Effect of Obesity on Angiotensin II, Electrolytes and Lipid Profile Level's

A Thesis

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بِسْمِ اللهِ الرَّحْمَنِ الرَّحِيمِ

((وَقُل رَّبِّ زِدْنِي عِلْمًا))

صَدَقَ اللهُ العليُّ العَظِيمُ

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Supervisor Certification

We certify that this thesis entitled **(Effect of Obesity on Angiotensin II, Electrolytes and Lipid Profile Level's)** has been prepared under our supervision at the Department of chemistry and Biochemistry, College of Medicine, University of Babylon, in partial requirements for the degree of master in Clinical Biochemistry.

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Date   /   / 2022

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DEDICATION

I would like to dedicate this thesis to ……………

To everyone who fell martyr in defense of the homeland

To my martyr brother….Adel

To the person who gave me strength and pride... my dear father

To the person who facilitates the difficulties in my study and inspired and encourage me to do this study….. My dear mother.

To my beloved Husband, who were supporting and encouraging me to believe in myself.

To the piece of my eyes…… My dear Children

To my bond, my consolation, my joy and my honor ….My dear brothers & Sisters

To everyone who supported, and encouraged me to do my study

Rasha majid

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Summary

Obesity is one of the most common health issues among children and adolescents in both industrialized and developing nations, It is form as a result of a combination of low energy expenditure (such as exercise) and a high calorie intake caused by multifaceted conditions such as inherited and environmental factors. Angiotensin II (Ang II) and electrolytes linked with obesity and related complications such as hypertension, metabolic disease, cardiovascular, and kidney disease.

Present study was designed to evaluate the Angiotensin II and explain the effects on electrolytes and some biochemical changes in obese individuals. To achieve this aims, 88 participants were involved in current study, 44 obese patients with ages range between (18-50) years compared with 44 apparently healthy individuals with age matching (as a control group). Serum angiotensin II was determined by enzyme linked immunosorbant assay (ELISA) method. While electrolytes (Na^+, K^+, Cl^-, iCa^{++}) estimated by GE300 electrolyte analyzer, Vitamin D_3 (Vit D_3) determined by High pressure liquid chromatography (HPLC), and total cholesterol, triacylglycerol and high density lipoprotein cholesterol (HDL-C) concentrations were determined by UV-visible spectrophotometric technique.

The results for obese patients in present study revealed that a significant ($p < 0.001$) increase in Angiotensin II concentration, with insignificant ($p > 0.005$) changes of electrolytes (Na^+, K^+, Cl^-) except highly significant ($p < 0.000$) decrease of ionized calcium (iCa^{++}), in addition to that Vitamin D_3 also, decrease significantly ($p < 0.003$), furthermore, (total cholesterol, triacyglycerol, very low density lipoprotein and low density lipoprotein) were increased significantly ($p < 0.000$) while high density lipoprotein was decreased significant ($p < 0.000$) in obese patients when compared to those of the controls group.

The results of linear regression analysis among obese patients shows positive correlations (r = 0.371, p < 0.013) of Angiotensin II concentration with sodium and positive correlations (r = 0.523, p < 0.000, r =0.656, p < 0.000) of body mass index (BMI) with sodium and triacylglycerol respectively. Also, the same analysis show negative correlations (r =-0.494, p < 0.001, r =-0.552, p <0.00) of body mass index with Vitamin D_3 and high density lipoprotein respectively. Furthermore, positive correlation (r =0.354 p < 0.018) of sodium with triacylglycerol was recorded.

This study was concluded that increase Angiotensin II in obese patients lead to retention of sodium, decrease potassium and dyslipidemia. In addition to that vitamin D_3 was strongly associated with decrease of ionized calcium and potassium. Also positive relationship between sodium and triacylglycerol play an important role in obesity development.

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LIST OF ABBREVIATION

Abbreviation	Description
Ang II	Angiotensin II
ACE	Angiotensin Converting Enzyme
ADH	Antidiuretic Hormone
AT1R	Angiotensin Receptor Type 1
AT2R	Angiotensin Receptor Type 2
BMI	Body Mass Index
BAT	Brown Adipose Tissue
Carbs	Carbohydrate
CVD	Cardio Vascular Disease
CKD	Chronic Kidney Disease
HDL	High Density Lipoprotein
IL-6	Interleukin -6
LDL	Low Density Lipoprotein
PG12	Prostaglandin 12
RAAS	Renin Angiotensin Aldosterone System
SNS	Sympathetic Nerves System
TNF	Tumor Necrosis Factor
UPC-1	Un Coupling Protien-1
VLDL	Very Low Density lipoprotein
WAT	White Adipose Tissue
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

AND

LITERATURES REVIEW

1. Introduction

Obesity is one of the most common health issues among children and adolescents in both industrialized and developing nations[1], It is a multifaceted condition caused by both inherited and environmental factors. Obesity develops as a result of a combination of low energy expenditure and a high calorie intake. This causes an excessive buildup of fat in the adipose tissue, which expands to handle the increased calorie intake and undergoes significant structural and cellular alterations[2-3]. Obesity includes abnormalities in clinical and laboratory tests that are associated with a higher risk of cardiovascular illnesses, type 2 diabetes (T2DM), abnormalities in electrolytes, and malignancies that have negative prognoses. Waist circumference (WC) and body mass index (BMI) are crucial factors in determining and evaluating obesity. The (BMI) is a popular measure of obesity that is calculated by dividing an individual's weight (in kilograms) on square of height (in meters). A BMI of 30 and above is considered obese. Unlike WC, it does not quantify the distribution of body fat and may not be correlated with the same level of obesity or associated health concerns in various people and communities; due to this fat measuring WC provides a straightforward, obvious, and useful method of identifying obesity-related health issues **[4– 6]** .

The adipose tissue acts as an endocrine organ producing a number of substances with an important role in the regulation of food intake, energy expenditure and a series of metabolic processes. The Ang II is known for its role in regulating blood pressure and fluid and electrolyte homeostasis. Angiotensin II (Ang II), a component of the renin-angiotensin system that found in adipocytes, has a beneficial effect on adipocyte development and regulates adiposity due to its lipogenic properties **[7-8].**

Electrolytes are charged substances that support muscle contractions, blood coagulation, bodily fluid balance, signal transduction, and acid-base balance. The body contains a variety of common electrolytes, such as potassium, sodium, magnesium, calcium, and chloride. While each of these electrolytes has distinct and important roles to play, the majority are specifically in charge of preserving the fluid balance in both the intracellular and extracellular environments and determining the electrophysiological characteristics of the myocardial membrane.

The development of electrolyte abnormalities is influenced by a number of factors, including nutrition, gastrointestinal tract absorption capacity, acid-base problems, drug use, concomitant infections, or acute and chronic illnesses, either separately or in combination. **[9– 10]**.

Though knowledge of the patterns of electrolytes disorders and related predictors in obese patients is crucial, derangements have not been well assessed, and limited data with conflicting results are available on electrolytes profiles in obese individuals. Therefore, this study aimed to evaluate the effect of Angiotensin II on electrolytes derangements and predictors among obese patients.

1.1 Obesity

1.1.1 Definition

Obesity results when energy intake chronically exceeds energy expenditure. Many factors are involved, including genetic, epigenetic, hormonal, and lifestyle factors that are beyond the scope of this review. Adipocyte number is believed to be tightly regulated and determined during childhood. However, during the development of obesity, adipose tissue can expand by either hypertrophy (an increase in adipocyte size) or

hyperplasia (an increase in adipocyte number due to the recruitment of new adipocytes) (figure 1-1) **[11,12].** When the capacity for adipocyte recruitment and hypertrophy is overwhelmed, fat accumulates in ectopic sites such as visceral depots, the liver, skeletal muscle, and pancreatic beta cells. These changes are accompanied by inflammation, insulin resistance and other features of the metabolic syndrome**[13].**
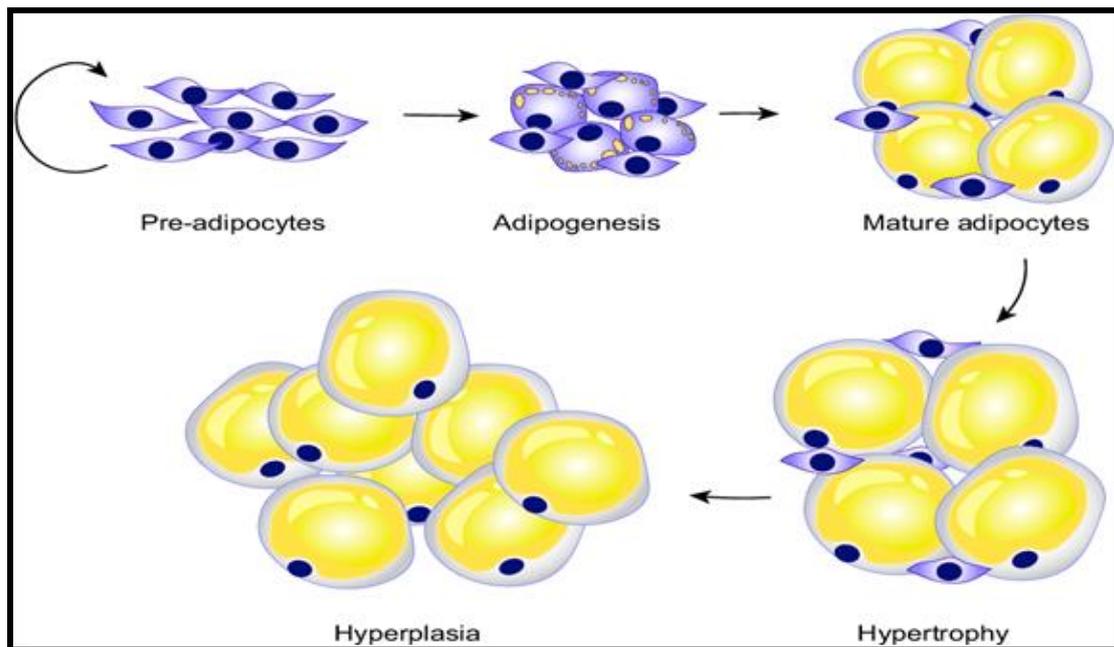
Figure (1-1): Adipose tissue changes in obesity[13]

1.1.2 Classification of Obesity

Individuals with adult-onset obesity have increased adipocyte size in general, whereas those with early-onset obesity have both adipocyte hypertrophy and hyperplasia**[14].** However, there is multiple classification of obesity:

1. Depending on the area of fat deposition, there are three forms of obesity.

a. Peripheral: accumulation of excess fat in the hips, buttocks and thighs (Gynoid type).

b. central: accumulation of excess fat in the abdominal area (Android type).

c. Combination of both peripheral and central obesity.

Abdominal area is considered the most dangerous area for the accumulation of fat because it is closely located to the vital organs and their blood supply.

2. Depending on the association with other disease ;(like caushing syndrome, hypothyroidism, polycystic ovary syndrome and insulinoma)**[15-17].**

3. Depending on the BMI is the most popular screening tool for determining if a person is overweight or obese. it is computed as weight in kilograms divided by height in meters squared, is the most extensively used way of measuring and identifying obesity. Obesity is defined as a BMI of 30 kg/m$_2$ or more in adults, and this is the WHO's standard measurement as in table (1-2) **[18].**

Table (1-2): Classification of BMI according WHO[18].

Definition	BMI category (kg/m^2)
Underweight	less than 18.5
normal weight	(18.5-24.9)
overweight	(25-29.9)
Obesity class 1	(30-34.9)
Obesity class 2	(35-39.9)
Obesity class 3	>40

1.1.3 Epidemiology

There are currently an estimated 2.1 billion overweight or obese people worldwide as a result of their rising prevalence, which increased by 47% in children and 28% in adults between 1980 and 2013, and by the year 2030, 58 percent of the adult population worldwide is anticipated to be overweight or obese[19]. Obesity and overweight are well-known risk factors for developing a variety of chronic diseases, including cardiovascular disease, diabetes, and cancer. Obesity is prevalent among adults worldwide, with 10.8% of men and 14.9 percent of women having a BMI of 30 kg/m^2 or more[20] .

According to the WHO, in 2016(39 %) of adults aged eighteen years and up were overweight or obese, while (13%) were obese. In countries where the majority of the overweight and obese people live, obesity causes more fatalities than underweight. According to a WHO working document in the Eastern Mediterranean Region, statistics on people from 16 different countries in the region showed that Kuwait, Egypt, the United Arab Emirates, Saudi Arabia, Jordan, and Bahrain had extremely high rates of overweight/ obesity[21].

Obesity and overweight prevalence in Iraqi provinces like Erbil and Basra were(33.4% and 40.9%)respectively [22].

Overweight and obesity are on the rise in low- and middle-income countries, particularly in metropolitan areas. One study in China found that the prevalence of age-adjusted obesity increased from 2.15 % to 13.99 % in both sexes over a 22-year period, with females increasing from 2.78 to 13.22 % and males increasing from 1.46 to 14.99 % [21].

1.1.4 Etiology and Risk Factors

Obesity and overweight are the fifth greatest causes of death worldwide, with around three million adults dying each year[23]. Excessive fat formation is the outcome of a positive energy balance caused by a combination of circumstances, including:

❖ Diet, increased intake of energy-dense foods, decreased intake of food rich in micronutrients, bioactive compounds and decreased physical activity (sedentary lifestyle)[24].

❖ Genetic, environmental, cultural, economic factors and some chromosomal aberrations (such as Prader-Willi syndrome)[25].

❖ Hormonal pathologies (such as Cushing's disease), hypothalamic lesions or tumors[26].

❖ Drugs (such as steroids and antidepressants)[27].

❖ Insufficient sleep, short sleep duration has been associated with weight gain in both children and adults in developed countries, such as the USA[28].

Global trade liberalization, economic expansion, and fast urbanization, which continue to fuel substantial changes in living environments, as well as in diets and lifestyles that promote positive energy balance ,are largely to blame for the rise in obesity and related chronic diseases globally[29].

1.1.5 Pathophysiology

A persistent mismatch between energy intake and output leads to the buildup of aberrant or excessive fat in adipose tissue. People who maintain modern lifestyles are frequently exposure to high levels of fats and nutrients, which may cause tissue malfunction and disease[30].

When the body receives more carbohydrate than can be used right away for energy or stored as glycogen, the excess is quickly converted to glycerol, which then produces triacylglycerol, which are then stored in the adipose tissue. Although the liver produces the majority of the triacylglycerol in humans, small amounts are also produced in the adipose tissue. Very low density lipoproteins (VLDL) are primarily responsible for carrying the triacylglycerol produced in the liver to the adipose tissue, where they are stored [31].

The accumulation of lipids, primarily triacylglycerol, in adipose tissue happens in combination with volume increases in skeletal muscle, liver, other organs and tissues[32]. Many amino acids can be converted into acetyl –CoA which in turn can then be synthesized into triacylglycerol. Therefore, when people have more protein in their diets than their tissue can use as proteins, a large share of the excess is stored as fat .In the past, adipose tissue was simply seen as a storage organ, but nowadays it has been revealed that adipose tissue not only serves as energy reserves but also as endocrine organ secreting important adipocytes and cytokines. Among adipocytes, leptin and adiponectin are major plasma proteins secreted by adipocytes, and the plasma concentrations of leptin are markedly increased while adiponectin is decreased in obese population [33].

As adipocytes play a key metabolic role as a source of fuel, they are required to respond acutely to changes in the nutritional levels. As a

result they are tightly regulated by both hormonal (such as insulin) and sympathetic such as adrenergic stimulation. During obesity, the adipocyte size increases (hypertrophy), and they are eventually unable to store excess lipids even with enhanced adipocyte proliferation (hyperplasia)[34] .

1.1.6 Adipose Tissue

The adipose tissue is made up of at least two types: white adipose tissue (WAT) and brown adipose tissue (BAT), each with its unique shape, distribution, gene expression, and function [35]. Obesity is caused by a mismatch between food intake and energy expenditure, as well as a mismatch between white adipose tissue, which serves as the main energy reservoir, and brown adipose tissue, which Its major function is to burn calories to produce heat to aid in maintaining body temperature[36].

The white adipose tissue is not only a source of energy, but also a secretory organ for a variety of chemicals with endocrine, paracrine, and autocrine effects [37]. Some of the chemicals released by adipocytes are involved in body weight management (leptin, adiponectin), local inflammation caused by obesity (TNF-, IL-6, and IL-1), and vascular function (Ang II).

Brown adipose tissue is an endocrine organ that secretes cytokines, hormones, and other substances like TNF-, adiponectin, and leptin as in figure (1-3). BAT, on the other hand, secretes a huge variety of other chemicals. Many of them are essential for cold adaptation and adrenergic stimulation, including fibroblast growth factor type 21 (FGF21) [38-39].
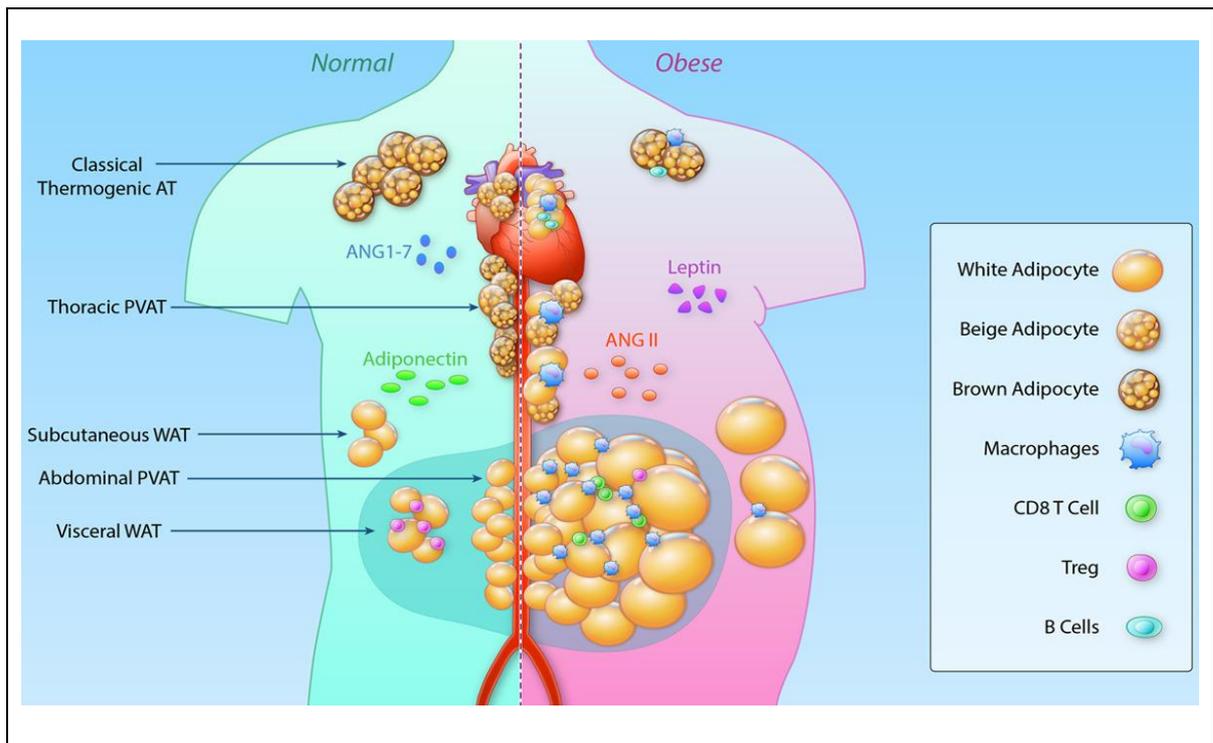
Figure(1-3): Adipose tissue types in obesity$[40]$

1.1.7 Complications

Obesity raises the chance of dying at any age. Being overweight or obese can have long-term negative impacts on one's health. The level of an individual's physical fitness affects how quickly their chance of developing health concerns. Overweight and obesity are associated with adipose tissue dysfunction, characterized by enlarged hypertrophied adipocytes, increased infiltration by macrophages and marked changes in secretion of adipokines and free fatty acids. This results in chronic vascular inflammation, oxidative stress, activation of the renin-angiotensin-aldosterone system and sympathetic overdrive**[40-42].**

Depending on the degree and duration of weight gain, obesity can progressively cause and/or exacerbate a wide spectrum of co-morbidities, including type 2 diabetes mellitus (T2DM), hypertension, dyslipidemia, cardiovascular disease (CVD), liver dysfunction, respiratory and

musculoskeletal disorders, sub-fertility, psychosocial problems, and certain types of cancer (figure 1-4)[42].
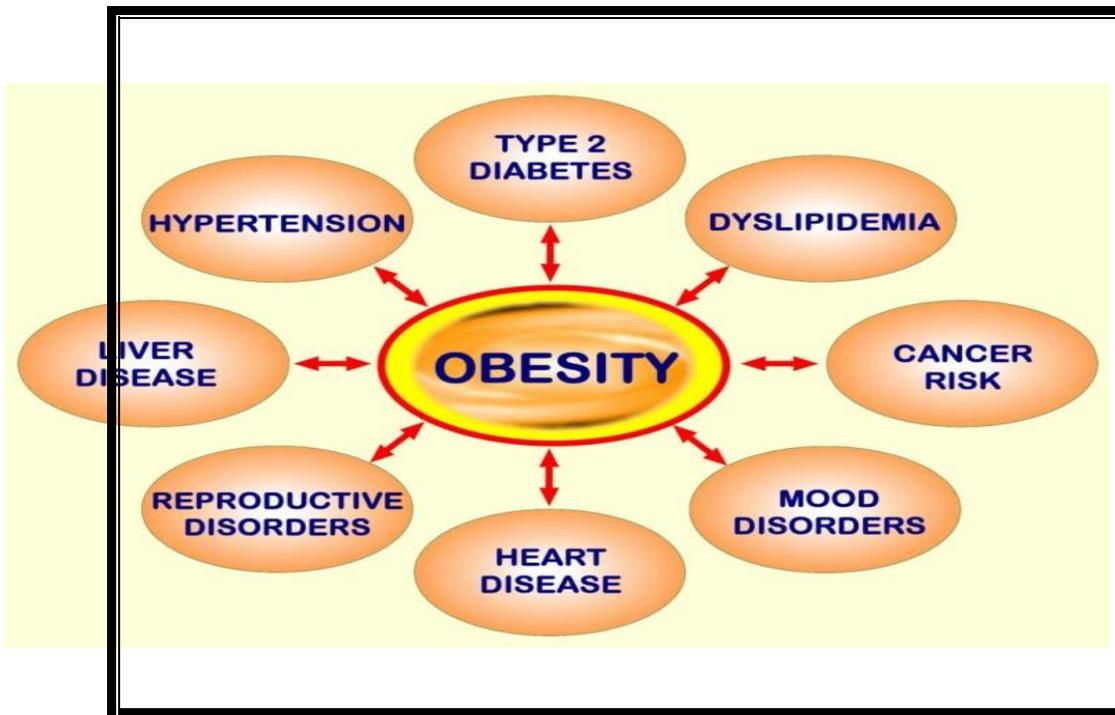
Figure (1-4): Complications of obesity[42]

1.1.8 Diagnosis of Obesity

1. Body Mass Index (BMI):

The degree of obesity can be described in a variety of ways, but the body mass index is the most relevant (BMI). A bmi of 19 to 25 kg/m2 is considered healthy weight. A BMI of 25–30 kg/m2 is considered overweight and is associated with a low risk. Except in body builders and other athletes, a BMI more than 30 kg/m2 is nearly invariably related with an increase in body fat and is synonymous with obesity[43].

2. Waist Circumference and Waist –hip ratio

The use of waist measurement as a risk indicator derives from the perception that abdominal obesity (accumulation of fatty tissue in the

abdomen) is "worse" than other types of fat accumulation (such as the buttocks or thighs) [44].

Another method of measuring abdominal obesity is the waist-to-hip ratio, which has been linked to an increased risk of cardiovascular disease in studies. The ratio should be 0.8 or less for women and 1.0 or less for men. (This indicates that the waist should be smaller or equal to the hips in men and should be narrower in women.) Since waist circumference alone may underestimate risk in smaller people, the waist-to-hip ratio is useful. You can better identify abdominal obesity by comparing waist and hip circumferences. **[46– 48].**

1.1.9 Treatment

The cornerstones of obesity treatment are diet and exercise. Additionally, it's critical to increase dietary fiber intake while decreasing the consumption of foods that are heavy in sugar and fat because these foods are energy-dense. Anti-obesity medications can be administered to suppress appetite or prevent the absorption of fat as a backup plan or in the event of failure. In extreme situations, surgery or the placement of an intra-gastric balloon are undertaken to lower the size of the stomach and/or the length of the colon, which results in quicker satiety and a decreased capacity to absorb nutrients from food[50]**.**

1.2 Angiotensin ll

1.2.1 Structure of Angiotensin ll

Ang II (H-Asp, Arg, Val, Tyr, Ile, His, Pro, Phe-OH) is an active octapeptide hormone that is synthesized from angiotensin I (ten amino acid).by the action angiotensin converting enzyme (ACE) two amino

acids are removes from it is C-terminal to produce angiotensin II as in figure (1-5) **[51, 52]**.
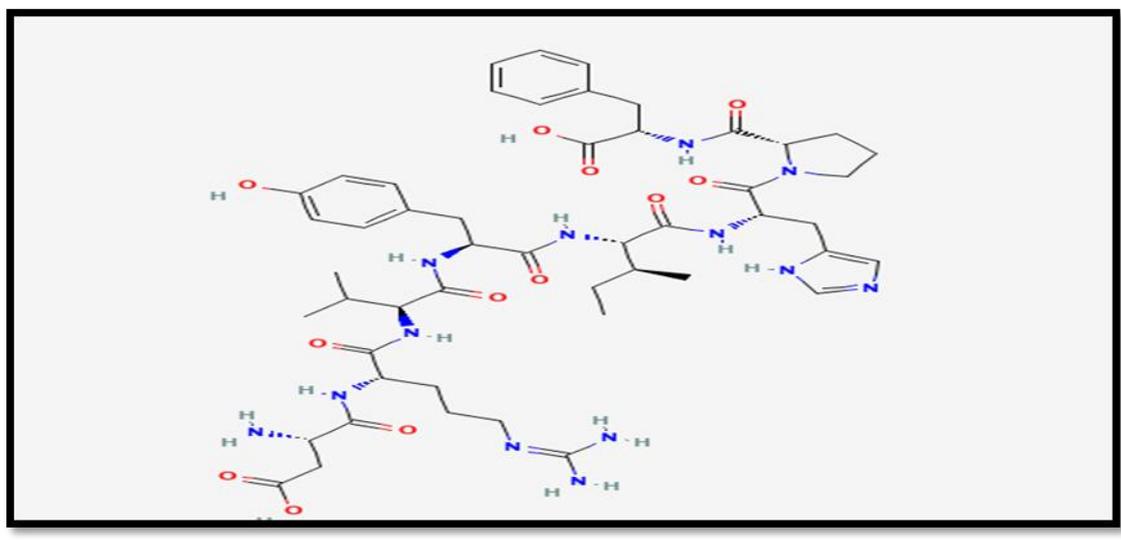
Figure (1-5):Structure of angiotensin II [52].

1.2.2 Function of Angiotensin ll

Angiotensin II is the major active peptide of RAS system, with a circulation half-life of 30 seconds and a tissue half-life 15 to 30 minutes. Ang II functions in the heart, brain, kidneys, adrenal cortex, blood vessels, and adipose tissue after it is generated[53]**.**

Angiotensin II has a various direct effect, like vasoconstriction (reduced vascular capacity), increased aldosterone production (act to enhance salt retention), increased thirst, and stimulate release of the antidiuretic hormone (ADH) (lower water excretion), increased sympathetic nervous system activity**[54].**

Chronic elevation of Ang II levels (systemic or renal) induce pathogenic factors, including proinflammatory cytokines releasing by immune cells activation ,growth factor, oxidative stress and mechanical stress by high blood pressure[55]**.**it also promotes aldosterone secretion

from the cortex of adrenal gland[56]. Aldosterone maintains sodium-potassium homeostasis by stimulating kidney proximal tubules to increase sodium reabsorption, hence, retaining sodium and excretion potassium. Also, Ang II can stimulate lipogenesis, thus increasing the bulk of adipose tissue[57].

Angiotensin II, which is generated locally in adipose tissue, may have autocrine effects on adipose tissue growth. Angiotensin II stimulates adipocyte development and differentiation both directly and indirectly by stimulating lipogenesis and prostaglandin (PGI2) synthesis [58].

Through its action on the sympathetic nervous system and direct actions on the stromal vascular cells in the adipose tissue, angiotensin II can regulate blood flow to the adipose tissue, hence controlling adipose tissue function to some extent [59].

1.2.3 Angiotensin II receptors

In obese people, considerable angiotensin II (AngII) release from abdominal subcutaneous adipose tissue has been conclusively demonstrated. Several (RAS) proteins have been found in adipose tissue. Renin, Angiotensinogen, Angiotensin I, Angiotensin II, Angiotensin type I (AT1) and type 2 (AT2) receptors, (ACE), and other Angiotensin II-producing proteases such as chymase are among them. cathepsin D and G, and tonin [60].

Ang II has a strong affinity for two different RAS receptors, AT1R and AT2R. The vast majority of Ang II physiological effects, such as cellular differentiation and proliferation, vasoconstriction, decreased vascular compliance, cardiac contractility, increased renal tubule sodium (Na+) reabsorption, aldosterone, vasopressin, and endothelia secretion,

salt aversion, thirst, and sympathetic nervous system activation, are mediated by AT1Rs. However, AT2R's pathophysiologic function is still unclear[61].

The binding of Ang II with this receptors lead to activates these receptors, and promote of Ang II to act in many body organs, including (kidney, heart, blood vessels, brain, and lymphatic organs)through binding and activation of receptors[63, 64].

1.3 Electrolytes

Electrolytes are electrically charged substances that play a key role in acid-base balance, blood coagulation, and bodily fluid and muscle contractions in physiology. It is typically advised to consume enough vitamins and minerals to maintain health, preferably as part of our normal food consumption. Electrolytes commonly referred to as sodium, potassium, calcium, and magnesium, have a variety of biological impacts on the human body, and their imbalances have a significant effect on one's health. The composition and function of bones, muscle, neural activities, oxygen transport, acid-base homeostasis, and many other biological processes depend on electrolyte balance. Even minor variations in blood electrolyte content can cause severe health issues and even increase mortality [65-66].

The extracellular fluids volume and osmotic pressure are mostly controlled by the cation sodium ($Na+$) in this fluid. Additionally, it can be found in the intracellular fluid, where it supports enzymatic activities and the potential of the cell membrane. Dietary sodium is typically ingested via table salt, along with chloride ($NaCl$) [66].

One of the etiologies of chronic disease, including obesity and high blood pressure, is a diet heavy in sodium, calories, and lipids[67]. Many studies have found that a high-sodium diet increases adipose tissue mass, which leads to changes in insulin and glucose metabolism, which promote fat storage[68].

The most prevalent cation in intracellular fluid is potassium (K^+), which is together with sodium involved in the distribution of body fluids, regulation of the acid-base balance, nerve impulse transmission and muscle contraction. Additionally, potassium regulates the creation of proteins, nerve impulses, and the conversion of blood sugar to glycogen. Although extracellular potassium only makes about 2% of the cell's total potassium content, both spikes and declines can cause significant neuromuscular or muscle dysfunction **[70-71]**.

Potassium consumption can be influenced by the activation of the renin-angiotensin system (RAS) in response to volume depletion, and increased potassium exchange with sodium in the distal tubule when potassium enters the cell leads to diuretic-induced alkalosis. In obese individuals, sodium retention in the body and volume expansion can be caused by increased sympathetic activity [71]. A higher Na^+ / K^+ ratio was identified in skeletal muscles in insulin-resistant obese patients, indicating reduced pump function. Additionally, several studies demonstrated a positive relationship between skeletal muscle Na^+/ K^+ and BMI[72].

Chloride is the most important extracellular anion in maintaining body fluid equilibrium. It regulates acid–base balance, contributes to the electro neutrality of urine and plasma, and may influence neuronal activity[73].

Calcium makes up about 1.5 percent to 2 percent of total body weight and is primarily found in bones and teeth to the tune of 99 percent, with the remaining 1% of total body calcium located in soft tissues and body fluids. It is also the most abundant mineral in the human body, and it plays a role in a variety of physiology processes including muscle contraction, hormone release, neurotransmitter release, glycogen metabolism, cell proliferation and differentiation, nerve impulse transmission, and skeleton structural support[74]. Calcium concentration is regulated by three hormones: parathyroid hormones, calcitriol, and calcitonin hormones. These hormones interact with the kidneys, intestines, and bones to increase calcium concentration. Extracellular and serum there are three different types of calcium:

1. ionized $Ca^{2+.}$

2. Complexes Ca^{2+} (5 percent of total calcium) to anions such lactate, citrate, bicarbonate, or phosphate.

3. protein-bound Ca^{2+} (45 percent of calcium).

Many studies have shown that intracellular Ca^{2+} regulates adipocyte energy storage, resulting in increased circulating calcitrophic hormones and/or parathyroid hormone, and that low Ca^{2+} diets increase adipocyte Ca^{2+} flow and so boost lipid storage[75].

1.4 Vitamin D

Vitamin D refers to the product found in food (vitamins D_2 and D_3) and generated in the skin under the effect of UV- light (vitamin D_3), whereas 1,25 $(OH)_2D_3$ is the metabolically active molecule [76]. Vitamin D3 (Calciferol), is a fat-soluble vitamin and vitamin D2 (ergocalciferol) are the two most common forms of this vitamin as in figure (1-6)[77].
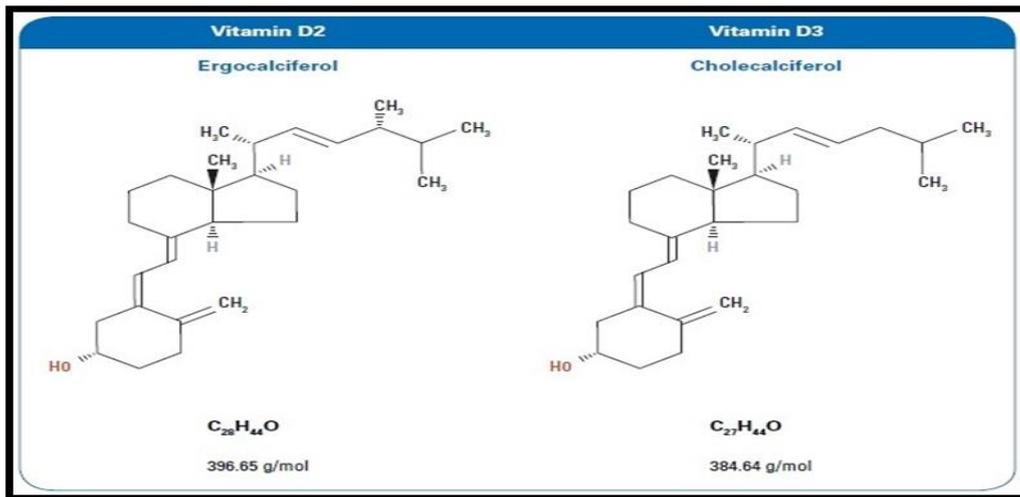
Figure (1-6): Chemical structure for vitamins D$_2$ and D$_3$[77].

Vitamin D is the only vitamin that the body can create, it is sometimes referred to as a hormone as well as a vitamin The sun (UVB- irradiation) causes a chemical reaction in the skin that converts pro vitamin D to active vitamin D$_3$, which is then delivered to the liver and kidneys for further processing[78]. However, any excess vitamin D$_3$ is stored in adipose tissue Vitamin D$_3$ is naturally found in dairy products, cheese, butter, cream, fortified milk, fish (salmon, mackerel, sardines, herring, tuna), fish oils, egg yolks, oysters, fortified cereal, and fortified juices[76].

Nutritional importance of vitamin D$_3$ in maintaining good health, as well as the link between vitamin D$_3$ deficiency and a variety of diseases such as hypertension, coronary heart disease, cardiovascular disease, cancer, tuberculosis, respiratory tract infection, diabetes, and obesity, have all been well documented**[80-81]**. Some researchers recommend taking Vitamin D supplements with meals that contain either cholecalciferol (vitamin D$_3$) or ergocalciferol (vitamin D $_2$) because oily or fatty foods induce the flow of bile into the stomach, which increases

vitamin absorption. According to certain studies, vitamin D_3 may be more effective and superior to vitamin D_2 as in figure (1-7) [81]**.**
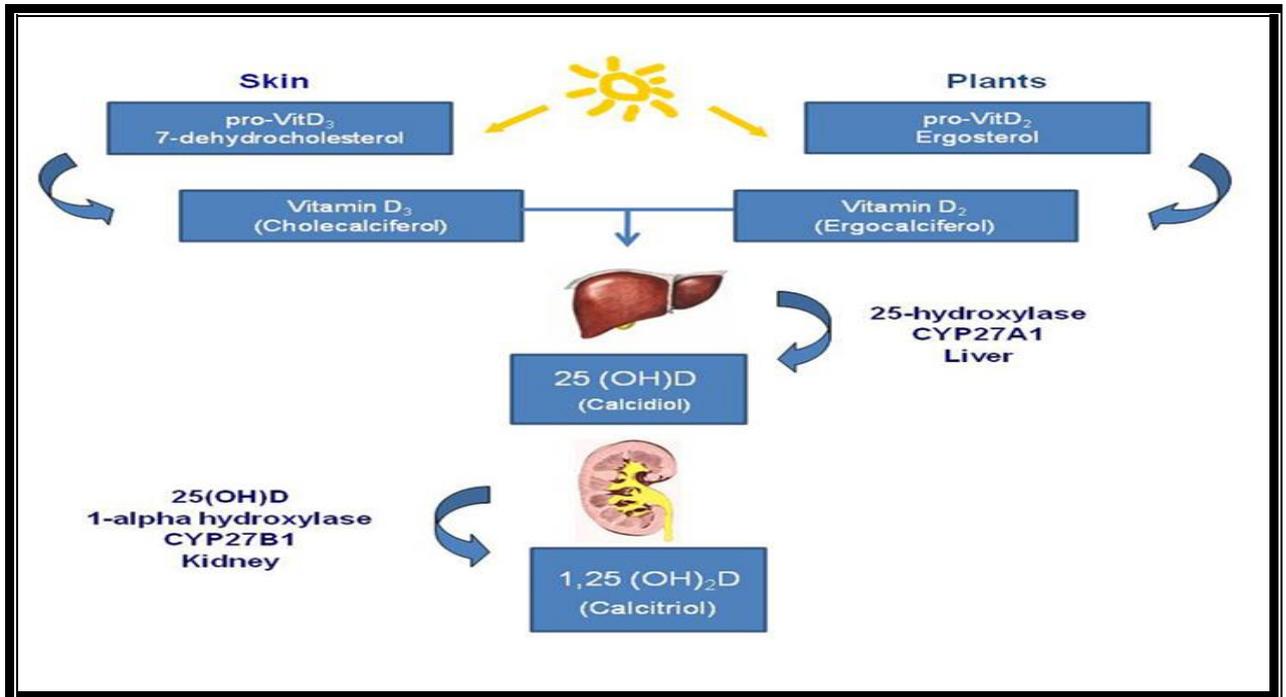
Figure (1-7): Metabolism of vitamin D$[81]$**.**

Vitamin D_3 affects calcium absorption from the intestine and promotes bone formation. It is regulate calcium absorption in the gut and keeps serum calcium and phosphate levels in check, allowing for normal bone mineralization and preventing hypokalemic titany (involuntary contraction of muscles, leading to cramps and spasms). It's also required for osteoblasts and osteoclasts to develop and repair bone. Bones can become thin, brittle, and deformed if they don't get enough vitamin D_3 as in figure (1-8) **[83-84].**
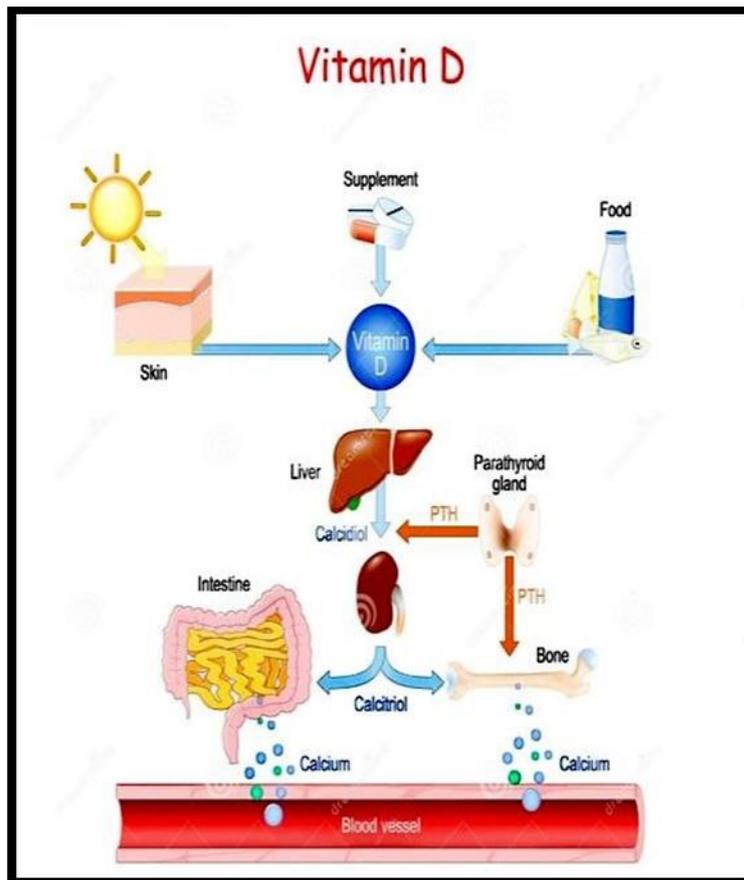
Figure (1-8): Vitamin D and calcium homeostasis[84].

Vitamin D_3 deficiency is common in people with obesity, with reported prevalence rates as high as 90%. The higher risk of deficiency is thought to be related to several factors. One contributor could be less sun exposure. In addition, since vitamin D is fat-soluble, people with higher amounts of body fat will 'sequester' vitamin D_3 in fat cells, leading to lower amounts circulating in the blood. Due to this, people with obesity typically need higher amounts of vitamin D_3 to maintain adequate levels or to correct a deficiency. **[85-86]**.

The active metabolite of vitamin D_3 has an effect on the kidneys by influencing congenital and acquired immunity, acting on adipose tissue and pancreatic beta-cells, altering insulin sensitivity, and enhancing lipid profile. Vitamin D_3 inhibits gluconeogenesis, enhances HDL cholesterol levels, promotes adipokine profile modifications, and increases leptin

levels in adipose tissue. Increased parathyroid hormone levels, reduced insulin sensitivity, faster lipogenesis, and fat mass accumulation are all symptoms of vitamin D deficiency **[87-89]**. Furthermore, previous studies observed a blunted tissue sensitivity to AngII in individuals with lower 25(OH)D levels, when compared with those with higher 25(OH)D, that was particularly evident among obese individuals [88]; these studies suggested higher tissue-RAS activity in obese individuals with low 25(OH)D concentrations [89]**,** this insufficiency may contribute to excess tissue-RAS activity and the development of HTN and CKD[90].

1.5 Lipid profile

Lipids are heterogeneous groups of compounds which are water insoluble and soluble in nonpolar solvents such as alcohol, ether, chloroform, benzene, etc. [91]. The major lipids present in the plasma are fatty acids, Triglycerides, cholesterol and phospholipids, all are transported in plasma as lipoprotein particles. Lipids are principle stored forms of energy full in many organisms. Phospholipids and cholesterols are major structural elements of biological membranes[92]**.** Other lipids, although present in relatively small quantities, play crucial roles as coenzymes, electron carriers, emulsifying agents in the digestive tract, hormones and intracellular messengers.

Fatty acids are aliphatic carboxylic acids compounds of varying lengths; they may be saturated or unsaturated. FAs may be esterified with glycerol to form "Glycerides", or they may be free non-esterified, called as free fatty acids, they are mainly bound to albumin in the blood. Cholesterol and triglycerides are water insoluble, thus they must be carried with proteins. Lipoproteins are complex particles having a central core of cholesterol esters and triglycerides, surrounded by free

cholesterol, phospholipids, and Apo lipoproteins, all of which help lipoprotein synthesis and function. Based on size, lipid composition, and Apo lipoproteins, plasma lipoproteins can be categorized into seven classes (chylomicrons, chylomicron remnants, VLDL, IDL, LDL, HDL and Lpa [94-96]. HDL is anti-atherogenic, while chylomicron remnants, VLDL, IDL, LDL, and Lpa are all pro-atherogenic. Apo lipoproteins have four major functions:

- Serving as structural proteins.

- Acting as ligands for lipoprotein receptors.

- Guiding lipoprotein synthesis.

- Activating or inhibiting enzymes involved in lipoprotein metabolism.

The integration of dietary lipids into chylomicrons in the intestine begins the exogenous lipoprotein route[95].Lipoprotein lipase metabolizes the triglycerides held in chylomicrons in muscle and adipose tissue, generating free fatty acids, which are then digested by muscle and adipose tissue, resulting in chylomicron remains. The liver then absorbs the chylomicron remains. The production of VLDL starts the endogenous lipoprotein pathway in the liver[96]. Lipoprotein lipase metabolizes the triglycerides carried by VLDL in muscle and adipose tissue, producing free fatty acids and IDL. IDL is converted into LDL, which is taken up by the LDL receptor in a variety of tissues, including the liver, which is the primary site of uptake. The production of nascent HDL by the liver and intestine is the first step in reverse cholesterol transport[97]. These small HDL particles can then take on cholesterol and phospholipids that are effluxes from cells, forming mature HDL(good cholesterol)as show in figure (1-9) [98-99].
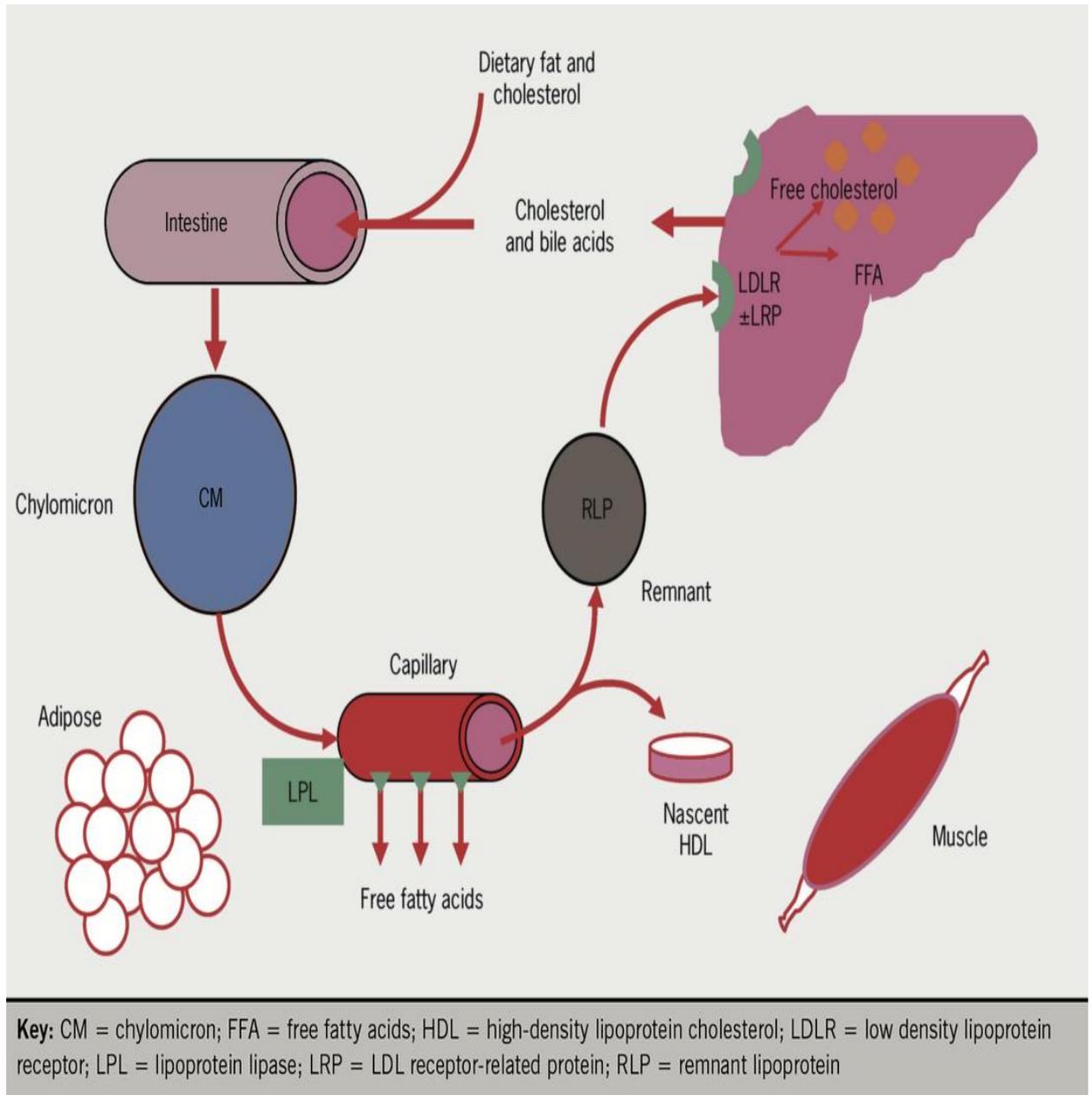
Figure (1-9): Metabolism of lipids [99] .

Aims of the Study:

The current study aims to analyze the role of:

1. The effect of Ang II in relation with electrolytes.

2. Serum vitamin D with lipid profile and it is effect on electrolytes.

As a biochemical changes in development of obesity.

2.1 Subjects:

This study was carried out from patients attended to obesity centers in private clinic and Imam Ali hospital in addition to some relative & medical staff in Baghdad province. All samples were collected from 1st of December 2021 until 20th of March 2022. The practical side of the study was performed at the laboratory of chemistry and biochemistry department in College of Medicine / University of Babylon.

2.1.1 Study Design: Case control study.

2.1.2 Study population

The current study was included 88 metabolically healthy subjects; the age was ranged between (18 - 50) years. These subjects were divided into two groups:

1**. Obese group (n = 44):** Obese individuals with (BMI>30).

2**. Control group (n = 44):** This group included 44 apparently healthy individuals with body mass index (BMI) between 18 and 25.
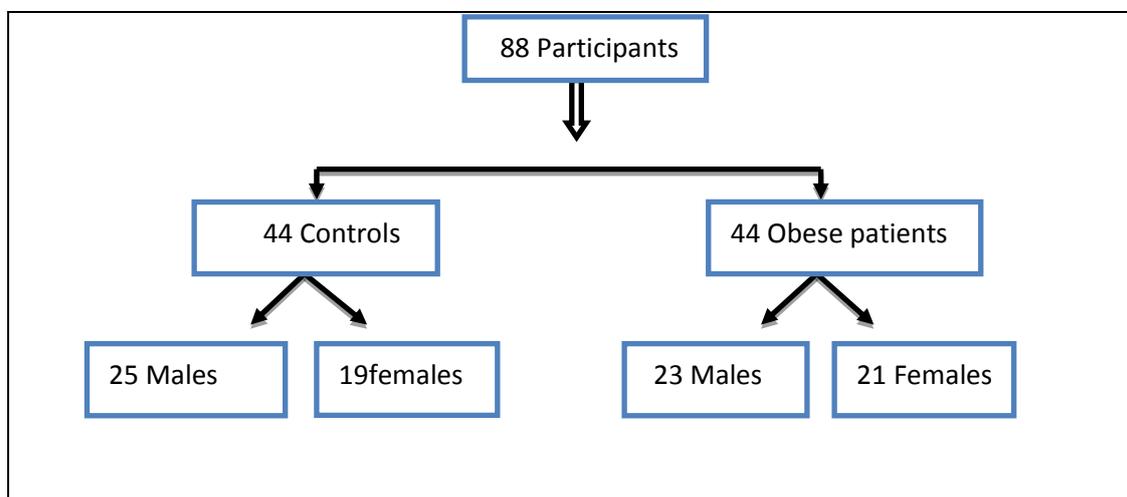
Figure (2-1): Simplified chart represents the study groups.

2.1.3 Ethical Approval

Depends on the following**:**

1. Approval of scientific committee of Babylon Medical College (University of Babylon, Iraq) and the Biochemistry Department in the same college.

2. Approval of scientific committee of Imam Ali hospital in Baghdad province, al-sadder city.

3. The objectives and methodology of this study were explained to all participants in the current study to gain their verbal acceptant.

2.1.4 Data collection

For this study, the inclusion and exclusion criteria are as follows:

1. Inclusion criteria: all the participants in present study are obese and non-obese adults, who are metabolically healthy subjects with average age between (18-50) years old, and (BMI >30) those who were accepted to participate in the current study.

2. Exclusion Criteria: Anyone who had no (diabetic disease,hyertention, pregnant, kidney disease, heart disease, thyroid disease).

2. Anthropometric measurements

Including:

1.Body Mass Index (BMI): Each participant's height and weight were also recorded, and BMI was calculated using this information[100,101]The following equation was used to compute BMI:

$$BMI=Weight\,(kg)\,/\,(Height\,(m))^2$$

The classification of (BMI) According to the World Health Organization (WHO):

Table (2-2): Classification of BMI according to WHO.

Definition	BMI category (kg/m^2)
Underweight	less than 18.5
normal weight	(18.5-24.9)
overweight	(25-29.9)
Obesity class 1	(30-34.9)
Obesity class 2	(35-39.9)
Obesity class 3	>40

2. Waist to Hip Ratio: The individual's waist and hip circumferences were measured with inextensible anthropometric tape while standing straight and relaxed, arms at sides, and feet near together. At the conclusion of normal expiration (measured slightly above the hipbones in adults).

Hip circumference was measured at the widest part of the hip. For all measurements the tape was positioned at a level parallel to the floor. All measurements were in centimeters (cm) .The risk level of WHR is summarized in Table below, according to the World Health Organization (WHO) [102].

Table (2-3): Levels of waist to hip ratio [102].

Definition	WHR
Low	0.80 or less
Moderate	0.81-0.85
High	0.86 or higher

2.1.5
Blood Samples Collection

(5 ml) of blood samples were aspirated without tourniquet from obese patients and control by using disposable syringe .blood push slowly in gel tube and let to clot at room temperature for (10-15)min, then centrifuged at 3000 rpm for 10 minutes .The serum was obtained put in eppendrof tubes (labeled) with number of sample then stored at -20°C until the time of examination.

2.2 Materials

2.2.1 Chemicals:

All chemicals and standard kits used in this study with their sources listed in the table (2 -4).

Table (2 – 4): Chemicals substances and standard kits

No	Chemicals and Kits	Origin
1	Acetonitrile for HPLC	France/Bio solve
2	Angiotensin ll ELISA kit	Elabscience
3	Cleaning solution	China
4	Deprotein solution	China
5	Electrolyte reagent DS-l (standard A+ standard B)	China
6	HDL-cholesterol kit	Biolabo SA (France)
7	Methanol for HPLC	France/Bio solve
8	PVC Electrode Activate Solution	China
9	Total cholesterol kit	Biolabo SA (France)
10	Triglyceride kit	Biolabo SA (France)
11	VIT D standard	India

2.2.2 Instruments and Equipment

The instrument and equipment's and sources used in this research were listed in table (2 – 5).

Table (2 – 5) Instruments and equipment:

No	Instruments	Origin
1	Autoclave	Germany
2	Blue and yellow tips	China
3	Deep freeze	GFL/Germany
4	Disposable Syringes	Emirate
5	Distiliter	GFL/Germany
6	Elisa (reader-washer-printer)	Biotic/ USA
7	Eppendrof tube(1.5)	China

8	Filter papers	China
9	Gel tube	Jordon
10	Higher performance liquid chromatography with column oven	Shimadzu /Japan
11	Incubator	Fisher cient/Germany
12	Macro Centrifuge	China
13	Micropipettes(5-50micron)	Germany
14	Multichannel micropipette	Germany
15	Sensitive balance	Sartorius/Germany
16	Sonicator for degassing	Germany
17	Spectrophotometer CECIL 7200	Cecil /UK
18	Vortex	CABB/China

2.3 Methods

2.3.1 Determination of Electrolytes (Sodium, Potassium, Chloride and Calcium) Concentrations:

The serum sodium, calcium, and potassium were determined by using electrolyte analyzer. This method is dependent upon the ion selective electrode (ISE) principle.

- **Principle**

An ion-selective electrode (ISE) is an indicator electrode that responds (produces a potential) when it is placed in a solution containing a certain ion. There is now a large variety of ISE available which selectively respond to particular cat ions and anions, and certain gases; pH electrodes are by far the best known. The ion-sensing part consists of a membrane (which may be plastic, glass or an ionic crystal) which has sites capable of adsorbing the analyte ion.

On other side of the membrane is a solution containing the ion of interest: one of these is the test solution, the other is a standard solution within the electrode itself. Inside the electrode body there is an electrical connection electrode – to monitor the response from the membrane. The relationship of the tested ion concentration and electrodes electric potential was described in equation:

E=Eo+ (RT/uF)*Ln (ax)

Where:

E: Electric potential of ion selective electrode during test

Eo: Standard electric potential of ion selective electrode

R: Gas constant (8.314/ j.mol)

T: absolute temperature (t+273 ºC)

F: Faraday constant (96487 C/mol)

Ax: Tested ions concentration in solution

The components of electrolytes kits and standard as show in tables (2-6), (2-7) respectively:

Table (2-6): Components of electrolytes kit

	Component	Volume
Electrolyte reagent DS-l	Standard A	390 ml
	Standard B	90 ml
DE protein solution	Diluent:0.1mol/L HCL	18 ml
	Protease: powder	100 mg
Cleaning solution	Nonionic Surface active	250 ml

Table (2-7): Main components of electrolytes standard

Electrolytes	Standard A	Standard B
K^+(mmol/L)	4.00	8.00
Na^+(mmol/L)	140.00	110.00
Cl^-(mmol/L)	100.00	70.00
Ca^{++}(mmol/L)	1.00	2.00
PH	7.20	7.60

- **Procedure:**

1. The instrument was turned on

2. Self-calibration was done by instrument.

3. From main screen, measure sample icon was selected.

4. Serum option was selected.

5. Serum sample was put under aspirated needle and sampling icon was pressed.

6. A volume of 160 μl was aspirated by needle; the result appeared on the screen and automatically for Na^+, K^+, Ca^{++} after 50 second.

7. Deprotinization and cleaning of instrument were undertaken after approximately 30 samples.

8. Internal quality control was carried out before starting analysis of samples and within batch of analysis to check the accuracy of the results.

Figure (2-8): Genrui E300 apparatuses to measure electrolytes

2.3.2 Determination of Angiotensin ll Concentration.

- **Principle:**

The quantitative ELISA (Sandwich technique) was used in present study the kit's micro titer strip wells have been coated with an A monoclonal antibody specific for human Ang ll. The samples and standards are placed in the wells labeling and human Ang ll binds to the immobilized antibody. After eliminating unbound compounds with a wash phase, an HRP-conjugated anti-human Ang ll antibody is added, which binds to human Ang ll caught by the Ab. During a wash step after incubation, unbound HRP-conjugated anti-human Ang ll antibody is removed. The wells are next filled with a chromogenic substrate solution, and color develops in proportion to the amount of Ang ll bound in the first phase. The reaction is completed when a colorful product is generated anda stop solution is added. At (450 nm), the color strength is determined spectrophotometric ally.

- **Standard preparation:**

Reconstitute the 120μl of the standard (400ng/L) with 120μl of standard diluent to generate a 200ng/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (200ng/L) 1:2 with standard diluent to produce 100ng/L, 50ng/L, 25ng/L and 12.5ng/L solutions. Standard diluent serves as the zero standards (0 ng/L). Dilutions of standard solutions suggested are as follow in table (2-9) and figure (2-10):

Table (2 – 9): Standard preparation steps for angiotensin ll

200ng/ml	Standard No.5	120μl Original Standard + 120μl Standard Diluent
100ng/ml	Standard No.4	120μl Standard No.5 + 120μl Standard Diluent
50ng/ml	Standard No.3	120μl Standard No.4 + 120μl Standard Diluent
25ng/ml	Standard No.2	120μl Standard No.3 + 120μl Standard Diluent
12.5ng/ml	Standard No.1	120μl Standard No.2 + 120μl Standard Diluent
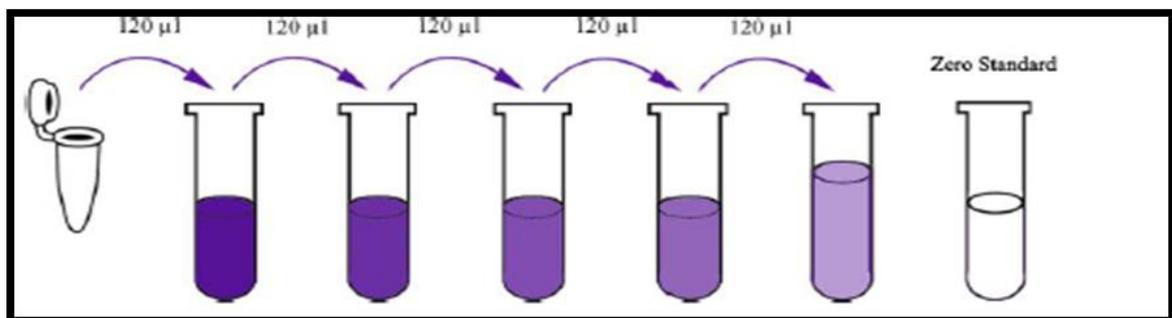
Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1

400ng/l	200ng/L	100ng/L	50ng/L	25ng/L	12.5ng/L

Figure (2-10): Concentration of standard angiotensin II

- ## Procedure:

1. Firstly all reagents, standard solutions and samples were prepared. The assay is performed at room temperature.

2. A volume 50μl of standard solution was added to standard well.

3. From samples 40μl was added to sample wells then 10μl anti-Ang-II antibody was added to sample wells after that 50μl streptavidin-HRP was added to sample wells and standard wells (Not blank control well).Mixed well the plate and covered with a sealer then at 37°C plate was incubated for 60 minutes.

4. The sealer was removed and the plate was washed 5 times with wash buffer. Soak wells with at least 0.35ml wash buffer for 30 sec to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent materials.

5. A volume of 50μl substrate solution was added to each well. Plate was incubated covered with a new sealer for 10 minutes at 37°C in the dark place.

6. To each well a volume of 50μl stop solution was added, the blue color had been will change into yellow immediately.

7. Determine the optical density (OD value) of each well immediately using a micro plate reader set to 450 nm within 10 minutes after

adding the stop solution, figure below demonstrated Elisa system that used to determine Ang II concentration.

Figure (2-11): Elisa system for angiotensin II measurement

- **Calculation**

A standard curve was plotted for the absorbance of each standard on the y-axis versus the concentration of these standards on the x-axis .In order to estimate the Ang ll concentration of each sample , first the horizontal line to the standard curve was extended, the point of intersection was found, a vertical line to the x-axis was extended and the corresponding sample concentration was read as shown in figure (2 – 12).

$y = 95.976x - 65.409$
$R^2 = 0.9906$
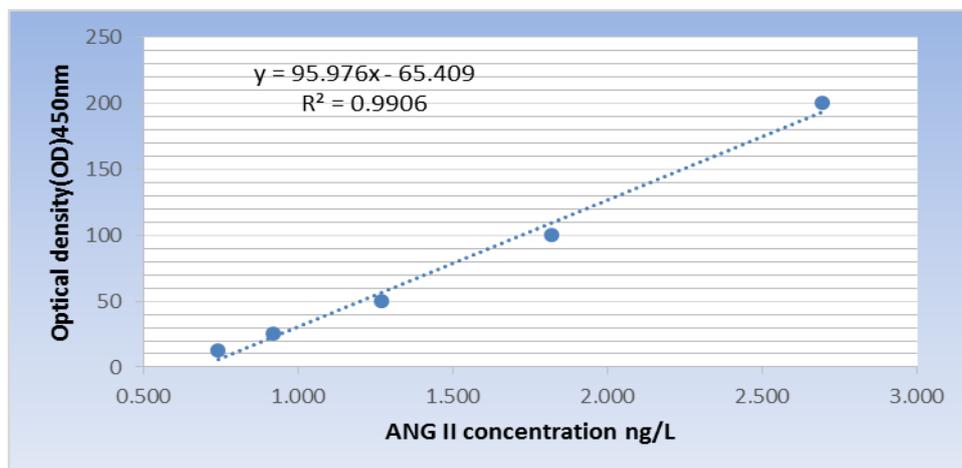
Figure (2-12): Standard curve for calculation of angiotensin ll concentration

2.3.3 Determination of Vitamin D_3 in High Performance Liquid Chromatography (HPLC).

1. Principle:

The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase. The specific intermolecular interactions between the molecules of a sample and the packing material define their time "on-column". Hence, different constituents of a sample are eluted at different times. Thereby, the separation of the sample ingredients is achieved [103].

2.General procedure[104]:

- **Sample preparation:** 400 microns were taken from the serum and treated w**ith** 400 microns of acetonitrile and placed in vortexes for 10 seconds for mixing, and then the sample was placed in Centrifuged for 10 minutes /10,000rpm at 25 C for separation .The upper layer (supernatant) used for Vit D_3 detection, and placed in the auto sampler unit of the HPLC. The extract was stable for at least 2 days at room temperature.

- **Mobile phase preparation:** In the sonicator poll solvents of acetonitrile and methanol mixed in container by (9:1 ratio) as mobile phase with isocratic mode. The mobile phase was delivered with flow rate 1mL/minute

- **Preparation and Analysis of Slandered Solution :**

Stocks and standards solution of Cholecalciferol (vitamin D_3): Stock solution was prepared by dissolving 1mg of cholecalciferol standard in 10 ml of methanol the intentional concentration (100 ppm). Serial standard solutions were prepared by using 10, 20, 30, and 40 (ppm). The stock and standard solutions were stored in brown flask with screw cap to avoid exposure to light and air at –4ºC in refrigerator.
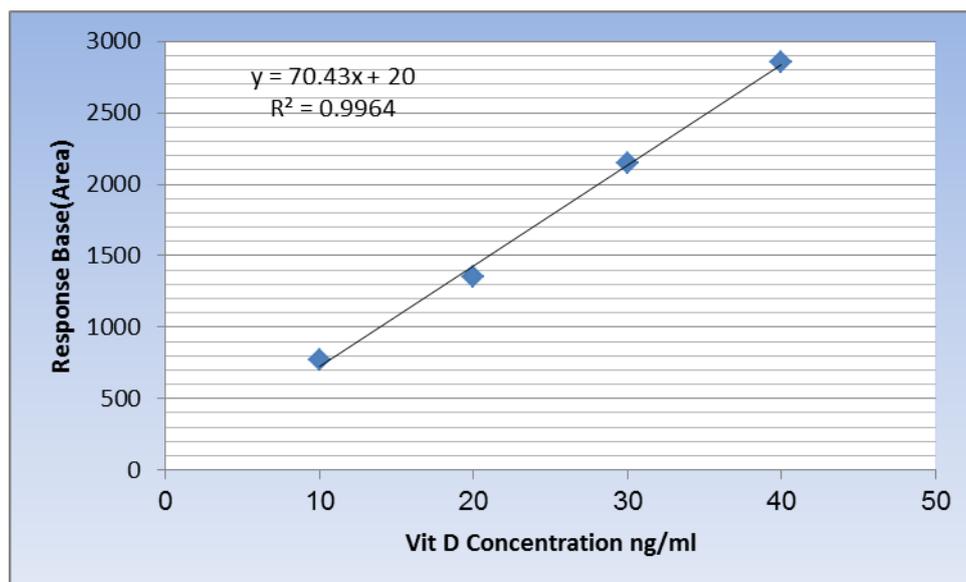
Figure (2-13): Standard curve for calculation Vitamin D_3 concentration

Chromatographic analysis

HPLC system equipped with a UV detector was used for the chromatographic analysis. The analyses were separated on a C18 - ODS (4.6 × 250mm analytical column with 5.0 μm particle size) .The mobile phase consisted of 90% acetonitrile and 10% methanol used in isocratic elution mode with a flow rate of 1.0 ml/min. Samples were injected using a six-port injection valve equipped with a 100 μl loop. Results were read at a wavelength of 264 nm.

C-Sample =C standard *A sample/A standard * D.F/Wt. or v of sample

C standard: Concentration of standard (ng/ml)

A sample: Area under the peak of sample

A standard: Area under the peak of standard.

D.F: Dilution factor used according to the method.

Wt. or V: Weight or Volume of taken sample.

Figure (2-14): High pressure liquid chromatography for determined vitamin D$_3$ concentration

2.3.4. Lipid profile: Total cholesterol, high density lipoprotein (HDL)-cholesterol, and triglycerides were measured with enzymatic colorimetric test, Low-density lipoprotein (LDL) cholesterol level was calculated by the Friedewald formula:

"Total cholesterol—HDL cholesterol—(triglycerides/5)"

2.3.4.1 Determination of Serum Total Cholesterol:

- **principle**

Cholesterol concentration was established by an enzymatic procedure in accordance

 with the method expressed by (Allain C. *et al.)* **[105]**,as exhibited in the following reactions:

Cholesterol esters + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol +Fatty acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholesten 4 one 3+H_2O_2

H_2O_2 + Phenol +PAP $\xrightarrow{\text{Peroxidase}}$ Quinonimine (pink) +4H_2O
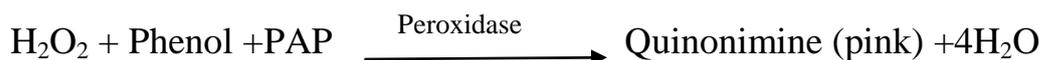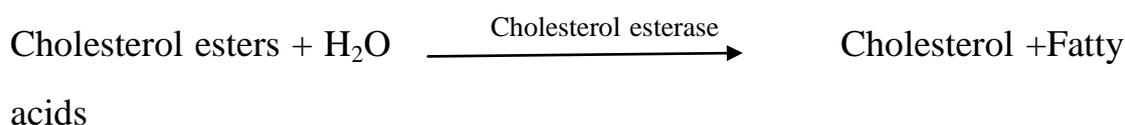
Table (2-15): Components of the total cholesterol kit

Reagents	Composition	
Reagent 1	Phosphate buffer	100 mmol/L
(Buffer)	Chloro-4-phenol	5.0 mmol/L
	Sodium chloride	2.3 mmol/L
	Triton x 100	1.5 mmol/L
	Preservative	

Reagent 2	Cholesterol oxidase	100 IU/L
(Enzymes)	Cholesterol esterase	170 IU/L
	Peroxidase	1200 IU/L
	Phenol aminophenazone(PAP)	0.25mmol/L
	Polyethylene glycol 6000	
Reagent 3 (Standard)	Cholesterol 200 mg/dL	5.17 (mmol/L)

- **Procedure**

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (Buffer), gently mixed up until complete dissolutionn (approximately 2 minutes) to prepare work reagent. The assay was acted as in table (2-16):

Table (2-16): procedure of cholesterol.

Reagents	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Deionized water	10	-	-
Standard	-	10	-
Sample	-	-	10

The tubes were mixed and then let stable for 5 minutes   at 37 C or 10 minutes at room temp. Absorbance was recorded at 500 nm (480 – 520) nm against the blank. The color is constant for 1 hour.

- **Calculation:**

Cholesterol (mg/dL) = sample absorbance/standard absorbance X 200

2.3.4.2 Determination of Serum HDL-Cholesterol.

The principle

LDL, VLDL and Chylomicron from specimens were precipitated by Phospho tungstic acid and magnesium chloride. HDL-cholesterol obtained in the supernatant after centrifugation is then measured with TC reagent. The components of HDL-c as show in table (2-17):

Table (2-17): Components of the high density lipoprotein cholesterol kit

Reagents	Composition	
Reagent 1 (Precipitant)	Phosphotungstic acid Magnesium chloride Ph. 6.2	13.9mmol/L 490mmol/L
Reagent 2 (Standard)	Cholesterol	100 mg/dL (2.58mmol/L)

- **Procedure:**

1. A volume of 0.5 ml of samples (serum) was added into clean plain tubes.

2. A volume of 50   of precipitant was added. The tubes were mixed, then let stand for 10 minutes at room temperature. Centrifuge 15 minutes at 2000 xg then the next procedure was applied, which include the measurement of cholesterol in the supernatant as show in table (2-18).

The tube were mixed, then let stand for 5 minutes at 37 °C or 10 minutes at room temperature. Record absorbance at 500 nm (480-520) against blank. The color is stable for 1 hour.

- **Calculation:**

HDL-cholesterol (mg/dl) = sample absorbance/standard absorbance X 100

(Standard concentration = 100 mg/dL)

Table (2-18): procedure of high density lipoprotein cholesterol

Reagents	Blank	Standard	Sample
Reagent	1 ml	1ml	1ml
Distilled water	25	-	-
Standard	-	25	-
Supernatant	-	-	25

2.3.4.3 Determination of Serum Triacylglycerol:

Principle: Triacylglycerol concentration was determined by an enzymatic procedure corresponding to the method expressed by Fossati P. And Principle method associated with Tinder reaction, as show in the following reaction:

Triglycerides $\xrightarrow{\text{Lipase}}$ Glycerol + free fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol-3-phosphate + ADP

$$\text{Glycerol-3-P} + O_2 \xrightarrow{\text{Glycerol-3-phosphate Oxidase}} \text{Dihydroxyacetone-p} + H_2O_2$$

$$H_2O_2 + \text{4-chlorophenol} + \text{PAP} \xrightarrow{\text{Peroxidase}} \text{Quinonimine(pink)} + 4H_2O_2$$
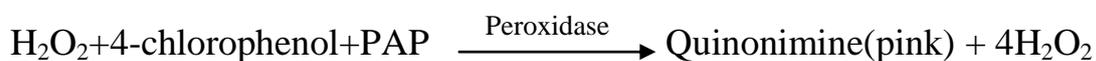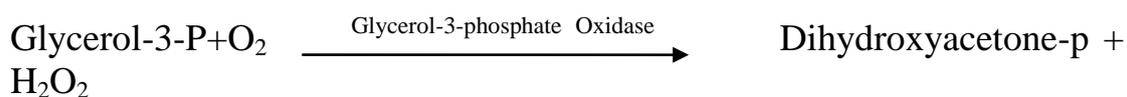
The absorbance of the colored complex (quinonimine), proportional to the amount of triglycerides in the specimen.

Table (2-19): Components of serum triacylglycerol kit.

Reagents	Composition	
Reagent 1 (Buffer)	PIPES	100mmol/L
	Magnesium chloride	9.8mmol/L
	Chloro-4-phenol	3.5mmol/L
	Preservative	
Reagent 2 (Enzymes)	Lipase	1000 IU/L
	Peroxidase	1700 IU/L
	Glycerol-3-p-oxidase	3000 IU/L
	Glycerol Kinase	660IU/L
	Phenol aminophenazone	0.5 mmol/L
	Adenosine triphosphate	1.3 mmol/L
Reagent 3 (Standard)	Glycerol equivalent to triglycerid	200 mg/dL

- **Procedure:**

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (Buffer), mixed gently until complete dissolution (approximately 2 minutes) to prepare work reagent .The procedure was carried out as in table (2-20):

Table (2-20): Procedure of triacylglycerol

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Deionized water	0.01 ml	-	-
Standard	-	0.01ml	-
Sample	-	-	0.01ml

The tubes was mixed, then let stand for 5 minutes at 37 ℃ or 10 minutes the tubes were mixed, then let stand for 5 minutes at 37 ℃ or 10 minutes at room temperature. Absorbance was recorded at 500 nm (480-520) against the blank .the color is stable for 1 hour.

- **Calculation:**

Triglyceride (mg/dl) = sample absorbance/standard absorbance X 200

2.3.4.4. Determination of Serum VLDL

Concentration of VLDL-C was determined by dividing the triglyceride value, obtained in section (2.3.6) by 5.

$$VLDL(mmol/L) = TGs/5$$

2.3.4.5 Determination of Serum LDL

Concentration of LDL was calculated by using Friedewald equation.

LDL-cholesterol (mg/dl) =Total-cholesterol – HDL-cholesterol – TG/5

Notes: If the TG >400 mg/dL this equation not applied.

Figure (2-21): CECIL system to calculate lipid profile

2.2.5 Statistical Methods

Statistical analysis was done by using Software Package for Social Science (SPSS-24., Standard version).The results were presented as mean and standard error. Continuous variables were tested for normality according to the t-test to determine the significant differences between the groups. Correlation coefficient (r) was used to find the relationship between two continuous variables. A p-value of ≤ 0.05 was considered as significant.

Receiver operating characteristic(ROC) curve was used to evaluate the diagnostic value of angiotensin II in obese patients. The sensitivity and specificity of biochemical parameter and calculate the optimal cutoff according to "Youden Index" by select the point that is closest to the top-left corner of the ROC curve giving equal weight to sensitivity and specificity when picking a cut-off point is a typical practice. This idea is often referred to as the Youden Index[106].The area under the curve (AUC) provides a useful tool to compare different biomarkers as Table (2- 21).

Table (2-21) List of AUC ranges and their classification levels

AUC Range	Classification Level

0.90 - 1.00	Excellent
0.80 - 0.90	Good
0.70 - 0.80	Fair
0.60 - 0.70	Poor
0.50 - 0.60	Failure

From the experimental work we can summarize the following features:

3.1 Demographic Features of the Study Group

Table (3-1) shows the findings of demographic data of studied groups (obese and control). When comparing the obese patients to the control groups, the results demonstrate no significant differences ($p > 0.05$) in age, while a significant ($p < 0.000$) changes present in BMI, and WHR. Means $\pm$SE of controls and patients for age were (33.75 $\pm$1.564, 35.27 $\pm$1.449), for BMI (23.02 $\pm$0.25, 35.05 $\pm$1.09) and for WHR (0.82 $\pm$0.007, 0.94 $\pm$.001) respectively

Table (3-1): Demographic Features of the Study Group.

Variable	Study groups	No.	Means ±SE	P- Value
Age (years)	Control	44	33.75 ±1.564	0.477
	Ob. Patients	44	35.27 ±1.449	
BMI Kg/m²	Control	44	23.02 ±0.25	0.000
	Ob. Patients	44	35.05 ±1.09	
Waist/ Hip ratio	Control	44	0.82 ±0.007	0.000
	Ob. Patients	44	0.94 ±.001	

BMI (Body mass index), **WC** (waist circumference), **SE** (Standard error), ob. (obese)

3.1.1. Age

Table **(3 – 1)** expressed no significant changes in age between control and obese patients. This age matching helps to eliminate variations in parameter results that may occur as a result of a large age difference **[106]**.

3.1.2 Waist /Hip Ratio (WHR) and Waist Circumference (WC):

According to the results of the data analysis mean ± SE of WHR for studied group control and obese patients were **(0.82 ±0.007, 0.94 ±.001)** respectively with statistically significant differences **(p < 0.05)** as in table **(3-1).**

A waist-to-hip ratio by WHO abdominal obesity in men and women **(0.90, 0.85)** respectively. For both sexes, a ratio greater than 1.0 indicates a substantially higher risk of health issues**[107]**. According to research by (McKeigue *et al*.2012), central adiposity, or body fat that is concentrated around the belly, increases the risk of heart disease and hypertension compared to peripheral adiposity, which is body fat that is distributed elsewhere. As a result, measuring waist circumference is a reliable obesity indicator. For instance, men who have a waist circumference greater than 102 cm and women who have a waist circumference bigger than 88 cm will both be considered obese**[108].**

3.2 Biochemical Results:

3.2.1 Estimation of Electrolytes in Study Groups

From table(**3-2**) The mean±SE of electrolytes (Na^+, K^+, Cl^-) level for control group **(316.81±0.596, 15.817±0.169, and 351.12±.1.152)** mg/dl respectively when compared with obese patients**(318.25±1.355, 15.493±0.249, 352.80±1.875)** mg/dl respectively that show no significant($p<0.05$) differences between them. While the mean±SE of iCa in studied groups control and patients were **(4.4980±0.539, 3.3800±0.119)** respectively with a highly significant **($p<0.000$)** differences between them.

Table (3-2): Mean±SE of Electrolytes in Control and Obese Groups

Variable	Groups	No.	Mean±SE	P-value
Sodium Mg/dl	Control	44	318.25±1.355	0.68
	Ob. Patients	44	316.81±0.596	
Potassium Mg/dl	Control	44	15.817±0.169	0.287
	Ob. Patients	44	15.493±0.249	
Chloride Mg/dl	Control	44	351.12±.1.152	0.464
	Ob. Patients	44	352.80±1.875	
iCa Mg/dl	Control	44	4.4980±0.539	0.000
	Ob. Patients	44	3.3800±0.119	

iCa: ionized calcium   Ob: obese

Electrolyte abnormalities are more prevalent in obese patients, which may be brought on by hyperglycemia, shifting osmotic fluids, total body deficits, sweating, diarrhea, or vomiting loss, which causes either high or low electrolyte levels in the body[109].Excessive weight gain initially leads to renal vasodilatation and an increase in RBF and GFR prior to kidney injury. Excessive sodium reabsorption by the kidneys leads to sodium retention and an increase in extracellular fluid volume causing an

increase in blood pressure associated with weight gain. In the early stages of obesity, the Blood Pressure may not be sensitive to salt; therefore, high blood pressure may not be significantly exacerbated by high salt intake **[3– 5]**.

At least three mechanisms have important roles in causing the increased renal sodium reabsorption and hypertension that are associated with rapid increases in adiposity and excessive weight gain: renal compression, stimulation of the renin-angiotensin-aldosterone system (RAAS) and stimulation of the SNS. These mechanisms and other factors that contribute to the development of obesity-induced hypertension**[112].**

The results of current study for electrolytes(Na^+, K^+, Cl^-)show non-significant differences, these results agreement with (L Hulthe´n *et al*,2014) he suggests that some obese persons with or without a family history of hypertension may have normal blood pressure and electrolytes levels**[113].**and these result disagrees with(Ebrahimi et al.2018) who studied the association between intracellular electrolytes and obesity indices and found increase in level of sodium and a lower level of potassium was observed in obese patients due to high sodium diet **[114].**

The disparity might be due to the fact that, the previous studies focused on subjects with renal problems or they focus on who are known to have a loss of renal-concentrating ability (electrolyte disturbance in admitted diabetes), patients have insulin resistance with uncontrolled blood sugar were at a greater risk to develop hypernatremia, since the inability of kidney to maintain control of homeostatic mechanisms involving stimulation of thirst, secretion of Antidiuretic Hormone (ADH), and renal handling.

The results of present study showed also significant p-value lowering iCa^{+2} in obese group when compared with control groups ($p<0.000$). Free

calcium is a more meaningful index than total calcium and provides a better indication of calcium status [8].

The calcium-body weight effect's mechanism and amplitude are both unknown. Low calcium intake enhances dihydroxy vitamin D_3 and PTH, and these calcitropic circulating chemicals, in turn, stimulate adipocyte calcium uptake, according to (Zemel *et al*.2012)[115]. High intracellular calcium levels, as previously stated, increase lipogensis while inhibiting lipolysis. Dietary calcium, according to this theory, enhances lipolysis while preserving thermogenesis, resulting in faster weight reduction. Increased intracellular Ca^{2+} causes lipogenic expression and lipogenesis to be stimulated, lipolysis to be suppressed, and adipocyte lipid metabolism and triacylglycerol storage to be suppressed [116].

Increased calcitriol production in response to low-calcium diets is also thought to firstly contribute to Ca^{2+} influx in human adipocytes and obesity. A second possible mechanism is the encouragement of increased fecal energy losses due to the development of calcium and fat no absorbed complexes[117].

3.2.2 Estimation of Angiotensin II and Vit D in Studied Groups.

From the experimental work data measured by ELISA and HPLC techniques we can summarize the following:

The Mean±SE of angiotensin-II for studied groups (control and patients) were **(31.0989±1.26403, 40.4555±2.47906)** respectively, with p-value **(0.001)** as summarized in table (3-3). While vit D_3 decrease significantly (p<0.003) in obese patients compared to control group, also the Mean±SE for control and obese patients groups Vit D_3 level were (25.20±0.45, 11.53±0.46) respectively.

Table (3-3): Mean ± SE for Angiotensin II and Vit D in Studied Group.

Variable	Groups	No.	Mean±SE	P-value
AngII(ng/ml)	Control	44	31.0989±1.26403	0.001
	Ob. Patients	44	40.4555±2.47906	
Vit D(ng/ml)	Control	44	25.20±0.455	0.003
	Ob. Patients	44	11.53±0.465	

Ang II: angiotensin II

Actually obesity causes the renin-angiotensin-aldosterone system (RAAS) to become activated. Sympathetic stimulation, adipokine production in the RAAS by visceral fat, and hemodynamic changes are all implicated in RAAS activation in obesity. This lead to promote many different inflammatory processes in the vasculature, heart, brain, liver, kidney and lung. In terms of alterations in hemodynamics, obesity causes increased renal plasma flow, GFR, glomerular pressure, and filtration fraction, as well as net afferent dilatation. Excess excretory load, increased energy intake, and tissue turnover could all contribute to these alterations[118].

AngII (the RAAS component that is biologically active) play a role in regulation of blood pressure, fluid, and electrolyte homeostasis. In the kidney, angiotensin II has at least three major functions: auto regulation of GFR, salt excretion decrease via direct and indirect actions on renal tubular cells, and growth control of AT1 receptor-expressing renal cells[119].

In a high concentration Angiotensin II can diminish GFR at larger doses by reducing the filtration surface area and sensitizing the afferent arteriole to the tubuloglomerular feedback's constricting signal. Thus, depending on the underlying disease, the net effect of angiotensin II on GFR differs. This is show by (JR Henegar,*et al* 2001) when examine the histologic and functional changes that occur in the kidney of dog in the early stages of obesity caused by a high-fat diet and found hemodynamic alteration and hormonal changes that could contribute to renal

remodeling in obesity and elevated plasma Ang II and insulin levels may contribute to glomerular structural**[120].**

The results of current study support previous research that suggests Increased local production of Ang II in adipose tissue was first shown in human with obesity caused by genetics or food **[121].**

On the other hand the results of the vit D_3 level above in table(3-4)there was decrease significantly (p<0.003)in obese patients compared to control group. These findings agreement with previous research that suggests decrease Vit D_3 in obese patients**[122].**

Although the exact mechanism causing low serum calcidiol levels in obese people is unknown There are four processes that have been proposed in the literature to explain why people with obesity have low vitamin D levels: (1) Obese people get less sun exposure than lean people; (2) negative feedback from an elevated 1,25 (OH)D concentration in obese people lowers 25 (OH)D concentrations; (3) vitamin D_3 is stored in adipose tissue; (Wortsman *et al*.2000)**[123].** Provided the first substantial, persuasive evidence that vitamin D (as a fat-soluble vitamin) can get sequestered within adipose tissue. (4) lower 25(OH)D concentration is simply due to volumetric dilution**[124].**

Vitamin D_3 deficiency and excessive fat accumulation have mutually negative effects as a result of excessive metabolic processes, enzymatic disorders, and decreased activity of alpha-hydroxylase, the key enzyme in the biotransformation of calciferol in a fat-infiltrated liver, resulting in the accumulation of inactive forms and reduced vitamin D bioavailability**[125].**and this is in accordance with the results revealed in this study

3.2.3 Estimation of Lipid Profile

Uv-visible spectrophotometer used to measure the lipid profile for obese and control groups. The results show a significant increase (**p< 0.00**) in Triglyceride, T-cholesterol, VLDL-cholesterol, and LDL-cholesterol in obese patients. The level of HDL-Cholesterol concentration in the obese patients was significant decrease (p<0.00) Compared to the control group as listed in table **(3-4).**

Table (3-4): Mean±SE of Lipid Profile in Control and Obese Groups

Variable	Groups	No.	Mean±SE	P-value
Tri (mg/dl)	Control	44	110.84±2.004	**0.000**
	Ob. patients	44	173.18±2.603	
Chol (mg/dl)	Control	44	171.39±2.159	**0.000**
	Ob. patients	44	193.23±3.381	
HDL (mg/dl)	Control	44	67.66±0.640	**0.000**
	Ob. patients	44	48.61±1.029	
LDL (mg/dl)	Control	44	80.93±2.101	**0.000**
	Ob. patients	44	109.64±3.163	
VLDL (mg/dl)	Control	44	22.17±0.405	**0.000**
	Ob. patients	44	34.84±0.542	

P-value (<0, 05) significant, **Tri** (triglyceride), **Chol**(cholesterol), **HDL**(high density lipoprotein)

LDL (low density lipoprotein), **VLDL** (very low density lipoprotein).

The results of current study is agreement with (Waleed.S.Mohamed) who found that obesity is associated with various detrimental changes in lipid metabolism including increased serum concentrations of total cholesterol, and LDL, Triacylglycerol and decrease HDL**[126].**

Adipose tissue, particularly visceral adipose tissue, is the most major contributor to obesity-related dyslipidemia. Which induced lipolysis to release fatty acid and increased transport to the liver and the generation of extremely low-density lipoprotein (VLDL), Increased synthesis of VLDL in the liver can impede lipolysis of chylomicrons, which drives hypertriglyceridemia, and decreased mRNA expression or activity of lipoprotein lipase (LPL) in adipose tissue and skeletal muscle[127].

Also, dyslipidemia with obesity may be result from up regulating lipoprotein lipase and fatty acid synthase (FAS) expression and blocking lipolysis via AT2R, AngII increases lipogenesis in mature adipocytes and when AngII binding to AT1R may decrease lipolytic enzymes and lipolysis [128].

3.3 The Correlation Among all Variables in Obese Patients Group:

Linear regression analysis was used to determine the degree of correlation between the variables in this study. As indicated in the table **(3-5)** that indicated positive relationship (r =0.371, p0.013) between Ang II and serum sodium and significant negative correlation(r=-0.494, p<0.001) between Ang II with serum vit D_3 also showed positive correlation(r=0.354, p=0.18) between Na and Triacylglycerol, While BMI showed positive correlation with Sodium, Triacylglycerol (r=0.523, p=0.000 r= 0.656,p= 0.000)respectively and also negative correlation with Vit D_3 ,HDL-c(r=-0.494, p<0.001 r= -0.552,p= 0.000)respectively.

**Table (3-5): Correlation between variables in obese group**

		BMI	AngII	Vit-D	Na	K	Tri	HDL	LDL
BMI	r	1	0.182	-0.434	0.532	-0.004	0.656	-0.552	0.122
	P-value		0.237	0.003	0.000	0.978	0.000	0.000	0.429
AngII	r	0.182	1	0.001	0.347	0.082	0.110	-0.208	0.030
	P-value	0.237		0.993	0.012	0.598	0.476	0.174	0.845
Vit-D	r	-0.494	0.001	1	0.035	0.462	-0.214	0.046	-0.105
	P-value	0.001	0.993		0.822	0.002	0.163	0.766	0.497
Na	r	0.523	0.372	0.035	1	0.089	0.354	-0.287	-0.056
	P-value	0.000	0.013	0.822		0.567	0.018	0.059	0.716
K	r	-0.004	0.082	0.462	0.089	1	0.061	0.172	0.131
	P-value	0.978	0.598	0.002	0.567		0.696	0.264	0.396
Tri	r	0.656	0.110	-0.214	0.354	0.061	1	-0.291	0.092
	P-value	0.000	0.476	0.163	0.018	0.696		0.055	0.551
HDL	r	-0.552	-0.208	0.046	-0.287	0.172	-0.291	1	0.000
	P-value	0.000	0.174	0.766	0.059	0.264	0.055		1.000
LDL	r	0.122	0.030	-0.105	-0.056	0.131	0.092	0.000	1
	P-value	0.429	0.854	0.497	0.716	0.396	0.551	1.000	

3.3.1 Correlation between all Variables and BMI in obese Patient Group.

In the current study, significant negative correlation between $vitD_3$ and BMI was found, this is in accordance with results of previous studies with(Brock *et al*.,) who reported that BMI >30 kg/is one of the major factors that decreases vitamin D_3 levels in obesity **[129].**and also supporting these finding by(Khosravi, *et al*.:2018) that founded weight,

waist circumference, and BMI all fell dramatically after 6 weeks of taking 50,000 international units per week of Vitamin D supplementation, while serum Vitamin D increased show figure(3-6)[130].

$$y = -0.7415x + 44.647$$
$$r = 0.434$$
$$p = 0.003$$
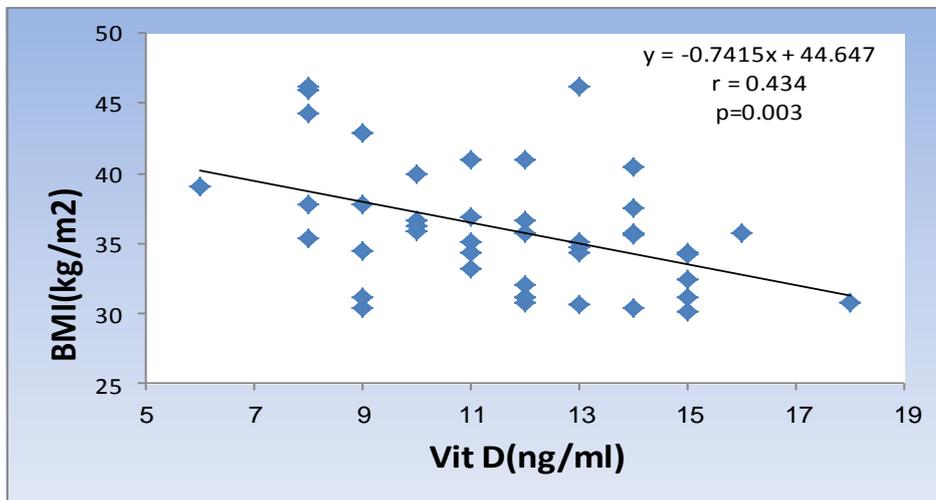
Figure (3-6): Correlation between BMI and vitamin D$_3$ in obese group

The results of current study showed that significant positive correlation of BMI with triacylglycerol (r= 0.656, p= 0.000) and significant negative correlation with HDL-C(r= -0.552,p= 0.000)in obese patients (figure 3-7, 3-8)respectively. This result agrees with(Kalkhoff, *et al.*)[131]. that studied the relationship between body weight and serum lipid levels Their findings revealed that HDL-C levels were considerably lower in patients with a high BMI [132].

$$y = 2.5371x + 81.886$$
$$r = 0.656$$
$$p = 0.000$$
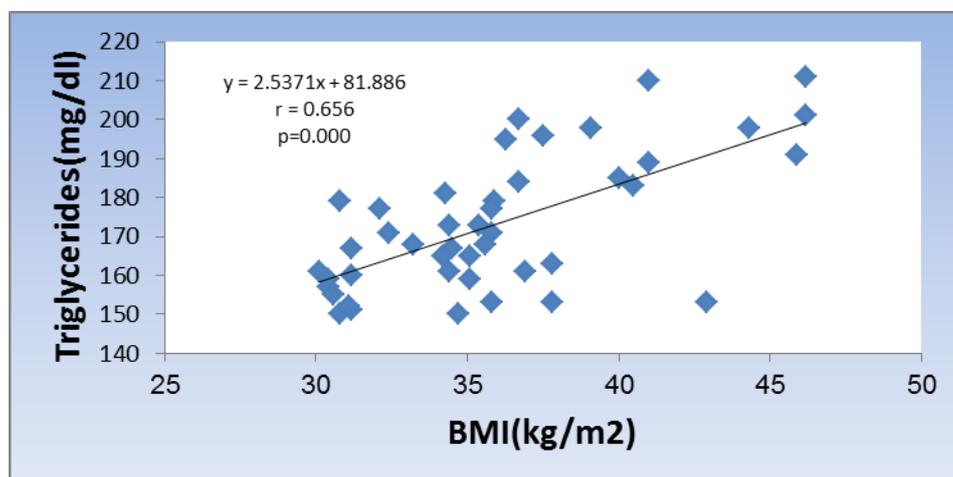
Figure (3-7): Correlation between BMI and triacylglycerol in obese group
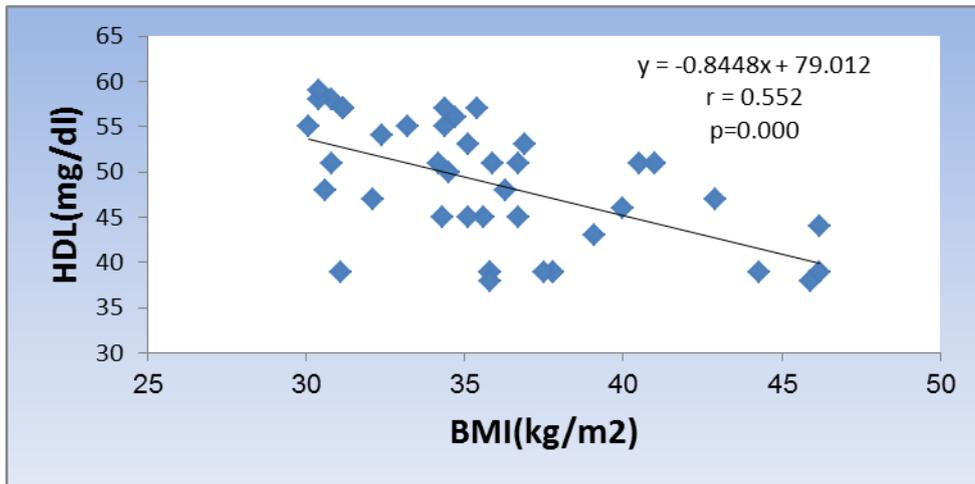
Figure (3-8): Correlations between BMI and high density lipoprotein in obese group

The results of these study also showed positive correlation of BMI and Sodium(r=0.523, p=0.000) in obese group in figure (3-9). It's worth noting that our findings were in line with those of other studies conducted in different populations. In Australian children, a higher incidence of obesity was linked to salt imbalance. Interestingly, a follow-up study of Hispanic and Latino Americans found a link between sodium and obesity indices like BMI that was independent of calorie intake[133].
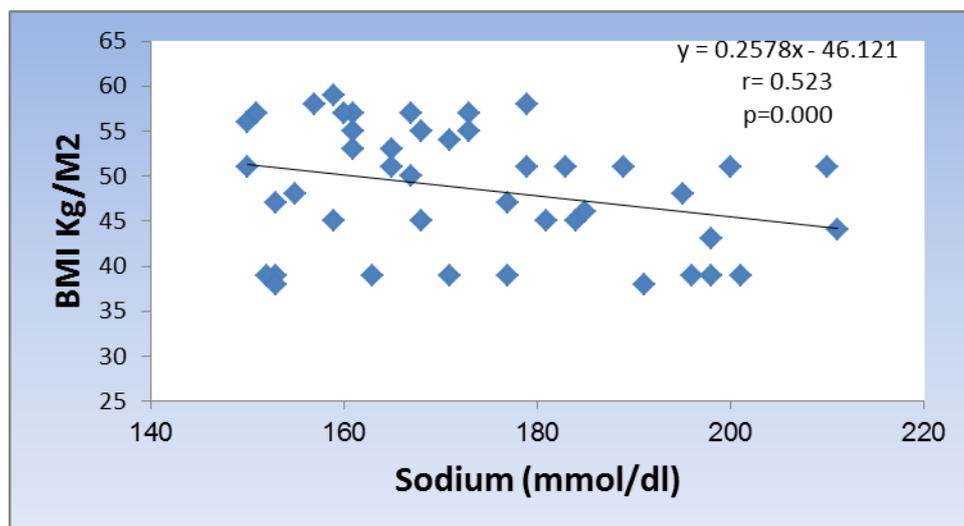
Figure (3-9): Correlation between BMI and sodium in obese group

3.3.2 Correlation Between Angiotensin II and Electrolytes, Vitamin D₃, Lipid profile in Obese Group.

The results of linear regression analysis in this study demonstrate a positive relationship (r =0.371, p0.13) between Ang II and serum sodium Level in obese patients as show in figure (3-10).These data support the theory, that elevated AngII most likely from adipose tissue ,causes Na retention ,which may play a key role in the pathogenesis of obesity-related hypertension[134].
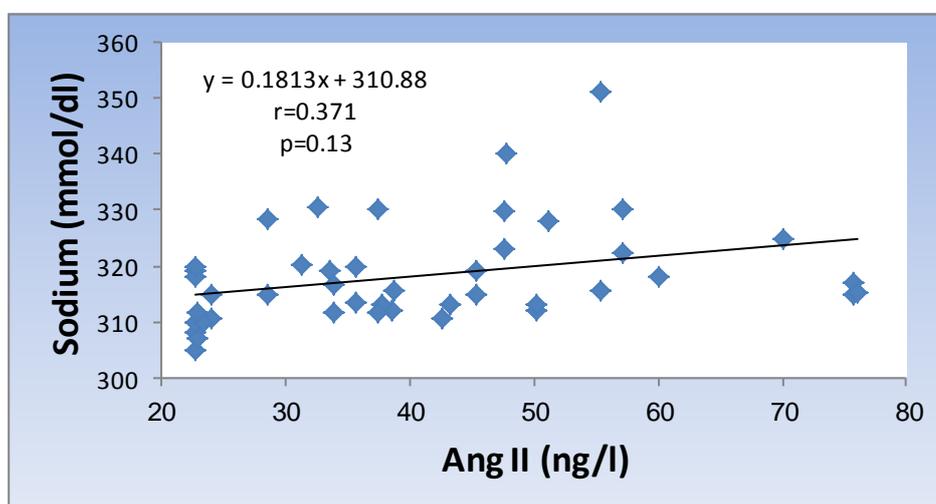
Figure (3-10): Correlation between angiotensin II and sodium in obese group

The result of present study express positive correlation between sodium and triacylglycerol (r=0.354, p=0.18) in obese patients as show in figure (3-11). These support by experimental study for high sodium diet in obese rats and control that show the obese rats had increased lipid buildup in adipocyte tissue. Other researches have suggested that a higher sodium level may cause fat formation in adipocyte tissue, as well as a change in lipid balance in the body.
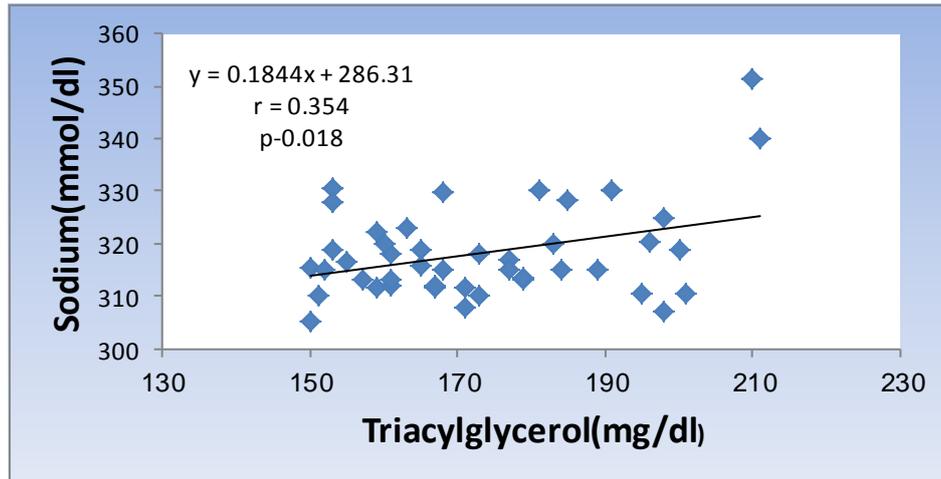
Figure (3-11): Correlation between sodium and triacylglycerol in obese group.

In the current study, significant negative correlation(r=-0.99, p<0.001) between serum vit D_3 and Ang II in obese group as show in figure (3-12).

These result agreement with(Forman *et al*.2010) investigated the relationship between 25(OH)D and the renin-angiotensin system in 184 people with normal blood pressure in a cross-sectional research [88]. Vitamin D_3 deficit and insufficiency were associated with increased plasma Ang II levels and a trend toward higher plasma renin activity when compared to vitamin D_3-sufficient individuals.
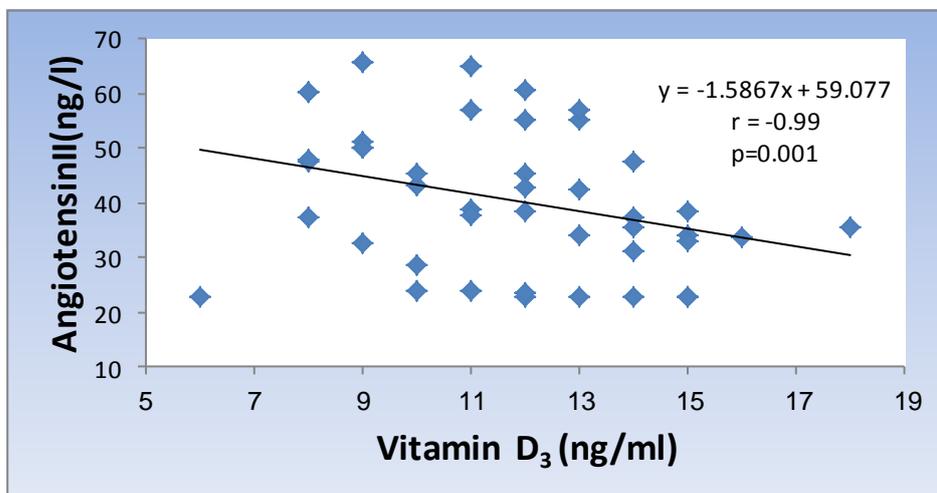
Figure (3-12): Correlation between angiotensin II and vitamin D₃ in obese group

3.2. Receiver Operating Characteristic (ROC) Analysis of the Angiotensin II:

ROC curve for the sensitivity and specificity of angiotensin ii for diagnosis of obesity at (Cut-off point was $\geq$ 32.4 (ng/l)), AUC (Area under the curve) of 0.73 and P= <0.001, the sensitivity and the specificity was (60.0 %, 75 %) respectively, as shown in figure (3-15).

AUC indicated that angiotensin II consider as apoor diagnostic marker in obesity.

ROC Curve

Diagonal segments are produced by ties.
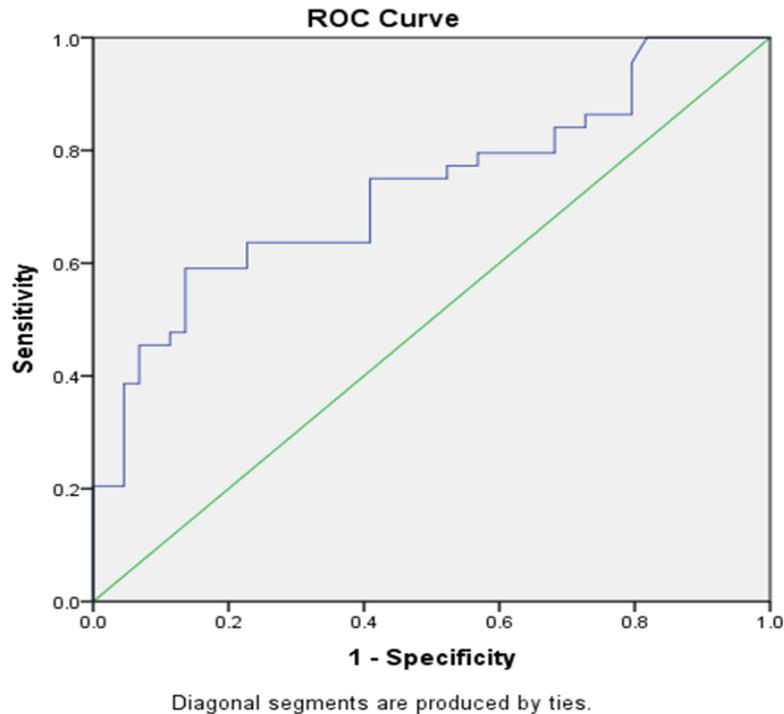
Figure (3-15) : Receiver operating characteristic (ROC)curve of angiotensin II of obese group.

Conclusions

This study concluded that:

1. Angiotensin II   increased in obese patients and no effect on electrolytes (Na^+, K^+, Cl^-).

2. Lipid is deranged.

3. Obesity is associated with decrease iCa that result from vit D_3 deficiency.

4. Increase Ang II lead to retention of sodium in obese patient that lead to obesity development.

Recommendations:

1. Increasing awareness and health education about obesity and its risks and ways of spreading it, changing the sedentary lifestyle and reducing fast food because of their impact and role in the development of obesity, especially in children.

2. A similar study with a bigger sample size in other regions of Iraqi patient from different regions.

3. A comparable study should be carried out, with additional biochemical signals to determine the risk for development of obesity such as fibroblast growth factor 21 (FGF21).

4. Opening specialized centers for fat people provides medical advice, therapy, and follow-up for obese people, assisting them in resolving their problems under the supervision of medical professionals.

5. It is crucial that there be school-based initiatives to encourage young children to engage in physical activity and consume a nutritious diet, as young obese children grow into fat adults

References:

[1]   W. H. Albuali, 'Evaluation of oxidant-antioxidant status in overweight and morbidly obese Saudi children', *World J. Clin. Pediatr.*, vol. 3, no. 1, p. 6, 2014.

[2]   J. J. Fuster, N. Ouchi, N. Gokce, and K. Walsh, 'Obesity-induced changes in adipose tissue microenvironment and their impact on cardiovascular disease', *Circ. Res.*, vol. 118, no. 11, pp. 1786–1807, 2016.

[3]   E. Gjermeni *et al.*, 'Obesity–An Update on the Basic Pathophysiology and Review of Recent Therapeutic Advances', *Biomolecules*, vol. 11, no. 10, p. 1426, 2021.

[4]   S. A. Shabu, 'Prevalence of overweight/obesity and associated

factors in adults in Erbil, Iraq: A household survey', *Zanco J. Med. Sci. (Zanco J Med Sci)*, vol. 23, no. 1, pp. 128–134, 2019.

[5]   E. Silvestris, G. de Pergola, R. Rosania, and G. Loverro, 'Obesity as disruptor of the female fertility', *Reprod. Biol. Endocrinol.*, vol. 16, no. 1, pp. 1–13, 2018.

[6]   T. Mekonnen, W. Animaw, and Y. Seyum, 'Overweight/obesity among adults in North-Western Ethiopia: a community-based cross sectional study', *Arch. public Heal.*, vol. 76, no. 1, pp. 1–6, 2018.

[7]   L. Yvan-Charvet and A. Quignard-Boulangé, 'Role of adipose tissue renin–angiotensin system in metabolic and inflammatory diseases associated with obesity', *Kidney Int.*, vol. 79, no. 2, pp. 162–168, 2011.

[8]   A. Saiki *et al.*, 'Circulating angiotensin II is associated with body fat accumulation and insulin resistance in obese subjects with type 2 diabetes mellitus', *Metabolism*, vol. 58, no. 5, pp. 708–713, 2009.

[9]   P. K. Cabandugama, M. J. Gardner, and J. R. Sowers, 'The renin angiotensin aldosterone system in obesity and hypertension: roles in the cardiorenal metabolic syndrome', *Med. Clin.*, vol. 101, no. 1, pp. 129–137, 2017.

[10] P. Santhanam, J. I. Shapiro, and Z. Khitan, 'Association between dietary potassium, body mass index, and proteinuria in normotensive and hypertensive individuals: Results from the Modification of Diet in Renal Disease study baseline data', *J. Clin. Hypertens.*, vol. 19, no. 5, p. 558, 2017.

[11] A. Catalano *et al.*, 'Incidence of hypocalcemia and hypercalcemia

in hospitalized patients: Is it changing?', *J. Clin. Transl. Endocrinol.*, vol. 13, pp. 9–13, 2018.

[12] C. B. Turer, H. Lin, and G. Flores, 'Prevalence of vitamin D deficiency among overweight and obese US children', *Pediatrics*, vol. 131, no. 1, pp. e152–e161, 2013.

[13] A. Ataey, E. Jafarvand, D. Adham, and E. Moradi-Asl, 'The relationship between obesity, overweight, and the human development index in world health organization eastern mediterranean region countries', *J. Prev. Med. Public Heal.*, vol. 53, no. 2, p. 98, 2020.

[14] S. Camacho and A. Ruppel, 'Is the calorie concept a real solution to the obesity epidemic?', *Glob. Health Action*, vol. 10, no. 1, p. 1289650, 2017.

[15] C. Guillermier *et al.*, 'Imaging mass spectrometry demonstrates age-related decline in human adipose plasticity', *JCI insight*, vol. 2, no. 5, 2017.

[16] A. B. Engin, 'Adipocyte-macrophage cross-talk in obesity', *Obes. lipotoxicity*, pp. 327–343, 2017.

[17] T. Tchkonia *et al.*, 'Obesity-associated oxidative stress: strategies finalized to improve redox state', *Int. J. Mol. Sci.*, vol. 14, no. 5, pp. 10497–10538, 2013.

[18] P. Sharma *et al.*, 'Epidemiological correlates of hepatitis C infection-A case control analysis from a tertiary care hospital', *J. Fam. Med. Prim. Care*, vol. 11, no. 5, pp. 2099–2105, 2022.

[19] M. Ferro *et al.*, 'The emerging role of obesity, diet and lipid

metabolism in prostate cancer', *Futur. Oncol.*, vol. 13, no. 3, pp. 285–293, 2017.

[20] C. S. Oxlund *et al.*, 'Body mass index, intensive blood pressure management, and cardiovascular events in the SPRINT trial', *Am. J. Med.*, vol. 132, no. 7, pp. 840–846, 2019.

[21] C. L. Ogden *et al.*, 'Prevalence of obesity among adults, by household income and education—United States, 2011–2014', *MMWR. Morb. Mortal. Wkly. Rep.*, vol. 66, no. 50, p. 1369, 2017.

[22] S. DVandevijvere *et al.*, 'Global trends in ultraprocessed food and drink product sales and their association with adult body mass index trajectories', *Obes. Rev.*, vol. 20, pp. 10–19, 2019.

[23] T. Omer, 'The causes of obesity: an in-depth review', *Adv Obes Weight Manag Control*, vol. 10, no. 4, pp. 90–94, 2020.

[24] R. Pérez-Escamilla *et al.*, 'Dietary energy density and body weight in adults and children: a systematic review', *J. Acad. Nutr. Diet.*, vol. 112, no. 5, pp. 671–684, 2012.

[25] G. V. P. Coutinho *et al.*, 'Intrauterine protein restriction combined with early postnatal overfeeding was not associated with adult-onset obesity but produced glucose intolerance by pancreatic dysfunction', *Nutr. Metab. (Lond).*, vol. 10, no. 1, pp. 1–9, 2013.

[26] Y. Winter, R. Sankowski, and T. Back, 'Genetic determinants of obesity and related vascular diseases', *Vitam. Horm.*, vol. 91, pp. 29–48, 2013.

[27] C. A. Stratakis, 'Cushing syndrome in pediatrics', *Endocrinol. Metab. Clin.*, vol. 41, no. 4, pp. 793–803, 2012.

[28] S. R. Patel and F. B. Hu, 'Short sleep duration and weight gain: a systematic review', *Obesity*, vol. 16, no. 3, pp. 643–653, 2008.

[29] R. C. Guerra, A. Zuñiga-Muñoz, V. Guarner Lans, E. Díaz-Díaz, C. A. Tena Betancourt, and I. Pérez-Torres, 'Modulation of the activities of catalase, Cu-Zn, Mn superoxide dismutase, and glutathione peroxidase in adipocyte from ovariectomised female rats with metabolic syndrome', *Int. J. Endocrinol.*, vol. 2014, 2014.

[30] B. Bjørndal, L. Burri, V. Staalesen, J. Skorve, and R. K. Berge, 'Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents', *J. Obes.*, vol. 2011, 2011.

[31] E. Standl, 'Dysglycemia and abdominal obesity', *Curr. Vasc. Pharmacol.*, vol. 10, no. 6, pp. 678–679, 2012.

[32] D. Serra, P. Mera, M. I. Malandrino, J. F. Mir, and L. Herrero, 'Mitochondrial fatty acid oxidation in obesity', *Antioxid. Redox Signal.*, vol. 19, no. 3, pp. 269–284, 2013.

[33] I. Harman-Boehm *et al.*, 'Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity', *J. Clin. Endocrinol. Metab.*, vol. 92, no. 6, pp. 2240–2247, 2007.

[34] S. Bryan, B. Baregzay, D. Spicer, P. K. Singal, and N. Khaper, 'Redox-inflammatory synergy in the metabolic syndrome', *Can. J. Physiol. Pharmacol.*, vol. 91, no. 1, pp. 22–30, 2013.

[35] J. P. Lewis and A. R. Shuldiner, 'Genetics of the metabolic complications of obesity', *Prog. Mol. Biol. Transl. Sci.*, vol. 94, pp.

349–372, 2010.

[36] X. Yang, S. Enerbäck, and U. Smith, 'Reduced expression of FOXC2 and brown adipogenic genes in human subjects with insulin resistance', *Obes. Res.*, vol. 11, no. 10, pp. 1182–1191, 2003.

[37] A. Gómez-Hernández, N. Beneit, S. Díaz-Castroverde, and Ó. Escribano, 'Differential role of adipose tissues in obesity and related metabolic and vascular complications', *Int. J. Endocrinol.*, vol. 2016, 2016.

[38] E. Hondares, M. Rosell, F. J. Gonzalez, M. Giralt, R. Iglesias, and F. Villarroya, 'Hepatic FGF21 expression is induced at birth via PPARα in response to milk intake and contributes to thermogenic activation of neonatal brown fat', *Cell Metab.*, vol. 11, no. 3, pp. 206–212, 2010.

[39] J. Villarroya, R. Cereijo, and F. Villarroya, 'An endocrine role for brown adipose tissue?', *Am. J. Physiol. Metab.*, vol. 305, no. 5, pp. E567–E572, 2013.

[40] D. V Chartoumpekis, I. G. Habeos, P. G. Ziros, A. I. Psyrogiannis, V. E. Kyriazopoulou, and A. G. Papavassiliou, 'Brown adipose tissue responds to cold and adrenergic stimulation by induction of FGF21', *Mol. Med.*, vol. 17, no. 7, pp. 736–740, 2011.

[41] J. A. N. Dorresteijn, F. L. J. Visseren, and W. Spiering, 'Mechanisms linking obesity to hypertension', *Obes. Rev.*, vol. 13, no. 1, pp. 17–26, 2012.

[42] I. Kyrou, H. S. Randeva, C. Tsigos, G. Kaltsas, and M. O. Weickert, 'Clinical problems caused by obesity', *Endotext*

[Internet], 2018.

[43] P. Adab, M. Pallan, and P. H. Whincup, 'Is BMI the best measure of obesity?', *Bmj*, vol. 360. British Medical Journal Publishing Group, 2018.

[44] L. Hu *et al.*, 'Prevalence of overweight, obesity, abdominal obesity and obesity-related risk factors in southern China', *PLoS One*, vol. 12, no. 9, p. e0183934, 2017.

[45] I. Janssen, M. Shields, C. L. Craig, and M. S. Tremblay, 'Prevalence and secular changes in abdominal obesity in Canadian adolescents and adults, 1981 to 2007–2009', *Obes. Rev.*, vol. 12, no. 6, pp. 397–405, 2011.

[46] F. J. Félix-Redondo *et al.*, 'Prevalence of obesity and associated cardiovascular risk: The DARIOS study', *BMC Public Health*, vol. 13, no. 1, pp. 1–10, 2013.

[47] Z. Kalani, T. Salimi, and M. Rafiei, 'Comparison of obesity indexes BMI, WHR and WC in association with Hypertension: results from a Blood Pressure Status Survey in Iran', *J. Cardiovasc. Dis. Res.*, vol. 6, no. 2, pp. 72–77, 2015.

[48] S. Ajeganova, M. L. Andersson, I. Hafström, and B. S. Group, 'Association of obesity with worse disease severity in rheumatoid arthritis as well as with comorbidities: a long-term followup from disease onset', *Arthritis Care Res. (Hoboken).*, vol. 65, no. 1, pp. 78–87, 2013.

[49] N. T. T. Tran *et al.*, 'The importance of waist circumference and body mass index in cross-sectional relationships with risk of

cardiovascular disease in Vietnam', *PLoS One*, vol. 13, no. 5, p. e0198202, 2018.

[50] A. M. Angelidi, M. J. Belanger, A. Kokkinos, C. C. Koliaki, and C. S. Mantzoros, 'Novel Noninvasive Approaches to the Treatment of Obesity: From Pharmacotherapy to Gene Therapy', *Endocr. Rev.*, vol. 43, no. 3, pp. 507–557, 2022.

[51] H. Asada *et al.*, 'The crystal structure of angiotensin II type 2 receptor with endogenous peptide hormone', *Structure*, vol. 28, no. 4, pp. 418–425, 2020.

[52] K. A. Carpenter, B. C. Wilkes, and P. W. Schiller, 'The octapeptide angiotensin II adopts a well-defined structure in a phospholipid environment', *Eur. J. Biochem.*, vol. 251, no. 1-2, pp. 448–453, 1998.

[53] M. Flores-Muñoz, 'Investigating the role of Angiotensin 1-9 in cardiomyocytes hypertrophy'. University of Glasgow, 2010.

[54] T. Morgan, 'Renin, angiotensin, sodium and organ damage', *Hypertens. Res.*, vol. 26, no. 5, pp. 349–354, 2003.

[55] W. C. De Mello and E. D. Frohlich, 'On the local cardiac renin angiotensin system. Basic and clinical implications', *Peptides*, vol. 32, no. 8, pp. 1774–1779, 2011.

[56] E. M. V De Cavanagh, F. Inserra, M. Ferder, and L. Ferder, 'From mitochondria to disease: role of the renin-angiotensin system', *Am. J. Nephrol.*, vol. 27, no. 6, pp. 545–553, 2007.

[57] E. H. Bae *et al.*, 'Murine recombinant angiotensin-converting enzyme 2 attenuates kidney injury in experimental Alport

syndrome', *Kidney Int.*, vol. 91, no. 6, pp. 1347–1361, 2017.

[58] A. C. T. Ng *et al.*, 'Impact of epicardial adipose tissue, left ventricular myocardial fat content, and interstitial fibrosis on myocardial contractile function', *Circ. Cardiovasc. Imaging*, vol. 11, no. 8, p. e007372, 2018.

[59] T. Rönn *et al.*, 'A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue', *PLoS Genet.*, vol. 9, no. 6, p. e1003572, 2013.

[60] C. Rüster and G. Wolf, 'The role of the renin-angiotensin-aldosterone system in obesity-related renal diseases', in *Seminars in nephrology*, 2013, vol. 33, no. 1, pp. 44–53.

[61] Y. Molla and M. Sisay, 'The role of Angiotensin II type 2 receptors (AT2Rs) in the regulation of cardio-renal and neuroprotective activities: Potential therapeutic implications', *J. Drug Deliv. Ther.*, vol. 7, no. 2, pp. 110–118, 2017.

[62] J. Stegbauer and T. M. Coffman, 'New insights into angiotensin receptor actions: from blood pressure to aging', *Curr. Opin. Nephrol. Hypertens.*, vol. 20, no. 1, p. 84, 2011.

[63] D. J. Tai *et al.*, 'Cardiovascular effects of angiotensin converting enzyme inhibition or angiotensin receptor blockade in hemodialysis: a meta-analysis', *Clin. J. Am. Soc. Nephrol.*, vol. 5, no. 4, pp. 623–630, 2010.

[64] A. N. Onyiriuka and E. E. Oyenusi, 'Prevalence of abnormal serum sodium and potassium concentration in paediatric new onset type 1 diabetes with ketoacidosis: A retrospective study from two Nigerian

Teaching Hospitals', *Sri Lanka J. Diabetes Endocrinol. Metab.*, vol. 8, no. 1, 2018.

[65] T. M. Kear, 'Fluid and Electrolyte Management Across the Age Continuum.', *Nephrol. Nurs. J.*, vol. 44, no. 6, 2017.

[66] S. Lear *et al.*, 'Corrigendum to: Long-term outcomes in young patients with atrioventricular block of unknown aetiology In the originally published version of this manuscript, errors were noted and listed in this corrigendum. In the Funding section, the section should read', *Eur Hear. J*, vol. 39, pp. 3021–3104, 2018.

[67] F. A. Al-Quraishe and A. T. Ghadhban, 'Estimation The Trace Elements And Their Association With Insulin Resistance In Obese Adults.', *Syst. Rev. Pharm.*, vol. 12, no. 7, pp. 2294–2298, 2021.

[68] C. A. Grimes, D. P. Bolhuis, F. J. He, and C. A. Nowson, 'Dietary sodium intake and overweight and obesity in children and adults: a protocol for a systematic review and meta-analysis', *Syst. Rev.*, vol. 5, no. 1, pp. 1–6, 2016.

[69] J. S. Chae *et al.*, 'Mild weight loss reduces inflammatory cytokines, leukocyte count, and oxidative stress in overweight and moderately obese participants treated for 3 years with dietary modification', *Nutr. Res.*, vol. 33, no. 3, pp. 195–203, 2013.

[70] D. Shin, H.-K. Joh, K. H. Kim, and S. M. Park, 'Benefits of potassium intake on metabolic syndrome: The fourth Korean National Health and Nutrition Examination Survey (KNHANES IV)', *Atherosclerosis*, vol. 230, no. 1, pp. 80–85, 2013.

[71] L. S. S. Mariosa *et al.*, 'Abdominal obesity is associated with

potassium depletion and changes in glucose homeostasis during diuretic therapy', *J. Clin. Hypertens.*, vol. 10, no. 6, pp. 443–449, 2008.

[72] M. S. Djurhuus, A. Vaag, and N. A. H. Klitgaard, 'Muscle sodium, potassium, and [3H] ouabain binding in identical twins, discordant for type 2 diabetes', *J. Clin. Endocrinol. Metab.*, vol. 86, no. 2, pp. 859–866, 2001.

[73] C. A. Pfortmueller, D. Uehlinger, S. von Haehling, and J. C. Schefold, 'Serum chloride levels in critical illness—the hidden story', *Intensive care Med. Exp.*, vol. 6, no. 1, pp. 1–14, 2018.

[74] L. P. Ewoldt, 'The relationship between calcium intake and body mass index in the participants of HUB City Steps', 2013.

[75] D. M. de Oliveira Freitas, H. S. D. Martino, S. M. R. Ribeiro, and R. de C. G. Alfenas, 'Calcium ingestion and obesity control', *Nutr. Hosp.*, vol. 27, no. 6, pp. 1758–1771, 2012.

[76] B. M. Thomson and P. J. Cressey, *Determination of vitamin D in foods: Current knowledge and data gaps*. Ministry for Primary Industries, 2014.

[77] K. J. Allen *et al.*, 'Vitamin D insufficiency is associated with challenge-proven food allergy in infants', *J. Allergy Clin. Immunol.*, vol. 131, no. 4, pp. 1109–1116, 2013.

[78] Y. C. Li, J. Kong, M. Wei, Z.-F. Chen, S. Q. Liu, and L.-P. Cao, '1, 25-Dihydroxyvitamin D 3 is a negative endocrine regulator of the renin-angiotensin system', *J. Clin. Invest.*, vol. 110, no. 2, pp. 229–238, 2002.

[79] A. Gupta, 'Vitamin D deficiency in India: prevalence, causalities and interventions', *Nutrients*, vol. 6, no. 2, pp. 729–775, 2014.

[80] S. Y. Alkass and N. I. Mohammed, 'Free Vitamin D Status Among Apparently Healthy Adults Living in Duhok Governorate', 2021.

[81] A. C. Ross, C. L. Taylor, A. L. Yaktine, and H. B. Del Valle, 'Committee to review dietary reference intakes for vitamin D and calcium', *Food Nutr. Board*, 2011.

[82] DRI, 'Institute of Medicine, Food and Nutrition Board, Dietary Reference Intakes: energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids'. The National Academy Washington, 2005.

[83] A. C. Ross, B. H. Caballero, R. J. Cousins, K. L. Tucker, and T. R. Ziegler, *Modern nutrition in health and disease*. Wolters Kluwer Health Adis (ESP), 2012.

[84] F. J. Ruiz-Ojeda, A. Anguita-Ruiz, R. Leis, and C. M. Aguilera, 'Genetic factors and molecular mechanisms of vitamin D and obesity relationship', *Ann. Nutr. Metab.*, vol. 73, pp. 89–99, 2018.

[85] H. D. Abdulkader and A. J. Al-Saffar, 'Assessment of Vitamin D Level in a Sample of Iraqi Obese Women', *IRAQI J. Med. Sci.*, vol. 19, no. 2, 2021.

[86] A.-J. A. L. Zainel, H. Qotba, A. Al Nuaimi, and M. Syed, 'Vitamin D status among adults (18–65 years old) attending primary healthcare centres in Qatar: a cross-sectional analysis of the Electronic Medical Records for the year 2017', *BMJ Open*, vol. 9, no. 8, p. e029334, 2019.

[87] A. Tomaschitz *et al.*, 'Independent association between 1, 25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin–angiotensin system: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study', *Clin. Chim. acta*, vol. 411, no. 17–18, pp. 1354–1360, 2010.

[88] J. P. Forman, J. S. Williams, and N. D. L. Fisher, 'Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans', *Hypertension*, vol. 55, no. 5, pp. 1283–1288, 2010.

[89] M. A. Beydoun, A. Boueiz, M. R. Shroff, H. A. Beydoun, Y. Wang, and A. B. Zonderman, 'Associations among 25-hydroxyvitamin D, diet quality, and metabolic disturbance differ by adiposity in adults in the United States', *J. Clin. Endocrinol. Metab.*, vol. 95, no. 8, pp. 3814–3827, 2010.

[90] A. Vaidya, B. Sun, C. Larson, J. P. Forman, and J. S. Williams, 'Vitamin D3 therapy corrects the tissue sensitivity to angiotensin ii akin to the action of a converting enzyme inhibitor in obese hypertensives: an interventional study', *J. Clin. Endocrinol. Metab.*, vol. 97, no. 7, pp. 2456–2465, 2012.

[91] J. Vekic, A. Zeljkovic, A. Stefanovic, Z. Jelic-Ivanovic, and V. Spasojevic-Kalimanovska, 'Obesity and dyslipidemia', *Metabolism*, vol. 92, pp. 71–81, 2019.

[92] K. R. Feingold and C. Grunfeld, 'Introduction to lipids and lipoproteins', 2015.

[93] H. O. Ahmed, M. A. H. Marif, M. A. A. Omer, A. F. Hamasur, S. H. Ahmed, and K. M. Abddalqadir, 'The life styles causing overweight or obesity: Based on 5 years of experience in two

centers in Sulaimani Governorate, Kurdistan Region/Iraq', *Int. J. Surg. Open*, vol. 11, pp. 22–29, 2018.

[94] Y. Xu, N. Wang, H.-Y. Tan, S. Li, C. Zhang, and Y. Feng, 'Function of Akkermansia muciniphila in obesity: interactions with lipid metabolism, immune response and gut systems', *Front. Microbiol.*, vol. 11, p. 219, 2020.

[95] J. Aron-Wisnewsky, M. V Warmbrunn, M. Nieuwdorp, and K. Clément, 'Metabolism and metabolic disorders and the microbiome: The intestinal microbiota associated with obesity, lipid metabolism, and metabolic health—Pathophysiology and therapeutic strategies', *Gastroenterology*, vol. 160, no. 2, pp. 573–599, 2021.

[96] C. Castaño, S. Kalko, A. Novials, and M. Párrizas, 'Obesity-associated exosomal miRNAs modulate glucose and lipid metabolism in mice', *Proc. Natl. Acad. Sci.*, vol. 115, no. 48, pp. 12158–12163, 2018.

[97] E. Lee, S.-R. Jung, S.-Y. Lee, N.-K. Lee, H.-D. Paik, and S.-I. Lim, 'Lactobacillus plantarum strain Ln4 attenuates diet-induced obesity, insulin resistance, and changes in hepatic mRNA levels associated with glucose and lipid metabolism', *Nutrients*, vol. 10, no. 5, p. 643, 2018.

[98] E. Lee, S.-R. Jung, S.-Y. Lee, N.-K. Lee, H.-D. Paik, and S.-I. Lim, 'Consumption of mung bean (Vigna radiata L.) attenuates obesity, ameliorates lipid metabolic disorders and modifies the gut microbiota composition in mice fed a high-fat diet', *J. Funct. Foods*, vol. 64, p. 103687, 2020.

[99] C. S. Conn *et al.*, 'The major cap-binding protein eIF4E regulates

lipid homeostasis and diet-induced obesity', *Nat. Metab.*, vol. 3, no. 2, pp. 244–257, 2021.

[100] C. B. Weir and A. Jan, 'BMI classification percentile and cut off points', 2019.

[101] M. T. Provencher *et al.*, 'Body mass index versus body fat percentage in prospective national football league athletes: overestimation of obesity rate in athletes at the national football league scouting combine', *J. Strength Cond. Res.*, vol. 32, no. 4, pp. 1013–1019, 2018.

[102] D. Bojanic, M. Ljubojevic, D. Krivokapic, and S. Gontarev, 'Waist circumference, waist-to-hip ratio, and waist-to-height ratio reference percentiles for abdominal obesity among Macedonian adolescents', *Nutr. Hosp.*, 2020.

[103] A. J. Mahrath, 'Isolation and Identification of Green Pigments from Waste Pineapple Peels', *Microscopy*, 2022.

[104] F. Keyfi, S. Nahid, A. Mokhtariye, S. Nayerabadi, A. Alaei, and A.-R. Varasteh, 'Evaluation of 25-OH vitamin D by high performance liquid chromatography: validation and comparison with electrochemiluminescence', *J. Anal. Sci. Technol.*, vol. 9, no. 1, pp. 1–6, 2018.

[105] S. K. Knutson *et al.*, 'Selective Inhibition of EZH2 by EPZ-6438 Leads to Potent Antitumor Activity in EZH2-Mutant Non-Hodgkin LymphomaLymphoma Tumor Regression with EZH2 Inhibitor', *Mol. Cancer Ther.*, vol. 13, no. 4, pp. 842–854, 2014.

[106] U. Branavan, K. Muneeswaran, W. S. S. Wijesundera, A.

Senanayake, N. V. Chandrasekharan, and C. N. Wijeyaratne, 'Association of Kiss1 and GPR54 Gene Polymorphisms with Polycystic Ovary Syndrome among Sri Lankan Women', *BioMed Research International*, vol. 2019. 2019, doi: 10.1155/2019/6235680.

[107] T. Anothaisintawee, N. Sansanayudh, S. Thamakaison, D. Lertrattananon, and A. Thakkinstian, 'Neck circumference as an anthropometric indicator of central obesity in patients with prediabetes: a cross-sectional study', *Biomed Res. Int.*, vol. 2019, 2019.

[108] M. Labib, 'The investigation and management of obesity', *J. Clin. Pathol.*, vol. 56, no. 1, pp. 17–25, 2003.

[109] P. Kughapriya and J. Evangeline, 'Evaluation of serum electrolytes in Ischemic Heart Disease patients', *Natl. J. Basic Med. Sci.*, vol. 6, no. 4, pp. 1–14, 2016.

[110] C. A. Schiavon *et al.*, 'Effects of bariatric surgery in obese patients with hypertension: the GATEWAY randomized trial (gastric bypass to treat obese patients with steady hypertension)', *Circulation*, vol. 137, no. 11, pp. 1132–1142, 2018.

[111] J. E. Hall, M. W. Brands, and J. R. Henegar, 'Angiotensin II and long-term arterial pressure regulation: the overriding dominance of the kidney.', *J. Am. Soc. Nephrol. JASN*, vol. 10, pp. S258-65, 1999.

[112] M. E. Hall, J. M. do Carmo, A. A. da Silva, L. A. Juncos, Z. Wang, and J. E. Hall, 'Obesity, hypertension, and chronic kidney disease', *Int. J. Nephrol. Renovasc. Dis.*, vol. 7, p. 75, 2014.

[113] L. Hulthen, M. Aurell, S. Klingberg, E. Hallenberg, M. Lorentzon, and C. Ohlsson, 'Salt intake in young Swedish men', *Public Health Nutr.*, vol. 13, no. 5, pp. 601–605, 2010.

[114] R. Ebrahimi, N. J. Alipour, and S. Emamgholipour, 'The association between intracellular electrolytes and obesity indices', *Arch. Med. Lab. Sci.*, vol. 4, no. 2, pp. 1–8.

[115] M. B. Zemel, H. Shi, B. Greer, D. Dirienzo, and P. C. Zemel, 'Regulation of adiposity by dietary calcium', *FASEB J.*, vol. 14, no. 9, pp. 1132–1138, 2000.

[116] H. Shi, D. DiRienzo, and M. B. Zemel, 'Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice', *FASEB J.*, vol. 15, no. 2, pp. 291–293, 2001.

[117] H. Shi, Y.-D. HALVORSEN, P. N. ELLIS, W. O. WILKISON, and M. B. ZEMEL, 'Role of intracellular calcium in human adipocyte differentiation', *Physiol. Genomics*, vol. 3, no. 2, pp. 75–82, 2000.

[118] T. Thethi, M. Kamiyama, and H. Kobori, 'The link between the renin-angiotensin-aldosterone system and renal injury in obesity and the metabolic syndrome', *Curr. Hypertens. Rep.*, vol. 14, no. 2, pp. 160–169, 2012.

[119] T. Fujita, 'Mineralocorticoid receptors, salt-sensitive hypertension, and metabolic syndrome', *Hypertension*, vol. 55, no. 4, pp. 813–818, 2010.

[120] J. R. Henegar, S. A. Bigler, L. K. Henegar, S. C. Tyagi, and J. E. Hall, 'Functional and structural changes in the kidney in the early

stages of obesity', *J. Am. Soc. Nephrol.*, vol. 12, no. 6, pp. 1211–1217, 2001.

[121] I. Hainault, G. Nebout, S. Turban, B. Ardouin, P. Ferré, and A. Quignard-Boulangé, 'Adipose tissue-specific increase in angiotensinogen expression and secretion in the obese (fa/fa) Zucker rat', *Am. J. Physiol. Metab.*, vol. 282, no. 1, pp. E59–E66, 2002.

[122] S. J. Mohammed, 'Association between Vitamin D and body weight in Iraqi population: Case-control study', *J Obes Weight Loss Ther*, vol. 8, no. 377, p. 2, 2018.

[123] J. Wortsman, L. Y. Matsuoka, T. C. Chen, Z. Lu, and M. F. Holick, 'Decreased bioavailability of vitamin D in obesity', *Am. J. Clin. Nutr.*, vol. 72, no. 3, pp. 690–693, 2000.

[124] I. Zakharova *et al.*, 'Vitamin D insufficiency in overweight and obese children and adolescents', *Front. Endocrinol. (Lausanne).*, vol. 10, p. 103, 2019.

[125] E. Hyppönen and C. Power, 'Vitamin D status and glucose homeostasis in the 1958 British birth cohort: the role of obesity', *Diabetes Care*, vol. 29, no. 10, pp. 2244–2246, 2006.

[126] W. S. Mohamed, M. A. Hassanien, and K. E. L. Sayed Abokhosheim, 'Role of ghrelin, leptin and insulin resistance in development of metabolic syndrome in obese patients', *Endocrinol Metab Synd*, vol. 3, no. 122, pp. 1017–2161, 2014.

[127] U. J. Jung and M.-S. Choi, 'Obesity and its metabolic complications: the role of adipokines and the relationship between

obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease', *Int. J. Mol. Sci.*, vol. 15, no. 4, pp. 6184–6223, 2014.

[128] P. A. Tyurin-Kuzmin, N. I. Kalinina, K. Y. Kulebyakin, A. V Balatskiy, V. Y. Sysoeva, and V. A. Tkachuk, 'Angiotensin receptor subtypes regulate adipose tissue renewal and remodelling', *FEBS J.*, vol. 287, no. 6, pp. 1076–1087, 2020.

[129] K. Brock, R. Cant, L. Clemson, R. S. Mason, and D. R. Fraser, 'Effects of diet and exercise on plasma vitamin D (25 (OH) D) levels in Vietnamese immigrant elderly in Sydney, Australia', *J. Steroid Biochem. Mol. Biol.*, vol. 103, no. 3–5, pp. 786–792, 2007.

[130] Z. S. Khosravi, M. Kafeshani, P. Tavasoli, A. H. Zadeh, and M. H. Entezari, 'Effect of Vitamin D supplementation on weight loss, glycemic indices, and lipid profile in obese and overweight women: A clinical trial study', *Int. J. Prev. Med.*, vol. 9, 2018.

[131] R. K. Kalkhoff, A. H. Hartz, D. Rupley, A. H. Kissebah, and S. Kelber, 'Relationship of body fat distribution to blood pressure, carbohydrate tolerance, and plasma lipids in healthy obese women', *J. Lab. Clin. Med.*, vol. 102, no. 4, pp. 621–627, 1983.

[132] G. Y. Serbest, 'Fazla kilolu olan polikistik over sendromlu hastalarda serum obestatin düzeylerine diyetin etkisinin değerlendirilmesi', 2012.

[133] C. A. Grimes, L. J. Riddell, K. J. Campbell, F. J. He, and C. A. Nowson, '24-h urinary sodium excretion is associated with obesity in a cross-sectional sample of Australian schoolchildren', *Br. J. Nutr.*, vol. 115, no. 6, pp. 1071–1079, 2016.

[134] S. Engeli *et al.*, 'The adipose-tissue renin–angiotensin–aldosterone system: role in the metabolic syndrome?', *Int. J. Biochem. Cell Biol.*, vol. 35, no. 6, pp. 807–825, 2003.

[135] G. Fruhbeck, J. Gómez-Ambrosi, F. J. Muruzábal, and M. A. Burrell, 'The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation', *Am. J. Physiol. Metab.*, vol. 280, no. 6, pp. E827–E847, 2001.

[136] M. G. Jameel, E. S. Saleh, and M. H. AL-Osami, 'VITAMIN D AND OXIDATIVE STRESS IN OBESE IRAQI SAMPLE WITH FIBROMYALGIA SYNDROME'.

Questionnaire

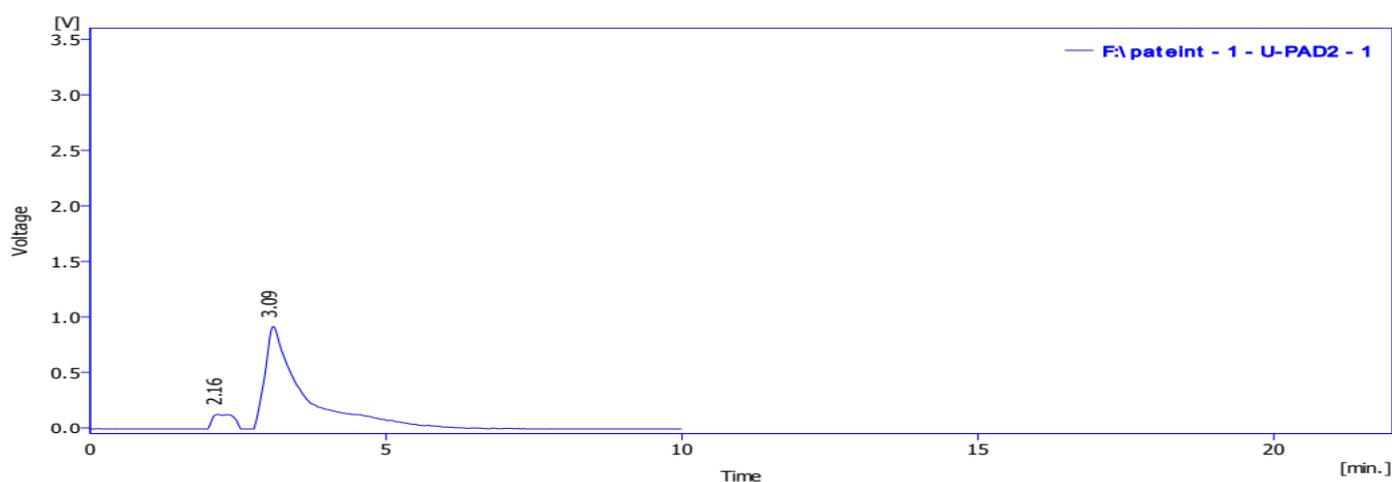| Number:	Phone number:	
Name patient:		
Age:		
Gender	**Male**	**Female**
Weight:	**Height:**	**BMI:**
Waist	**Hip:**	**W/H:**
Address:		
Family history:		
Educational level:		
Smoking habits:		
Medical history (D.M, hypertension, CVD, CKD, Asthma, Liver disease, cancer):		
occupational status:		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: pateint	Amount	: 0
Sample	: pateint	ISTD Amount	: 0
Inj. Volume [mL]	: 0.05	Dilution	: 1

Autostop	: 10.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change

Result Table (Uncal - F:\pateint - 1 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.160	343.168	36.002	49.6	33.3	0.23	
2	3.092	348.725	72.178	50.4	66.7	0.08	
	Total	691.894	108.180	100.0	100.0		

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: Control 1
Sample	: control 1
Inj. Volume [mL]	: 0.05

Amount	: 0
ISTD Amount	: 0
Dilution	: 1

Autostop	: 10.00 min
Detector 1	: U-PAD2 - 1
Subtraction Chromatogram	: (None)

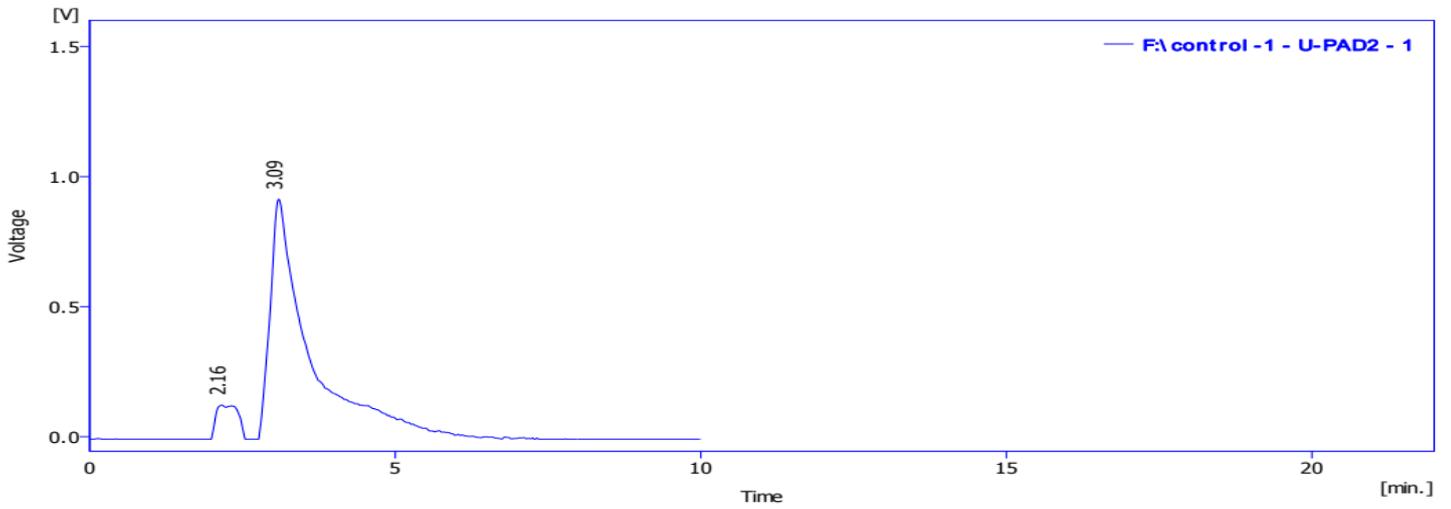|	
External Start	: Start - Restart, Down
Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching	: No Change

Result Table (Uncal - F:\control -1 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.160	354.647	36.581	21.6	17.0	0.24	
2	3.092	1288.950	179.178	78.4	83.0	0.13	
	Total	1643.597	215.759	100.0	100.0		

الخلاصة

السمنة هي واحدة من أكثر المشاكل الصحية شيوعًا بين الأطفال والمراهقين في كل من الدول الصناعية والنامية ، ولكنها حالة متعددة الأوجه ناتجة عن عوامل وراثية وبيئية على حد سواء ، وتتطور السمنة نتيجة لمزيج من الإنفاق المنخفض للطاقة (مثل التمارين الرياضية). وتناول كمية عالية من السعرات الحرارية. الانجيوتنسين 2 واضطراب الالكترولايت(الصوديوم والبوتاسيوم والكلورايد والكالسيوم) يرتبط بالسمنة والمضاعفات ذات الصلة مثل ارتفاع ضغط الدم ، وأمراض التمثيل الغذائي ، وأمراض القلب والأوعية الدموية ، وأمراض الكلى.

صُممت هذه الدراسة لتقييم تأثير الانجيوتنسين 2 والإلكتروليتات وبعض التغيرات البيوكيميائية لدى الأفراد الذين يعانون من السمنة . لتحقيق هذا الهدف ،اختير ثمانية وتمانون شخصا أربعة وأربعين منهم يعانون من السمنة تتراوح أعمارهم بين (20-55) عامًا ،و أربعة وأربعين فردًا سليمًا ظاهريا في اعمار متطابقة للمجموعتين (مجموعة السمنه ومجموعة السيطرة)،وتم جمع نماذج الدم من مجاميع مرضى السمنة والسيطرة واخذ المصل واستخدامه في تحديدِ الانجيوتنسين 2 بطريقة الاليزا، وايضا تم تقدير الالكترولايت بواسطة الالكترولايت انالايزر،في حين تم قياس تركيز(فيتامين د3) بواسطة جهاز كروماتوجرافيا سائله عالية الضغط واخيرا تم قياس الدهون الثلاثيه والكوليسترول والدهون الحميده بواسطة مقياس الطيف الضوئي.

اظهرت نتائج التحليل الاحصائي زيادة معنويه في مستوى الانجيوتنسين 2 والدهون الثلاثية والكوليسترول والبروتينات الدهنية واطئة الكثافة في مرضى السمنه بالمقارنة مع الاصحاء. كذلك شوهد نقصان حاد في تركيز فيتامين د3 والكالسيوم والبروتينات الدهنية عالية الكثافة في مصول البدناء عند مقارنتهم مع الاصحاء. اما بالنسبة لنتائج الصوديوم والبوتاسيوم والكلورايد لم تظهر اي تأثير معنوي.

أظهرت نتائج تحاليل الانحدار الخطي علاقة موجبة (0.371 = r، $p < 0.013$)بين تركيز الانجيوتنسين 2 والصوديوم بينما كانت هناك علاقة موجبه (0.523 = r، $p < 0.000$، 0.656 = r، $p < 0.000$) لمعيار وزن الجسم مع الصوديوم والدهون الثلاثية في مجموعة مرضى السمنة.كما أظهر نفس التحليل ارتباطات سلبية ($-0.494, p < 0.001$ = r ، $0.462, p < 0.002$ = r، -0.552 = r، $p < 0.00$) لمعيار وزن الجسم مع فيتامين د3 والدهون الحميدة والبوتاسيوم على التوالي في مجموعة السمنة، وأيضا كانت هناك علاقة موجبة r $p < 0.018$ (0.354 =) للصوديوم مع الدهون الثلاثية في مجموعة مرضى السمنة.

توصلت هذه الدراسة إلى أن السمنة مرتبطة بزيادة الانجيوتنسين 2 مما ادى الى احتباس الصوديوم في مرضى السمنه .وتم الاستنتاج ايضا ان انخفاض فيتامين د3 ادى الى ارتفاع الدهون الثلاثية والكوليسترول والبروتينات الدهنية منخفضة الكثافة ونقصان البروتينات الدهنية عالية الكثافة والبوتاسيوم.

وكذلك استنتجت الدراسة إلى أن السمنة مرتبطة بانخفاض iCa الناتج عن نقص (فيتامين د3). بالاضافه الى وجود علاقة قوية بين ارتفاع الصوديوم وارتفاع الدهون الثلاثية وحدوث السمنة.

جمهورية العراق

وزارة التعليم العالي

والبحث العلمي

جامعة بابل/ كلية الطب

فرع الكيمياء الحياتية السريرية

تأثير السمنة على نسبة هرمون الانجيوتنسين 2 والالكترولايت والدهون بالدم

رسالة

مقدمة الى مجلس كلية الطب في جامعة بابل

كجزء من متطلبات نيل درجة الماجستير

في العلوم /الكيمياء الحياتية السريرية

من قبل

رشا ماجد عبد الزهرة لفته

بكالوريوس تحليلات مرضية(2010)

إشراف

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