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THE ROLE OF GHRELIN IN OVERWEIGHT AND OBESITY

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THE ROLE OF GHRELIN IN OVERWEIGHT AND OBESITY

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November 2022

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Wadhah Ihsan Ibrahim IBRAHIM

ABSTRACT

THE ROLE OF GHRELIN IN OVERWEIGHT AND OBESITY

Wadhah Ihsan Ibrahim IBRAHIM

Master of Science in Chemistry

Advisor: Assoc. Prof. Dr. Şevki ADEM

November 2022

In the current study, the main goal was to search in the change of the metabolic functions in overweight and obese people by taking ghrelin, glucose, triglycerides, cholesterol, HDL, LDL, and VLDL with the activities of ALT, AST, and ALP as parameters in the study. Forty people were inserted to each of the three groups of the study (control, overweight, and obese) with matched age and different body mass index according to their category. The obtained results showed a significant reduction of ghrelin in obese and overweight compared to control. Moreover, the ghrelin level was significantly lower in obese compared to overweight. Glucose level was increased in obese compared to control and overweight. The levels of triglycerides and VLDL were increased significantly in obese and overweight compared to control. Moreover, the levels of the last two parameters were significantly higher in obese compared to overweight. The cholesterol and LDL levels were significantly higher in obese and overweight compared to control. Nevertheless, HDL level was decreased in obese and overweight compared to control. The activities of ALT, AST, and ALP were increased significantly in obese compared to control and overweight. Clearly, obesity induce significant change on the metabolic functions in people.

2022, 61 pages

Keywords: Ghrelin, BMI, Lipid profile, Obesity, Liver function, Overweight

ÖZET

AŞIRI KİLO VE OBEZİTEDE GHRELİN'İN ROLÜ

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Tez Danışmanı: Doç. Dr. Şevki ADEM
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Bu çalışmada asıl amaç, ALT, AST ve ALP aktiviteleri ile ghrelin, glukoz, trigliseridler, kolesterol, HDL, LDL ve VLDL alarak aşırı kilolu ve obez kişilerde metabolik fonksiyonların değişimini araştırmaktı. Çalışmadaki parametreler. Çalışmanın üç grubunun (kontrol, fazla kilolu ve obez) her birine, kategorilerine göre eşleştirilmiş yaş ve farklı vücut kitle indeksine sahip kırk kişi yerleştirildi. Elde edilen sonuçlar, kontrole kıyasla obez ve fazla kilolu kişilerde ghrelin'de önemli bir azalma olduğunu gösterdi. Ayrıca, obezlerde ghrelin seviyesi aşırı kilolulara göre anlamlı derecede düşüktü. Obezlerde kontrol ve aşırı kilolulara göre glukoz düzeyi yükselmiştir. Obez ve aşırı kilolu kişilerde trigliserit ve VLDL seviyeleri kontrole kıyasla önemli ölçüde arttı. Ayrıca, son iki parametrenin seviyeleri, aşırı kilolu ile karşılaştırıldığında obezlerde anlamlı olarak daha yüksekti. Kolesterol ve LDL seviyeleri, kontrole kıyasla obez ve aşırı kilolu kişilerde anlamlı derecede yüksekti. Bununla birlikte, kontrole göre obez ve fazla kilolularda HDL düzeyi azalmıştı. ALT, AST ve ALP aktiviteleri obezlerde kontrol ve aşırı kilolulara göre anlamlı olarak arttı. Açıkça, obezite insanlarda metabolik fonksiyonlar üzerinde önemli değişikliklere neden olur.

2022, 61 sayfa

Anahtar Kelimeler: Ghrelin, BMI, Lipid profili, Obezite, Karaciğer fonksiyonu, Fazla kilo

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Wadhah Ihsan Ibrahim IBRAHIM

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CONTENTS

LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees Celsius
dL	Deciliter
Eq	Equivalent
g	Gram
kDa	Kilodalton
kg	Kilogram
L	Liter
m	meter
mg	Milligram
mL	Milliliters
ng	Nanogram
nm	Nanomoles
rpm	Revolution per minute
µL	Microliter

LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine amino transferase
ASP	Acylation stimulating protein
AST	Aminotransferase
BEE	Basal energy expenditure
BMI	Body mass index
BRFSS	Behavioral risk factor surveillance system
CVD	Cardiovascular diseases
DIO	Diet-induced obesity
EI	Energy intake
GH	Growth hormone
GHSR1a	Growth hormone secretagogue type 1a receptor
GOAT	Ghrelin o-acyltransferase
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
NAFLD	Non-alcoholic fatty liver disease
NHANES	National health and nutrition examination survey
NIH	National institutes of health
TEE	Total energy expenditure
TGF	Transforming growth factor
TOS	The obesity society
VTA	Ventral tegmental area
WHO	World health organization

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Public health officials recognize obesity as a leading cause of many serious illnesses (Chan and Woo 2010). One modeling research found that for every 2% rise in a population's BMI, life expectancy drops by 1%. (Peto *et al.* 2010). People who are overweight or obese may be advised to make a variety of lifestyle adjustments, including food, exercise, attitude, and sometimes even medication or even surgical weight loss. Helping patients achieve long-term weight reduction is a major responsibility for both primary care physicians and interdisciplinary teams (Bray *et al.* 2013). Patients put their faith in their primary care physicians for guidance on weight management, yet several obstacles stand in the way of proper counseling and treatment for those who are overweight or obese. Although a holistic lifestyle intervention is essential for patients who are overweight, many doctors and other health professionals lack the requisite skills in behavioral counselling and multidisciplinary teamwork (Dietz *et al.* 2015). However, genetics have a role in determining whether or not a person will be overweight or obese. As a result, effective evidence-based treatments are frequently not implemented as intended (Farooqi and O'Rahilly, 2000). The restricted scope of such treatments may also be a result of the challenges associated with making long-term changes in behavior. Long-term exposure to an obesogenic environment, with its many and constant temptations, and a surge in hunger-stimulating hormones after major weight loss may be to blame (Semlitsch *et al.* 2019).

Scientists think ghrelin contributes to the development of obesity. While one would assume that obese people's fasting ghrelin levels would be higher, the opposite is true; plasma ghrelin levels have been shown to correlate negatively with BMI in a number of published research that concentrate on obesity. Positive energy balance in obese people may have caused this physiological anomaly (Wang *et al.* 2022).

In turn, insulin resistance, which is linked to obesity, is thought to be a primary reason why people with diabetes struggle to lose weight. That's why it seems sense that a diet plan that helps people lose weight and improves their insulin resistance would also help

cut down on the number of cases of type 2 diabetes among the obese. Carbohydrates are getting a lot of attention these days when it comes to nutrition. Carbohydrates are directly linked to insulin resistance because they have the largest effect on postprandial glucose excursions and are the major trigger for postprandial insulin release. Cereals are a major contributor of dietary carbs in Western nations (Minicozzi *et al.* 2013).

Some disorders may be linked to the way fat is distributed in the body, according to previous research. It's common practice today to take pharmaceuticals to reduce weight and regulate blood lipids, but these therapies seldom work and often come with unwanted side effects (Zare *et al.* 2014).

Although the precise biochemical mechanisms linking obesity to these diseases remain unclear, it is known that an increase in triglyceride stores is associated with a linear increase in the production of cholesterol, which is associated with an increase in cholesterol secretion in bile and an increased risk of gallstone formation and the development of gall bladder diseases (Klop *et al.* 2013). People who are overweight tend to have lower levels of HDL, which may contribute to their increased vulnerability to cardiovascular disease and heart attacks (Khan and Khaleel 2016).

A research found that the blood level of ALP in obese people was considerably greater than in those who were not overweight (Sultan 2010). Recent research suggests that ALP is expressed in different tissues in addition to adipose tissue, which is responsible for regulating intracellular fat storage in preadipocytes during adipogenesis (such as liver, bone, bile duct, kidney, intestinal mucosa, and placenta) (Ali *et al.* 2013). Extra adipose lipid deposits inside cells are a predicted consequence of the increased ALP activity seen in obese people. Adipose tissue responds by releasing an abundance of ALP into the bloodstream (Ali *et al.* 2006). So far, however, there has been no large-scale clinical investigation done to determine whether or whether elevated blood ALP levels are linked to weight gain and obesity.

Research has shown a link between high levels of ALT and AST and abdominal obesity (Adams *et al.* 2008). Most previous research evaluated the correlations by just

addressing a few liver enzymes, leading to contradictory findings. Only a small number of research have looked at how maximal levels of hepatic enzymes correlate with abdominal and total obesity in adults (Ali *et al.* 2021).

The main purpose of the study is to evaluate the level of ghrelin in the serum of overweight and obese Iraqi people and to estimate the possibility of using ghrelin as marker in the prognosis of obesity risks. Further investigations are intended to find the correlation between ghrelin and glucose, lipid profile, and liver function in the serum of obese and overweight people.

2. LITERATURE REVIEW

2.1 Obesity

When extra fat accumulates to dangerous levels, we call it obesity (Sung 2013). The many contributing factors to its persistence lend credence to the idea that this is a highly intricate chronic condition. Alterations in fat accumulation are often brought on by an oversupply of calories relative to the body's needs (overnutrition). This may set in motion complicated feedback mechanisms in response to food consumed or fat cell activity, including connections between environmental, genetic, and neuroendocrine variables (Ouchi *et al.* 2011). Obesity now has a much clearer link to systemic inflammation than ever before (Lackey and Olefsky 2016).

For this reason, indicators of overweight or obesity try to quantify the quantity or percentage of fat tissue in the body, since adipose tissue is crucial to understanding obesity and its related health implications. Epidemiological studies were the first to use BMI to measure and categorize obesity; their results informed the current criteria of obesity as a BMI of 30 kg/m^2 and overweight as a BMI of 25-29.99 kg/m^2. While body mass index (BMI) is helpful for gauging overall health risk, it may not be as informative for particular people due to variations in fat distribution. Standardizing waist circumference and the waist-to-hip ratio was motivated by the association between visceral fat and health problems. More than 94 cm in males and 80 centimeters in women is associated with an elevated health risk (Knowles *et al.* 2011). Since obesity is a complicated, chronic inflammatory disorder, these tests do not diagnose metabolic status but rather the risk of comorbidities.

Metabolic disease research has shown a link between eating too much and the activation of innate immune system organs involved in energy balance, a process known as obesity-induced inflammation. Many different tissues and organs, not only fat cells, are affected by the inflammation that is a hallmark of obesity (Saltiel and Olefsky 2017). Studies conducted over the last two decades have shown the importance of adipose tissue's ability to synthesize both pro- and anti-inflammatory molecules in the control of

inflammation and immunity (eg leptin, adiponectin, cytokines, and chemokines). The effect of fat mass on immunological function varies according to the type and location of fat tissue, with visceral fat (associated with central adiposity) producing more endocrine secretions responsible for inflammation than peripheral fat cells (Matsuzawa 2008).

Body mass index (BMI) for age growth charts from the Centers for Disease Control and Prevention (CDC) may be used to get a rough idea of the prevalence of pediatric obesity. People that are overweight fall between the 85th and 95th percentiles. If your BMI is more than the 95th percentile, you are considered obese. In children and adolescents, like in adults, the rate of obesity varies by both race and gender. Hispanic children and adolescents had a higher obesity rate than white, black, or Asian youth between 2011 and 2014. Asian kids and teens have the slimmest average waistlines. There is a 70 percent correlation between adolescent obesity and adult obesity (Anekwe *et al.* 2020).

2.1.1 Body mass index

By calculating a person's BMI, it is feasible to compare their weight to that of others in the same or other groups without using identical scales for each. Although there is a positive correlation between BMI and body fat percentage, this association is also modified by gender, age, and race (Jackson *et al.*, 2002), with some data suggesting that South Asians have a higher BMI-adjusted % body fat than other ethnicities (Jackson *et al.* 2009). Obesity in adults in the United States was defined by the results of the second National Health and Nutrition Examination Survey (NHANES II) as a body mass index (BMI) of 27.3 kg/m^2 or more for women and a BMI of 27.8 kg/m^2 or more for men (Najjar *et al.* 1987). The 85th percentile for both sexes between the ages of 20 and 29 was used to establish the body mass index (BMI) thresholds for these groups. The World Health Organization's (WHO) criteria for overweight and obesity were approved in 1998 by the National Institutes of Health (NIH) Expert Panel on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. (Ryan 2014). According to the World Health Organization's (WHO) categorization, which mostly

pertained to people of European ancestry, individuals with a higher body mass index (BMI) than those with a normal weight (BMI of 18.5 - 25 kg/m^2) were at a greater risk for contracting various chronic diseases. However, owing to a greater predisposition for central fat distribution, Asians are more likely to acquire these disorders at lower body mass index (BMI) ranges than non-Asians (see below). Body mass index (BMI) cutoffs of 18.5–23 kg/m^2 (showing acceptable risk), 23–27.5 kg/m^2 (imparting increased risk), and 27.5 kg/m^2 (or more) have been recommended by the World Health Organization for use in deciding whether or not to implement therapeutic intervention in Asians (constituting severe risk) (Consultation 2004).

2.1.2 Epidemiology of obesity

Prevalence increases have been seen in every age group, across all demographics and socioeconomic levels, according to studies looking at the trends in obesity (Boutari and Mantzoros 2022). The percentage of American adults who are overweight or obese rose from 30.5% in 1999 to 42.4% in 2018. For example, adults aged 20–39 had a prevalence of obesity of 40%, those aged 40–59 of 45%, and those aged 60+ of 43%.

Data on the prevalence of obesity in the United States are collected through the National Health and Nutrition Examination Survey (NHANES), whose sample is selected using a complex, multistage probability design, and through annual phone calls from state health departments in collaboration with the CDC (the Behavioral Risk Factor Surveillance System [BRFSS]) (Fryar and Carroll 2021). To paraphrase the National Health and Nutrition Examination Survey, in 2017-2018, 42.4% of American adults were overweight or obese after adjusting for age (NHANES). Because of the self-report bias inherent to phone surveys, BRFSS estimates of the prevalence of obesity are typically lower than NHANES estimates (30.9% in 2019). Even more so, it is predicted by the Centers for Disease Control and Prevention that 31.4% of American adults will be obese this year. As a result, determining the root of the obesity epidemic is crucial when analyzing prevalence statistics. It would be interesting to evaluate and report on the incidence of obesity in the United States and abroad following the COVID-19

epidemic, which killed an estimated 15 million people over the last two years (estimates vary from 12 to 22 million) (Wanga *et al.* 2021).

An alarming percentage of Europeans (59%) are overweight or obese, and over a third of children (32%) are also overweight or obese, according to a recent World Health Organization report. This has led many to question what can be done (Boutari and Mantzoros 2022).

2.1.3 Obesity as disease and increased comorbidity risk

The Obesity Society (TOS) recognizes obesity as a legitimate public health crisis, classifying it as a disease that is both a risk factor for and a direct cause of a number of other, more severe chronic conditions (Müller and Geisler 2017).

Obesity is a complex condition that has many potential causes, including but not limited to changes in eating habits and lifestyle, genetics, the hypothalamus, iatrogenic factors, and endocrine dysfunction (Vallgarda *et al.* 2017).

Adiposopathy, also known as "sick fat," is the underlying cause of obesity. It is defined as "pathologic adipose tissue anatomic/functional disturbances promoted by positive caloric balance in genetically and environmentally susceptible individuals that result in adverse endocrine and immune responses that may cause or worsen metabolic disease" (Bays *et al.* 2011).

Adiposopathy is associated with the release of hormones like leptin as well as proinflammatory proteins like the plethora of cytokines, and it has been linked to metabolic illness (Bays *et al.* 2008).

Obesity may be a primary condition due to the fact that adiposopathy is the defining factor in metabolic dysregulation (Bays and Ballantyne 2006). Obesity in children and adolescents has been associated to a variety of metabolic disorders, including

atherosclerosis, hypertension, dyslipidemia, diabetes type II, hyperandrogenemia in women, and hypoandrogenemia/hyperestrogenemia in males (Bays and Ballantyne 2006).

Pathogenic immunological and adiposopathic endocrine responses may have deleterious consequences on the cardiovascular system and other organs. The development of atherosclerotic risk factors including type 2 diabetes mellitus and dyslipidemia have been associated to adiposopathy (Bays 2005).

Obesity is associated with an increased risk of several illnesses and a shortened lifespan, making its diagnosis and treatment very important.

The following list is not exhaustive; obesity is a known cause of or risk factor for well over 200 different chronic illnesses (COS, 2008). CVD, hypertension (risk of being affected by hypertension increases threefold in subjects with obesity), dyslipidemia, coronary heart disease, gallbladder stones (risk of suffering from gallbladder stones increases threefold in subjects with obesity), and type 2 diabetes mellitus. Weight gain is associated with an increased risk of numerous cancers, including those that appear after menopause (breast, endometrial, prostate, colorectal, and adenocarcinoma) (Marmot *et al.* 2007). Diabetes, cardiovascular disease, cancer, and polycystic ovary syndrome are only few of the diseases that have been related to childhood obesity (Costacurta *et al.* 2012, Beamish *et al.* 2015).

2.1.4 The pathophysiology of obesity

A failure to maintain a constant rate of energy expenditure is what leads to obesity, as stated by the first law of thermodynamics. What provide this fuel for our bodies are the three macronutrients: carbs, protein, and fat (Badman and Flier 2005). In order to get energy, your body needs carbohydrates as a starting point. Carbohydrates are turned into fat when the body receives more than it needs. When glucose supplies are limited or nonexistent, the body turns to stored fat for fuel. Lipolysis is the breakdown of fat

into free fatty acids and glycerol. So, the human body agrees with the first rule of thermodynamics, which states that energy can neither be generated nor destroyed, but can only be transformed. Adipose tissue is where the majority of the body's excess energy is kept after being transformed from its original form into a chemical (Lowell and Spiegelman 2000). When EI increases more rapidly than TEE, adipose tissue swells, causing an increase in fat mass, muscle mass, and body fat percentage (Ravussin 2005). Changes in total energy expenditure (TEE), the sum of BEE and EEA, which includes thermogenesis-generated energy expenditure, may lead to weight swings (EET) (Hill *et al.* 2003).

However, the nervous system, the gastrointestinal tract (including the liver and pancreas), and the adipocyte all play a role in regulating energy expenditure and intake (Cypess *et al.* 2009). Adipocytes are specialized cells that regulate the body's endocrine and metabolic systems, store excess energy, and release it when needed. Adipocytes may grow to be as much as 20 times their normal size, and their volume can increase by up to 1,000 times. Each adipocyte may only be able to store around 1.2 grams of triglycerides. Lipoprotein lipase (LPL) and acylation stimulating protein (ASP) are enzymes involved in this esterification process, which is stimulated by insulin and chylomicrons. Triglyceride concentrations in adipocytes are typically lower than 0.6g per cell. Taking into account that the typical human being has between 30 and 60 109 adipocytes, each of which stores 0.5 g of triglycerides, we can calculate that this individual has an average fat volume of 15 kg, or 135,000 kcal (López-Jaramillo *et al.* 2005).

Common metabolic and physiological repercussions of obesity that lead to comorbid illnesses and diseases are shown in Figure 2.1.

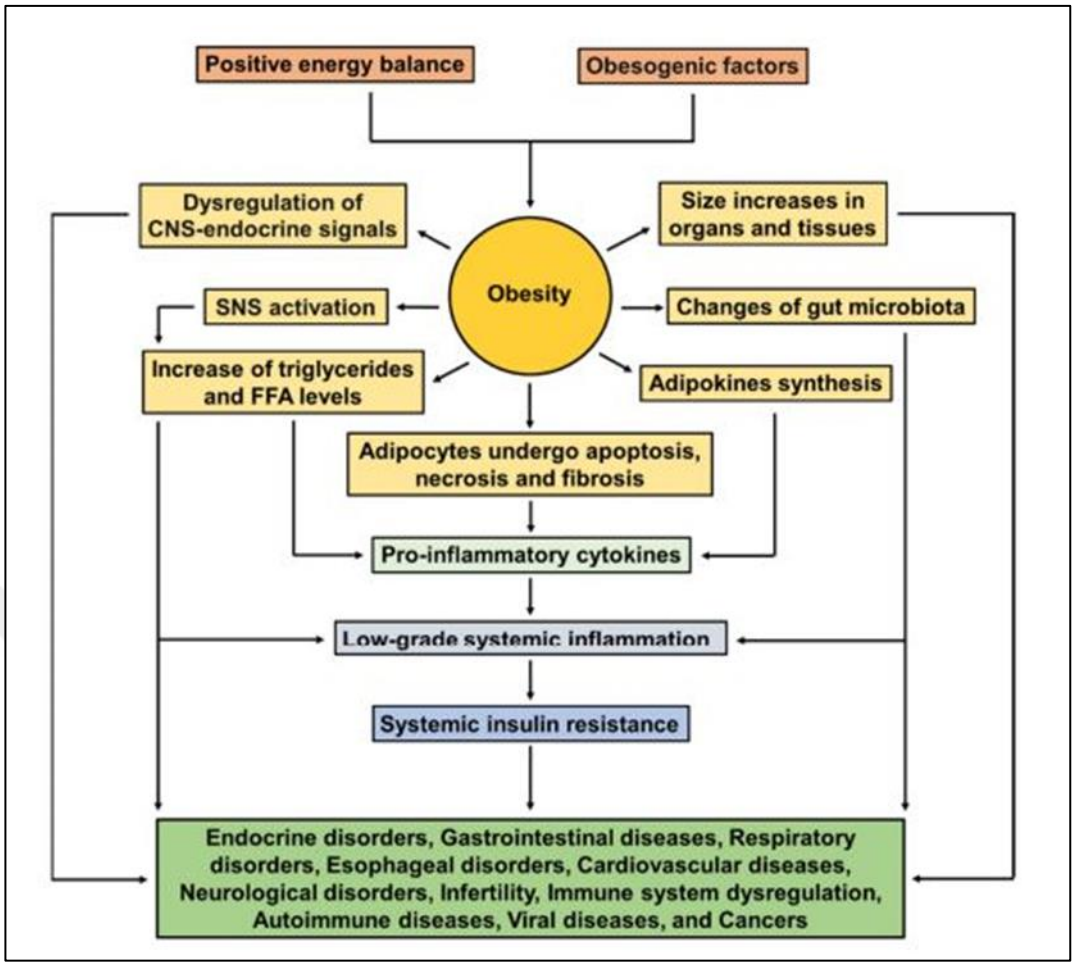

Figure 2.1 Explaining the causes and effects of obesity's pathological mechanisms (Gutiérrez-Cuevas *et al.* 2021)

2.1.5 Etiology

An excess of calorie intake over energy expenditure via thermogenesis, physiological functions, and physical activity is the simplest explanation for the processes that contribute to weight growth and obesity. Extra calories are stored as triglycerides in the fat cells of the adipose tissue or, more accurately, in the adipose organ, which subsequently grow and may also multiply by differentiating from precursors. This chapter will explain how environmental, lifestyle, genetic, and neuro-hormonal variables influence the metabolic response to increasing calorie intake and highlight the extraordinary individual heterogeneity across people (Berthoud *et al.* 2017).

2.2 Obesity and Inflammatory Markers

Metabolic inflammation, also known as chronic low-grade inflammation, is connected with obesity and its risk factors, including cardiovascular disease, atherosclerosis, insulin resistance, and more, suggesting that it may be a common pathway via which many illnesses develop (Xu *et al.* 2021). Adipose tissue, or fat, has various functions in the body's metabolism, including acting as a complicated secretory organ. Its role as a triacylglycerol reservoir affects a wide variety of physiological processes, including energy expenditure, hunger, insulin sensitivity, bone metabolism, the reproductive and endocrine systems, inflammation, and immunology. Both cardiovascular disease and diabetes risk are significantly increased when visceral adiposity is present, even when a high body mass index is not present (BMI). Higher correlations with visceral adiposity have been observed, although the underlying biochemical and physiological mechanisms remain elusive. One theory is that this is because molecules produced by visceral fat, unlike those produced by subcutaneous white adipose tissue, have direct access to the portal circulation and hence impact the liver, and that this is more prevalent in obese individuals (Yadav *et al.* 2017).

Proteins termed adipokines are secreted by adipocytes and have been shown to have a significant impact in a number of inflammatory disorders. Adiponectin, leptin, resistin, visfatin, interleukin 6 (IL-6), and tumor necrosis factor alpha are all examples of adipokines (Engin 2017). More than 50 distinct adipokines exist, each with a unique inflammatory function. Adipose tissue that is linked with a higher body mass index is more likely to release pro-inflammatory adipokines, whereas adipose tissue that is associated with a lower body mass index is more likely to secrete anti-inflammatory adipokines. The production of several adipokines, including tumor necrosis factors (TNFs), interleukin (IL)-6, leptin, angiotensin II, visfatin, and resistin, has been linked to the stimulation of inflammation (Vella *et al.* 2017). Adiponectin, TGF-, IL-4, IL-10, IL-13, IL-1Ra, and IL-1 are all examples of adipokines that have been shown to have anti-inflammatory effects (Maiborodina *et al.* 2021).

2.3 Autophagy and Nutrient Status in Obesity

Increased food consumption and decreased energy expenditure contribute to the inhibition of autophagy in obesity, which in turn is caused by elevated mTOR signaling (Galluzzi *et al.* 2014). Overnutrition reduces levels of NAD^+, a critical substrate for the metabolic processes of glycolysis and oxidative phosphorylation, which provide the majority of the energy used by cells. When nutrients are few, NAD^+ accumulates at the cost of NADH, activating the sirtuin family of deacetylases to induce autophagy (Houtkooper *et al.* 2012). Low energy charge, as indicated by a drop in ATP levels, may also trigger autophagy by inhibiting mTOR (Lapierre *et al.* 2015). Rotenone, an inhibitor of the respiratory chain, achieved this by reducing mitochondrial ATP production and setting off mitophagy (despite inhibiting autophagic flux). Epigenetic alteration has a role in regulating autophagy in response to under or over feeding (Mader *et al.* 2012).

2.4 Genetic Predisposition to Obesity

Adiposity has been proven to have a high heritability. Multiple studies of twins have demonstrated a very high (>50-70%) concordance rate for obesity among identical twins, even when they were not reared together. This is indicative of a substantial family "clustering" of the disorder (Stunkard *et al.* 1990). Whether this is due to differences in metabolic rate or issues with portion management is presently unclear. Although obese individuals have higher metabolic rates than lean participants because of their increased muscle mass, one sophisticated investigation has demonstrated that the variation in weight gain from overfeeding is quite minor between pairs but much larger across pairs. The results of this study imply that a person's tendency to gain weight when overfed may be dictated by their genes (Bouchard *et al.* 1990). Recent studies suggest that people who successfully adjust for overfeeding by increasing their metabolic rate are less thrifty in response to deprivation, and that this has no connection with obesity, so it is unclear whether or not these reactions contribute to overall weight gain in the population (Weyer *et al.* 2001).

2.5 Ghrelin

Ghrelin, a peptide consisting of just 28 amino acids, was only recently identified as the hunger hormone in the bloodstream. In addition to its role as a hormone in the endocrine system, it also acts as a neurotransmitter in the brain. The molecule in question goes by a few different names, including growth hormone secretagogue and motilin-related peptide (Kojima *et al.* 2001). Following its first discovery in 1996, GHSR1a was again found in 1999, this time by Kojima in Japan. The name "ghrelin" comes from the Proto-Indo-European root ghre, which means "hunger," in reference to the peptide's function as a growth hormone-releasing molecule (from whence we derive the English word "grow") (Inui *et al.* 2004).

Non-octanoylated ghrelin is known as des-acyl ghrelin, whereas the octanoylated version is known as acyl ghrelin (nonoctanoylated form). Ghrelin O-acyltransferase (GOAT) catalyzes the third carbon octanoylation of 20% of ghrelin (Figure 2.2), a change necessary for ghrelin to carry out its physiological actions (Du *et al.* 2018). Ghrelin in its acyl form increases growth hormone secretion, but the nonoctanoylated and inactive form, known as des-acyl ghrelin, does not (GH). Recently, it was shown that des-acyl ghrelin has a physiological role distinct from that of ghrelin. Multiple tissues and organs, as well as malignancies and their metastases, include the GHSR 1a and 1b proteins, which are both encoded by the same gene on chromosome 3q26.31 (aranac and Gucev, 2017). Multiple organs and tissues express ghrelin and its receptors. The hypothalamus, brain, stomach, kidney, thyroid, lung, heart, pancreatic islets, ovaries, testicles, and sebaceous glands are all examples of such organs and tissues (Elham *et al.* 2015). The anterior pituitary, the pancreatic islets, the adrenal gland, the thyroid, the heart, the arcuate nucleus, the hippocampus, the substantia nigra pars compacta, the ventral tegmental area (VTA), and the raphe nuclei all express GHSR1a at high levels (Elham *et al.* 2015, Akalu *et al.* 2020), cortex, and parafascicular thalamic region (Shi *et al.* 2017). The vast distribution of ghrelin receptors provides evidence for the hormone's extensive physiological effects. Ghrelin plays an important role in several physiological processes.

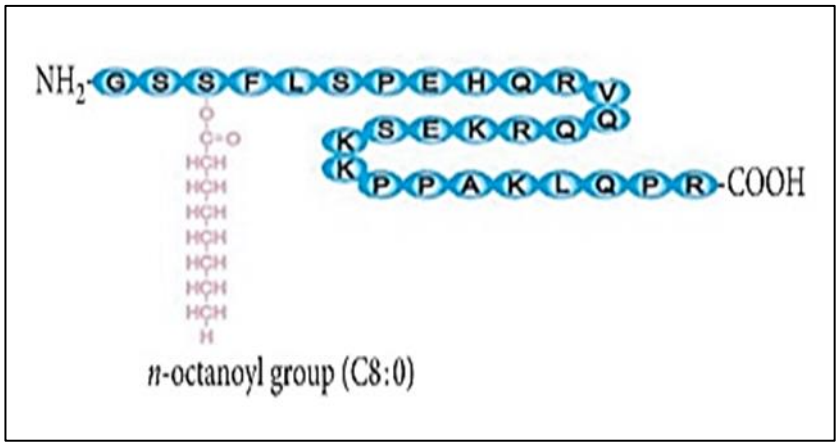

Figure 2.2 Ghrelin's molecular make-up (Akalu *et al.* 2020)

2.5.1 Ghrelin and Obesity

Weight gain happens when caloric intake surpasses energy expenditure. Neuronal responses to metabolic stresses in the hypothalamic areas that control eating behavior and energy balance are blunted in those with diet-induced obesity (DIO). Furthermore, DIO immunization protects ghrelin-producing cells from the satiating effects of calorie restriction and food intake (Zigman *et al.* 2016). Obese people, in contrast to normal-weight controls, had lower levels of the hunger hormone ghrelin in their bodies and in their blood plasma as a physiological reaction to a positive energy balance. Obese persons, contrary to popular belief, do not have a similar drop in ghrelin levels after eating as thin ones. Increased levels of the hunger hormone ghrelin have been linked to weight increase in weight-cyclers (Hooper *et al.* 2010). It was also shown that the concentrations of the two isoforms differed; slim controls had lower DAG concentrations, which caused the AG/DAG ratio to increase. Researchers found a correlation between AG levels and both BMI and waist circumference. DIO causes a ghrelin resistance due to insufficient signaling along the neuroendocrine ghrelin axis and a decreased NPY/AgRP response to plasma ghrelin. Therefore, overeating may have a role in the development of DIO (Sato *et al.* 2014). Inflammation in the nodose ganglia of high-fat diet-fed rats has been linked to peripheral ghrelin resistance, and DIO has been shown to both block the release of GH and change the permeability of the blood-brain barrier (Rhea *et al.* 2017).

Short-term effects on weight reduction are controlled by ghrelin, which mediates hunger and prompts eating. In addition, ghrelin levels fluctuate in response to the body's energy needs and storage. Furthermore, exogenous treatment of ghrelin changes food intake and affects neuronal activity in the brain that regulates body weight. All three functions may be linked to sustained weight reduction; however the evidence is mixed on the subject (Cummings 2006). After 1 year of diet-induced weight reduction, some studies show a sustained rise in ghrelin concentrations, which may be related in part to physiological causes for weight rebound (Nymo *et al.* 2018). Still others report a reversion to pre-hyperghrelin levels, suggesting that this hormone may only be causal in the short term (Iepsen *et al.* 2016).

2.5.2 Ghrelin and stress-induced obesity

When a person's physical equilibrium is disturbed, a cascade of neuroendocrine reactions takes place within the hypothalamic-pituitary-adrenal axis (HPA-axis) that leads to a number of physiological and behavioral adaptations (including arousal, increased respiration rate, and glucose utilization, among others) (Charmandari *et al.* 2005). Many mental and physical stress-induced illnesses, including as depression and obesity, formerly thought to be unaffected by transient reactions to stress have been related to exposure to chronic or recurrent stressors (Ota *et al.* 2014). Obesity caused by homeostatic, metabolic, and behavioral alterations due to chronic stress is called stress-induced obesity (Foster *et al.* 2009). Ghrelin seems to have a dual role in stress-induced obesity through the HPA-axis by increasing both ACTH and plasma ghrelin levels in response to prolonged or chronic stress (Van-Loenen *et al.* 2022).

2.6 Obesity-associated insulin resistance

The alarming increase in obesity prevalence over the last several decades has had devastating consequences for public health (NCD Risk Factor Collaboration 2016). Obesity dramatically increases the risk of serious health problems such as hypertension, cancer, and type 2 diabetes. Fundamental to the origin of these metabolic problems is the insulin resistance associated with obesity. Insulin resistance is defined as

intracellular impairments in insulin function. Hypercoagulability and increased release of inflammatory proteins are potential outcomes of hepatic insulin resistance, which also encourages abnormal glucose synthesis, atherogenic dyslipidemia, and steatosis. There are currently few therapeutic alternatives for treating hepatic insulin resistance effectively. Understanding the underlying mechanisms of obesity and insulin resistance is essential for the creation of new therapies. Quantifying mRNA and protein levels is possible with the use of transcriptomic and classical proteomic methods, respectively. However, enzyme activity is what ultimately decides physiology, and this activity is frequently tightly controlled by post-translational changes that cannot be detected by either transcriptome or conventional proteomic research (Zierath 2017).

2.7 Obesity and Changes in Fat and Glucose Metabolisms

Long-term overeating used to be self-limiting since it would eventually prevent people from being physically strong enough to hunt, gather, and defend themselves (Pessayre *et al.* 2001). Obesity rates have skyrocketed because people in developed nations can now afford to both eat fatty foods and laze around doing nothing for the first time in history (Pessayre *et al.* 2001). If current trends continue, the percentage of obese Americans might reach 40% by 2025 (Pessayre *et al.*, 2001), with a current prevalence of 22.5%. Adipocytes become swollen with fat due to obesity, but the condition also alters the body's fat redistribution and glucose metabolism (Fig. 2). Obesity also causes insulin resistance, which is detrimental since insulin is a pancreatic hormone that helps cells take in and use glucose by mediating the transport of glucose receptors from the inside of adipocytes and muscle cells to the plasma membrane (Shulman 2000). Insulin resistance causes an increase in blood glucose levels in the obese, which causes pancreatic -cells to secrete more insulin (the insulin-secreting cells of the pancreas). This compensatory increase, however, is either inadequate or fails inadvertently in some persons, leading to diabetes (Pessayre *et al.* 2001).

2.8 Dyslipidemia

Not only do greater FFA levels in obesity lead to impaired glucose utilization and increased hepatic glucose production, but they also lead to other negative repercussions. Increased FFAs also influence lipid metabolism by upping the creation of very low-density lipoprotein by the liver, decreasing HDL-cholesterol levels, and raising the amount of tiny, dense LDL particles (Boyer *et al.* 2018). These smaller particles are more atherogenic because they are able to enter the artery wall more easily and are more susceptible to oxidation and glycation. Atherogenic risk may be increased even when there is no discernible rise in LDL cholesterol level due to the existence of smaller LDL particles. These alterations in lipoprotein profile are linked to an increased risk of CHD when considered together. A comparison between women with low and high abdominal obesity shows how obesity affects lipid metabolism (Alwash *et al.* 2021).

These two groups had an average abdomen fat thickness of 107 and 187 cm2, respectively. Women who were not overweight averaged 50 cm2 of abdominal fat. Women with extreme abdominal obesity had abnormally high levels of triglycerides and low levels of high-density lipoprotein cholesterol. Obese women had a little rise in LDL-cholesterol, but their LDL particles were denser and more atherogenic than normal-weight women's (Pi-Sunyer 2002).

2.9 Obesity and Non-alcoholic Fatty Liver Disease

Poor dietary habits, less physical activity, and more sedentary lifestyles have contributed to a rise in the incidence of obesity in recent decades, especially in the abdominal region (Pang *et al.* 2008). People who are overweight, have diabetes, high blood pressure, and are married are at a higher risk of developing non-alcoholic fatty liver disease (NAFLD) (Alam *et al.* 2018). Increased risk of nonalcoholic fatty liver disease (NAFLD) is associated with both a high waist circumference (WC) and a high body mass index (BMI), with a stronger association between abdominal obesity and NAFLD (Pang *et al.* 2008).

Meta-analyses and epidemiological research have shown that, in addition to overall obesity, abdominal obesity is a strong predictor of metabolic disease (Frank *et al.* 2013). Previous research has also pointed to abdominal obesity as an essential feature of metabolic syndrome. As a result, it's clear that a lack of attention to abdominal obesity as a potential risk factor for NAFLD would be unwise. In people of normal weight, abdominal obesity is still a significant predictor of NAFLD (Machado and Cortez-Pinto 2011).

2.10 Liver Function

2.10.1 Alanine amino transferase

In most laboratories, hepatotoxicity is measured by measuring the serum alanine aminotransferase (ALT) activity level (Amacher, 2002). It has been established as the reference clinical chemistry marker of liver injury because to its high sensitivity and low false-negative and false-positive rates in identifying liver histopathologic damage. Despite some inconsistency, serum ALT levels have great therapeutic utility. In early human studies, histopathologic data is often lacking, hence additional markers are sought to supplement the information supplied by serum ALT enzymatic signals. ALT is an essential enzyme because of its role in gluconeogenesis and amino acid metabolism. The enzymes alanine aminotransferase and aspartate aminotransferase catalyze the reductive transfer of an amino group from alanine or aspartate to alpha-ketoglutarate, resulting in glutamate and the intermediates pyruvate or oxaloacetate. When damaged, hepatocytes release their contents, including the enzymes ALT and AST, into the blood and the liver. Serum levels of ALT and AST are elevated in comparison to those of control persons because of the released enzymes entering the circulation. AST is mostly expressed in the liver, although it is also found in the heart, brain, and skeletal muscle. The liver has the greatest ALT enzyme activity, whereas skeletal muscle and the heart have far lower levels. Injuries to other tissues outside the liver, most often skeletal muscle, have been associated with elevations in serum enzymatic levels of ALT and AST. Although other, more accurate indications of liver

damage may be employed, ALT is often used for this purpose in the pharmaceutical industry (Ozer *et al.* 2008).

2.10.2 Aminotransferase

The transamination reaction is catalyzed by AST. The mitochondrial isoenzyme and the cytoplasmic isoenzyme of AST are genetically separate isoforms. When compared to the liver, skeletal muscle, and kidneys, the heart has the highest AST level of any organ (Gowda *et al.* 2009).

Serum ALT levels between 0 to 35U/L are considered normal. Myocardial infarction causes widespread tissue necrosis, which is accompanied by elevated levels of mitochondrial AST, as is the case with chronic liver disorders that cause liver tissue deterioration and necrosis. The cytosolic isoenzyme of AST is primarily responsible for AST activity in the circulation, whereas the mitochondrial isoenzyme is mostly responsible for AST activity in the liver (Thapa and Walia 2007). A diagnosis of liver cell necrotic type illness or alcoholic hepatitis relies heavily on the ratio of mitochondrial AST to total AST activity. In addition to having increased ALT values, patients with cirrhosis and other liver problems typically have elevated AST levels (Powles *et al.* 2015).

2.10.3 Alkaline phosphatase

Among the several hydrolases, alkaline phosphatase (ALP) is the one responsible for dephosphorylating proteins and nucleotides. In most nonhuman primate species, ALP is the most reliable indicator of hepatobiliary damage. When the patency of the bile duct is diminished, serum ALP levels rise; as a result, ALP is commonly employed in both animal and human clinical settings as a marker of cholestatic liver damage. The elevated activity of ALP, like that of ALT, is not limited to hepatobiliary damage; it may also be seen in situations of bone development and illness, in response to glucocorticoid therapy, and after stimulation of microsomal enzymes. Since an intestinal isoform of

ALP causes a reduction in ALP activity during fasting or anorexia and a temporary rise postprandially, caution must be used when interpreting fluctuations in ALP outside of clinical situations, especially in rodents. Isoforms of alanine aminotransferase (ALP) may be measured in order to distinguish between ALP originating in the liver, the bone, or the intestines, however in nonhuman primates, this information is not diagnostically useful compared to total ALP (Aulbach and Amuzie 2017).

3. MATERIALS AND METHODS

3.1 Study Design

The study included 140 samples collected from the sixteenth of April to the twenty-second of August at Al-Jumhouri Hospital - Kirkuk - Iraq.

The microbes in the study were classified into three groups:

- Group 1: 50 Overweight patients
- Group 2: 50 Obesity patients
- Group 3: 40 healthy people (Control group)

In addition, samples were selected based on BMI and age, but cases using therapies or suffering from chronic diseases or cancer were excluded.

3.2 Sample Collection

As we mentioned earlier that samples were collected from Al-Jumhuri Hospital in Kirkuk, we took samples from patients from the Obesity Control Center department. Following the physician's order, we took 5 mL of venous blood, incubated the samples at room temperature for 10 minutes, and then centrifuged them at 3700 rpm for 7 minutes. From whence is the serum taken? Four eppendorf tubes hold the gel tube. Finally, after serum separation, all samples were incubated and stored at 20 °C.

3.3 Materials

3.3.1 Instruments

Research equipment is listed in Table 3.1.

Table 3.1 Used Equipment and Utilities

No	Instruments and Tools	Origin
1	Oven	Germany
2	Centrifuge	Germany
3	Refrigerator	Turkey
4	Electronic balance	China
5	Pipette tips and automatic micropipettes that can dispense volumes from 10 to 1000 μL	China
6	ELISA reader	Germany
7	Gel tube	Lebanon
8	ELISA washer	Germany
9	Incubator	Germany
10	Spectrophotometer	Japan
11	Deep Freeze	Italy
12	Water bath	Germany
13	Disposable syringes	UK
14	Chemistry Analyzer	SPINREACT, Spain
15	Ependroff tubes	England
16	Cobas c111	Roch/ Germany

3.3.2 Kits

In Table 3.2, the researchers detail the many kits they put to use throughout the research.

Table 3.2 Kits used in this study

No	Kits	Lot. No.
1	Glucose	REF 1129005
2	Human Ghrelin (GHRL) ELISA Kit	MBS165622
3	Triglycerides	REF 20767107 322
4	VLDL	Triglycerides/5
5	Cholesterol	REF 03039773 190
6	Determination of alkaline phosphatase	MD41233
7	HDL-Cholesterol	REF 07528566 190
8	Determination of aspartate aminotransferase	MD41264
9	LDL-Cholesterol	REF 07005717 190
10	Determination of alanine aminotransferase	REF 1001171
11	Body Mass Index	Weight/$(Height)^2$

3.4 Methods

3.4.1 Human ghrelin (GHRL) ELISA kit

The Enzyme-Linked Immunosorbent Assay concept lies at the heart of this kit's assay (ELISA). To speed up the process, human GH antibody has already been put onto the plate. When GH is introduced to a sample, it binds to the antibodies that have been coated on the wells. The sample is incubated with GH, and then a biotinylated human GH Antibody is added.

Next, Streptavidin-HRP is added to the mixture, and it binds to the biotinylated GH antibody. A washing procedure follows incubation, removing unbound Streptavidin-HRP. After adding the substrate solution, a color change occurs that is directly proportional to the concentration of human GH. Absorbance is taken at 450 nm after the addition of an acidic stop solution to end the process.

Assay Procedure:

1. Follow the protocol to the letter and have your reagents, standards, and samples ready. Before using any reagent, make sure it has reached room temperature. The test is done in a room temperature environment.
2. Find out how many test strips will be necessary. To use, just insert strips into frames. The recommended storage temperature for the unused strips is between 2 and 8 degrees Celsius.
3. To the standard well, add 50 μL of the standard. Antibody should not be added to the standard well since biotinylated antibody already exists in the standard solution.
4. To the sample wells, add 40 μL of sample, followed by 10 μL of anti-GH antibody. Finally, to the sample wells and standard wells, add 50 μL of streptavidin-HRP (Not blank control well). Combine in a harmonious manner. Put some kind of sealant on the plate. The recommended incubation time is 60 minutes at 37 degrees Celsius.
5. The plate must be sealed and washed five times with wash buffer once the sealer has been removed. We recommend soaking wells in wash buffer (at least 0.35 ml) for 30 seconds to 1 minute for each wash. Automatic washing requires aspirating all wells and washing them five times with wash buffer, each time filling the wells to the brim. Remove excess liquid from the dish by blotting it with paper towels.
6. Incubate the substrates in a substrate solution A and B of 50 μL each. Put the sealed plate into a dark 37°C incubator for 10 minutes.
7. When you put 50 μL of Stop Solution in each well, the blue will quickly turn to yellow.
8. The optical density (OD) of each well must be measured at 450 nm using a microplate reader within 10 minutes after injecting the stop solution.

3.4.2 Glucose

Glucose oxidase (GOD) catalyzes the Trinder reaction, in which glucose is oxidized to D-gluconate and hydrogen peroxide. 1,2. When phenol and 4-aminoantipyrine (4-AA) are oxidized by hydrogen peroxide in the presence of peroxidase, red quinoneimine dye is generated, the concentration of which is proportional to the amount of glucose in the sample (POD).

3.5 Statistics

The data was statistically analyzed in SPSS version 26.0, Excel 2016 was used to plot the relationships between parameters. The independent sample ANOVA test was performed to compare the averages of obese and overweight individuals with the healthy group. Pearson's test was used to verify association, and receptor characteristic analysis was used to assess the sensitivity of diagnostic tests (ROC).

4. RESULTS AND DISCUSSION

4.1 Age

Table 4.4 individuals with obesity (28.80±6.24 year) and overweight (29.18±7.44 year) were at near non-significant (P=0.380) age values, when referenced to lean control individuals (30.78±7.21 year), as showed in the Figure 4.1.

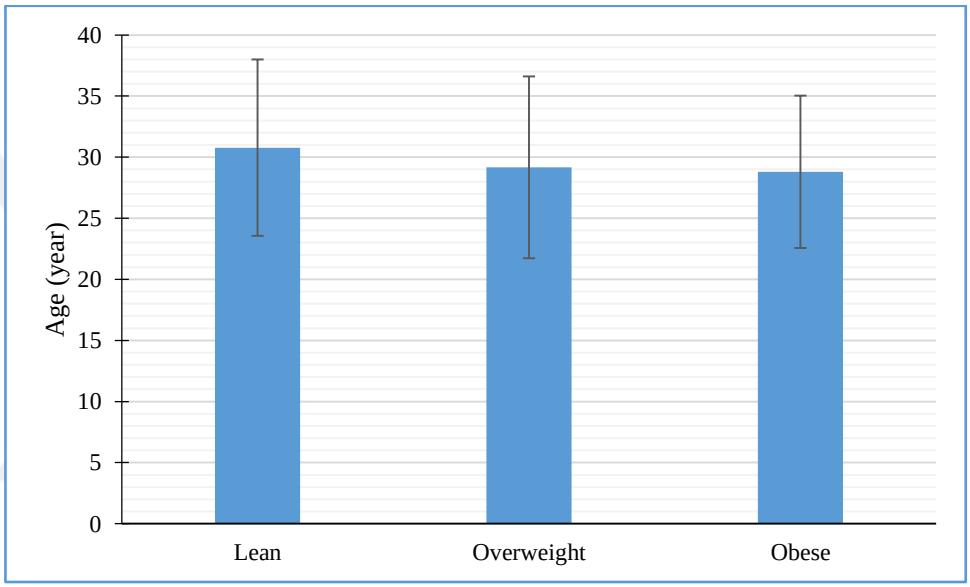

Figure 4.1 Age distribution's mean (■) and standard deviation (error bar) among people who are lean, overweight, or obese

4.2 Body Mass Index

The BMI was obtained in lean control individuals as 22.61±1.13 kg/m^2, in overweight individuals as 27.51±1.24 kg/m^2, and in obese individuals as 36.23±3.64 kg/m^2. The differences were highly significant (P<0.001) among the three groups, as seen in the Figure 4.2. Moreover, 44% of obese individuals were at the class I of obesity, 42% were at the class II of obesity, and 14% were morbidly obese individuals.

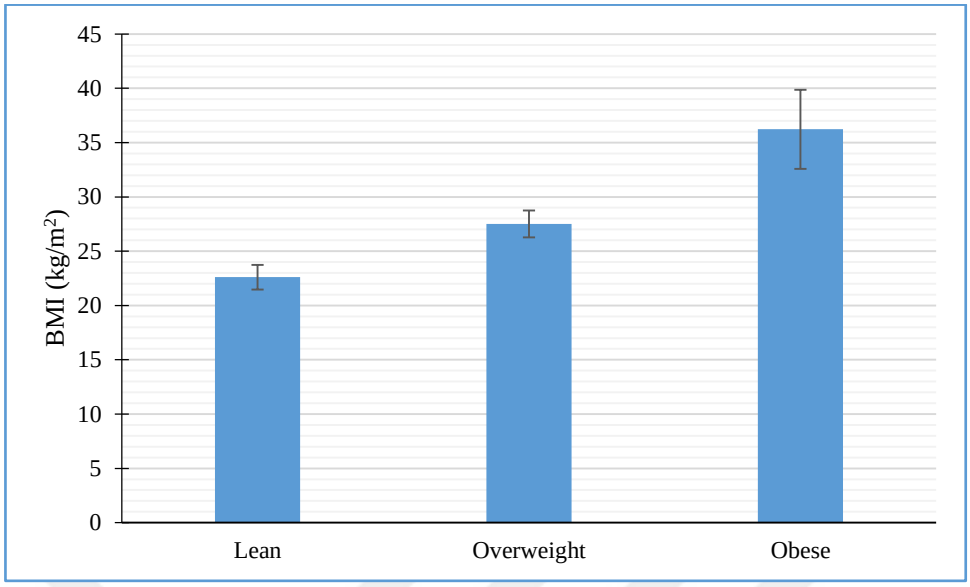

Figure 4.2 Body Mass Index (BMI) mean (■) and standard deviation (error bar) for healthy, overweight, and obese adults

4.3 Gender

All of the three groups (lean control, overweight, and obese) were contained equal numbers of males and females in the ratio of 50%, as shown in the Figure 4.3.

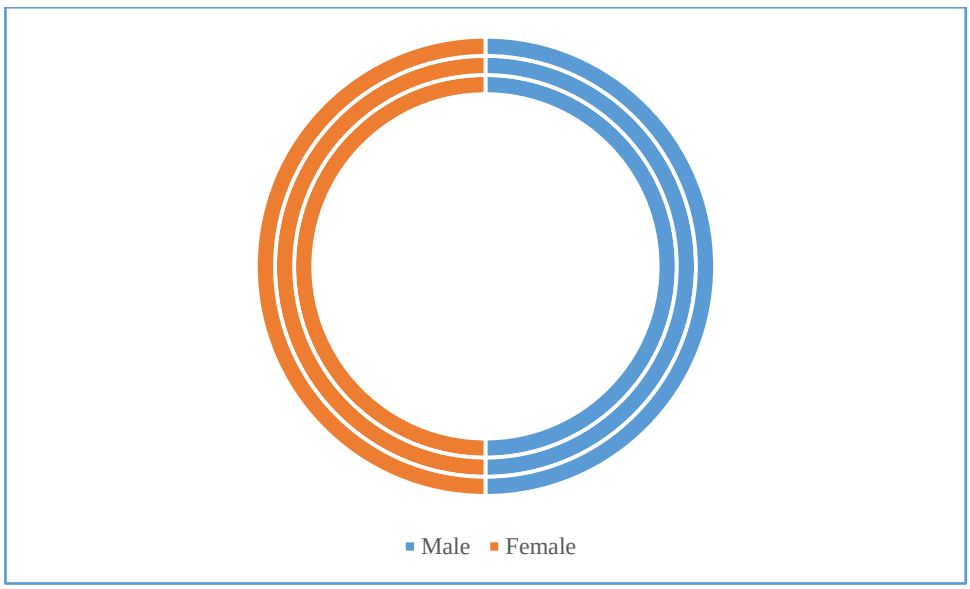

Figure 4.3 Pi chart of gender distributional percentage in lean control (inner circle), overweight (middle circle), and obese (outer circle)

4.4 Fasting Ghrelin

Significant (P<0.001) low serum concentration of ghrelin was observed in individuals with overweight (3.98±0.93 ng/mL) and obesity (2.38±1.11 ng/mL), when referenced to lean control (7.09±1.44 ng/mL). Also, the lowest concentration of ghrelin was seen in obese individuals, which was significantly (P<0.001) lower than that in overweight individuals, as seen in the Figure 4.4.

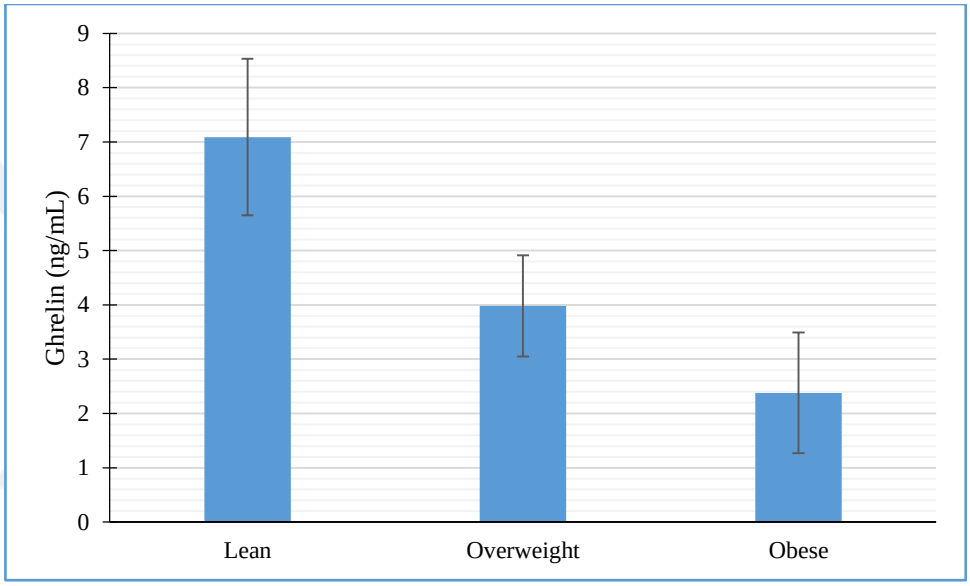

Figure 4.4 Values of ghrelin for people who are lean, overweight, and obese, together with their respective means (■) and standard deviations (error bar)

4.5 Fasting Glucose

Significant (P<0.001) high serum concentration of fasting glucose was observed in individuals with obesity (104.28±17.15 mg/dL), when referenced to overweight (93.32±7.37 mg/dL) and lean control (90.78±7.10 mg/dL). But the concentration of fasting glucose was seen in overweight and lean individuals in near values, which was non-significantly (P>0.05) higher in overweight than that in lean control individuals, as seen in the Figure 4.5.

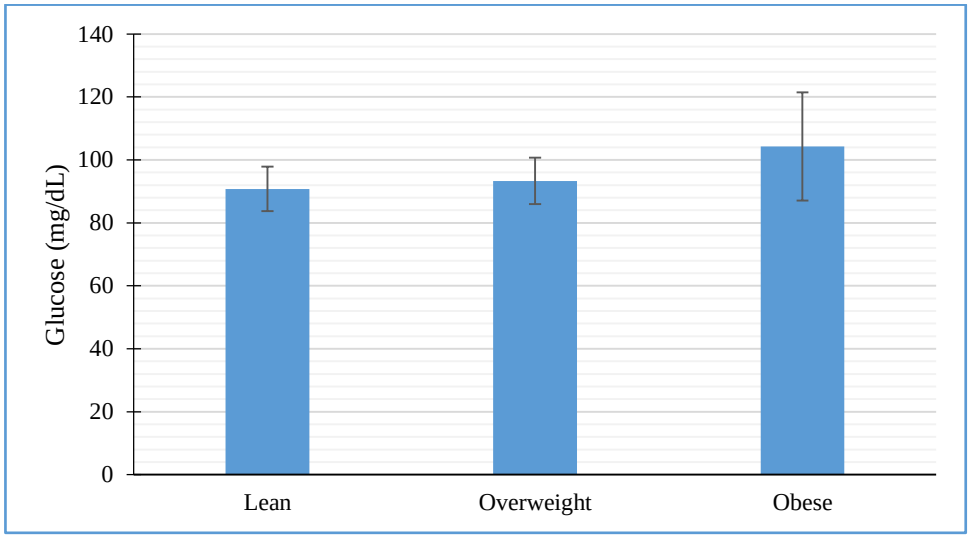

Figure 4.5 Fasting glucose means (■) and standard deviations (error bar) for lean, overweight, and obese people

4.6 Triglycerides

Significant (P<0.001) high serum concentration of TGs was observed in individuals with overweight (134.99±30.82 mg/dL) and obesity (167.28±35.81 mg/dL), when referenced to lean control (97.48±8.18 mg/dL). Also, the highest concentration of TGs was seen in obese individuals, which was significantly (P<0.001) higher than that in overweight individuals, as seen in the Figure 4.6.

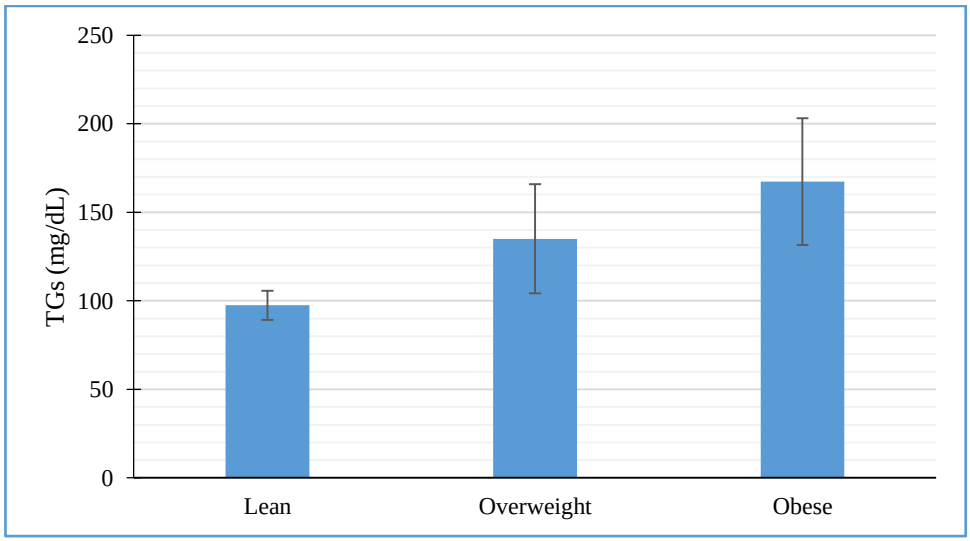

Figure 4.6 The average (■) and standard deviation (error bar) of fasting glucose in slim, plus-sized, and chubby people

4.7 Total Cholesterol

Significant (P<0.001) high serum concentration of TC was observed in individuals with overweight (243.17±62.88 mg/dL) and obesity (235.69±41.11 mg/dL), when referenced to lean control (138.06±23.28 mg/dL). But the concentration of TC was seen in overweight and obese individuals in near values, which was non-significantly (P>0.05) higher in overweight than that in obese individuals, as seen in the Figure 4.7.

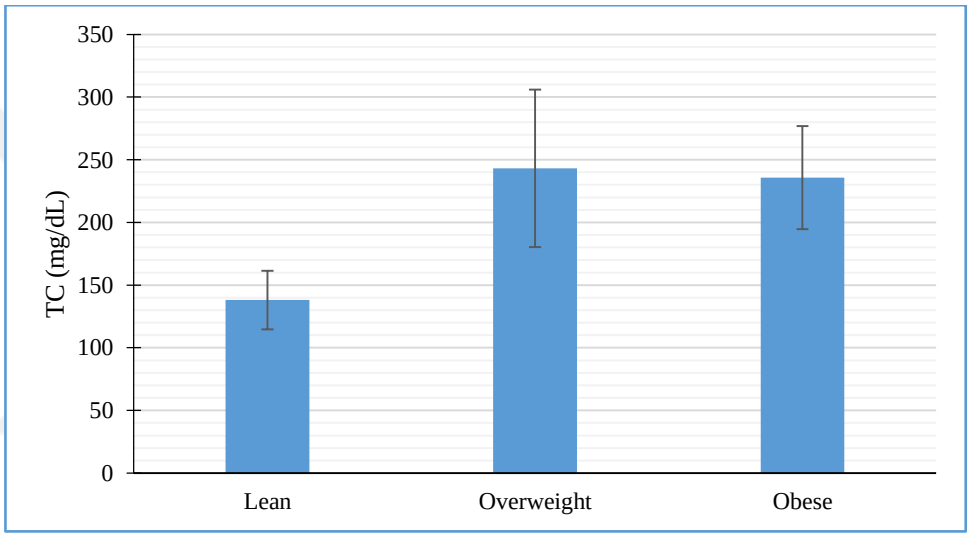

Figure 4.7 Standard deviation (error bar) and mean (■) TC for normal-weight, overweight, and obese people

4.8 High Density Lipoprotein

Significant (P<0.001) low serum concentration of HDL was observed in individuals with overweight (47.43±10.01 mg/dL) and obesity (40.74±7.75 mg/dL), when referenced to lean control (57.37±11.77 mg/dL). Also, the lowest concentration of HDL was seen in obese individuals, which was significantly (P<0.001) lower than that in overweight individuals, as seen in the Figure 4.8.

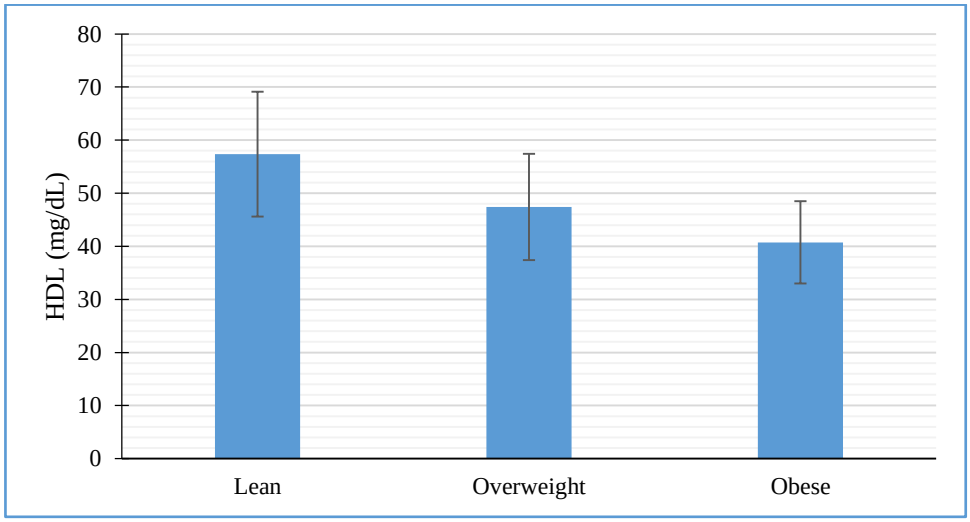

Figure 4.8 High density lipoprotein (HDL) distributions, means (■), and standard deviations (error bar) for persons of varying body mass indexes

4.9 Low Density Lipoprotein

Significant (P<0.001) high serum concentration of LDL was observed in individuals with overweight (168.74±59.11 mg/dL) and obesity (161.50±41.66 mg/dL), when referenced to lean control (61.19±24.21 mg/dL). But the concentration of LDL was seen in overweight and obese individuals in near values, which was non-significantly (P>0.05) higher in overweight than that in obese individuals, as seen in the Figure 4.9.

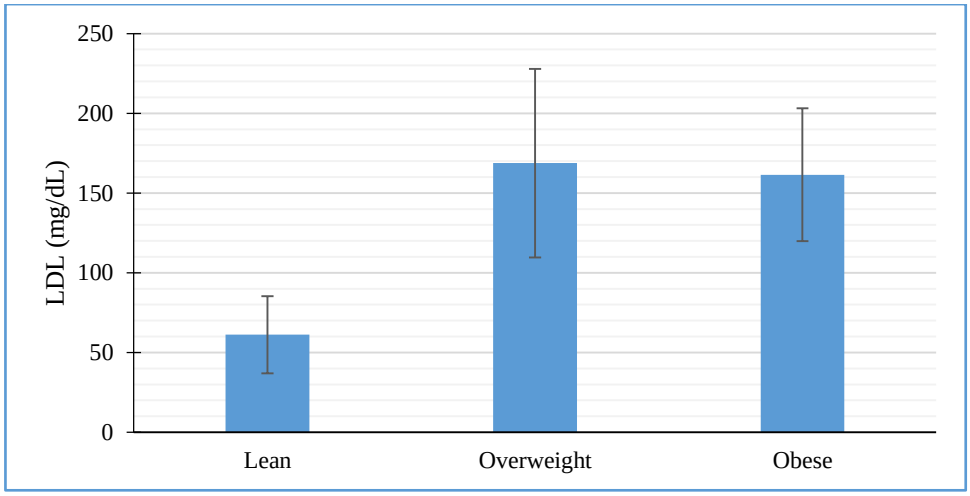

Figure 4.9 Normal, Overweight, and Obese Individuals' Mean and Standard Deviation of Low-Density Lipoprotein (LDL)

4.10 Very Low-Density Lipoprotein

Significant (P<0.001) high serum concentration of VLDL was observed in individuals with overweight (27.00±6.16 mg/dL) and obesity (33.46±7.16 mg/dL), when referenced to lean control (19.50±1.64 mg/dL). Also, the highest concentration of VLDL was seen in obese individuals, which was significantly (P<0.001) higher than that in overweight individuals, as seen in the Figure 4.10.

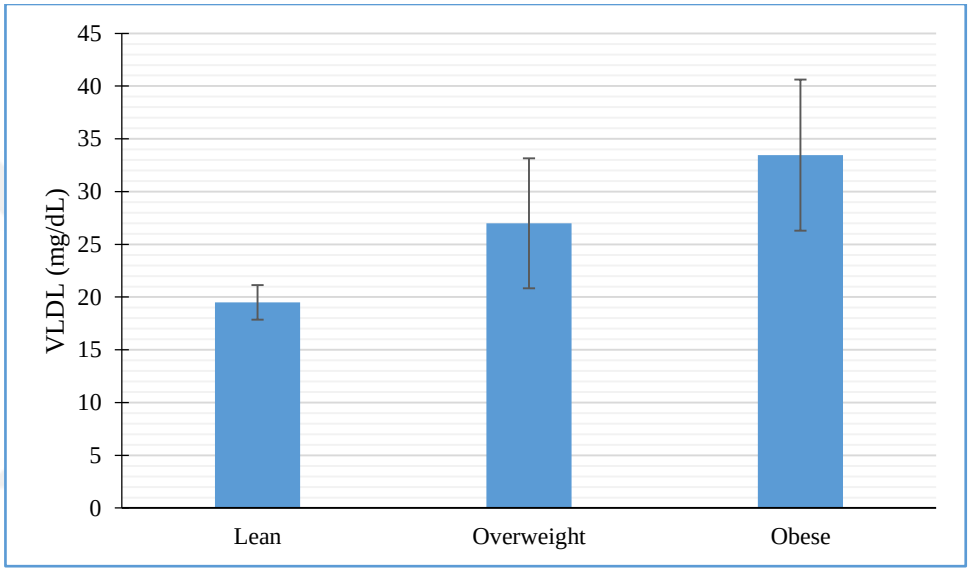

Figure 4.10 Very low-density lipoprotein (VLDL) mean (■) and standard deviation (error bar) in healthy, overweight, and obese adults

4.11 Alanine Aminotransferase

Significant (P<0.001) high serum activity of ALT was observed in individuals with obesity (31.02±13.96 IU/L), when referenced to overweight (21.24±4.70 IU/L) and lean control (21.53±4.72 IU/L). But the activity of ALT was seen in overweight and lean individuals in near values, which was non-significantly (P>0.05) lower in overweight than that in lean control individuals, as seen in the Figure 4.11.

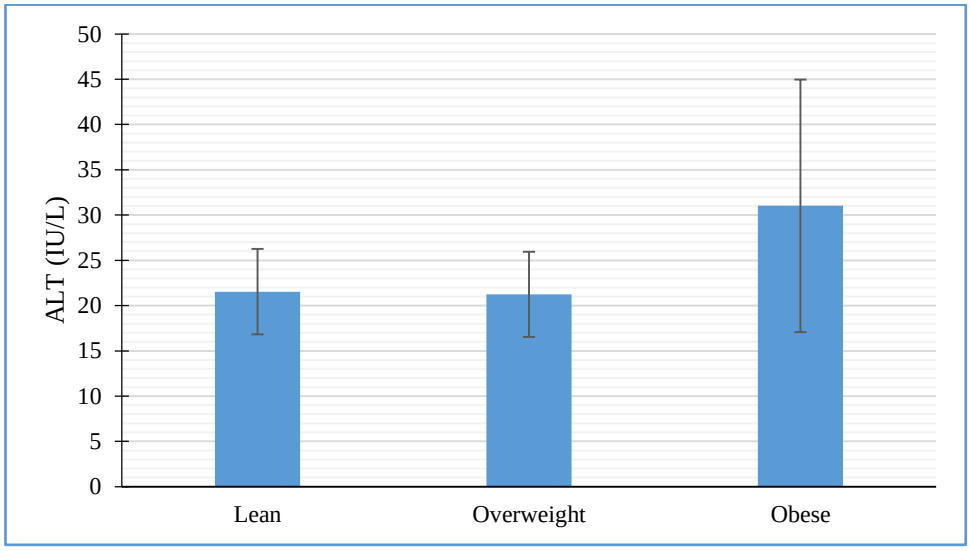

Figure 4.11 Normal, overweight, and obese people's ALT averages (■) and standard deviations (error bars)

4.12 Aspartate Aminotransferase

Significant (P<0.001) high serum activity of AST was observed in individuals with obesity (31.60±13.31 IU/L), when referenced to overweight (22.76±4.72 IU/L) and lean control (22.80±4.95 IU/L). But, the activity of AST was seen in overweight and lean individuals in near values, which was non-significantly (P>0.05) lower in overweight than that in lean control individuals, as seen in the Figure 4.12.

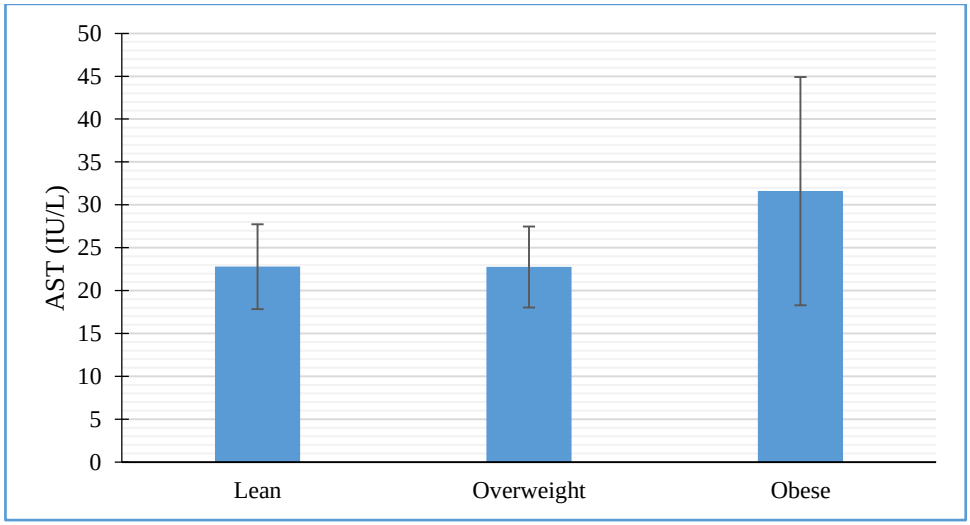

Figure 4.12 Variation from the Mean (■) and Standard Deviation (error bar) of AST in People of Various BMIs

4.13 Alkaline Phosphatase

Significant (P<0.001) high serum activity of ALP was observed in individuals with obesity (182.74±33.13 IU/L), when referenced to overweight (120.10±20.15 IU/L) and lean control (117.00±22.80 IU/L). But the activity of ALP was seen in overweight and lean individuals in near values, which was non-significantly (P>0.05) higher in overweight than that in lean control individuals, as seen in the Figure 4.13.

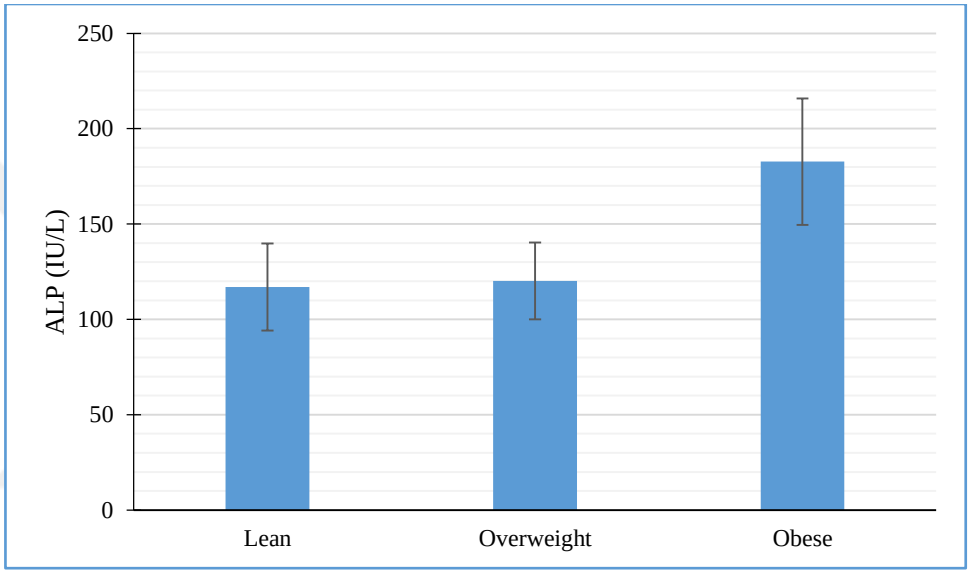

Figure 4.13 Comparison of mean alp (■) and standard deviation (error bar) between people of normal, overweight, and obese weights and weight loss patients

4.14 Correlation in Individuals with Overweight

Overweight individuals' body mass index (BMI) and ghrelin levels, as well as their relationships to the other variables in this research, are shown in Table 4.1.

The levels of serum ghrelin are inversely proportional to body mass index (BMI) in individuals who are overweight, as illustrated in Figure 4.14. BMI was also correlated with HDL negatively (Figure 4.15).

Table 4.1 Correlation in overweight individuals

PARAMETER	BMI		GHRELIN	
	r	*P*	r	*P*
Ghrelin	-0.674**	0.0001	-	-
Age	0.020	0.889	0.042	0.773
Fasting glucose	-0.076	0.601	-0.056	0.697
TGs	-0.120	0.406	0.171	0.235
TC	0.048	0.740	0.130	0.368
HDL	-0.330*	0.019	0.150	0.297
LDL	0.120	0.408	0.095	0.512
VLDL	-0.120	0.406	0.171	0.235
ALT	0.011	0.939	0.119	0.412
AST	-0.238	0.096	0.316*	0.025
ALP	0.265	0.063	-0.325*	0.021

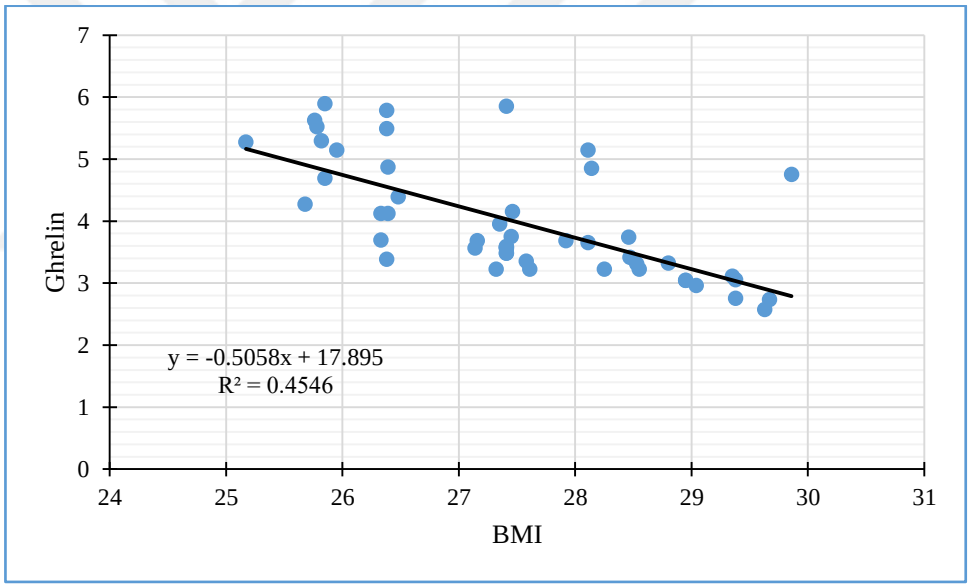

Figure 4.14 Correlation of BMI and ghrelin in overweight individuals

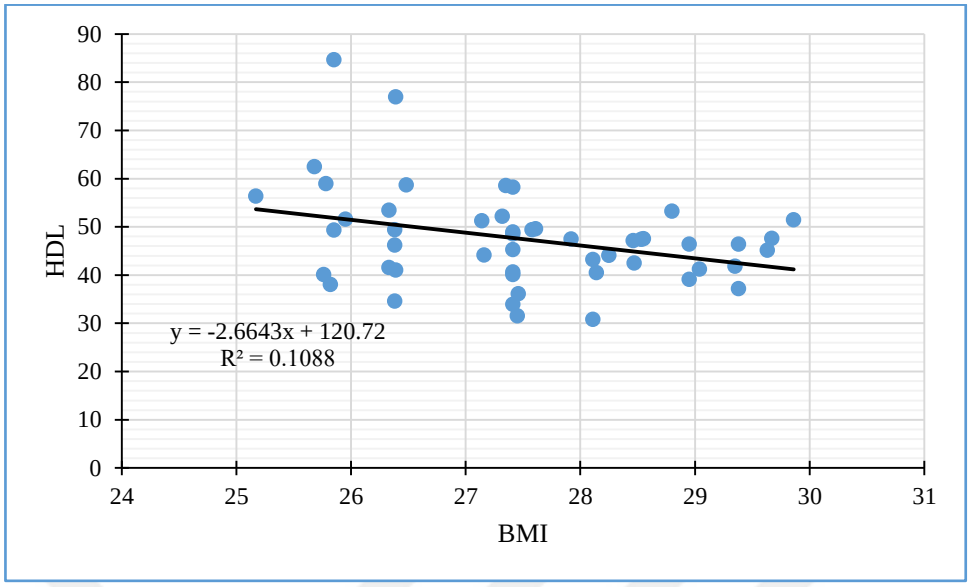

Figure 4.15 Correlation of BMI and HDL in overweight individuals

Individuals with overweight have shown to have a positive correlation between the ghrelin level and the activity of AST, as shown in the Figure 4.16. Fasting ghrelin was also correlated with ALP negatively (Figure 4.17).

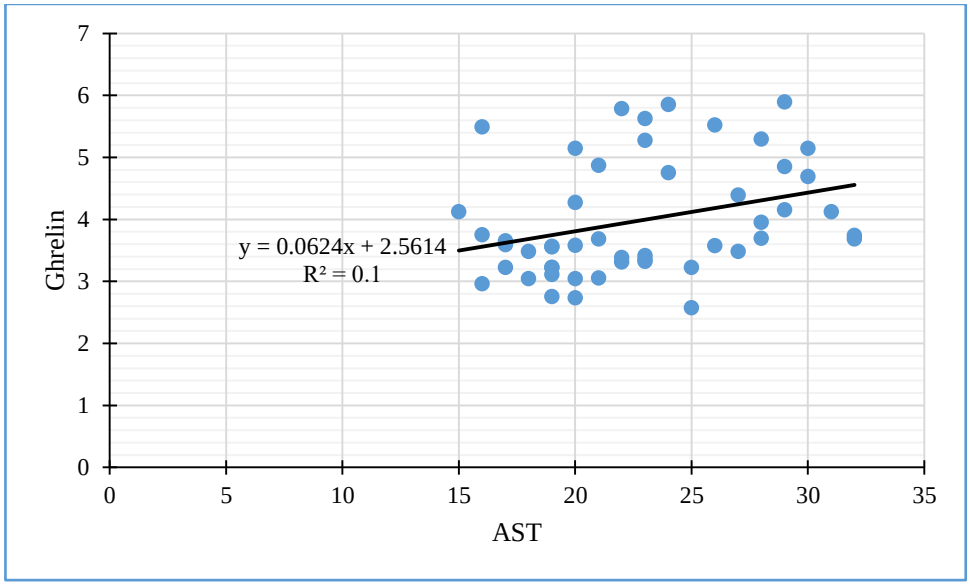

Figure 4.16 Correlation of ghrelin and AST in overweight individuals

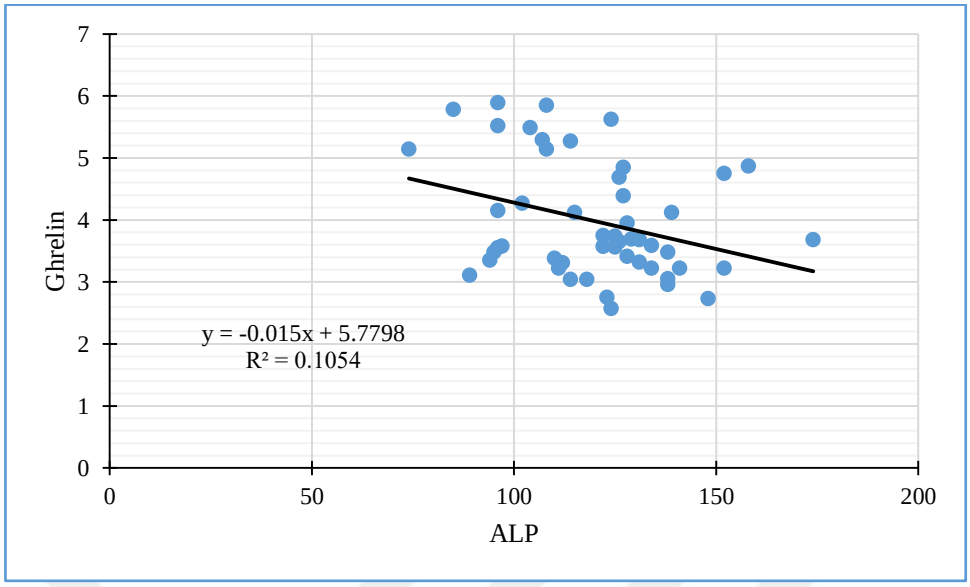

Figure 4.17 Correlation of ghrelin and ALP in overweight individuals

4.15 Correlation in Individuals with Obesity

Table 4.2 displays the results of a test examining the relationship between body mass index and ghrelin, as well as the other variables in this investigation.

Table 4.2 Correlation in obese individuals

PARAMETER	BMI		GHRELIN	
	r	*P*	r	*P*
Ghrelin	-0.826**	0.0001	-	-
Age	0.061	0.672	0.006	0.969
Fasting glucose	0.179	0.214	-0.193	0.179
TGs	0.382**	0.006	-0.291*	0.041
TC	0.117	0.417	-0.133	0.356
HDL	-0.023	0.872	-0.081	0.575
LDL	0.054	0.707	-0.066	0.647
VLDL	0.382**	0.006	-0.291*	0.041
ALT	0.613**	0.0001	-0.556**	0.0001
AST	0.435**	0.002	-0.401**	0.004
ALP	0.820**	0.0001	-0.646**	0.0001

Obese people tend to have higher than average concentrations of serum ghrelin (Figure 4.18), triglyceride (Figure 4.19), very low-density lipoprotein (VLDL) (Figure 4.20), alanine aminotransferase (ALT) (Figure 4.21), aspartate aminotransferase (AST) (Figure 4.22), and alkaline phosphatase (ALP) (Figure 4.23).

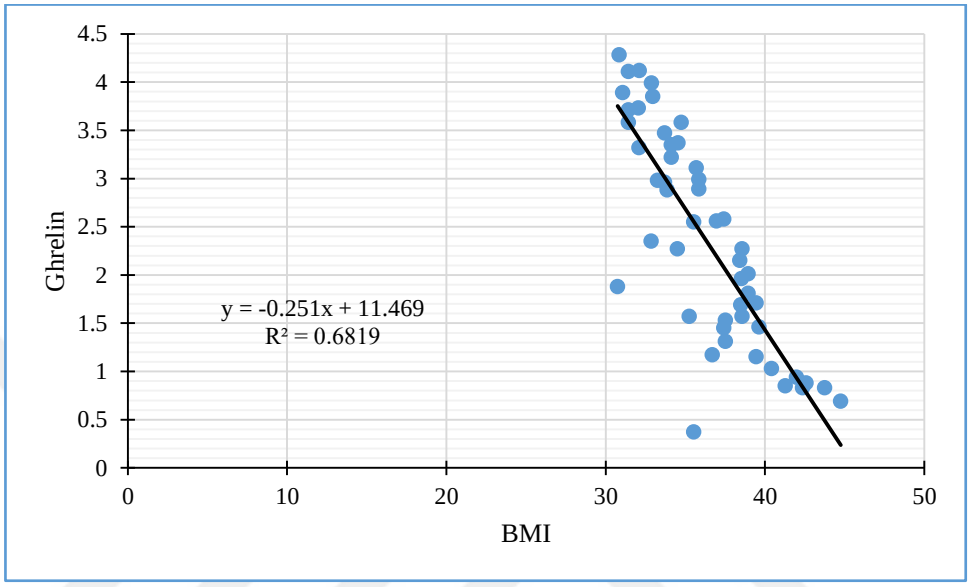

Figure 4.18 Correlation of BMI and ghrelin in obese individuals

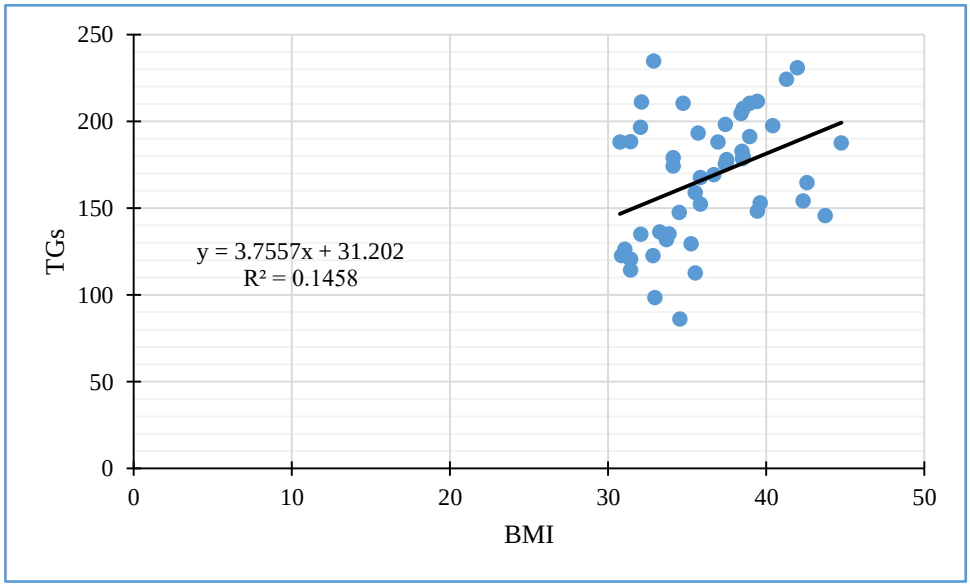

Figure 4.19 Correlation of BMI and TGs in obese individuals

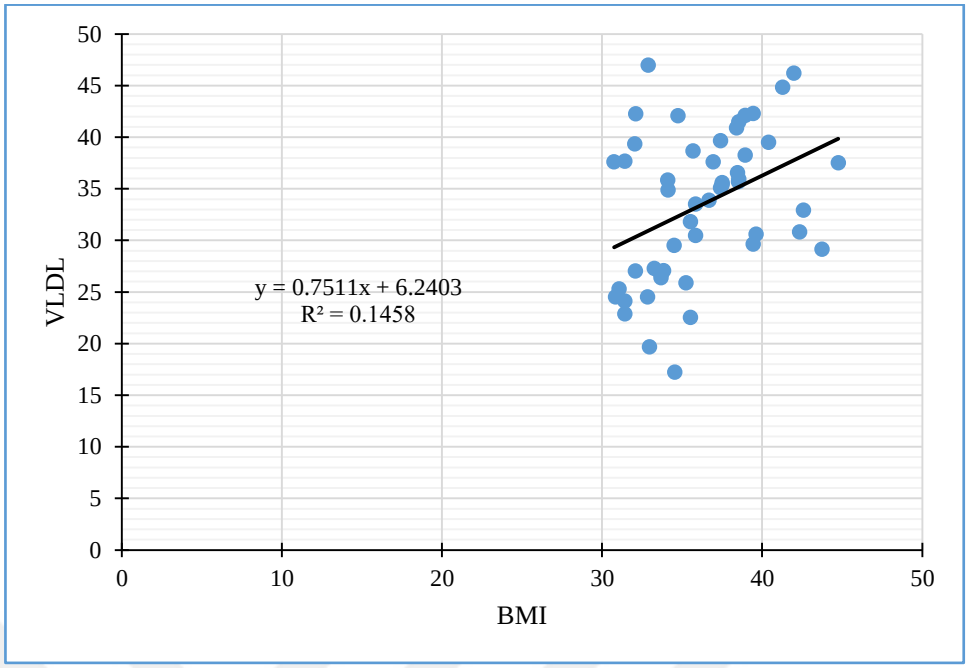

Figure 4.20 Correlation of BMI and VLDL in obese individuals

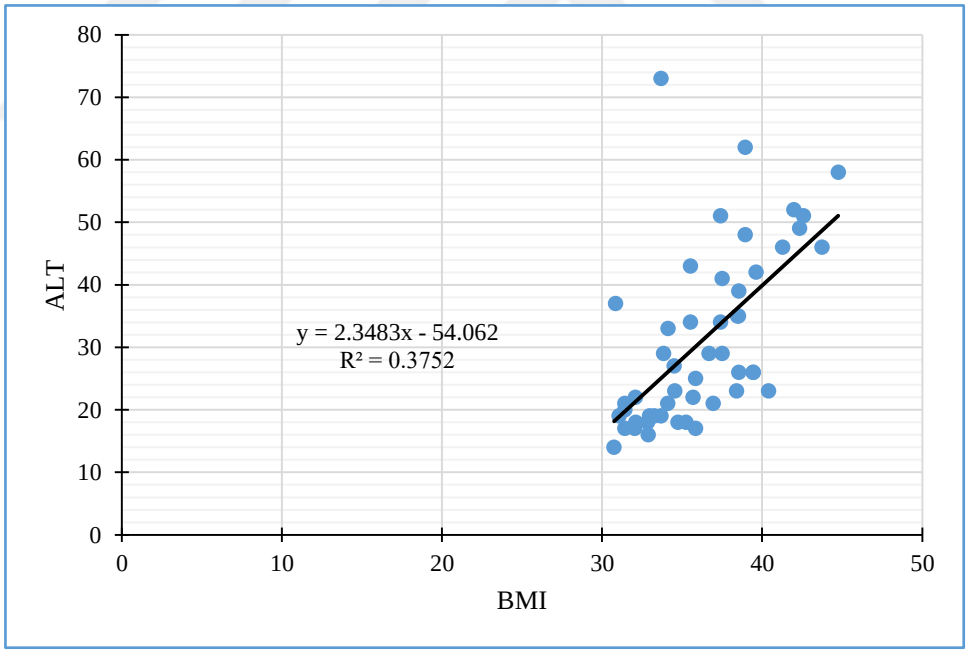

Figure 4.21 Correlation of BMI and ALT in obese individuals

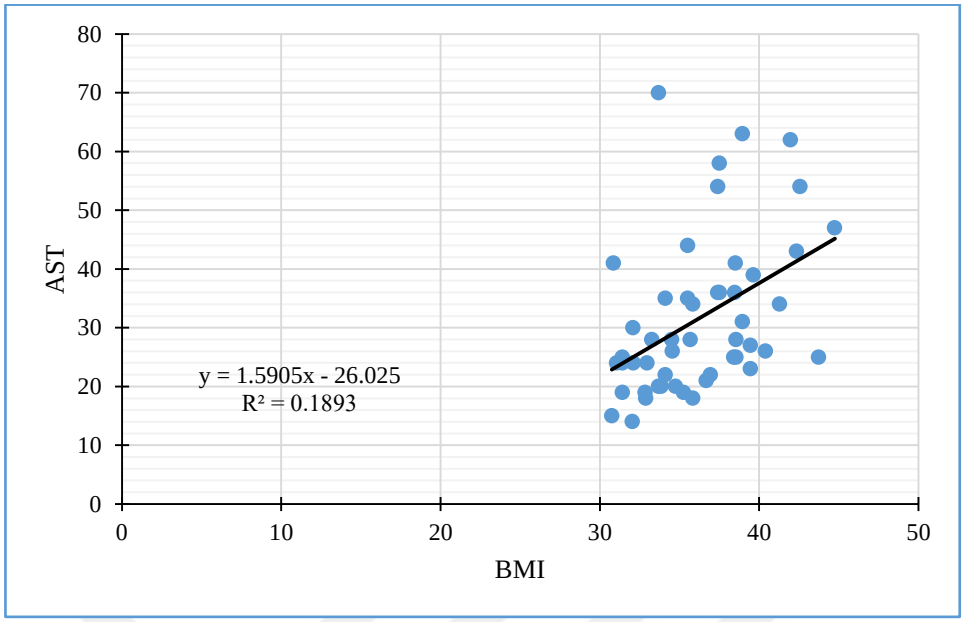

Figure 4.22 Correlation of BMI and AST in obese individuals

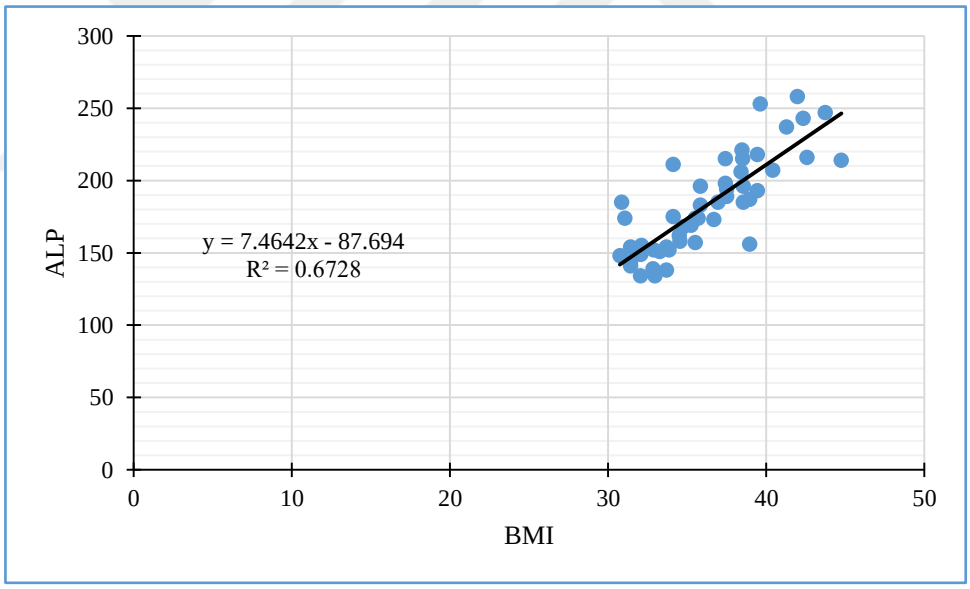

Figure 4.23 Correlation of BMI and ALP in obese individuals

Individuals with obesity have shown to have a negative correlation between the serum ghrelin levels and TGs (Figure 4.24), between ghrelin levels and VLDL (Figure 4.25), between ghrelin levels and ALT activity (Figure 4.26), between ghrelin levels and AST activity (Figure 4.27), and between ghrelin levels and the ALP activity (Figure 4.28).

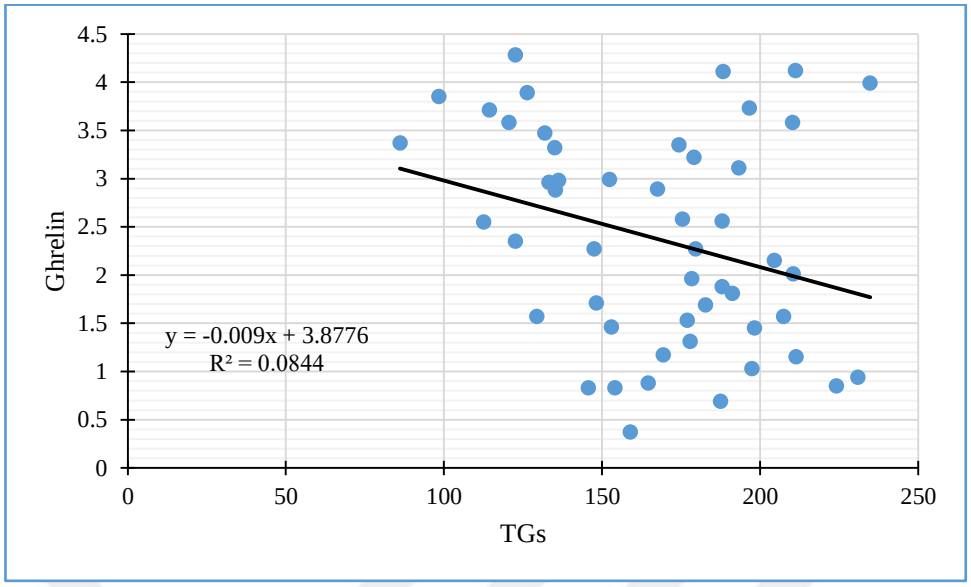

Figure 4.24 Correlation of ghrelin and TGs in obese individuals

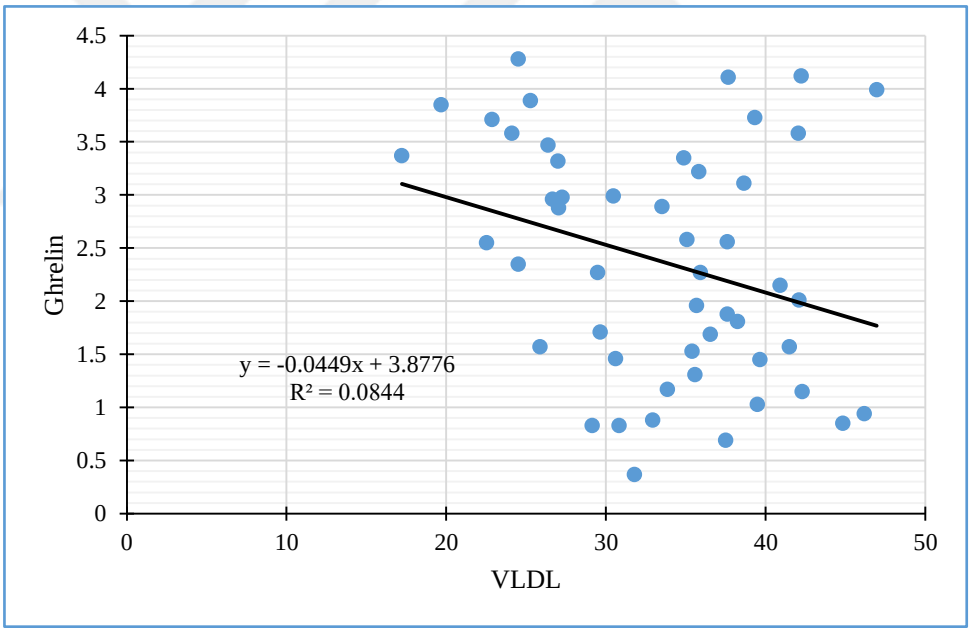

Figure 4.25 Correlation of ghrelin and VLDL in obese individuals

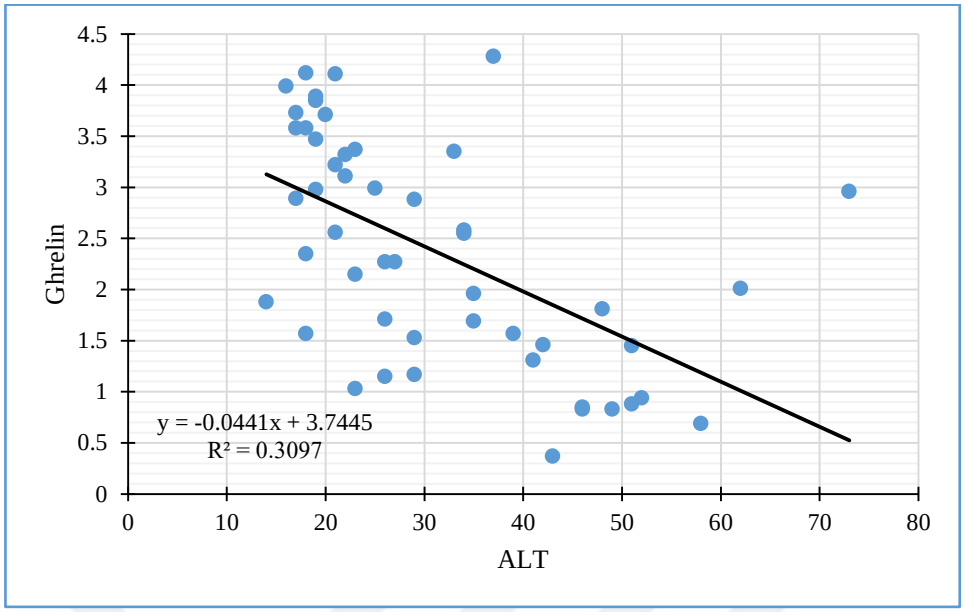

Figure 4.26 Correlation of ghrelin and ALT in obese individuals

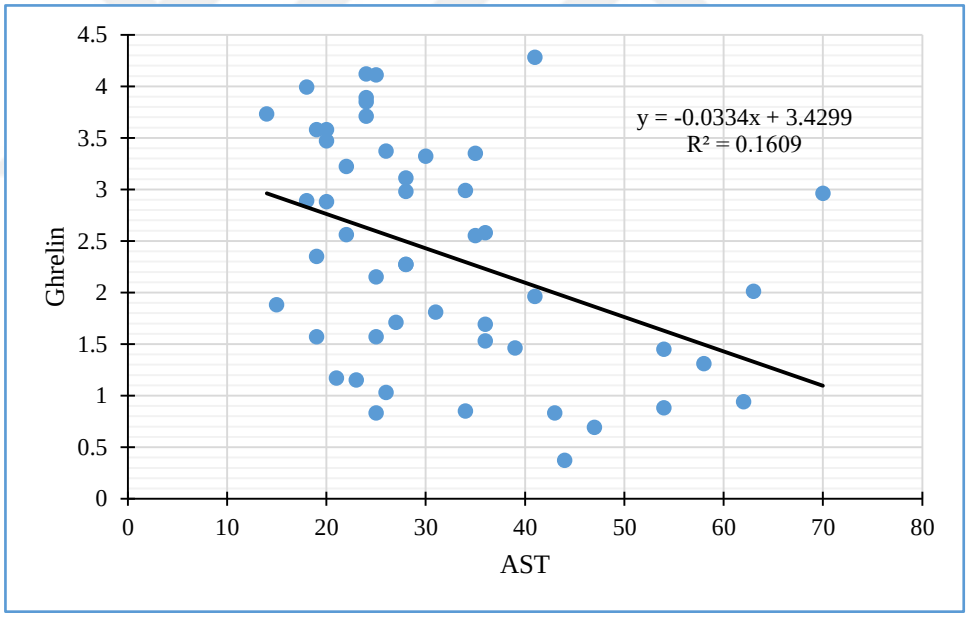

Figure 4.27 Correlation of ghrelin and AST in obese individuals

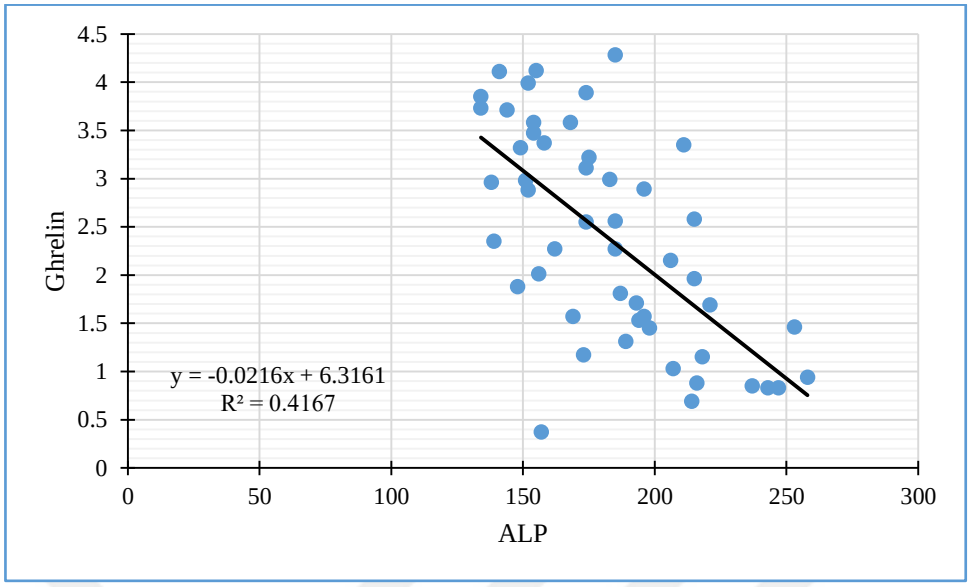

Figure 4.28 Correlation of ghrelin and ALP in obese individuals

4.16 Receiver Operating Characteristics

Ghrelin was tested for the prognosis of overweight and obesity by using the ROC curve test. The area under the curve (AUC) and cut-off value of ghrelin in overweight and obesity prognosis are listed in Table 4.3 with the sensitivity and specificity.

Table 4.3 ROC results of ghrelin (ng/mL) in the prognosis of overweight and obesity

PARAMETER	OVERWEIGHT	OBESITY
AUC	0.959	1.0
Standard error	0.017	0.0001
P-value	0.0001	0.0001
Cut-off value	5.17	4.52
Sensitivity %	90%	100%
Specificity %	84%	100%

As can be shown in Figure 4.29, ghrelin has a high area under the curve for detecting obesity (AUC=0.959, P<0.001), as evidenced by the ROC analysis in Table 4.4. Furthermore, the cut-off value of ghrelin for the prognosis of overweight was found to be 5.17 ng/mL, which indicates that when ghrelin is obtained in individuals at a

level lower than 5.17 ng/mL, they may be able to refer to weight gain within the limits of overweight with 90% sensitivity and 84% specificity.

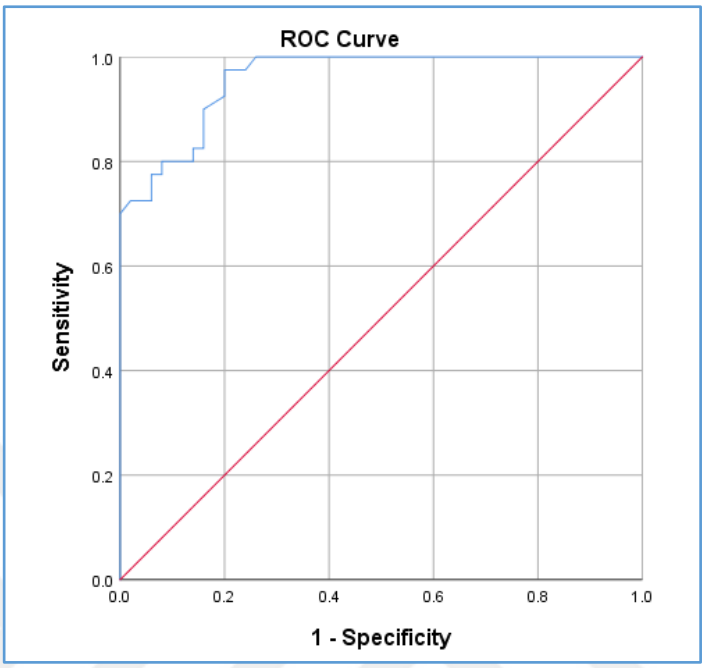

Figure 4.29 The curve of ROC for ghrelin in the prediction of overweight

Table 4.4's ROC analysis demonstrates that ghrelin has high diagnostic accuracy for obesity (AUC=1.0, P<0.001), as can be shown in Figure 4.30. More importantly, the cut-off value of ghrelin for the prognosis of overweight was determined to be 4.52 ng/mL, which indicates that when ghrelin is obtained in individuals at a level lower than 4.52 ng/mL, they may be able to refer to weight gain within the limits of overweight with a sensitivity of 100% and a specificity of 100%.

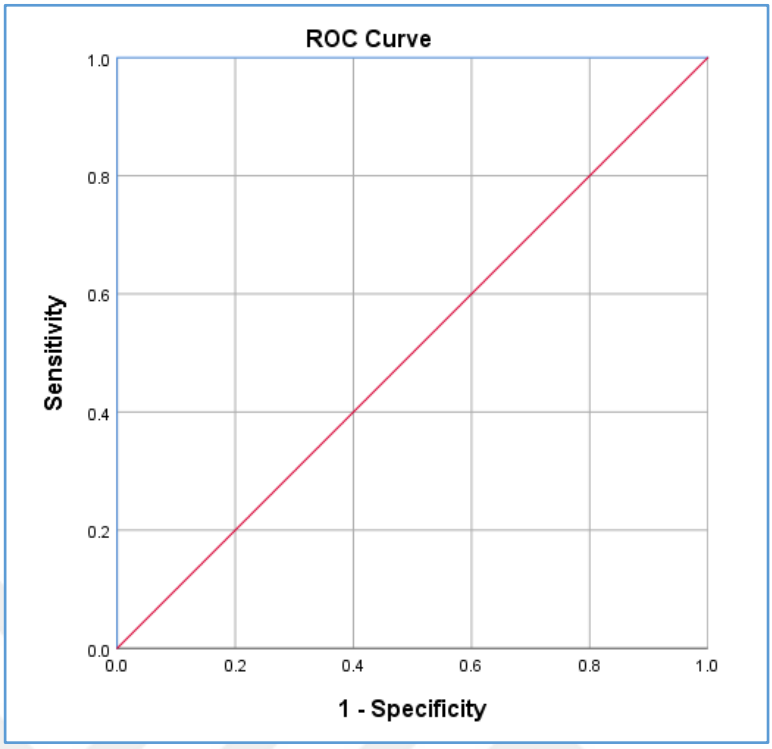

Figure 4.30 Obesity prognosis using ghrelin's ROC curve

Table 4.4 The mean and SD for all parameters used in this study between obesity and overweight and the control group

Parameters	**Mean ±SD**			**P-Value**
	Lean Control	overweight	obesity	
Age (year)	30.78±7.21	29.18±7.44	28.80±6.24	0.380
BMI (kg/m^2)	22.61±1.13	27.51±1.24	36.23±3.64	<0.001
Ghrelin (ng/mL)	7.09±1.44	3.98±0.93	2.38±1.11	<0.001
Fasting glucose (mg/dL)	90.78±7.10	93.32±7.37	104.28±17.15	<0.001
Triglycerides (mg/dL)	97.48±8.18	134.99±30.82	167.28±35.81	<0.001
ALT (IU/L)	21.53±4.72	21.24±4.70	31.02±13.96	<0.001
Total Cholesterol (mg/dL)	138.06±23.28	243.17±62.88	235.69±41.11	<0.001
AST (IU/L)	22.80±4.95	22.76±4.72	31.60±13.31	<0.001
HDL (mg/dL)	57.37±11.77	47.43±10.01	40.74±7.75	<0.001
ALP (IU/L)	117.00±22.80	120.10±20.15	182.74±33.13	
LDL (mg/dL)	61.19±24.21	168.74±59.11	161.50±41.66	<0.001
VLDL (mg/dL)	19.50±1.64	27.00±6.16	33.46±7.16	<0.001

5. DISCUSSION

Participants' ages were selected in accordance with a study paradigm in which the impact of participants' ages on the outcome measures is nullified. In this research, subjects were stratified into three groups according to their body mass index (BMI), with those with BMIs below 25 kg/m2 serving as the lean control group. Health issues such as insulin resistance (Kahn and Flier, 2000), high blood pressure (Taay and Mohammed, 2020), and liver dysfunction (Schiavo *et al.*, 2018) have all been linked to an overweight or obese person's BMI.

For those who are overweight or obese, ghrelin levels are inversely related to body mass index. In comparison to slim people, Korek *et al.* found that those who are overweight had much lower ghrelin levels (Korek *et al.* 2013). The research was conducted by (Korek *et al.*, 2013). Studies by Subramanian *et al.* showed that low ghrelin levels in obese people were inversely related to their body mass index (BMI) (Subramanian *et al.* 2004). (Subramanian *et al.*, 2004) The ghrelin levels of obese children and adolescents were found to be low by Reinehr *et al.*, and they did not significantly increase when the children and adolescents lost weight (Reinehr *et al.* 2008). Reported by Reinehr *et al.* 2008. According to studies conducted by Monti *et al.*, those who are overweight or obese have significantly decreased ghrelin levels, and this decrease is correlated negatively with the increase in BMI in both the obese and overweight as well as in the lean and healthy (Monti *et al.* 2006). Based on their findings, Bouillon-Minois *et al.* concluded that ghrelin is a stress mediator, with the impact being more noticeable in those who were already overweight or obese. Ghrelin has also been proposed for usage in helping obese and overweight people cut down on their food intake (Bouillon-Minois *et al.* 2021).

Obese persons tend to have lower levels of good cholesterol (HDL) and higher levels of bad ones (fasting glucose, triglycerides, cholesterol, low-density lipoprotein, and very low-density lipoprotein). In their study of obese women, Sadiq and Jassim found similar outcomes, with the authors attributing the lipid profile anomalies to complete adipocyte storage of lipids and decreased expression of lipoprotein lipase (Sadiq and Jassim,

2021). Elevated fasting glucose and lipid abnormalities were also documented by Taay *et al.* in their investigation of obese women. The authors state that insulin resistance linked to obesity is to blame for the rise in fasting glucose (Taay *et al.* 2021). Consistent glucose and lipid profile findings have been reported in previous research (Bhatti *et al.* 2001, Schmatz *et al.* 2017, Tahir *et al.* 2014).

Obese people tend to have higher than average levels of ALT, AST, and ALP. The ALT and AST findings are consistent with those of other research efforts (Saleh, 2008; Isra'a, 2010; nanir, 2020; AL-Samarrai *et al.*, 2012. It is well known that the elevated lipids associated with obesity may lead to liver damage by overloading the organ. Damage to the liver may occur through many pathways, one of which is the entry of free fatty acids stored in visceral fat depots directly into the portal circulation. The lipolytic capacity of visceral adipose tissue is higher than that of the more common subcutaneous adipose tissue (Marchesini *et al.* 2008). Increased body fats in both men and women, as observed by Bekkelund and Jorde, have an effect on ALT (Bekkelund and Jorde, 2019). In addition, Khan *et al.* found that the serum of obese people had significantly elevated ALP activity (but not ALT or AST) (Khan *et al.* 2015.

6. CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

1. Ghrelin was reduced significantly in the serum of overweight and obese individuals.
2. Ghrelin was correlated negatively with the BMI in overweight and more strongly in obese individuals.
3. Glucose was elevated significantly in obese individuals.
4. Lipid profile abnormalities were seen in both overweight and obese individuals.
5. The activities of ALT, AST and ALP were elevated significantly in the serum of obese individuals.
6. BMI was correlated positively with TGs, VLDL, ALT, AST and ALP in obese individuals, and negatively with HDL in overweight individuals.
7. Ghrelin was correlated negatively with TGs, VLDL, ALT, AST and ALP in obese individuals, and with ALP in overweight individuals.
8. Ghrelin is an excellent sensitive marker in the prognosis of overweight and obesity.

6.2 Recommendations

1. Enhancing ghrelin level in overweight and obese individuals which may increase the weight loss.
2. Eliminating the abnormalities of lipid profile in obese individuals to reduce the cardiovascular risks.
3. Controlling the activity of ALP in obesity.
4. Study the link between ghrelin and inflammatory events in overweight and obese individuals.

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