipidaemia

A relationship between adoption of a healthy diet and a decreased risk of CVD in a cohort study has been found (Hlebowicz et al., 2013), likewise, a balance of lipid profile when subjects consumed a low-fat diet (Schwingshackl, 2013). However, high consumption of carbohydrate than fat resulted in high HDL-cholesterol (Yanai, 2015).

A 2018 study done in India showed diet is an independent variable for causing metS (Katip, 2018). Other studies observed that lipid profiling is related to changes in the number of meals consumed in a day or irregular eating habits (Erem et al., 2008; Sierra-Johnson et al., 2008).
The dietary pattern has been shown to be associated with dyslipidaemia (Na et al., 2015; Asghari et al., 2016). A Mediterranean diet characterized by high intake of vegetables, fruits, whole grains, fish, legumes, nuts, and olive oil was positively associated with high-density lipoprotein cholesterol (HDL-C) in a Spanish population aged 40–55 years (Penalvo et al., 2015). Another study regarding the Dietary Approaches to Stop Hypertension (DASH) diet, which is high in whole grains, vegetables, fruits, legumes, and low-fat dairy products, reduced low-density lipoprotein cholesterol (LDL-C), and total cholesterol compared with a control diet in healthy adults aged >21 years (Chiu et al., 2016). A study of Northern Chinese adults aged 20–74 years showed that a snacking pattern with high intake of biscuits, fried chips, liquid beverages, sweets, and ice cream was positively associated with serum total cholesterol, LDL-C, and triglyceride (TG) (Na et al., 2015).

2.2.2.3 Sedentary Lifestyle

2.2.2.3.1 Obesity

According to some studies, the time spent sitting is an independent risk factor for the development of obesity, T2DM and CV disease (Cochrane et al., 2017; Wilmot et al., 2012). Results from a meta-analysis showed sedentary behaviour to be associated with a “significantly increased risk of developing diabetes (112%) and CV disease (147%), as well as an increased risk of CV mortality (90%) and all-cause mortality (49%)” (Wilmot et al., 2012). Thus, total energy expenditure is significantly influenced by the time the individual spends sitting, and sedentary behaviour may negatively influence energy balance and long-term body composition. Moreover, time spent watching TV has been shown to be linked with more food consumption and increased weight gain (Saeidifard et al., 2018). In a meta-analysis, Saeidifard et al. (2018) analysed “the difference in energy
expenditure while sitting versus standing and revealed a significantly higher energy expenditure (mean difference of 0.15 kcal/minute, 95% confidence interval [CI] 0.12–0.17) while standing”. In women, the difference was lower (0.1 kcal/minute) than in men (0.19 kcal/minute). The authors concluded that “substitution of sitting with standing could be a potential solution to prevent weight gain in the long term”.

2.2.2.3.2 Dyslipidaemia

Physical inactivity (PI) has without question become one of the main (CV) risk factors and has been linked to a 20 - 30% rise in relative risk for early all-cause mortality. PI is accountable for 3.2 million deaths globally each year (WHO, 2014; Lim et al., 2012). In addition, PI is also a major factor in overweight and obesity development and their associated diseases (Shiroma et al., 2012; Lee et al., 2012). The occasions for inactive behaviours e.g. watching television or using electronic digital devices are ever-present in our daily lives. Added to this are the technological advancements that have led to automation in the several workplaces further reducing most forms of jobs to mainly sedentary activities. Across 32 European countries, the average self-reported sitting time ranged from 3.2 - 6.8 hours per day (Bennie et al., 2013). The outcome of a recent study using tri-axial accelerometers to objectively measure sedentary time confirmed an association of sedentary time with CV risk, independent of individual physical activity levels. In contrast, higher numbers of less than 5 minutes standing bouts were significantly associated with a lower CV risk (Vasankari et al., 2017).
2.2.2.4 Physical Activity

2.2.2.4.1 Obesity

There is a growing body of evidence documenting the positive health impacts of physical activity at all ages (American Physical Activity Guidelines, 2017). The health benefits of exercise training are well-established (Pedersen & Saltin, 2015). High levels of moderate-to-vigorous physical activity are strongly associated with successful long-term weight loss maintenance (Swift et al., 2014) and current guidelines recommend 300 minutes/week of moderate intensity (or 150 minutes/week of vigorous intensity) to prevent weight gain and sustain weight loss (ACSM’s Guidelines for Exercise Testing and Prescription, 2014). The classic United States Department of Health and Human Services Physical Activity Guidelines for Americans recognizes the continued health benefits of regular physical activity among the rapidly growing older population (Federal Interagency Forum on Aging-Related Statistics, 2016). A sustained increase of physical activity is associated with a substantial reduction of obesity and, furthermore, increases life expectancy and life quality globally. In fact, national physical activity guidelines are typically the same for adults and older adults who have no limiting chronic conditions (Lee et al., 2012).

The exact amount and type of physical activity, appropriate for different persons with specific conditions, remains a debate (Whitehead & Blaxton, 2017). However, from the guidelines, inactivity must be avoided as much as possible at any time. For older adults, at least 150 min of weekly moderate-intensity physical activity (e.g. brisk walking) is recommended. Furthermore, recommendations to engage in a vigorous-intensity activity (e.g., jogging, swimming laps) are irrespective of age. The guidelines recognize special circumstances relevant to some older adults and include suggestions that older adults
should be as physically active as their abilities and conditions allow (American Physical Activity Guidelines, 2017).

2.2.2.4.2 Dyslipidaemia

Achieving a normal BMI through physical training (Lee et al., 2010; Friedenreich et al., 2011) leads to an improvement in lipid profile (Kraus, 2009). In addition, mortality and morbidity are reduced even in obese adults (Moore et al., 2012). There is a balance of lipid profile in physical training even when there is no weight loss hence this can be used to predict the protective effect of exercise over mortality in obese persons (Zaros et al., 2009; Balducci et al., 2010). The response of the lipid profile to physical exercise could be mediated by its chronic effect over inflammatory and anti-inflammatory agents related to insulin resistance (Balducci, 2010; Teixeira, 2011).

Despite this benefit to serum lipids, there is an absence of widely accepted parameters (e.g. intensity, volume and time) to the prescription of exercise interventions targeting dyslipidaemia (Kraus, 2009). Physical activity practice for long periods of time prevents dyslipidaemia (Fernandes, 2011; Lima et al., 2014). Adults who are physically active and were engaged in sporting activities in their youthful days have reduced the probability to report dyslipidaemia, independent of weight and age (Fernandes, 2011). Overweight or obesity and lack of exercise were also associated with poor dyslipidaemia control (Zhang et al., 2017).
2.2.2.5 Alcohol Consumption

2.2.2.5.1 Obesity

Ten per cent of the calorie intake among adults who drink can be attributed to alcohol (Gatineau et al., 2012). Alcohol provides 7 kcal/g which is close to 9 kcal/g fat (the highest energy dense macronutrient) hence alcohol can increase the amount of energy consumed in a day leading to obesity (Gatineau et al., 2012). Though the energy content of alcohol is mostly not considered when assessing daily calorie consumption, it has an association with weight gain when consumed in large quantities (Gatineau et al., 2012).

2.2.2.5.2 Dyslipidaemia

It has been acknowledged that chronic and excessive alcohol intake and obesity are associated with the incidence of many illnesses, including CHD, fatty liver and other chronic diseases (Xie et al., 2010; Xu et al., 2011; Strandberg, 2012). However, these diseases are all linked to plasma lipid levels. Feinman et al. in 2012 believed that alcohol consumption has an influence on lipid metabolism and therefore body weight and composition are affected. This may result in hyperlipidaemia and further to atherosclerosis.

2.3 CONSEQUENCES OF OBESITY AND DYSLIPIDAEMIA

The risk associated with obesity is not just about the amount of fat but also about fat distribution that determines the risk associated with cardiovascular risk factors and MetS. Abdominal or visceral fat or central obesity is associated with increased incidence of cardiovascular risk factors. Morbidity includes T2DM, impaired glucose tolerance, hypertension and dyslipidaemia (Musunuru, 2010). Obesity, in addition, is associated
with CVD’s and cancer (which is often overlooked), places a burden on health care cost (Colditz and Peterson, 2018) and has broader economic impacts (Cawley & Wen, 2018).

Dyslipidaemia is a risk factor for CHD, including myocardial infarction and stroke (Feigin et al., 2016). According to multiple global estimates, elevated TC remains a significant risk factor for overall disability and premature death (Murray et al., 2015). Lipid metabolic changes and obesity are both strong and intensive risk factors for developing complications, first of all, microvascular ones (Shin et al., 2013; Seo et al., 2012). Therefore, it is very important to properly correct metabolism disorders of lipoprotein and obesity (Shin et al., 2013; Choi & Ginsberg, 2011).

2.4 TREATMENT AND PREVENTION OF OBESITY AND DYSLIPIDAEMIA

It is important to recognize that obesity presents a complex set of challenges and are not expected to be amenable to simple solutions. Responses to obesity should not only consider immediate, short-term actions but also establish a long-term vision. These should involve much more than a mere headline prevalence figure and include the elimination of social and other inequalities, as well as put a strong focus on environmental drivers of diet and physical activity. The needs of people with severe obesity, who may benefit greatly from surgical or other treatments should all the same not be forgotten and adequate provision should be made for this within health services (Rutter, 2018).

2.4.1 Dietary Change and Physical Activity

In relation to treatment, there are reviews that focus on dietary change (Cawley & Wen, 2018), and physical activity (Jakicic et al., 2018; Strath & Rowley, 2018). The evolving global epidemic of obesity may well take another 10 to 20 years truly to reverse in high-
income countries and will require concerted efforts by low- and middle-income countries. Although no country has yet succeeded at reversing the epidemic, there are important and encouraging signs of progress. As understanding of the problem develops, it is becoming increasingly apparent that obesity is not, fundamentally, a problem of individuals making poor decisions but one which everyone faces in an environment of abundance, convenience, and choice in which many people need to apply appreciable effort to maintain a healthy weight. Effective responses to obesity over the coming years will thus place increasing emphasis on changing the environments in which people live to make it easier, and more appealing, to consume a healthy diet and engage in regular physical activity (Rutter, 2018).

2.4.2 Surgical Approach: Bariatric Surgery

Surgical approaches, such as bariatric surgery, produce significant weight loss and ameliorate associated cardiovascular complications and T2DM in morbidly obese patients (Abdeen & le Roux, 2016; Sinclair et al., 2018). However, surgical procedures are invasive, expensive and have their own inherent risks and side-effects, including increased alcohol use, which suggests that these procedures facilitate an addiction transfer or exchange of palatable food reinforcers for an alternate reinforcer such as alcohol (Lent et al., 2013).

2.4.3 Public Health Policies

Policy responses to obesity should not only address short-term treatment but also long-term prevention, with the most important impacts of actions, especially those to tackle childhood obesity, which may manifest themselves for many decades (Rutter, 2018).
In addition, policy responses and other interventions should take adequate account of the complex nature of the problem, acting at different levels of the system, and in multiple domains over time (Johnston et al., 2014). This will entail actions that move beyond direct effects on individuals to reshaping the system itself, such as reconfiguring agricultural subsidies to promote the production of healthy foods or prioritizing compact urban development that minimizes motorized transport and encourages active mobility (Rutter, 2018). Feedback within the system can be addressed by changing pricing signals through taxes or subsidies, which may go well beyond directly influencing the cost of products bought by consumers (Rutter, 2018). Also, several reviews that focus on the management of obesity, including an overview of the impact of public health policies to prevent obesity and promote dietary change (Cawley & Wen, 2018). Actions should also reflect the chronic nature of the condition much more than they tend to now. Short term impacts, such as those from intensive weight management interventions, are helpful, but the biggest impacts will result from changes that persist over the long term, resulting in a lower burden of excess weight over the life course at both the individual and the population level (Rutter, 2018).

2.4.4 Pharmacotherapy

Since lifestyle interventions and bariatric surgery for obesity treatment have limitations, other options such as pharmaco-therapeutic approaches are important (Gadde et al., 2018). During the past two decades, many anti-obesity drugs have been discovered, marketed and afterwards withdrawn from the market. Although these obesity therapeutics were efficacious at the onset of treatment, long term use was accompanied by adverse side-effects. However, these have not prevented the search for new, effective and safe
therapeutic interventions as obesity and its associated health issues have continued to increase (Narayanaswami & Dwoskin, 2017).

Weight management through pharmacotherapy should be viewed as supporting lifestyle intervention rather than a replacement. Drug therapy acts physiologically by modifying energy regulation, often by lowering appetite, creating a sense of fullness with a smaller meal, and an extended period of fullness after the small meal. Naturally, all medications have their risks and side effects and thus, weighing the risks and benefits of therapy with the risks and severity of the disease, needs to be balanced (Lee & Dixon, 2017). Understanding of the several weight-loss medications available, indications, contraindications and limitations in specific patient populations allows general practitioners (GPs) to decide if, and which, pharmacotherapy is appropriate when managing patients with obesity. However, the use of these medications should be terminated if there are safety or tolerability concerns, or if clinically meaningful weight loss (>5%) is not attained. On the other hand, if satisfactory weight loss is attained and no tolerability concerns arise for the medication, continued use remains an option since medications may support lifestyle management and play a useful role in the management of obesity in the long-term (Lee & Dixon, 2017).

Among dyslipidaemia patients using lipid-lowering drugs, participants with regular exercise were more likely to have their dyslipidaemia controlled than those who were not exercising. Additionally, obesity also seems to be associated with the rising prevalence of dyslipidaemia, as several studies have suggested a positive correlation between BMI and dyslipidaemia. Moreover, since obesity is concomitant with adipose tissue dysfunction, ectopic fat accumulation, especially in the liver, and inflammation, it favours
the development of dyslipidaemia (Dias et al., 2018). Because of the increased risk of CVD with comorbidities, guidelines have recommended simultaneous treatment of dyslipidaemia (Mancia et al., 2013). In response, pharmacological treatments for weight loss and balancing of lipid profile have become common. Orlistat (Xenical) which works by reducing the absorption of dietary fat, is approved for long-term use and therefore accessible (Dias et al., 2018), as also is Sibutramine, a serotonin and noradrenergic reuptake inhibitor. Medications including statins such as atorvastatin, fluvastatin, lovastatin, pravastatin, pitavastatin, and simvastatin, fibrates, such as gemfibrozil and fenofibrate and niacin have been developed for controlling lipid profiles (Kobayashi et al., 2008; Wat et al., 2016).

2.5 MS01 METABOLIC PILL

The MS01 dietary supplement of interest in this study is a combination of nutraceuticals and has been developed to regulate metabolism. It contains a proprietary ingredient(s): alpha lipoic acid and, ginseng, citrulline (citrulline malate), leucine, riboceine (D-ribose-L-cysteine), and pantetheine. Also, the dietary supplement has vitamin D2, thiamine, vitamin B6, vitamin B12, and potassium.

2.5.1 Alpha Lipoic Acid (ALA)

ALA is a naturally occurring antioxidant compound that can be found in some entrails and in vegetables such as spinach, broccoli, and tomatoes (Fernández-Galilea et al., 2013). ALA has been established in many studies as a protector against oxidative agents and damaged cells which is described in diabetes mellitus patients (Gupta et al., 2013), and positively influences the regulation of glucose (Morakinyo et al., 2013), and reduces blood lipids (TC, LDL, and TG) (Kandeil et al., 2011). It has been shown that ALA
significantly reduces body weight gain in humans (Carrier & Rideout, 2013; Ratliff et al., 2013). In addition, ALA has been shown to be effective in reducing symptoms of diabetic polyneuropathy without serious adverse effects (Han et al., 2012; Xu et al., 2013). The potential health implications of ALA have been investigated in clinical practice in countries such as Germany and Korea (Okanović et al., 2015), with multi-centric trials currently ongoing in Europe and North America (Okanović et al., 2015). These studies have included products containing ALA in a very wide range of doses, ranging from 50-1800 mg/day (Carrier & Rideout, 2013; Cicek et al., 2013). ALA supplementation for 12 months significantly decreased serum levels of common markers of inflammation in ablated patients (Sardu et al., 2017). Furthermore, dietary supplementation with ALA for 10 weeks significantly improved systemic inflammation and cardiovascular disease-related risk factors in healthy overweight women (Huerta et al., 2016). Experimental and pathological studies suggest that ALA supplementation plays an essential role in mitochondrial bio-energetic reactions (Gomes & Negrato, 2014; Koufaki, 2014). Also, ALA supplementation in another study led to significant weight reduction while maintaining normal lipid profile of the rats (Seo et al., 2012). In one human study, it was observed that ALA caused significant weight loss in obese subjects and this led to the conclusion it may be effective as an adjunct therapy for managing obesity (Koh et al., 2011).

2.5.2 Ginseng

Ginseng is considered a tonic and a panacea in traditional Chinese medicine and has been used to treat many diseases for over 2000 years in Asian countries (Kakisaka et al., 2012). It is believed that ginseng can improve health and increase metabolism. The physiologic and pharmacologic functions of ginseng have been discovered gradually, and include
promotion of haematopoiesis, modulation of immune functions, anticancer activity, protection against circulatory shock, and regulation of cellular metabolic processes. It has also been reported that ginseng improves hyperglycaemia in animal and human studies. In addition, ginseng extracts have anti-obesity and anti-hyperglycemic activities in obese animal models. Ginseng has been widely studied as an alternative medicine for T2D treatment (Li et al., 2018).

The bioactive compounds of ginseng include polyacetylenes, phenolics, polysaccharides and various ginsenosides (Lee et al., 2012). Ginsenosides are believed to be the main bioactive fraction of ginseng and are classified into two groups via the aglycones structures: 20(S)-protopanaxatriol (ginsenosides Re, Rg1, Rg2, Rh1) and 20(S)-protopanaxadiol (ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Rg3). Currently, almost all ginsenosides have been reported to display several bioactivities including anti-ageing, neuroprotective, anti-cancer, radioprotective, anti-amnestic, and anti-diabetic effects (Li et al., 2018).

2.5.3 Citrulline
The health properties of watermelon have earned it a recommendation by the American Heart Association as a heart-healthy food. In addition to lycopene, watermelon is the cucurbit crop containing the highest concentration of L-citrulline (citrulline). Citrulline is a non-proteinaceous, non-essential physiologically active amino acid and has relevance in mammalian metabolism. It is an intermediate metabolite that has sparked much human health research in the past forty years. This spike of citrulline in human-health with a consumer desire for functional foods has pointed scientists to watermelons as a natural source of citrulline (Perkins-Veazie et al., 2012).
Citrulline is also found in most cucurbits, including bitter melon, cucumber, muskmelon, pumpkin, bottle gourd, dishrag gourd, and wax gourd (Hartmen et al., 2018). Because of the health benefits of citrulline (and lycopene, the red-pigmented carotenoid), and because they can be obtained from a natural food source, watermelon offers a natural and cheap alternative to citrulline health supplements, which are currently produced by bacterial fermentation (mutant B. subtilis auxotrophs) (Fish, 2014).

Citrulline has been administered orally in the form of citrulline-malate and was found to affect many areas of human health, including skeletal and muscle performance (Perez-Guisado & Jakeman 2010), diabetes (Wu et al., 2007), pharmacology (Thibault et al., 2011), immunology (Sureda et al., 2009), and neurology (Sase et al., 2013). As a member of the L-arginine metabolic family, roles of citrulline include aiding in muscle recovery during exercise (Tarazona-Diaz et al., 2013), and benefiting vascular health, such as improving blood pressure (Figueroa, 2011), and increasing vasodilation in many tissues of the body.

2.5.4 Leucine

Dietary proteins strongly influence metabolic health through their effect on appetite, weight gain, and adiposity (Gosby et al., 2014). Levels of the branched-chain amino acid L-leucine (Leucine) are likely to represent a physiological signal of protein availability in the control of appetite and metabolism. In humans and rodents, circulating leucine levels rapidly increase following the ingestion of a protein dense meal (Luiking et al., 2016). As a functional amino acid, leucine may regulate many important physiological functions, including protein and energy metabolism, cell proliferation and apoptosis, in
different tissues and cells (Mao et al., 2013; Xiao et al., 2016; Toneto et al., 2016). In mammalian tissues and cells, “leucine not only acts as the supply of substrate and energy for functional protein synthesis, it also regulates some physiological functions by affecting intracellular signalling pathways such as mammalian target of rapamycin (mTOR), adenosine 50-monophosphate-activated protein kinase (AMPK) and extracellular regulated protein kinases (ERK) signalling pathway” (Zhang et al., 2014; Ren et al., 2016; Dodd & Tee, 2012). Previous studies have shown that leucine supplementation regulates energy metabolism in human duodenal mucosa, and leucine deprivation affects the phosphorylation of translation-relative factors in muscle cells (Goichon et al., 2013).

2.5.5 Riboceine

Riboceine is a unique molecule that combines ribose and cysteine. Riboceine once ingested will be absorbed, enter the bloodstream and deliver cysteine and ribose to the cells supporting glutathione production as well as providing ribose, an integral part of ATP. Riboceine significantly outperformed other means of glutathione enhancement (Gehlot & Goyal, 2007). Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system’s ability to readily detoxify the reactive intermediates or to repair the resulting damage, thus, causing disruptions in normal mechanisms of cellular signalling and cell death (Chandra et al., 2015). One of the biological systems (antioxidants) that detoxifies reactive oxygen species or that repairs the resulting damages caused by free radicals is glutathione. Sometimes these free radicals overpower the biological systems, thus, the body may need external supplements to complement the production of antioxidants (Chandra et al., 2015). Riboceine is one of the synthetic antioxidants that help cells produce glutathione
on-demand. The active ingredient of riboceine is D-Ribose-L-Cysteine. Whole glutathione consumption cannot be effective because it would be destroyed in the digestion process before reaching the cell. The ribose component of the Riboceine solves these challenges by effectively protecting and delivering the fragile cysteine molecule, enabling the cells to produce glutathione when the cells need it most (Falana et al., 2017).

2.5.6 Pantetheine

The mechanism of action of pantetheine in its role as a lipid-lowering agent is yet to be clarified. It has been postulated that pantetheine acts by inhibiting the enzymes acetyl-CoA carboxylase and HMG-CoA reductase, thus modifying lipoprotein metabolism (Evans et al., 2014). Pantetheine is a disulphide derivative of pantothenic acid and precursor of coenzyme A (CoA). Cytoplasmic CoA stimulates oxidation of acetate at the expense of fatty acids and cholesterol synthesis and CoA increases Krebs cycle activity, hence, reducing cholesterol and fatty acid synthesis from acetate. Fatty acid and cholesterol synthesis are decreased by 50% and 80%, respectively. Pantetheine increases arterial cholesteryl esterase activity leading to the removal of arterial cholesterol esters and reduces fatty streak formation and intimal, endothelial thickening, lipid deposition, LDL peroxidation and endothelial dysfunction (Kota et al., 2013).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN
An independent samples design was used. The animals were assigned into equally sized groups and each group received a different treatment. Each group received only one treatment, this prevented treatment cross-contamination between the groups. Comparison between the groups was then made.

3.2 EXPERIMENTAL DESIGN

3.2.1 Animals and Experimental Groups
Twenty-four male Sprague Dawley rats were used in this study. The animals were acquired from the Animal House, Centre for Plant Medicine Research (CPMR), Mampong-Akwapim, Eastern Region, Ghana. The rats were age-matched (4 weeks old) and randomly divided into 4 groups (2 control groups and 2 treatment groups) of 6 animals each. Animal models were used because this is the first time the dietary supplement is being tested in Ghana and results from the animal study may lead to experimental trials on human subjects for a further study on the effectiveness of the dietary supplement.

The groups were set up as included below.

Control groups:
1. Group 1 - Normal control diet (NCD) - negative diet control.
2. Group 2 - High-fat diet (HFD) - negative treatment control.

Treatment groups (dietary supplement i.e. MS01 administered):
3. Group 3 - High-fat diet + 4 mg/ml (low dose) per kg body weight of rat of the MS01 dietary supplement (MS01/L).

4. Group 4 - High-fat diet + 8 mg/ml (high dose) per kg body weight of rat of the MS01 dietary supplement (MS01/H).

The rats were coded and housed individually in single stainless-steel cages with softwood shavings as bedding. Each rat was fed with standard commercial pellet (AGRIMAT, Accra), and given water *ad libitum* in a controlled environment (ambient temperature of 25 ± 1 °C, relative humidity 60 - 70%, and 12- hour light-dark cycle) at the Research Laboratory, Department of Pharmacology and Toxicology, University of Ghana. The rats were made to acclimatize with laboratory conditions for 7 days prior to the experiment. The study was conducted over a period of 30 days during which the body weights of the animals were taken at the beginning of the study then followed with weight measurements once a week on every Monday of the study period. All the 6 rats from each group were sacrificed after the 30 - day period. Prior to the sacrifice, enough blood was taken from each rat for biochemical analysis.

### 3.2.2 Diet

After the 7 days acclimatization period, the rats were provided with feed (Tables 3.1 and 3.2) (15 g/rat/day) and any leftover from each day weighed to determine the amount consumed. All experimental diets (for groups 1, 2, 3 and 4) were prepared to have the same baseline composition but diets for groups 2, 3 and 4 were of high caloric value in the form of vegetable oil fortification (Ainuson, 2013). The caloric content of the HFD (Table 3.3) was considered due to the brief duration (30 days) of the study compared to other studies, including a study done within 4 - 12 weeks (Kamisah *et al*., 2006).
The feeds were formulated based on what is required as recommended by the International Laboratory Rodent Diet (5001* Laboratory Rodent Diet, 2005 Appendix-A). During this period, weights of the rats were taken weekly.
Table 3.1: Composition of the diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Normal diet (NCD)</th>
<th>High fat diet (HFD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ distilled water</td>
<td>+ MS01/L 4 mg/kg</td>
</tr>
<tr>
<td>Soya bean</td>
<td>11.40</td>
<td>11.40</td>
</tr>
<tr>
<td>Maize</td>
<td>49.20</td>
<td>49.20</td>
</tr>
<tr>
<td>Groundnuts</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>12.80</td>
<td>12.80</td>
</tr>
<tr>
<td>Fish mill</td>
<td>10.50</td>
<td>10.50</td>
</tr>
<tr>
<td>Shrimp mill</td>
<td>6.10</td>
<td>6.10</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>-</td>
<td>12.05</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

*NCD = Normal control diet, HFD = High fat diet, MS01/L = High fat diet + 4 mg/kg MS01 dietary supplement, MS01/H = High fat diet + 8 mg/kg MS01 dietary supplement. Composition of diet for the different groups: fortified diet with vegetable oil for treatment groups and normal control diet for control group (Kamisah et al., 2006).
### Table 3. 2: Proximate analysis of feed

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Units</th>
<th>Results (NCD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>AOAC 925.10 (1990) 15th Editions</td>
<td>g/100 g</td>
<td>9.2</td>
</tr>
<tr>
<td>Ash</td>
<td>AOAC 923.03 (2000) 15th Editions</td>
<td>g/100 g</td>
<td>7.3</td>
</tr>
<tr>
<td>Fat</td>
<td>AOAC 920.39 (2000) 15th Editions</td>
<td>g/100 g</td>
<td>4.9</td>
</tr>
<tr>
<td>Protein</td>
<td>AOAC 984.13 (1990) 15th Editions</td>
<td>g/100 g</td>
<td>24.5</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>By difference</td>
<td>g/100 g</td>
<td>54.1</td>
</tr>
<tr>
<td>Energy</td>
<td>At water factor</td>
<td>Kcal/100 g</td>
<td>2,024</td>
</tr>
<tr>
<td>Calcium</td>
<td>Permanganate Titration</td>
<td>mg/100 g</td>
<td>1210.7</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Molybdenum Blue Calorimetric</td>
<td>mg/100 g</td>
<td>1010.2</td>
</tr>
</tbody>
</table>

*NCD = Normal control diet. The composition of diet for the different groups: fortified diet with vegetable oil for treatment groups and normal control diet for the control group (Kamisah et al., 2006).*
Table 3.3: Composition of formulated feed for rats to assess the effect of the MS01 dietary supplement on their weight gain and lipid profile.

<table>
<thead>
<tr>
<th>Ingredients (calories/100 g)</th>
<th>Normal diet (NCD) + distilled water</th>
<th>High fat diet (HFD) + MS01/L 4 mg/kg</th>
<th>+ MS01/H 8 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya bean</td>
<td>471</td>
<td>471</td>
<td>471</td>
</tr>
<tr>
<td>Maize</td>
<td>86</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>Groundnuts</td>
<td>585</td>
<td>585</td>
<td>585</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>339</td>
<td>339</td>
<td>339</td>
</tr>
<tr>
<td>Fish mill</td>
<td>290</td>
<td>290</td>
<td>290</td>
</tr>
<tr>
<td>Shrimp mill</td>
<td>253</td>
<td>253</td>
<td>253</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>-</td>
<td>884</td>
<td>884</td>
</tr>
<tr>
<td>Total calories</td>
<td>2,024</td>
<td>2,908</td>
<td>2,908</td>
</tr>
<tr>
<td>Total grams</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*NCD = Normal control diet, HFD = High fat diet, MS01/L = High fat diet + 4 mg/kg of the MS01 dietary supplement, MS01/H = High fat diet + 8 mg/kg of the MS01 dietary supplement. The composition of diet for the different groups: fortified diet with vegetable oil for treatment groups and normal control diet for the control group (Kamisah et al., 2006).
3.2.3 Blood Sample Collection

Baseline sera lipid measurements were not taken because groups 3 and 4 (treatment groups) were compared with group 1 (negative diet control) and group 2 (negative treatment control) to assess differences amongst groups after the treatment. Thus, only post-treatment sera lipid measurements were required.

After 30 days, the rats in each group were anaesthetized with chloroform and blood was drawn from the heart (cardiac puncture) by a chemical pathologist. The blood samples (4 ml) were collected into gel-separator tubes and allowed to clot at room temperature (20°C). The samples were centrifuged at 3000 rpm for 10 min to separate sera which were transferred into Eppendorf tubes, labelled and stored in a laboratory freezer at −80°C until analysed. The stored samples (sera) were thawed to room temperature of 20°C before assaying. Rats were later euthanized through a physical method of cervical dislocation.

3.3 SUPPLEMENT ADMINISTRATION

Animals to receive the MS01 dietary supplement (groups 3 and 4) fasted for 12 hours before the administration of the dietary supplement. The MS01 dietary supplement was poured out of the capsule, mixed evenly with distilled water, and administered to rats via oral gavage every morning between 8 am to 9 am for 30 days. The dietary supplement was given to treatment groups in two different doses: 4 mg/ml/kg as low dose and 8 mg/ml/kg as a high dose.

3.4 BIOCHEMICAL ANALYSIS

Sera lipid profile comprising of total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)
were analysed with an automated blood chemistry analyser machine (Mindray Bio-
Medical Electronics Bs-200e, Shenzhen, China).

3.5 RAT'S ORGAN COLLECTION
The rats were immediately euthanized after the experiment. The thoracic cage and abdominal cavity were dissected for the following organs; heart, kidney, and liver. These organs were rinsed with 0.9% NaCl solution (Seo et al., 2012), dried on filter paper and individually weighed with an electronic scale (LAB-KITS, 2010). The respective organs were stored for further studies. The organs were harvested in the presence of a veterinary technician.

3.6 STATISTICAL ANALYSIS
The results were presented as mean ± standard error of the mean (SEM) and means of continuous variables compared by paired t-tests with SPSS version 20. Two-way ANOVA followed by a Bonferroni’s post hoc test to monitor changes in weight among the groups along time-course of treatment and One-way ANOVA followed a Dunnett’s multiple comparison tests to assess the changes in weight. A level of p<0.05 was considered statistically significant.

3.7 ETHICAL ISSUES
Ethical clearance was obtained from the Ethics and Protocol Review Committee of the University of Ghana Medical School, Korle-Bu. This experiment was done adhering to protocol and maintaining quality assurance in concordance with good laboratory practice (GLP). All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (N.R.C., 1996).
CHAPTER FOUR

4.0 RESULTS

4.1 WEIGHT OF RATS OVER STUDY PERIOD

The changes in body weight of the Sprague Dawley (SD) rats after 30 days is presented in Figure 4.1 (A and B) and Table 4.1. Rats in group 2 (fed with HFD plus distilled water) showed a significant increase in weight (99.08% weight gain, p<0.001) compared to the rats fed with normal diet (Group 1) after the 30-day period. Rats administered MS01 showed a significant reduction in weight (84.87% and 84.83%, p<0.001 for MS01/L and MS01/H, respectively) compared to the rats fed with high-fat diet (group 2). However, the decrease in weight observed for rats administered with the dietary supplement (MS01) was not found to be dose-dependent (MS01/L vs MS01/H p>0.05). It is noteworthy, however, that there was no statistically significant difference (p>0.05) among groups with respect to feed intake over the period: 14.74 ± 5.60; 13.52±1.59; 13.17±1.59 and 12.72±1.86 for NCD, HFD (with distilled water), MS01/L and MS01/H groups respectively.
Figure 4.1: Changes in weight of rats during the 30-day period

(A) Time-course event following treatment of rats with NCD (normal control diet), HFD (high fat diet), HFD+ALA_LD (high fat diet and treated with low dose of alpha lipoic acid supplement) and HFD+ALA_HD (high fat diet and treated with a high dose of alpha lipoic acid) for a continuous 30 days duration (B) The total weight calculated as area under the curves (AUCs) from (A). Data are mean ± SEM (n=6). **P<0.01, ***P<0.001 compared to HFD (a two-way ANOVA followed by a Bonferroni’s post hoc test); +++P< 0.001 compared to HFD (a One-way ANOVA followed a Dunnett’s multiple comparison tests).
Table 4.1: Weight gain and total feed intake of the rats after 30 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal diet NCD + distilled water</th>
<th>High fat diet HFD + MS01/L 4 mg/kg</th>
<th>+ MS01/H 8 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial bodyweight (g)</td>
<td>34.55±7.90</td>
<td>33.98±6.90</td>
<td>33.85±2.90</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>68.05±7.20</td>
<td>62.82±14.10</td>
<td>62.43±5.05</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>33.50±7.20</td>
<td>28.84±14.10</td>
<td>28.58±5.05</td>
</tr>
<tr>
<td>% Weight gain</td>
<td>96.96%</td>
<td>84.87%</td>
<td>84.43%</td>
</tr>
<tr>
<td>Feed intake (g)</td>
<td>14.74 ± 5.60&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>13.52±1.59</td>
<td>13.17±1.59</td>
</tr>
</tbody>
</table>

Data presented in % is for weight gain among groups after 30 days. Data presented as mean ± SEM (n=6) is for weight gain, feed intake among groups after and during 30-day period respectively. NCD- normal diet; HFD- high-fat diet; MS01/L – HFD-fed rats treated with a low dose of the MS01 dietary supplement; MS01/H – HFD-fed rats treated with high dose of MS01 dietary supplement. *** p<0.001 compared to NCD; ¥¥¥ p<0.001 compared to HFD; NS - not significant.

4.2 CHANGES IN WEIGHT OF RAT ORGANS

The organs (heart, liver, kidneys (both left and right)) tissue weight of rats fed with the control high-fat diet only, showed an increase compared to the rats fed with the normal diet but was not statistically significant (p>0.05). Moreover, the organs (heart, liver, kidneys) tissue weight of rats treated with the high dose of the dietary supplement was noted significantly small compared to the rats treated with a low dose of the dietary supplement and the rats that fed the control high fat diet only. Despite the difference in means for tissue weight of rat organs (heart, liver and kidneys) both treated and untreated groups, the rat body weight to organ ratio showed no statistically significant difference (p>0.05) among all groups. A summary of this is shown in Table 4.2.
Table 4.2: Changes in weight of organs after the 30-day period

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Mean organ weight (g)</th>
<th>Mean body weight (g)</th>
<th>Organ/body ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>NCD</td>
<td>0.30±0.02</td>
<td>68.05±7.20</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>HFD</td>
<td>0.35 ±0.04</td>
<td>88.33±7.0</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>MS01/L</td>
<td>0.30±0.06</td>
<td>62.82±14.10</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>MS01/H</td>
<td>0.26 ± 0.02*</td>
<td>62.43±5.05</td>
<td>0.004^NS</td>
</tr>
<tr>
<td>Liver</td>
<td>NCD</td>
<td>2.12 ±0.1</td>
<td>68.05±7.20</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>HFD</td>
<td>2.72 ±0.4</td>
<td>88.33±7.0</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>MS01/L</td>
<td>1.93 ±0.3</td>
<td>62.82±14.10</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>MS01/H</td>
<td>1.88 ±0.1***</td>
<td>62.43±5.05</td>
<td>0.030^NS</td>
</tr>
<tr>
<td>Right kidney</td>
<td>NCD</td>
<td>0.27±0.03</td>
<td>68.05±7.20</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>HFD</td>
<td>0.32±0.04</td>
<td>88.33±7.0</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>MS01/L</td>
<td>0.26±0.05</td>
<td>62.82±14.10</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>MS01/H</td>
<td>0.25±0.02*</td>
<td>62.43±5.05</td>
<td>0.004^NS</td>
</tr>
<tr>
<td>Left kidney</td>
<td>NCD</td>
<td>0.28±0.04</td>
<td>68.05±7.20</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>HFD</td>
<td>0.33±0.07</td>
<td>88.33±7.0</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>MS01/L</td>
<td>0.24±0.04*</td>
<td>62.82±14.10</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>MS01/H</td>
<td>0.24±0.03*</td>
<td>62.43±5.05</td>
<td>0.003^NS</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. NCD (n=6) normal control diet; HFD: high-fat diet; MS01/L: high-fat diet and treated with a low dose of the MS01 dietary supplement; MS01/H: high-fat diet and treated with a high dose of the MS01 dietary supplement. *p<0.02 compared to HFD-fed rats only; ***p<0.001 compared to HFD-fed rats only; NS not significant among all the groups.

4.3 SERA LIPID CONCENTRATION

After the 30-day treatment period, serum total cholesterol, triglyceride and HDL-cholesterol was found to be significantly higher (p<0.05) in rats on HFD (with distilled water) compared to rats fed with the normal diet (NCD) respectively. Also, the serum concentration of triglyceride was significantly increased (p<0.01) in the rats treated with MS01/L compared to rats treated with MS01/H and control HFD rats. The effect of the dietary supplement on treated groups was found not to be dose-dependent (MS01/L vs MS01/H p>0.05). However, no statistical difference (p>0.05) was observed in total cholesterol, HDL-cholesterol and LDL-cholesterol between groups fed with HFD (with
distilled water) and the MS01 treated groups (MS01/L and MS01/H). A summary of this is shown in Table 4.3.

**Table 4.3: Changes in lipid profile of rats after the 30-day period**

<table>
<thead>
<tr>
<th>Parameters (mmol/L)</th>
<th>Normal diet (NCD)</th>
<th>High fat diet (HFD)</th>
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<tr>
<td></td>
<td>+ distilled water</td>
<td>+ distilled water</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1.51 ± 0.18</td>
<td>2.08 ± 0.47&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Triglyceride</td>
<td>0.75 ± 0.22</td>
<td>1.56 ± 0.81&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>0.59 ± 0.64</td>
<td>0.98 ± 0.19&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>0.39 ± 0.67</td>
<td>0.39 ± 0.17&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM (n=6). NCD normal diet; HFD: high-fat diet; MS01/L: high-fat diet and treated with low dose of the MS01 dietary supplement; MS01/H: HFD-fed rats treated with high dose of the MS01 dietary supplement. *p<0.05 compared to NCD; **p<0.01 compared to HFD-fed rats treated with high dose of dietary supplement and control HFD-fed rats only; NS: not significant in TC between control HFD-fed rats and HFD fed rats treated with MS01. LDL-cholesterol in all groups and HDL-cholesterol between HFD-fed rats treated with high dose of dietary supplement and control HFD-fed rats only.
CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSIONS

5.1 DISCUSSION

The study by Kim and colleagues in 2014 established that the anti-obesity mechanism of ALA can be carried out using both humans and animals. In the present study, a total of twenty-four Sprague Dawley rats were randomly put into 4 groups of 6 rats each. Group 1 was fed with normal diet (NCD) and groups 2-4 were fed on high-fat diet (HFD) for 30 days. The rats on high-fat diet were treated with the dietary supplement (MS01), at low (group 3, MS01/L) and high (group 4, MS01/H) doses. The control HFD-fed rats received distilled water (group 2). The dietary supplement MS01 being studied has a proprietary blend of ALA, ginseng, citrulline (citrulline malate), leucine, riboceine (D-Ribose-L-Cysteine) and pantetheine; each of these individually affects metabolism. Also, the supplement has vitamin D2, thiamine, vitamin B6, vitamin B12, and potassium. Individual nutraceuticals including ALA have been proven to have an effect on metabolism. The ultimate accepted prescription of ALA in rats has not been definite and this was confirmed by Kim et al. in 2016 in a study conducted in humans using ALA. Hence, the dosage for this study in SD rats was a low dose of 4mg/ kg and a high dose of 8mg/kg of rat body weight. The MS01 dietary supplement was generally tolerated in this study and no adverse effects or death was observed among the rats. This was also established in other studies done with ALA by Yadav et al. in 2005, Ziegler et al. in 2006 and Koh et al. in 2011.

Dietary-induced obesity is the most common type of obesity (Wang et al., 2015). First of all the effect of the high-fat diet on weight gain was determined, hence, the high-fat diet (HFD) formulation on weight gain was successful. Body weight gain of rats fed with the
control high-fat diet (with distilled water) showed a significant increase in weight (99.08% weight gain, p<0.001) compared to the rats fed with the normal diet after the 30-day period. This higher weight gain is in accordance with other studies including, Li et al. 2018 who reported a significantly higher weight gain in mice fed on a high-fat diet compared to the mice on a normal diet after 8 weeks. Also, Kudo et al. reported a significant weight gain in rats fed with a high-fat diet after 9 weeks. The possibility of the weight gain is explained as ‘the hypothalamic inflammatory activation as a result of consuming a high-fat diet and obesity are thought to disturb anorexigenic and thermogenic signals and promote abnormal body weight control (Manousopoulou et al., 2016). Also, ‘the development of fat cells from adipogenesis includes morphological changes, the expression of many lipogenic enzymes, and extensive lipid accumulation (Rosen and B. M. Spiegelman, 2000), in which all contribute to the growth and expansion of adipose tissue, thus under chronic inflammation in the hypothalamus of mice, as a response to HFD, a sustained cycle of mechanism were observed’ (Thaler and Shwartz, 2010).

Moreover, the first objective of this study was to determine the effect of the dietary supplement MS01 on weight gain of SD rats fed with a high-fat diet. Treatment of rats with the dietary supplement MS01 showed a significant reduction in weight gain (84.87% and 84.83%, p<0.001 for low and high doses respectively) compared to the rats fed with the control high fat diet only. Several studies on the nutraceuticals involved in this study showed a significant weight loss or limited weight gain in rats fed with a high-fat diet including, Zhang et al. in 2008 reported that weight gain was hindered after feeding with ALA supplement and a high-fat diet. Also, Shen et al. in 2005 stated that mice treated with ALA supplement had a substantial 3.3g reduction of weight while the control group
gained a weight of about 3.6g, they established that reduction of weight was associated with reduced feed consumption. In studies done on ginseng, weight loss was significant (Xie et al., 2002; Dey et al., 2003; Lee et al., 2013), also, the body weight gain of high fat diet-fed mice treated with ginseng was lower than that of the untreated high fat diet-fed mice, which suggested that ginseng treatment prevented dietary-induced body weight gain (Li et al., 2018). These findings are in accordance with previous reports showing that ginseng extracts prevent obesity in high fat diet-fed mice and in an obese insulin-resistant rat model (Lim et al., 2008). Furthermore, it was found that ginsenoside Rg1 activated the AMPK pathway in high fat diet-fed mice, which was consistent with previous studies (Lee et al., 2012). AMPK plays an important role in the maintenance of glucose energy homeostasis in whole-body level and is a key regulator of obesity (Canto and Auwerx, 2009). AMPK activation induces cellular catabolism of lipids, protein and sugars via multiple downstream pathways (Lopez-Lluch, 2016). Specifically, AMPK impedes mTOR complex 1 (mTORC1) activity (Gwinn et al., 2008).

Gosby et al. in 2014 described dietary proteins as strong influencers in metabolic health through their effect on appetite, weight gain, and adiposity. Body weight gain also lowered significantly in a study done on L-Citrulline in SD rats (Kudo et al., 2017). Luiking et al. in 2016 reported that levels of the branched-chain amino acid leucine are likely to represent a physiological signal of protein availability in the control of appetite and metabolism. In humans and rodents, circulating leucine levels rapidly increase following the ingestion of a protein dense meal. In other studies, leucine may have regulated many important physiological functions, including protein and energy metabolism, cell proliferation and apoptosis in different tissues and cells (Mao et al., 2013; Xiao et al., 2016; Toneto et al., 2016). These studies support the effectiveness of
the MS01 dietary supplement on weight control as evidenced in the MS01 treatment
groups compared to the non-treatment groups, hence, in this study, results showed that
the dietary supplement MS01 can limit weight gain in dietary-induced obesity.

The feeds were formulated for the rats according to group specifications. Group-1 had a
normal diet (NCD) and group 2-4 had a high-fat diet (HFD). The caloric content of the
high-fat diet (HFD) (Table 3.1) was considered due to the brief duration (30 days) of the
study compared to other studies including one done in 4-12 weeks (Kamisah et al., 2006).
The high-fat diet formulation was successful and induced weight gain when compared
with the rats that fed with the normal diet (Table 4.1). Reduced feed intake was observed
in the MS01 treatment groups compared to the non-treated groups though there was no
statistically significant difference among the groups, the trend suggested that the MS01
dietary supplement suppressed their appetite. The suppression of appetite was previously
evident in a human study done by Kim et al. (2016) over a 12-week period and an earlier
study done by Kim et al. in 2008 when administered ALA. However, Shen et al. in 2005
and Zhang et al. in 2008 stated that mice treated with ALA that showed a reduction in
body weight was associated with reduced feed consumption. Kudo et al. in 2017 recorded
a reduced feed intake among SD rats fed with a high-fat diet treated with citrulline. Attele
et al. in 2002 established that ginseng affects appetite and increases metabolism. These
findings support the observed trend in the reduction of feed intake and weight gain of rats
in the MS01 treatment groups, nonetheless, the MS01 dietary supplement was effective in
controlling weight gain of the SD rats.

In the Black African population, dyslipidaemia is to a large extent similar to worldwide
averages (Shisana et al., 2013). In Africa, dyslipidaemia prevalence rates of between 14%
and 69% have been found using community-level assessments (Manning et al., 2016).
Therefore, the second objective of this study was to determine the effect of the dietary
supplement MS01 on serum lipids of SD rats. After the 30-day treatment period, serum total cholesterol, triglyceride and HDL-cholesterol were found to be significantly higher (p<0.05) in rats on high-fat diet only compared to rats fed with the normal diet respectively. Before this study, it was expected that the high-fat diet will increase serum LDL-cholesterol but rather reduced compared to the HDL-cholesterol. Even so, the increased triglyceride and total cholesterol in the high fat diet-fed rats were evident in studies done by Kudo et al. in 2017 and Li et al. 2018.

Also, serum concentration of triglyceride was significantly increased (p<0.01) in the rats treated with a low dose of MS01 compared to rats treated with a high dose of MS01 and rats fed with high fat diet only, which is not the expected results because, the dietary supplement MS01 was to significantly reduce the triglyceride levels of rats compared to the HFD-fed rats only, this was evident in a study by Teachey et al. in 2003 which reported that blood TG levels and free fatty acid levels were reduced in Zucker rats treated with ALA. Though ginseng has been proven to reduce serum triglyceride, there was no effect on triglyceride level in a study by Kudo et al. in 2017. However, no statistical difference (p>0.05) was observed in total cholesterol, HDL-cholesterol and LDL-cholesterol between groups fed with high-fat diet (control) and the MS01 treated groups (MS01/L and MS01/H).

In the majority of studies, alpha-lipoic acid led to significant reduction of serum total cholesterol, and HDL-cholesterol concentration in obese subjects (Choi and Ginsberg, 2011; Seo et al., 2012; Morakinyo et al., 2013; Kandeil et al., 2011). In this research, the cholesterol concentration did not differ significantly after the treatment with the dietary supplement in comparison with rats fed with the high-fat diet only at the end of the
treatment. Possible reasons for this could be that the rats in our study were treated with 4mg/kg (low) and 8mg/kg (high dose) daily, but in majority of other studies this dose was pretty higher (Koh et al., 2011; Carrier and Rideout, 2013; Han et al., 2012; Xu et al., 2013). Also considering the low dose of alpha-lipoic acid, the treatment period (30 days) was relatively short in our study. The effect of the dietary supplement on treated groups was found not to be dose-dependent (MS01/L vs MS01/H p>0.05). In a study done by Koh et al. in 2011, the anti-obesity effects of ALA appeared to be related to time and dosage. Kim et al. in 2016 stated that despite the visceral fat reduction in the ALA treatment group, lipid profiles were not significantly different between the control high-fat diet and the ALA treatment group in their study. Hence, significant changes could have been observed if this study had been carried out longer than the 30 days (Song et al., 2005; Kim et al., 2008), Koh et al. 2011 and Seo et al. 2012 have reported that ALA improved lipid metabolism. Yang et al. in 2008 also showed that plasma total cholesterol, triglycerides, and LDL-cholesterol levels reduced significantly when compared to the control group after feeding a 21.5% fat diet with 0.1% ALA to C57BL/6 mice after six weeks. Attele et al. in 2002 established that ginseng could reduce lipid profile. Hence results from this study did not show significant differences between lipid parameters in treated and untreated groups and might as well be due to the brief duration of the study. It is believed that ginseng can improve health and increase metabolism. The physiologic and pharmacologic functions of ginseng have been discovered gradually and include regulation of cellular metabolic processes (Li et al., 2018).
Evans and his group in 2014 postulated that pantetheine acts by inhibiting the enzymes acetyl-CoA carboxylase and HMG-CoA reductase, thus modifying lipoprotein metabolism. Kota et al., in 2013 stated cytoplasmic CoA stimulates oxidation of acetate at the expense of fatty acids, cholesterol synthesis and CoA increases Krebs cycle activity, however, reducing cholesterol and fatty acids synthesis from acetate; fatty acid and cholesterol synthesis are decreased by 50% and 80%, respectively. Pantetheine has been known to increase arterial cholesterol esterase activity leading to the removal of arterial cholesterol esters and reducing fatty streak formation and intimal, endothelial thickening, lipid deposition, LDL peroxidation, and endothelial dysfunction (Kota et al., 2013).

ALA supplementation in another study led to significant weight reduction while maintaining normal lipid profile of the rats (Seo et al., 2012). ALA is established in many studies as a protector against oxidative damage cells, which is described in diabetes mellitus patients (Gupta et al., 2013) and positively influences the regulation level of glucose (Morakinyo et al., 2013) and reduces blood lipids total cholesterol, LDL, and triglycerides (Kandeil et al., 2011). Therefore, the weak significance observed between the lipid profile and the MS01 dietary supplement was probably as a result of the dose administered and the brief duration of this study.

The organs (heart, liver, kidneys (both left and right)) tissue weight of rats fed with the control high-fat diet only, showed an increase compared to the rats fed with the normal diet but was not statistically significant (p>0.05). Moreover, the organs (heart, liver, kidneys) tissue weight of rats treated with the high dose of the dietary supplement was noted significantly small compared to the rats treated with a low dose of the dietary supplement and the rats that fed the control high fat diet only. Despite the difference in
means for tissue weight of rat organs (heart, liver and kidneys) both treated and untreated
groups, the rat body weight to organ ratio showed no statistically significant difference
(p>0.05) among all groups.

This is in agreement with a study done by Li et al., in 2018 on anti-obesity effects of
ginseng on high fat diet-fed mice, hence, the weight of the organs (heart, liver and kidney)
was unaffected but effectively prevented dietary-induced obesity development. Also,
there was a significant lower in organ adipose tissue weight in the L-Citrulline group but
no significant differences in weights of organ tissues between treatment and non-
treatment groups (Kudo et al., 2017).

However, the findings of our present study should be interpreted with an understanding
of a preliminary study with the following limitations. First, as the study duration (30 days)
was relatively short. Secondly, only a few rats (24) were used.
5.2 CONCLUSIONS

The MS01 dietary supplement reduced weight gain of rats fed on a high-fat diet but did not improve the lipid profile. Weight gain of rats fed with HFD compared to the restricted weight gain of the rats treated with MS01/L and MS01/H was statistically significant (p<0.001). There was no statistically significant difference among lipid profiles of the HFD group compared to both the MS01/L and MS01/H groups (p > 0.05).

It is recommended that:

1. In further studies, the duration of the study should be longer than 30 days with higher doses of the supplement in order to achieve a significant difference between treatment groups in the various parameters of the lipid profile

2. A different composition of the high-fat diet (HFD) can be tried.

3. Toxicity studies of the MS01 dietary supplement on organs to assess adverse effects must be carried out.
REFERENCES


Ortega, F. B., Sui, X., Lavie, C. J. and Blair, S. N. (2016). Body mass index, the most widely used but also widely criticized index: would a criterion standard measure of total body fat be a better predictor of cardiovascular disease mortality? Mayo Clinic Proceedings. 91(4):443–455.


WHO (2010). Global status report on non-communicable diseases. 176


APPENDICES

Appendix I: Reagents for Lipid Profile Analysis

Reagents for Total Cholesterol

Components and concentrations

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
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<tbody>
<tr>
<td>Phosphate buffer</td>
<td>100 mmol/L</td>
</tr>
<tr>
<td>Phenol</td>
<td>5 mmol/L</td>
</tr>
<tr>
<td>4-Aminoantipyrine</td>
<td>0.3 mmol/L</td>
</tr>
<tr>
<td>$R_1$ Cholesterol esterase</td>
<td>$&gt;150$ KU/L</td>
</tr>
<tr>
<td>Cholesterol oxidase</td>
<td>$&gt;100$ KU/L</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>5 KU/L</td>
</tr>
</tbody>
</table>

Reagents for Triglycerides

Components and Concentrations

<table>
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<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
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<td>Phosphate buffer</td>
<td>50 mmol/L</td>
</tr>
<tr>
<td>4-Cholesterol</td>
<td>5 mmol/L</td>
</tr>
<tr>
<td>$R_1$ ATP</td>
<td>2 mmol/L</td>
</tr>
<tr>
<td>$Mg^{2+}$</td>
<td>4.5 mmol/L</td>
</tr>
<tr>
<td>Glycerokinase</td>
<td>$\geq 0.4$ U/mL</td>
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<tr>
<td>Peroxidase</td>
<td>$\geq 0.5$ U/mL</td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td>$\geq 1.3$ U/mL</td>
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<td>4-Aminoantipyrine</td>
<td>0.25 mmol/L</td>
</tr>
<tr>
<td>Glycerol-3-phosphate-oxidase</td>
<td>$\geq 1.5$ U/mL</td>
</tr>
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### Reagents for HDL-C

**Components and concentrations**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good’s buffer</td>
<td>100 mmol/L</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>600 U/L</td>
</tr>
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<td>R1: Cholesterol oxidase</td>
<td>380 U/L</td>
</tr>
<tr>
<td>Catalase</td>
<td>600 KU/L</td>
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<td>HDAOS</td>
<td>0.42 mmol/L</td>
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<tr>
<td>Good’s buffer</td>
<td>100 mmol/L</td>
</tr>
<tr>
<td>4-aminoantipyrine</td>
<td>1.0 mmol/L</td>
</tr>
<tr>
<td>R2: Peroxidase</td>
<td>&gt;2.8 U/mL</td>
</tr>
<tr>
<td>Surfactant</td>
<td>&lt;2%</td>
</tr>
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### Reagents for LDL-C

**Components and Concentrations**

<table>
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<tr>
<th>Component</th>
<th>Concentration</th>
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</thead>
<tbody>
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<td>Good’s buffer</td>
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<tr>
<td>Cholesterol esterase</td>
<td>600 U/L</td>
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<tr>
<td>R1: Cholesterol oxidase</td>
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<td>Catalase</td>
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<td>TOOS</td>
<td>2 mmol/L</td>
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<td>Good’s buffer</td>
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<tr>
<td>4-aminoantipyrine</td>
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<tr>
<td>R2: Peroxidase</td>
<td>4 U/mL</td>
</tr>
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<td>Surfactant</td>
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Appendix II - Statistical analysis

Table of multiple comparisons: TC

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Group</th>
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<th>p-Value</th>
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<tbody>
<tr>
<td>Total cholesterol</td>
<td>NCD</td>
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<td>MS01/L</td>
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<td>HFD</td>
<td>NCD</td>
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<td>MS01/L</td>
<td>1.000</td>
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<tr>
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<td>MS01/H</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
<td>MS01/H</td>
<td>1.000</td>
</tr>
<tr>
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<tr>
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<td>MS01/L</td>
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Table of multiple comparisons: TG

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<td>MS01/L</td>
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<tr>
<td></td>
<td></td>
<td>MS01/H</td>
<td>1.000</td>
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<td>HFD</td>
<td>NCD</td>
<td>0.414</td>
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<td></td>
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<td>NCD</td>
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<td>HFD</td>
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<td>0.017</td>
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Table 4.2: Table of multiple comparisons: HDL Cholesterol

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<td>MS01/L</td>
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<tr>
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### Table of multiple comparisons: LDL- Chol

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### Table of multiple comparisons: Right kidney

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Summary

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<td>Total Cholesterol</td>
<td>1.51 ± 0.18</td>
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<td>2.05 ± 0.32</td>
<td>2.03 ± 0.58</td>
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<tr>
<td>Total Triglycerides</td>
<td>0.75 ± 0.22</td>
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<td>2.28 ± 1.09</td>
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<td>LDL-Cholesterol</td>
<td>0.39 ± 0.67</td>
<td>0.39 ± 0.17</td>
<td>0.36 ± 0.09</td>
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<tr>
<td>HDL-Cholesterol</td>
<td>0.59 ± 0.64</td>
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<td>0.92 ± 0.11</td>
<td>0.92 ± 0.29</td>
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<td>Heart</td>
<td>0.30 ± 0.02</td>
<td>0.35 ± 0.04</td>
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<td>Right Kidney</td>
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<td>Left Kidney</td>
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