Ministry of Higher Education and Scientific Research
University of Basrah
College of Science
Department of Biology

Physiological role of Visfatin hormone on lipid profile and glucose level in obese and diabetic women

A Thesis

Submitted to the Council of College of Science

University of Basrah in Partial Fulfillment of Requirement for the

Degree of Master of Science (MSc) in Biology / Physiology

Submitted By

Zahraa Mahmoud Hussain Al-Hejaj

B.Sc. in Biology, 2010

Supervised By

Asst. Prof. Dr. Hana Salman Kadhum

2021 **1443**

Supervisor's Recommendation

I certify that this thesis was carried under my supervision at the College of Science, University of Basrah, as a partial Requirement for the Degree of Master of Science in Biology (Physiology).

Signature:

Asst. Prof. Dr. Hana Salman Kadhum

Date: / / 2021

In view of the available recommendation, I forward this thesis for debate by the Examining Committee.

Signature

Prof. Dr. Nasir Abd Ali Almansour

Head of Biology Department

Date: / / 2021

بِسْمِ ٱللّٰهِ ٱلرحْمّٰنِ ٱلرحِيمِ

و منْ يتَّقِ اللّٰهَ يجْعلْ لَهُ مخْرجاً * و يرْزُقْهُ مِنْ حيْثُ لاَيحْتَسِبُ

و منْ يتَوكَّلْ علَى اللّٰهِ فَهُو حسْبُهُ إِنَّ اللّٰهَ بالِغُ أَمْرِهِ قَدْ جعلَ اللّٰهُ

لكِلِّ شيْء قَدْرا

صدق اللَّهُ العلي العظيم

DEDICATION

Imam Al-Hussain said:

"Knowledge is twenty-seven letters, all what the messengers brought is two letters, so people did not know until today except the two letters. So, if our standing took out the twenty-five letters, then he spread them among the people, and he added the two letters to them so that they were transmitted by twenty-seven letters".

**To the savior of mankind, to the owner of our era and time Imam Mahdi*

**To my family especially my Mother and my Father*

I dedicate this humble effort.

Zahraa

Acknowledgments

In the name of Allah, most gracious, most merciful. Praise be to Allah, the Lord of the worlds and prayers and peace be upon the most honorable Prophets and messengers Muhammad, my god bless him and upon his family, who enabled and helped me to complete this study.

I would like to express of my deep thanks and gratitude to a several people who had the greatest merit in completing this study.

First of all, I would like to thank my supervisor Asst. prof. Dr. Hana Salman Kadhum for choosing this subject, also I appreciate her guidance, continuous advice and her educational support throughout my study.

I also extend my sincere thanks and appreciation to the Dean of the College of Nursing, Prof. Dr. Abdul-Ameer Abdullah Almousawi, for giving me the opportunity to complete my postgraduate study.

I also thank all the people who encouraged me to apply for and complete my postgraduate study, especially Mrs. Ghassaq Salem, M.Sc. Iman Hadi and M.Sc. Kholoud Abdel-Kareem.

I would like to thank the Dean of the College of Science, Asst. Prof. Dr. Muwafaq F. Al-Shahwan and the Head of Department of Biology, Prof. Dr. Nasir Abd Ali Almansour for offering support to accomplish this research.

Also, I would like to thank the Postgraduate Unit at the College of Science and the Postgraduate Committee of the Department of Biology. Special thanks to Dr. Sabeeh Lafta and Dr. Adnan Almousawi.

I also would like to express my sincere love and deep thanks to the staff of the College of Nursing and the Department of Biology for their kindness and their agreement to participate in this research by donating the samples that necessary to conduct this study.

I also extend thanks and appreciation to Dr. Majed Hameed, the director of endocrinology center at the Al-Mawane Hospital for facilitating collection of samples from diabetes patients.

Also, I wish to express my most loving thanks to all my family, my husband and everyone who supported me and encouraged me to achieve this thesis. Also, I would like to express my endless love and thanks to my little souls, Redha and Zainab for giving me part of their time to complete the study.

Special thanks to my dear friends Sara Kareem and Zainab Nasser. To all other friends who are not mentioned by name, my hearty thanks and gratitude for their support.

Finally, I would like to extend my thanks and pride to all of my classmates for their continued cooperation and useful advice. I had the honor to know you and work with you all.

Zahraa

Summary

Visfatin is an adipokine secreted mainly by visceral adipose tissue and has been linked to obesity. Visfatin has insulin mimetic properties. Visfatin also play an important role in the development of several chronic diseases and inflammation. The study was aimed to evaluate of serum visfatin, adiponectin and leptin concentrations in Basrahian women of different body weights to determine the relationships with obesity and diabetes mellites in women in Basrah city.

In this study, 88 women of different body weights were chosen between October and February, from Basrah University staff and students, and from endocrinology Center affiliated to Al-Mawane Teaching Hospital aged from (25-55). The participants were divided according to their ages into three age groups. The first age group was between 25-35 years, the second age group was between 36-45 years and the third age group was between 46-55 years.

Anthropometric measurements were recorded for all participants, the participants were divided into two main groups according to their BMI. The first group I including 30 lean women with BMI range (18-24.9), the second group including 58 obese women with BMI more than 30, which in turn was divided into two subgroups; group II include 30 healthy obese women and group III which include 28 obese women with Diabetes Mellitus (DM).

Blood samples were collected to assay the biochemical parameters, including the levels of visfatin, adiponectin, leptin, insulin, glucose, Total cholesterol (T.C), Low-density lipoprotein-cholesterol (LDL-C), Triglyceride (T.G), High-density lipoprotein-cholesterol (HDL-C) and Very low-density lipoprotein (VLDL).

The results were showed that diabetic and obese women exhibited significantly higher visfatin (p=0.003) and leptin (p=0.0001) levels compared to lean women. Furthermore, diabetic and obese women showed significant increase of T.C (p=0.024), T.G (p=0.0001) and LDL-C (p=0.005) compared to lean women. However, diabetic and obese women had significantly lower HDL-C (p=0.003) compared to lean women. Whereas, no significant differences of adiponectin and insulin was found between groups.

In conclusion, the results of this study revealed that visfatin levels were increased in diabetic and obese women. This suggests that visfatin levels strongly associated with obesity and diabetes, therefore, it can be used as a marker for diagnosis and treatment of diabetes.

List of Contects

Chapter two: Materials and methods

Chapter three: Results

Chapter four: Discussion

Chapter five: Conclusions and recommendations

List of Figures

Chapter one: Introduction and literatures review

Chapter two: Materials and methods

List of Tables

Chapter two: Materials and methods

Chapter three: Results

List of Appendixes

List of Abbreviations

BMI ……..……..…. Body Mass Index

BAT ……...…… Brown Adipose Tissue

CVD ……..... Cardiovascular Disease

DM ……..……. Diabetes Mellitus

ELISA …… Enzyme-Linked Immune-Sorbent Assay

HDL-C ……... High Density Lipoprotein Cholesterol

HMW ……...…… High-Molecular-Weight

IDF ……...…... The International Diabetes Federation

IL-1β ……...……. Interleukin 1β

IL1Ra …… Interleukin 1Ra

IL-1 …… Interleukin 1

IL-6 ……. Interleukin 6

IL-10 ……. Interleukin 10

IL-12 ……. Interleukin 12

IR ……...…… Insulin Resistance

k-Da ……..…… kilo Dalton

LDL-C …… Low Density Lipoprotein-Cholesterol

LMW …… Low-Molecular-Weight

MMW ……... Middle-Molecular-Weight

NAD …….. Nicotinamide Adenine Dinucleotide

NAMPT ……... Nicotinamide Phosphoribosyl Transferase

NMN …… Nicotinamide Mono-Nucleotide

O.D ……. Optical density

PCOS ……. Polycystic Ovary Syndrome

PBEF ……...……. Pre-B cell Colony Enhancing Factor

PRPP ……..... Phosphoribosyl Pyrophosphate

T.C ……..……... Total Cholesterol

T.G ……...…….. Triglycerides

T1D ……... Type 1 Diabetes

T2D ……... Type 2 Diabetes

TNF-a ……..... Tumor Necrosis Factor-a

WAT …….....….. White Adipose Tissue

WHR ……..... Waist-Hip Ratio

WHO …… The World Health Organization

Chapter one

Introduction and Literatures Review

1. Introduction and Literatures Review

1.1 Introduction

Obesity is chronic medical condition characterized by excessive fat accumulation in body (Blüher, 2020). It is a serious global problem due to its negative effect on wellbeing and its relation to mortality and morbidity (Alnowihi *et al.,* 2020). It is one of the most important factors that lead to many metabolic complications include type 2 diabetes (T2D), insulin resistance (IR) and cardiovascular disease (CVD) (Barazzoni *et al.*, 2018). Obesity is a state when the number of calories provided by food exceeds the body 's intake (Lima *et al.*, 2017). It occurs as a result of excess adipose tissue accumulation on the body lead to the occurrence of hypertrophy and/or adipocyte hyperplasia (Gong *et al.,* 2019).

The spread of obesity globally has risen significantly among developing and, more recently, countries with medium and low income (Mancuso and Bouchard, 2019). The World Health Organization (WHO) reports that in 2016, about 2 billion people were overweight, with 650 million meeting the obesity criterion (Jiménez *et al.*, 2020). In the United States, the Middle East and Europe, in particular, the highest level of obesity is found and the lowest in East Asia and Sub-Saharan Africa (Balistreri *et al.,* 2010).

Obesity is measured by using Body Mass Index (BMI) that determined by dividing the weight of the person in kilograms by their square height in meters, therefore, individuals can be classified into three categories, normal (BMI= 18-24.9 kg / m2), overweight (BMI= 25-29.9 kg / m2) and obese (BMI= 30 kg / m2) (Berthoud and Klein, 2017).

There are many reasons that contribute to the evolution of obesity such as genetic variation, individual and environmental factors. Moreover, the prevalence of obesity is often influenced by racial disparities such as sex, age and race (Hales *et al.,* 2020; Alqarni, 2016).

Furthermore, obesity is a disorder in which there is excess adipose tissue (Mancuso and Bouchard, 2019). The main source of fatty acids (FFA) in the fasting state is adipose tissue which is used for energy use and heat production (Balistreri *et al.,* 2010). The visceral, subcutaneous, perivascular compartments, and bone marrow represent the main adipose tissue depots. In humans there are two types of adipose tissue: brown adipose tissue (BAT) and white adipose tissue (WAT) (de Farias Lelis *et al.,* 2019). Adipose tissue composed of mature adipocytes, preadipocytes and various cell type (Mancuso and Bouchard, 2019). The percentage of adipocytes in lean people does not change after 16 years of age, while in obese people it rises gradually in both volume (hypertrophy) and number (hyperplasia) (Nadulska *et al.*, 2017).

Adipose tissue also recognized as large endocrine and paracrine organ in human body which is secretes hundreds of bioactive molecules called adipokines (Zhang and Sairam, 2014). These molecules are proteins secreted mainly by adipocytes and have role in several function in the body including energy metabolism, glucose homeostasis, inflammation, insulin resistance, immunity, appetite and satiety (Unamuno *et al.,* 2018).

There are two types of adipokines secreted from adipose tissue: pro-inflammatory adipokines such as visfatin, leptin, resistin, Interleukin 6 (IL-6), Tumor necrosis factor-a (TNF-a) and anti-inflammatory adipokines such as vaspin, adiponectin, omentin. The pro-inflammatory adipokines are secreted in large quantities from adipocytes in the obese state in

compared with anti-inflammatory adipokines, which excreted in small quantities (Kong *et al.*, 2019).

Visfatin hormone, the subject of our current research, is one of the important adipokines secreted from adipose tissue. Visfatin was first described by Fokohara in 2005, this hormone is predominantly found in visceral fat of obese mice and humans (Makhoumi *et al.,* 2014). Visfatin has insulin mimic properties, its play an important role in the homeostasis of energy, glucose metabolism and inflammation by regulation the production of some inflammatory cytokines including TNF-a and IL-6 (Yu *et al.*, 2018). Visfatin is also implicated in the pathogenesis of multiple metabolic disorders such as obesity, diabetes mellitus (DM), blood pressure and IR (Zhang *et al.,* 2019).

1.2 The aim of the study

1. Evaluating visfatin levels in obese women and study its relationship with other hormones such as Adiponectin and Leptin.
2. Studying the relationship between visfatin, insulin and diabetes mellitus.
3. Establishing the relationship between visfatin and biochemical parameters.

1.3 literatures review

1.3.1 Obesity

1.3.1.1 Define

Obesity is medical state characterized by abnormal or extreme accumulation of fat in the body that may affect health. Obesity is generally caused by unbalancing between energy intake and energy output. Obesity is the sixth most significant risk factor contributing to the overall disease prevalence worldwide (Gunaid,2012; Seth *et al.*, 2021). It leads to the development of more than 20 serious and chronic diseases, such as hypertension, cardiovascular disease, diabetes, hyperlipidemia, some type of cancers in men and women (Kim *et al.*, 2016; Brinton and Trabert,2018), gall bladder disease, stroke and infertility, therefore, obesity is an important public health concern (Dağ and Dilbaz, 2015).

1.3.1.2 Epidemiology

According to WHO estimates, more than 1.9 billion individuals were overweight over the age of 18 in 2016 and 650 million of adults are obese. Overall, by 2016, approximately 13% of the world's adult population (11% of males and 15% of females) was obese, and about 41 million children under the age of 5 were overweight and obese. In fact, WHO revealed that between 1975 and 2016, the worldwide incidence of obesity nearly tripled (DeOliveira *et al.*, 2019).

In Arab community the prevalence of obesity and overweight has increased dramatically over the past six decades, especially in the Gulf nations (Iraq, Kuwait, Qatar, Saudi Arabia, and Bahrain). Currently, 66%–

75% of adults and 25%–40% of children in the Arab countries are either overweight or obese (DeNicola *et al.*, 2015) figure (1.1). In Iraq, the percentage of people who are overweight is about 60%, and those who suffer from obesity are 24% (Al-Kaseer *et al.*, 2018; Mansour *et al.*, 2012).

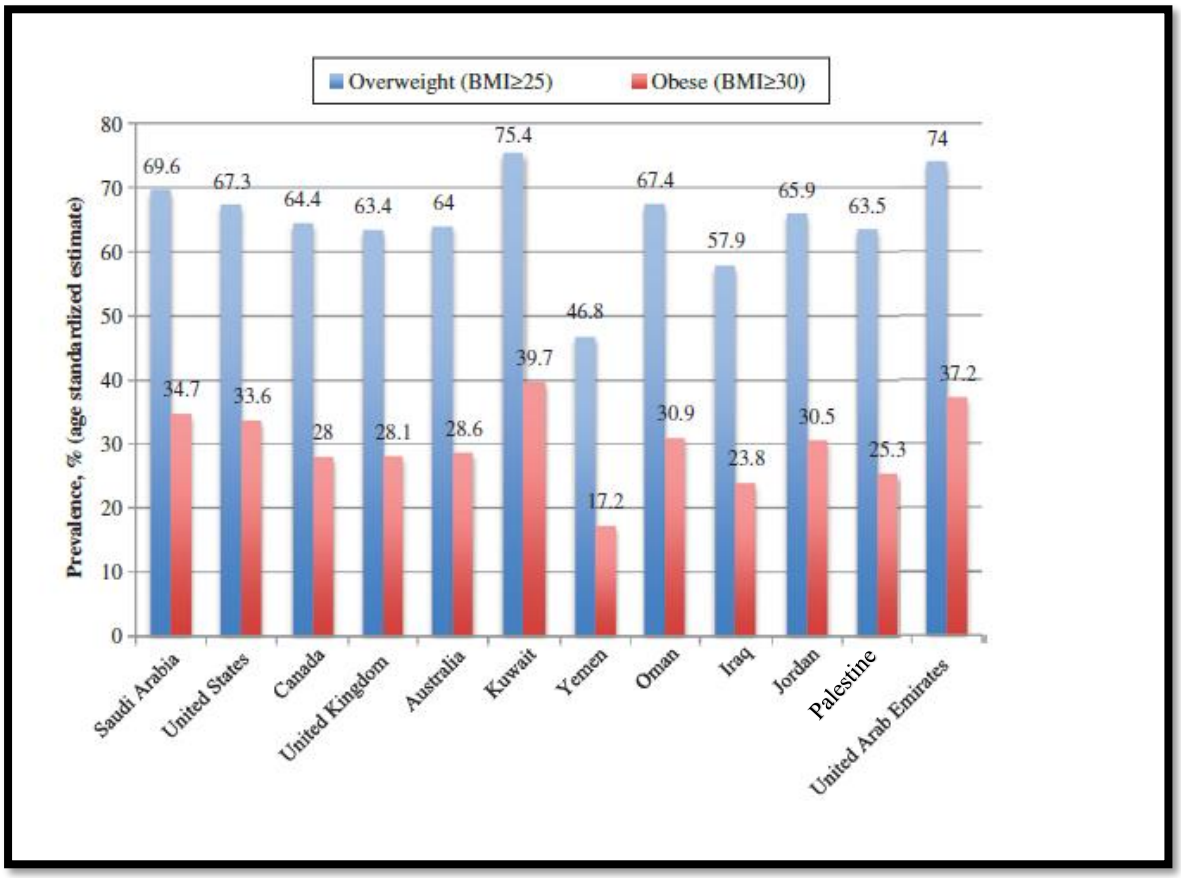


Figure 1.1 The prevalence of obesity in some Arab countries, depending on the body mass index (BMI) (DeNicola *et al.*, 2015).

1.3.1.3 Etiology

• Genetic variation: more than 360 genes are identified that have led to the growth of obesity (Nadulska *et al.,* 2017).

• Individual factors: where the civilization and changing lifestyle in the last 30 years led to appear of two phenomena, the first, increased the intake of processed and high-caloric foods (fast food) as well as reduced fruit, vegetables and fiber consumption, and the second the development of technology and transportation led to decrease of daily physical movement (Walker *et al.*, 2020).

• Environmental factors: long working hours, the role of social networks and peer are considered as environmental factors that increase the prevalence of obesity (Omer, 2020).

• Medical conditions and medications: medical conditions like polycystic ovary syndrome (PCOS) (Behboudi-Gandevani *et al.*, 2017) hypothyroidism, Cushing's disease and certain medications such as anti-depressants, anti-diabetic and anti-hypertensives are contributing to weight gain (Wharton *et al.,* 2018).

• Depression, Anxiety, stress (Gomez-de-Regil *et al.*, 2020).

1.3.1.4 Obesity-related diseases

Obesity has been linked to a higher risk of IR. Adipose tissue regulates metabolism by controlling the release of fatty acids, glycerol, proinflammatory cytokines, immune cells (macrophages and lymphocytes) and hormones like leptin, visfatin and adiponectin. The synthesis of these

molecules is increased in obesity, and this can influence IR in a variety of ways (Shimobayashi *et al.*, 2018).

At the point when IR occur, besides the irregularly function of β-cells, the overall effect of being unable to control the high glucose levels in the blood. As a result, defects in β-cell are very important in the development of T2D and its symptoms (Gupta *et al.*, 2020).

Moreover, obesity has been linked to dyslipidemia. It's characterized by elevated plasma triglycerides (TG), as well as a reduction in high density lipoprotein cholesterol (HDL-C). Given a strong link between dyslipidemia and atherogenesis, alterations in lipid profile caused by obesity play a significant role in increased cardiovascular mortality (Feingold and Grunfeld, 2018).

Furthermore, hypertension raises the risk of heart disease, stroke, and death. Obesity can be used to detect hypertension, since, an increase in arterial pressure has been linked to weight gain, and it is reported that adiposity is responsible for 60-70 percent of adult hypertension (Nurdiantami *et al.*, 2018).

Besides that, obesity and PCOS have been linked together for a long time. Obesity is common in women with PCOS, with up to 80% of them being obese. PCOS is described by a rise in androgen production, which may have an effect on fertility. On the other hand, obesity may be considered as complication of PCOS, given the prevalence of excess visceral fat in the abdomen (Barber *et al.*, 2019).

Additionally, obesity has been linked to the development with at least 13 common cancers such as breast, ovary, endometrial, esophageal, gastric, gallbladder, kidney, colon and rectum, liver, thyroid, and pancreas (Colditz and Peterson, 2018) figure (2.1).

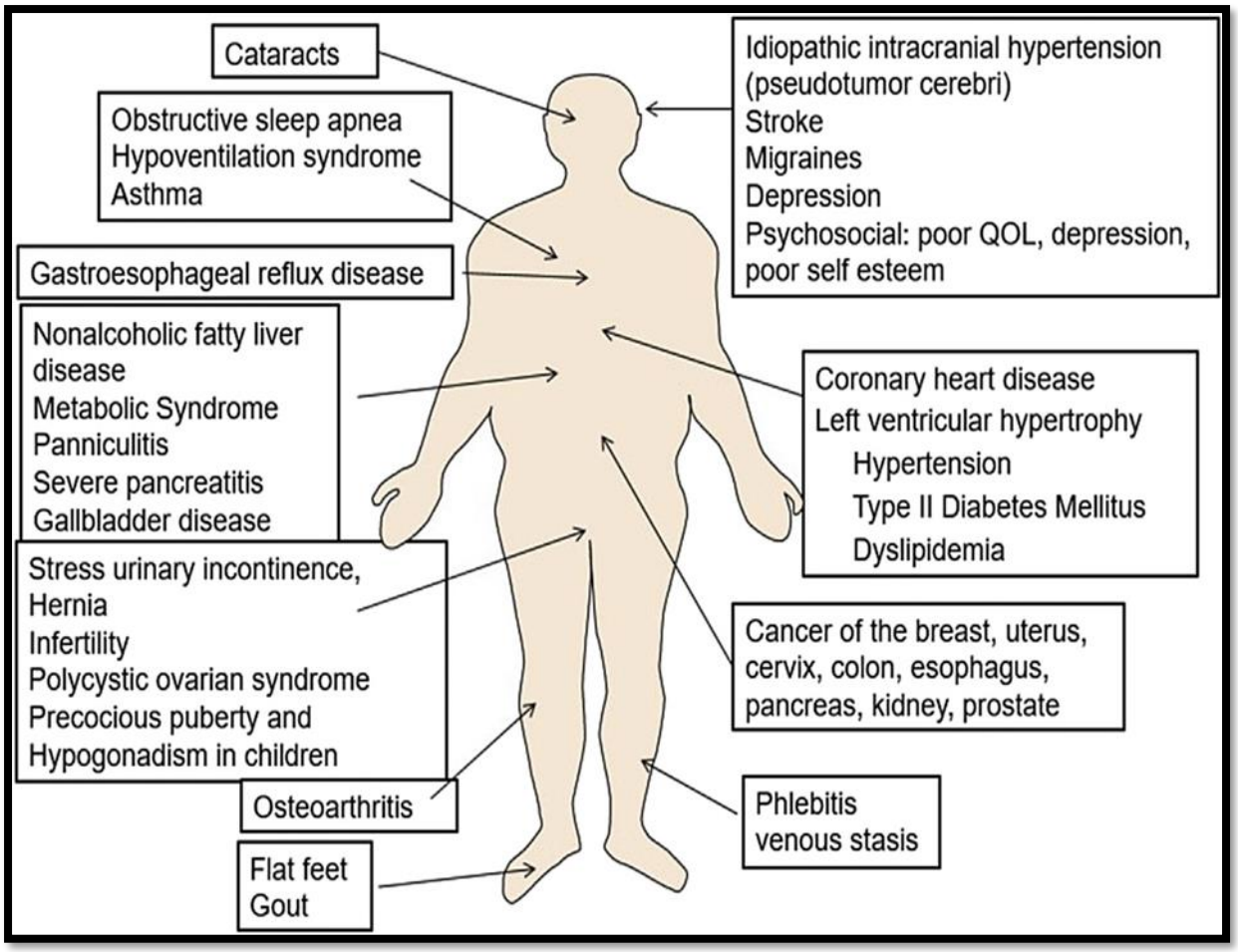


Figure 1.2 Obesity-related comorbidities (Upadhyay *et al.*, 2018).

1.3.1.5 Body Mass Index (BMI) as measurement for obesity

There are several methods that can be used to calculate the degree of obesity such as body mass index (BMI), waist-hip ratio (WHR), skin fold width and fat density, the first person used the body mass index as a measurement was Belgian mathematician in the 19th century (Doustjalali *et al.*, 2020). Despite BMI does not give a marker for fat distribution and fat content in the body but it is considered as one of the most important

anthropometric indexes that used medically to measure of total adiposity (Rathnayake *et al.*, 2020).

BMI is measured by dividing the individual's weight in kilograms to their square height in meters (Peterson *et al.*, 2016). Obesity is described by WHO as a body mass index equal to or greater than 30 Kg/m2 (Argolo *et al.*, 2018). The WHO has classified people based on BMI into several groups, underweight BMI < 18.5, normal weight BMI 18.5-24.9, over wight BMI 25-29.9, obesity BMI 30-39.9 and sever obesity BMI > 40 (Schetz *et al.*, 2019).

1.3.2 Adipose tissue

1.3.2.1 Location and functionality

Adipose tissue is the major unit for lipid storage in our body, which is used for energy production and heat generation to carry out vital activities (Goossens, 2017). Adipose tissue is loose connective tissue composed of preadipocytes, mature adipocytes, macrophage, fibroblasts, lymphocytes mast cells, and endothelial cells (Pereira and Alvarez-Leite, 2014).

According to morphology, location, and function, two major types of adipose tissue are found in mammals: brown adipose tissue (BAT) and white adipose tissue (WAT), they are play opposite roles, WAT is composed of massive spherical adipocytes containing a large amount of lipid droplet, it is acts as a lipid storage that used for energy. Besides, WAT play a role in hormone secretion and immune function (Cheng *et al.*, 2021). In contrast BAT consists of adipocytes with a great number of mitochondria and small amount of lipid, it is responsible for the production of heat (Lagowska and Jeszka, 2011; de Farias Lelis *et al.*, 2019).

Visceral, subcutaneous, bone marrow, and perivascular compartments are the major adipose tissue depots in the body, and depending on adipose tissue distribution there are two types of obesity, central obesity and peripheral obesity which is determined by some factors such as sex, age and race (Ouchi *et al.*, 2011) figure (1.4).

The number of adipocytes is usually limited in lean people (about 1-2 million adipocytes are found in the gram of adipose tissue in human) while people whose weight increases with time, the number of adipocytes will increase in size (hypertrophy) or number (hyperplasia) or both together (Lee *et al.*, 2013; Su and Peng, 2020) figure (1.3).

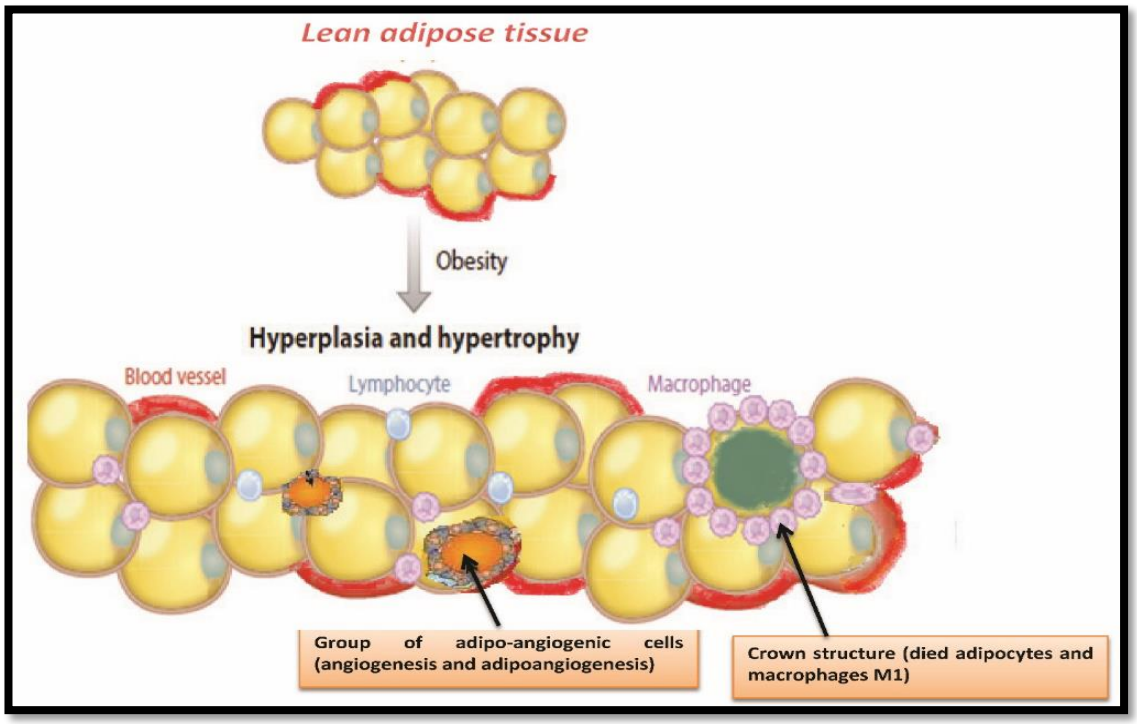


Figure 1.3 The difference between a normal adipocyte and enlargement adipocyte (Divella *et al.*, 2016).

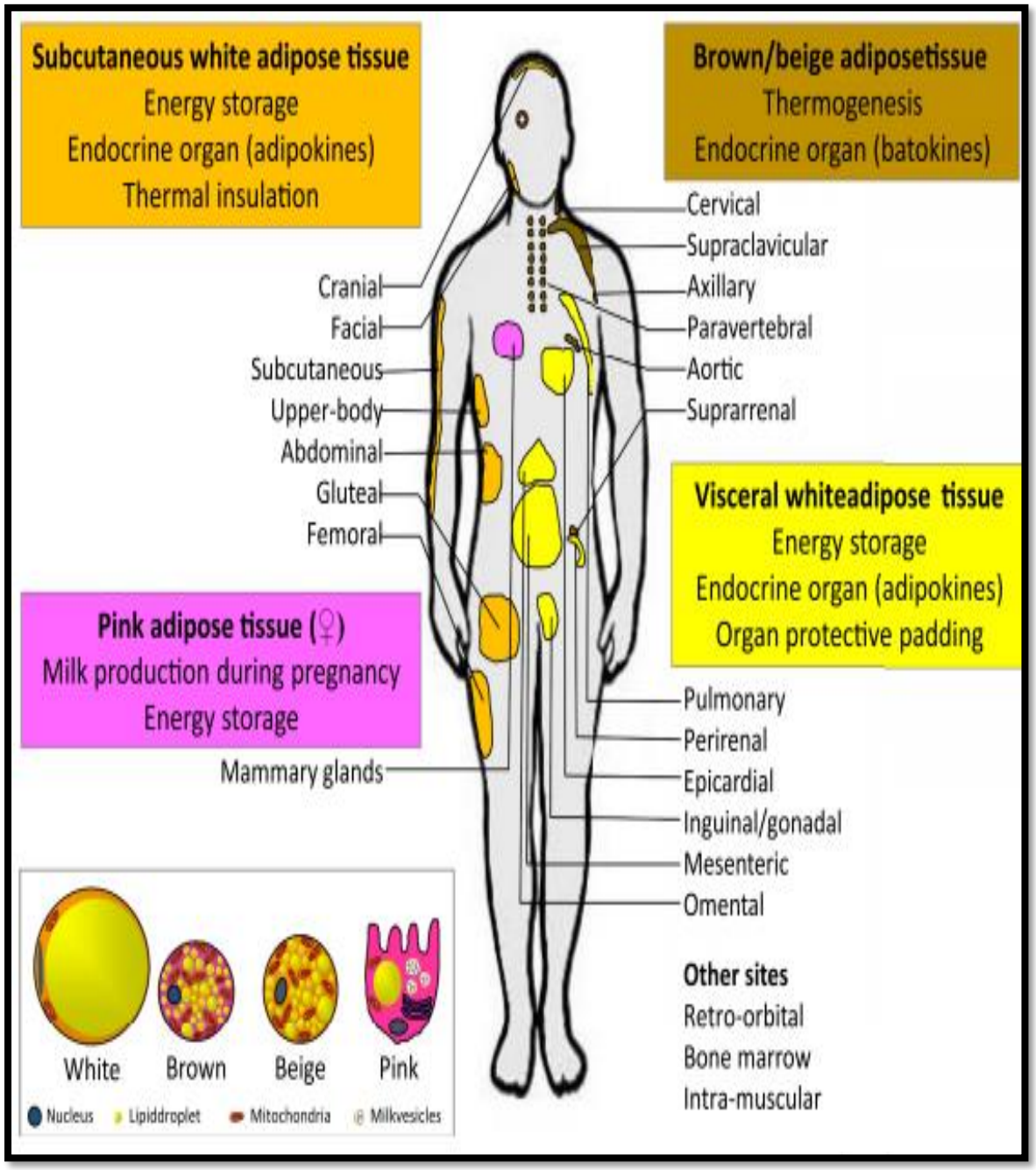


Figure 1.4 The anatomy and function of the major depots of human adipose tissue and the morphology of the various forms of adipocytes (Rodríguez *et al.*, 2020).

1.3.2.2 Adipose tissue as an endocrine organ

As seen earlier, adipose tissue is not only an energy-storing organ, but also an endocrine organ that can secrete a number of hormones and bioactive proteins molecules called adipokines (Torres *et al.,* 2019). Some of adipokines either hormones, cytokines or chemokines, adipocytes are swollen and unstable in the obese state, therefore, the secretion of adipokines will increase in large quantities, which in turn affects many metabolic processes (Pham and Park, 2020).

Adipokines function on various organs, such as the brain, kidney, pancreas, liver, and skeletal muscle to control metabolic homeostasis, adipokines act as network to control glucose and fat metabolism, insulin resistance, appetite, blood pressure, lead to inflammation and the activity of immune cells (Apostolopoulos *et al.*, 2016).

Two types of adipokines are secreted by adipocytes pro-inflammatory and anti-inflammatory adipokines, among adipocytokines, visfatin, leptin, resistin, chemerin, IL-6, IL-12, TNF-a are pro-inflammatory adipokines. Anti-inflammatory adipokines, such as adiponectin, vaspin, omentin and IL-10, play important defensive functions in inflammatory conditions, as opposed to pro-inflammatory adipokines (Ambroszkiewicz *et al.,* 2018) figure (1.5).

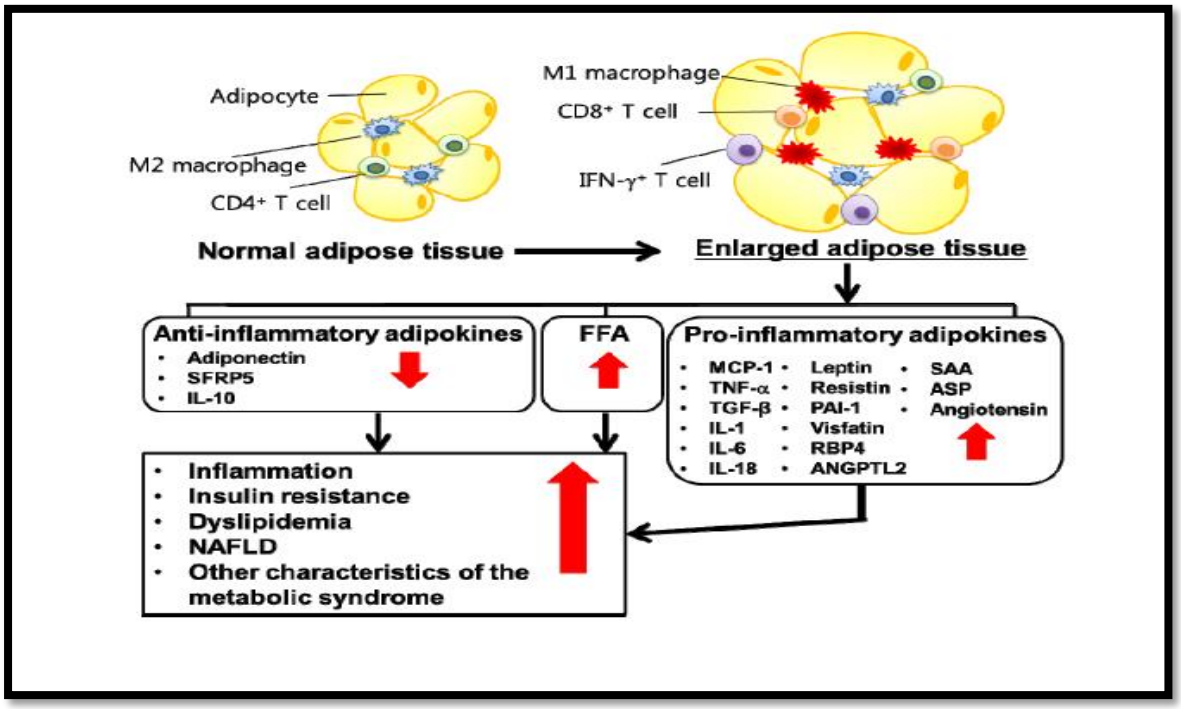


Figure 1.5 Inflammatory adipokine secretion from obese adipose tissue (Jung and Choi, 2014).

1.3.3 Visfatin

1.3.3.1 Discovery of visfatin

Visfatin was first described as cytokine named Pre-B cell Colony Enhancing Factor (PBEF) that secreted from human peripheral blood lymphocytes (Koch *et al*,2018). It also acts as nicotinamide phosphoribosyl transferase (NAMPT), an enzyme transforming nicotinamide to nicotinamide mono-nucleotide (NMN), a substrate of nicotinamide adenine dinucleotide (NAD) (Heo *et al.*, 2019).

In 2005 PBEF was discovered by Fukuhara as visfatin which is expressed in subcutaneous and visceral adipose tissue and they called it visfatin because it's mainly produced by visceral fat (Chang *et al.*, 2011).

In normal weight individuals, visfatin is mainly produced by subcutaneous adipose tissue, in comparison, high level of visfatin is synthesized by visceral adipose tissue in obese subjects (Olszanecka-Glinianowicz *et al.*, 2012). Visfatin also found in the liver, bone marrow skeletal muscles, brain, spleen, lung and kidney (Wu *et al.*, 2018). Visfatin has been found at value range between 10 to 282 ng/mL in human blood (Carbone *et al.*, 2011).

1.3.3.2 Structure of visfatin

Visfatin is a new adipokine hormone has molecular weight of 52 kilo Dalton (kDa), which is active as a dimer, each monomer contains 491 amino acids in humans. There are 19 β-strands and 13 β-helices in each monomer. The gene is found between 7q22.1 and 7q31.33 in humans on the long arm of chromosome 7 (Sonoli *et al.*, 2011) figure (1.6).

```
1   MNPAAEAEFN ILLATDSYKV THYKQYPPNT SKVYSYFECR EKKTENSKLR KVKYEETVFY
61  GLQYILNKYL KGKVVTKEKI QEAKDVYKEH FQDDVFNEKG WNYILEKYDG HLPIEIKAVP
121 EGFVIPRGNV LFTVENTDPE CYWLTNWIET ILVQSWYPIT VATNSREQKK ILAKYLLETS
181 GNLDGLEYKL HDFGYRGVSS QETAGIGASA HLVNFKGTDT VAGLALIKKY YGTKDPVPGY
241 SVPAAEHSTI TAWGKDHEKD AFEHIVTQFS SVPVSVVSDS YDIYNACEKI WGEDLRHLIV
301 SRSTQAPLII RPDSGNPLDT VLKVLEILGK KFPVTENSKG YKLLPPYLRV IQGDGVDINT
361 LQEIVEGMKQ KMWSIENIAF GSGGGLLQKL TRDLLNCSFK CSYVVTNGLG INVFKDPVAD
421 PNKRSKKGRL SLHRTPAGNF VTLEEGKGDL EEYGQDLLHT VFKNGKVTKS YSFDEIRKNA
481 QLNIELEAAH H
```

Figure 1.6 Amino acid sequence of visfatin human with 491 amino acids (Adeghate, 2008).

Visfatin has crystal structure, the crystalline phase of visfatin/PBEF/Nampt was identified first by Wang and Kim with regard to its enzymatic role in synthesizing NMN from nicotinamide and phosphoribosyl pyrophosphate (PRPP) (Sommer *et al.,* 2008).

The visfatin dimer exists in an asymmetric unit of the crystal, during column chromatography in solution it seems as a dimer. The two subunits are connected and they are very close to each other by a non-crystallographic 2-fold pseudo-symmetry axis. The structure suggests that dimerization is necessary for visfatin's catalytic activity, since, both subunits contribute to the NAMPT active site (Kim *et al.,* 2006) figure (1.7).

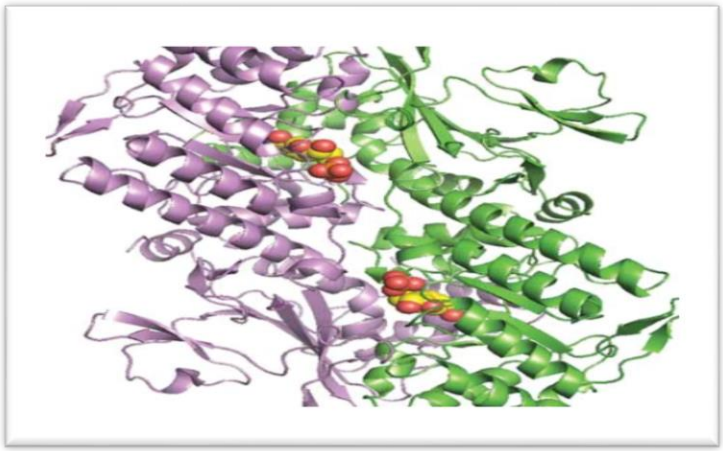


Figure 1.7 The crystal structure of mouse Nampt bond to NMN, monomers appeared in violet or green and the carbons substrate in yellow (Revollo *et al.,* 2007).

1.3.3.3 Functions of visfatin

In addition to adipocytes, visfatin is present in monocytes, lymphocytes, neutrophils. Macrophages also reported as a major source of visfatin, it has been observed that during inflammatory diseases and obesity, visfatin may play an important role in innate immunity (Thomas and Apovian, 2017). Visfatin involved in regulation the production of pro and anti-inflammatory cytokines such as IL-6, IL-1β, IL1Ra, IL-10 and TNF-a in human monocytes (Koka *et al.*, 2019).

Moreover, visfatin has characterized by insulin-mimic activity, it is bind to the same receptors that the insulin hormone binds to on cells but from other locations, therefore, it plays an important role in the balancing of glucose levels in blood and thereby performs an auxiliary function for insulin, especially in people with T2D (Rashad *et al.*, 2018). Insulin mimetic effect was observed through an experiment on cultured cells, in which visfatin decreased the level of plasma glucose in mice, while in another experiment in mice heterozygous with the mutant visfatin gene, higher plasma glucose concentrations was observed compared to wild-type individuals (Hug and Lodish, 2005).

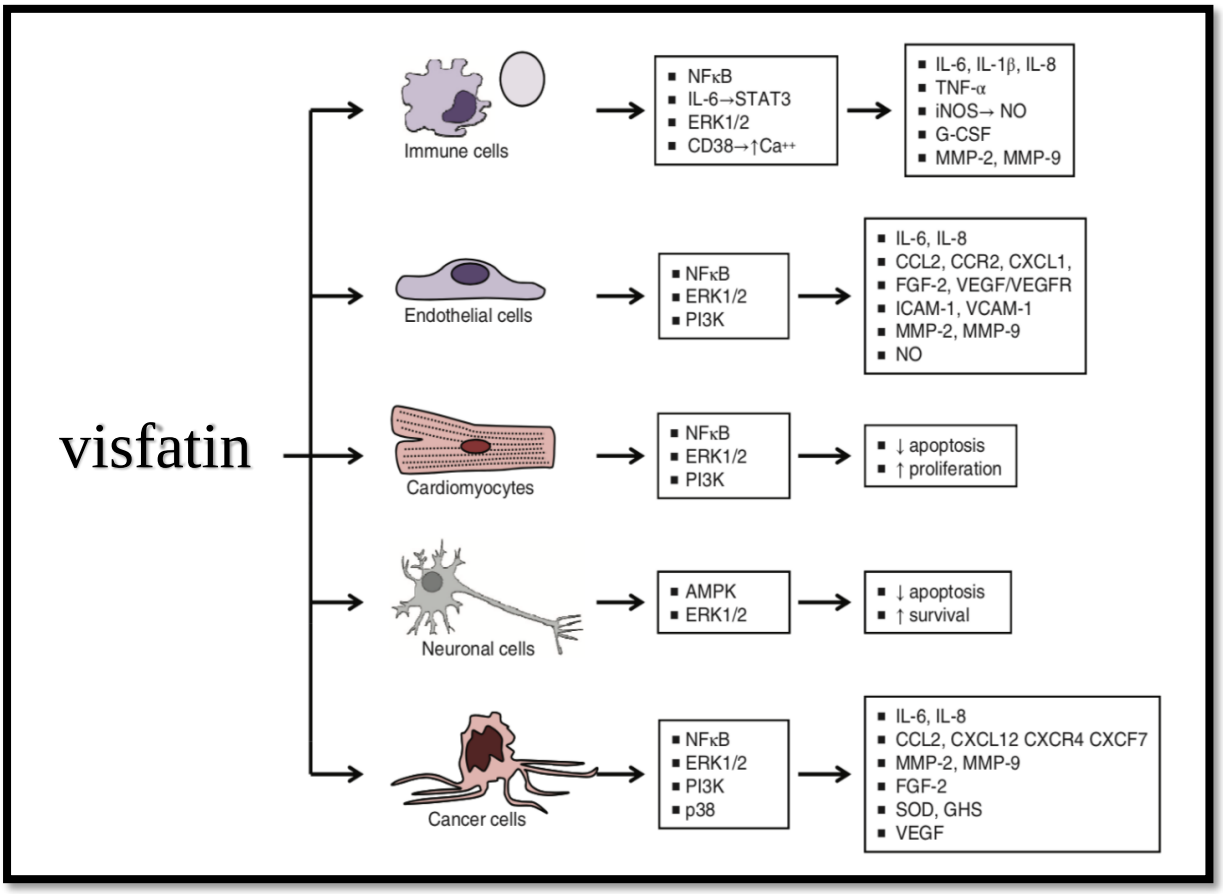


Figure 1.8 The effect of Visfatin hormone on different cell types (Zamora, 2019).

1.3.3.4 Visfatin and obesity

The excess of adipose tissue in obese patients is responsible for the secretion of high amounts of visfatin. It is noticed that plasma visfatin is higher in overweight and obese individuals (Bayani *et al.*, 2017). In the other hand several studies have proven that aerobic exercise and diet has a beneficial effect on visfatin concentrations and BMI variables involved in the obesity inflammatory process (Ghanbarzadeh and Omidi, 2017; Mir and Fathi, 2018; Moravveji *et al.*, 2019).

1.3.3.5 Visfatin and lipid profile

Relatively several studies demonstrate the positive relationship between plasma visfatin, HDL-C and TG in both males and females, with the exception of some studies in which the positive association between plasma visfatin, HDL-C and TG was found only in females, and in addition to this, negative effects of plasma visfatin on low density lipoprotein-cholesterol (LDL-C) were recorded (Stastny *et al.*, 2012).

1.3.3.6 Visfatin and diabetes

In recent years a significant increase in the prevalence of diabetes have been seen in almost all countries of the world, about 415 million persons living with diabetes worldwide. This is very important because the rising in the prevalence of diabetes would increase the number of acute and chronic complications in the overall population, with a significant impact on the quality of living, need for health care and the expense of the economy (Harding *et al.*, 2019). Diabetes mellitus (DM) is a chronic disease identified as a collection of metabolic disorders characterized by elevated blood glucose level, polyuria, polydipsia and polyphagia (Bascones-Martínez *et al.*, 2011; Ogurtsova *et al.*, 2017).

There are two main types of DM, the most common class of DM is type 2 diabetes (T2D) which is recognized by hyperglycemia, insulin resistance, and insulin deficiency, its generally known as non-insulin dependent DM. Interaction between genetic, environmental and behavioral factors results in T2D (Olokoba *et al.*, 2012).

Obesity is considered as one of the major risk factors for the development of T2D, it has been reported that approximately 90 % of T2D are overweight or obese (Urbanavičius *et al.*, 2013; Niu *et al.*, 2016).

Therefore, obesity and diabetes have been recognized by WHO as epidemic diseases of the 21st century (Kocot *et al.*, 2017).

The correlation between obesity and T2D has been recognized for long time, and the major explanation is that obesity is capable of inducing IR, the main pathophysiological feature of T2D (Zatterale *et al.*, 2020). IR is a physiological complication in which insulin activity becomes less receptive to the three main insulin-sensitive tissues (skeletal muscle, the liver, and adipose tissue). IR is characterized by severe glucose uptake failures and glycogen synthesis (Czech, 2017; Ormazabal *et al.*, 2018).

The other type of diabetes is known as type 1 diabetes mellitus (T1D), it's also referred to as autoimmune diabetes or insulin dependent DM, is a chronic disorder characterized by insulin deficiency which is caused due to pancreatic β-cell depletion and contributes to hyperglycemia. This disease is more common in children and adolescences than adults. The International Diabetes Federation (IDF) estimate that about 8.8 percent of the total population worldwide have diabetes, just 10-15 percent of all people have T1D (Atkinson *et al.*, 2014; Katsarou *et al.*, 2017).

1.3.4 Adiponectin

Adiponectin was discovered first time in 1995, as adipocyte complement related protein (Acrp30) with molecular weight of 30-kDa, another study identified adiponectin of mouse and referred to it as AdipoQ which is consists of 247 amino acids polypeptide. In 1996 adiponectin isolated from human plasma as small protein consist of 224 amino acids (Parida *et al.*, 2019), and it is found in lean subjects at circulating levels between 5 to 30 mg/L (Kumari *et al.*, 2019).

Two types of adiponectin receptors have been identified (AdipoR1 and AdipoR2) in human and mice, AdipoR1 is located at chromosomes

1p36.13-q41 and 1 E4, while AdipoR2 is located at chromosome 12p13.31 and 6 F1, respectively (Yamauchi *et al.*, 2014).

Furthermore, adiponectin can be found in three different oligomeric forms in plasma, low-molecular-weight (LMW), middle-molecular-weight (MMW) and high-molecular-weight (HMW) adiponectin (Wang and Scherer, 2016) figure (1.9).

Generally, adiponectin is an adipocyte derived hormone produced mainly by the adipose tissue, it also produced from different tissues including human osteoblasts, liver parenchyma cells, epithelial cells, placental tissue and myocytes (Achari and Jain, 2017).

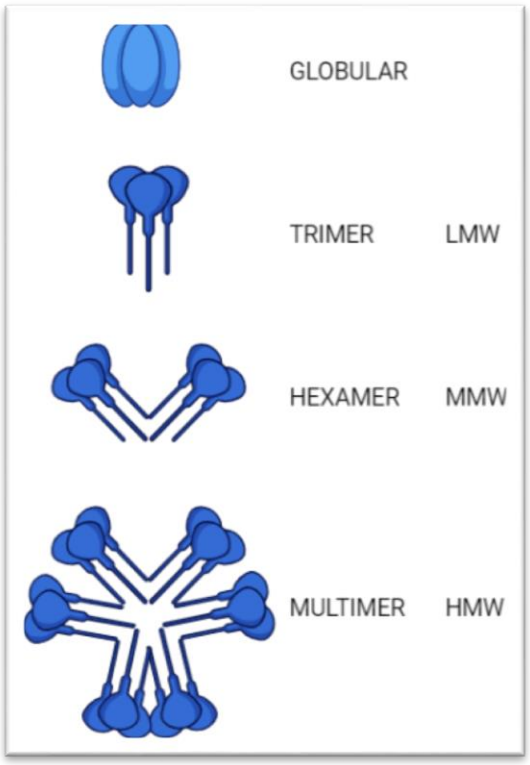


Figure 1.9 The structure of adiponectin in three different forms (Khoramipou *et al.*, 2021).

Adiponectin exerts its effect on target organs include kidney, liver, muscle, pancreas, blood vessels, bone, brain, adipose tissue and immune cells (Wang and Scherer,2016). Due to its beneficial effect on metabolic homeostasis, adiponectin has gained interest. This beneficial effect of adiponectin is due to its anti-inflammatory properties, since, adiponectin reduced the production of IL-6 and TNF-a as well as induced IL-10 and IL-1 antagonist (Vicente *et al.*, 2017).

Moreover, adiponectin increases insulin sensitivity, maintains glucose homeostasis, increases energy expenditure and improves fatty acid oxidation. Adiponectin also appearing to increase HDL-C in blood level and decrease TG (Yanai and Yoshida, 2019). Furthermore, it has been found that adiponectin decreased the dangers of metabolic syndrome, chronic kidney disorders, inflammations, atherosclerosis, some cancers and diabetic retinopathy (Yu *et al.*, 2020) figure (1.10).

Recent studies on obesity suggested that declining in the plasma level of adiponectin contributes to the increase in obesity-related diseases. Mouse models showed a high expression of adiponectin, which in turn led to increase health and longevity (Donoyama *et al.*, 2018; Larsen *et al.*, 2019; Askarpour *et al.*, 2020).

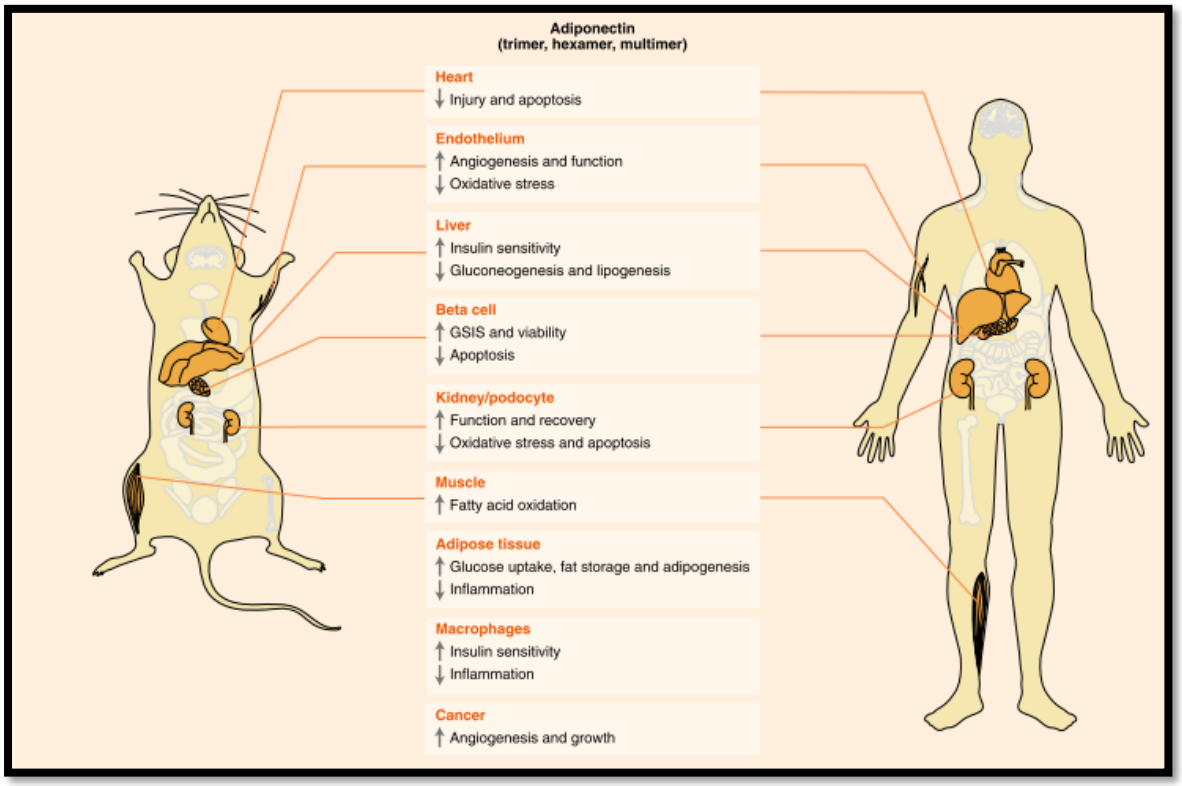


Figure 1.10 The target tissues and adiponectin biological activity (Straub and Scherer, 2019).

1.3.5 Leptin

One of the first adipocytokines described was leptin, it was discovered in 1994, Leptin is a non-glycosylated protein consist of 167 amino acids with 16-kDa, *ob/Lep* gene responsible for the secretion of leptin from WAT so that, its release in direct correlation to the mass of adipose tissue and food consumption, therefore, it is also known as the hormone of appetite (Ayman *et al.*, 2019; Amjad *et al.*, 2019).

Leptin is classically linked to controlling of energy homeostasis and food intake. However, many neuro-physiological functions also have been attributed to leptin, such as brain growth, neurogenesis, neural protection, stress and mood regulation, reproduction and reproductive (Forny-Germano *et al.*, 2019).

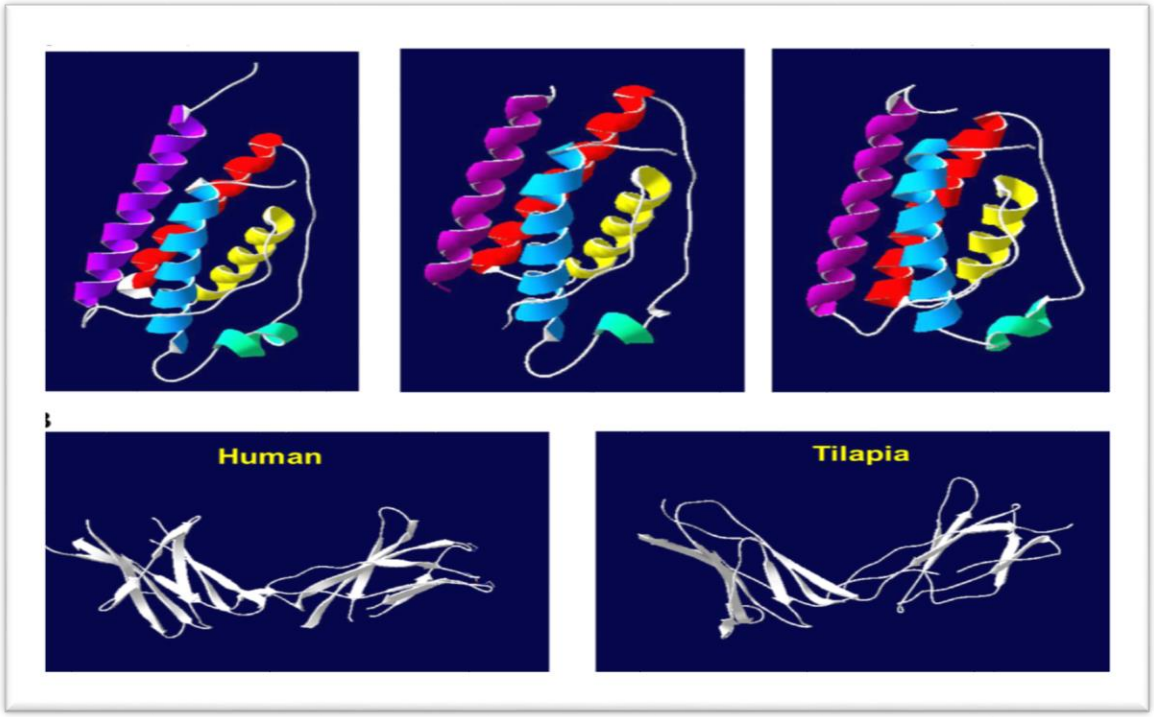


Figure 1.11 The three-dimensional arrangement of fish and human leptins. (a) the leptin-binding site of the leptin receptor (b) The secondary and tertiary structure of the protein (Shpilman *et al.*, 2014).

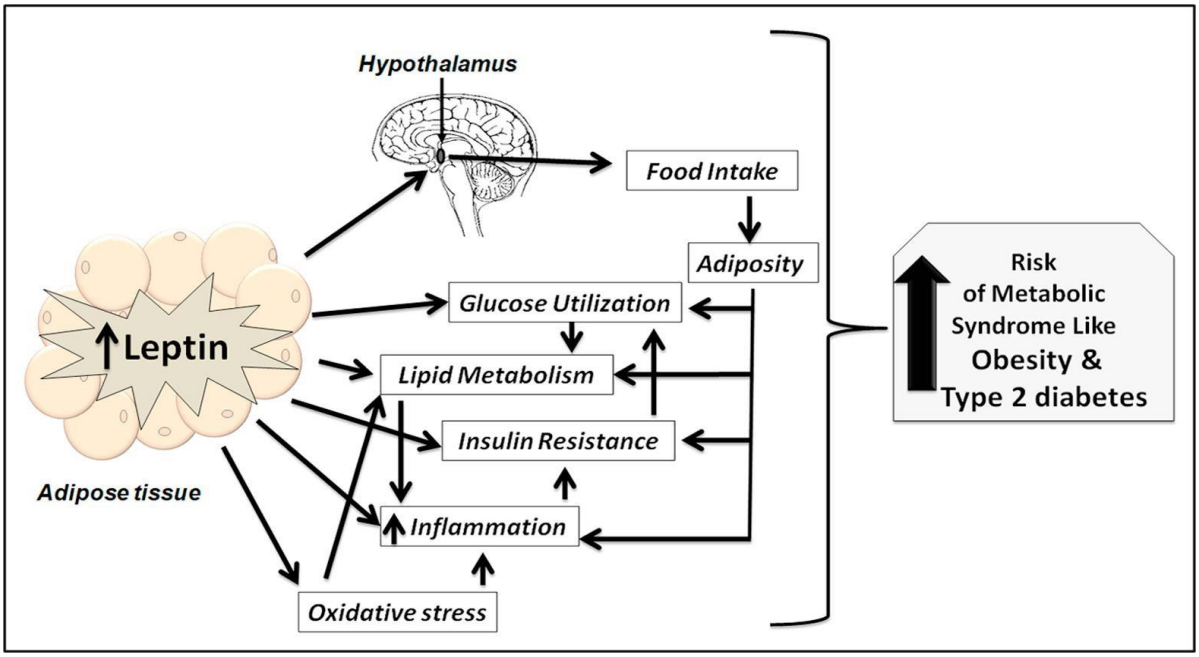


Figure 1.12 Leptin target organ and its effects (Ghadge and Khaire, 2019).

Moreover, leptin is pro-inflammatory adipokines, it is essential in cell-mediated immunity and cytokine interaction. It is structurally similar to the family of interleukins and reacts with other cytokines (Alipoor *et al.*, 2018).

However, several researches have demonstrated the association between leptin and metabolic syndrome, including hypertension, insulin resistance, visceral obesity and dyslipidemia. In general, the highest level of leptin was observed in female more than in male and in obese women more than lean women. (Duan *et al.*, 2020; Ali *et al.*, 2020; Turki *et al.*, 2020; Ahmed *et al.*, 2016). While other experimental studies have shown a decline in leptin levels and an increase in its sensitivity over a span of not less than two weeks during exercise (Fedewa *et al.*, 2018).

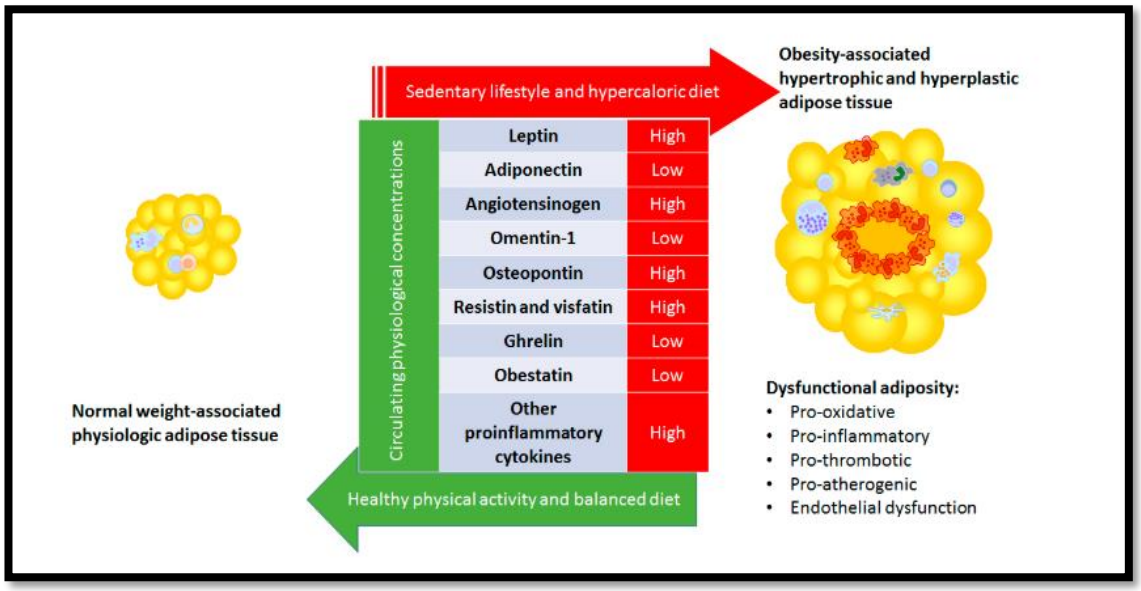


Figure 1.13 Major changes in adipokines concentrations that secreted from adipose tissue (Landecho *et al.*, 2019).

1.3.6 Insulin

Insulin is a vital polypeptide hormone that secreted entirely from pancreatic β-cells, it is the main glucose balance regulator in blood, which is decreases plasma glucose concentrations after the meal by rising glucose uptake and utilization from peripheral tissues, in addition, insulin regulates carbohydrate, lipid, and protein metabolism while also promoting cell division and growth (Kim and Park, 2017).

Human insulin is made up of 51 amino acids and has a molecular weight of 5.808 k-Da. Insulin gene is responsible for human insulin expression and it is present on chromosome 11p15.5 (Vakilian *et al.,* 2019). The actions of the insulin are beginning by binding to it is receptor on target cells surface. The receptor is an α2β2 heterodimer that binding to insulin with strong affinity, leads to altered in the permeability of plasma membrane of the cells, which is then leads to an increase in the entry of glucose into the cells (Saltiel, 2021).

In the case of obesity and T2D, insulin resistance was observed, it is described as a reduction in a target cell's metabolic reaction to insulin or a reduced effect of circulating insulin on blood glucose levels (Petersen and Shulman, 2018).

In contrast, the people without diabetes, prandial insulin makes up 50% of the total daily pancreatic output. Most of prandial insulin is released during the first hour after a meal. According to the recommendation of IDF, blood glucose levels should not exceed 8 mmol/l 2-hour after meal. While in people with either T1D or T2D diabetes treated with several daily treatments, insulin is supplied with meals to support mealtime glucose excursions, preferably 30 minutes before a meal (Slattery *et al.,* 2018).

Chapter two
Materials and methods

2.1 Materials

2.1.1 Chemicals

The chemicals were used in the current study illustrate in table 2.1 below:

Table 2.1 The chemicals used in this study and their sources.

Chemicals	Catalogue no.	Company	Country
Human Visfatin Elisa kit	RDEEH0651	MyBioSource	USA
Human Adiponectin Elisa Kit	RDEEH2593	MyBioSource	USA
Human Insulin Elisa kit	ME E-0900	LDN	Germany
Human Leptin Elisa kit	ME E-0300	LDN	Germany
Total cholesterol kit	0005168538190	Roche	Germany
Triglyceride kit	0008058687190	Roche	Germany
HDL-C kit	07529031001	Roche	Germany
LDL-C kit	0107005717190	Roche	Germany
Glucose kit	04773365001	Roche	Germany

2.1.2 Apparatus and instruments

The following laboratory apparatus and instruments were used in this study as shown in table 2.2 below:

Table 2.2 The laboratory apparatus and instruments were used in the study.

Apparatus	Company	Country
Digital body scale	DMK Kolding	China
Measuring tape	Ingco	China
Centrifuge	Gemmyco	Taiwan
Deepfreeze	Vistel	Korea
Elisa reader	Human	Germany
Incubator	Fisher scientific	USA
Micropipette (10-100 µl)	Human	Germany
Plate shaker	Fisher scientific	USA
Cobas Integra 400 plus	Roche	Germany
Serum separation tube (gel tube)	Song Bong	China
Eppendorf tube	Song Bong	China

2.2 Methods

2.2.1 Study population

In this study, a total of 88 Iraqi women samples aged from 25 - 55 years were collected from Basrah University's staff and students, and from endocrinology Center affiliated to Al-Mawane Teaching Hospital during the period from October 2020 to February 2021. A brief explanation of the project was explained to the participants before sample collecting. Written informed consent has been obtained from all participants before their inclusion. The medical histories of the study population and some required data such age and geographical area were obtained by direct interview with women by using a questionnaire (Appendix 1).

2.2.2 Study design

The participants were divided according to their ages into three age groups. The first age group was between 25-35 years, the second age group was between 36-45 years and the third age group was between 46-55 years, each age group include 10 women. Also, the participants were divided into two main groups according to their BMI. The first group I including 30 lean women with BMI range (18-24.9), the second group including 58 obese women with BMI more than 30, which in turn was divided into two subgroups; group II include 30 healthy obese women and group III which include 28 obese women with DM.

The exclusion criteria for healthy subject (group I and group II) were the presence of any chronic diseases, endocrine diseases, treatment with any medication, pregnancy, and irregular menstrual cycle. While the exclusion criteria for group III were the presence of any chronic diseases except DM,

treatment with any medication except medication of DM, pregnancy, and irregular menstrual cycle.

2.2.3 Anthropometric measurements

Anthropometric measurements, including body weight, height, and waist and hip circumferences, were measured. Weights and heights were recorded without shoes and heavy clothing. Body weight was taken to the nearest 0.1 kg by using digital bod

y scale and height was taken to the nearest 0.1 cm by measuring tape. Waist circumference was measured midway between the costal margins and the iliac crest, and the hip circumference was measured around the widest part of the buttocks. BMI values were calculated by dividing the person's weight in kilograms to height in meters square, and the waist-to-hip ratio (WHR) was calculated by dividing the waist circumference to the hip circumference in centimeters.

2.2.4 Serum preparation

Five ml of venous blood was collected in the morning between 8:00 and 10:00 after an overnight fasting, and placed in sterilized serum separation tube (gel tube). Leave it for a period (about 10 minutes) until the clot formation is occurred. After clot formation, the samples were placed in centrifuge (3500 rpm for 10 minutes at room temperature) to obtain the serum. The serum obtained were withdraw and placed in Eppendorf safe-lock tubes (1ml) which used for dividing the samples before storage in deepfreeze at (-20°) until the time of assay (Alnowihi *et al.*, 2020).

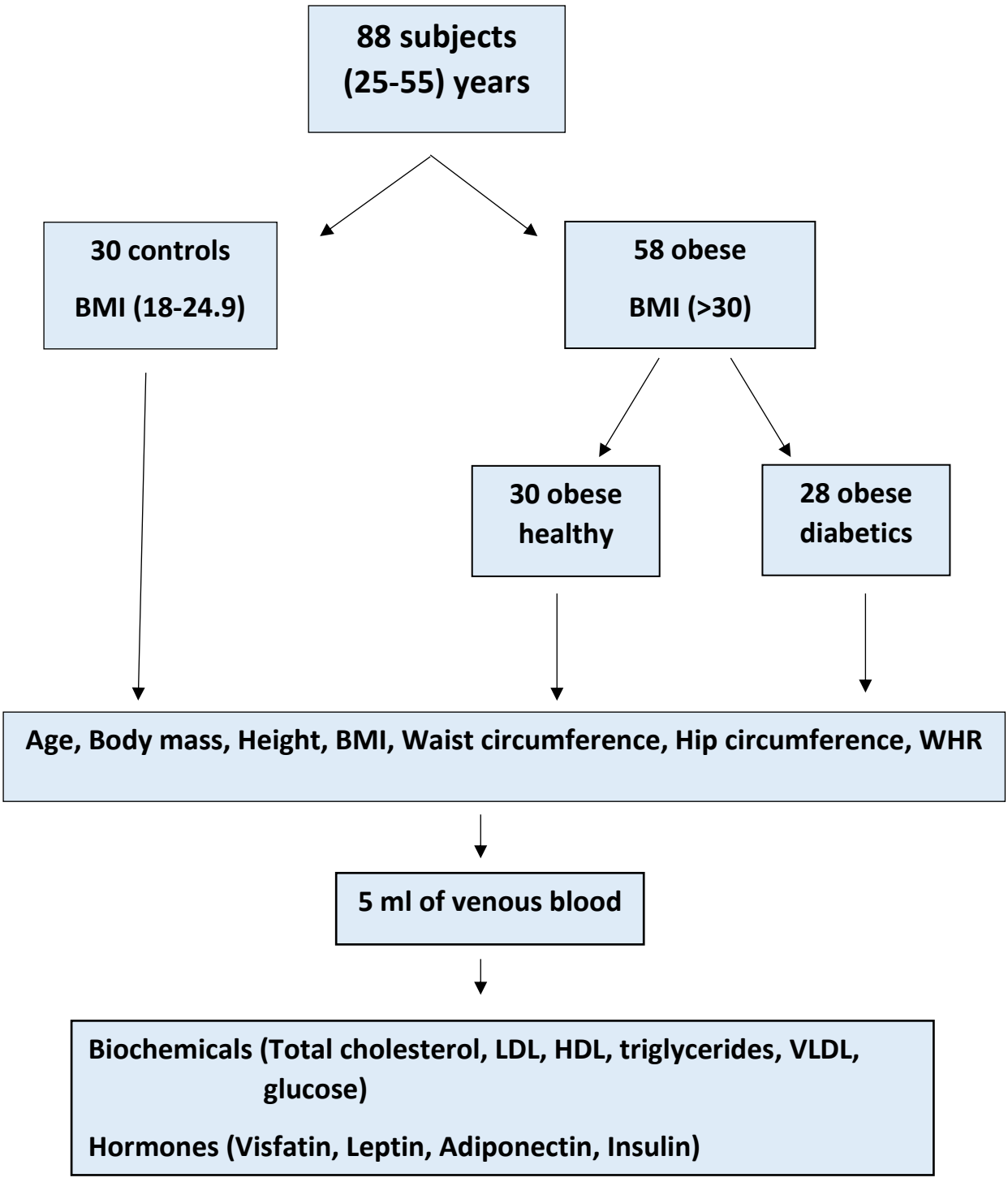


Figure 2.1 The study design

Figure 2.2. Centrifuge that used to separated blood samples.

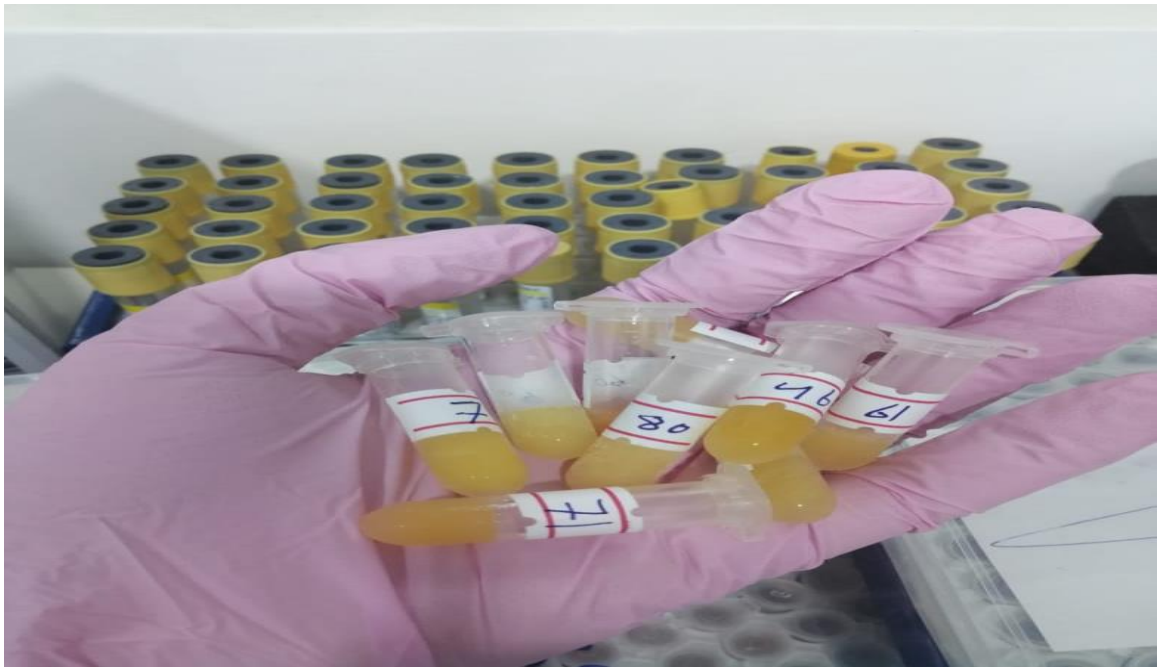


Figure 2.3 The serum placed in Eppendorf tubes before storage in deepfreeze.

2.3 Biochemical analysis and procedures

2.3.1 Visfatin concentration

Kit Components

Item	Specifications(48T/96T)	Storage
ELISA Microplate (Dismountable)	8×6 /8×12	4°C/-20°C
Lyophilized Standard	1 vial/2 vial	4°C/-20°C
Sample / Standard Dilution Buffer	10ml/20ml	4°C
Biotin-labeled Antibody (Concentrated)	60ul/120ul	4°C
Antibody Dilution Buffer	5ml/10ml	4°C
HRP-Streptavidin Conjugate (SABC)	60ul/120ul	4°C (shading light)
SABC Dilution Buffer	5ml/10ml	4°C
TMB Substrate	5ml/10ml	4°C (shading light)
Stop Solution	5ml/10ml	4°C
Wash Buffer (25X)	15ml/30ml	4°C
Plate Sealer	3/5pieces	
Product Description	1 copy	

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology (ELISA). Anti-visfatin antibody was pre-coated onto 96-well plates. The biotin conjugated anti-visfatin antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the visfatin amount of sample captured in plate. Read the optical density (O.D) absorbance at 450nm in a microplate reader, and then the concentration of visfatin can be calculated.

Reagent Preparation

The kit put at room temperature for 20 minutes before use.

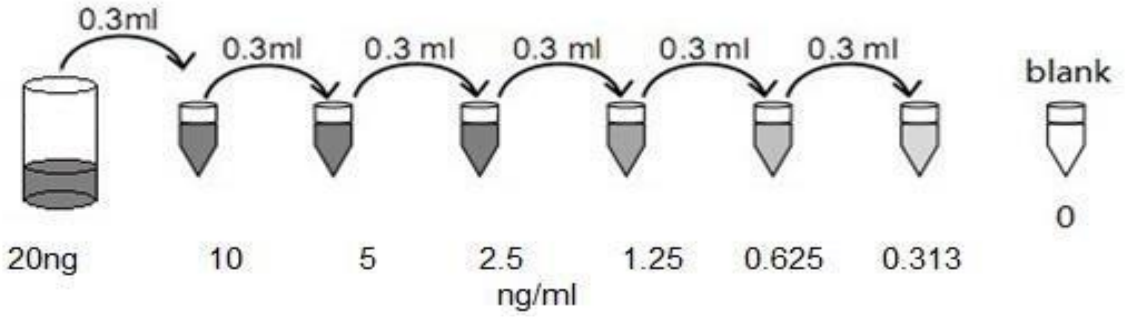
1. Wash Buffer:

30mL of Concentrated Wash Buffer diluted into 750 mL Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

2. Standard:

1) 20ng/ml of standard solution: 1 ml of sample / standard dilution buffer was added to one Standard tube, keep the tube at room temperature for 10 minutes and mix them thoroughly.

2) 10ng/ml→0.313ng/ml of standard solutions: Label 6 Eppendorf tubes with 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.313ng/ml, respectively. Add 0.3 ml of the Sample/Standard dilution buffer into each tube. Add 0.3 ml of the above 20ng/ml standard solution into 1st tube and mix them thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix them thoroughly, and so on.



3. Preparation of Biotin-labeled Antibody Working Solution

Prepare it within 1 hour before experiment.

1) Calculating required total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2) Diluting the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e., Add 1μl Biotin-labeled antibody into 99μl Antibody Dilution Buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

It was prepared within 30 minutes before experiment.

1) Calculating required total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2) Diluting the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e., Add 1µl of SABC into 99µl of SABC Dilution Buffer.)

5. Sample preparation

The sample was diluted with the provided dilution buffer. Dilution: 1:10. (i.e. Adding 10µl of sample into 90µl of Sample/Standard Dilution Buffer). The test sample must be well mixed with the dilution buffer.

Assay Procedure

Before adding reagents into wells, equilibrate TMB Substrate for 30 min at 37 °C.

1. The plate was washed 2 times before adding standard, sample and control (zero) wells.

2. Aliquot 0.1ml of 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.313ng/ml, standard solutions into the standard wells.

3. A 0.1 ml of Sample/Standard Dilution Buffer was added to the control (zero) well.

4. A 0.1 ml of properly diluted sample (serum) was added into test sample wells.

5. The plate was sealed with a cover and incubated at 37 °C for 90 minutes.

6. The cover was removed and discard the plate content, and plate washed 2 times with Wash Buffer.

7. A 0.1 ml of Biotin-labeled antibody working solution was added into above wells (standard, test sample and zero wells).

8. The plate sealed with a cover and incubated at 37°C for 60 min.

9. The cover removed and plate washed 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time.

10. A 0.1 ml of SABC Working Solution was added into each well, cover the plate and incubate at 37°C for 30 minutes.

11. The cover removed and plate washed 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minute each time.

12. 90µl of TMB Substrate was added into each well, cover the plate and incubate at 37°C in dark within 15-30 minutes. It is turn blue in the first 3-4 wells (with most concentrated visfatin standard solutions), the other wells may not display obvious color.

13. 50µl of Stop Solution was added into each well and mix them thoroughly. The color changes to yellow immediately.

14. The O.D. absorbance was read at 450 nm in Microplate Reader immediately after adding the stop solution.

All data were calculated according to a standard curve in comparison to an optical density of treated samples to evaluate the concentration, and expressed as (ng/ml). (Appendix 2).

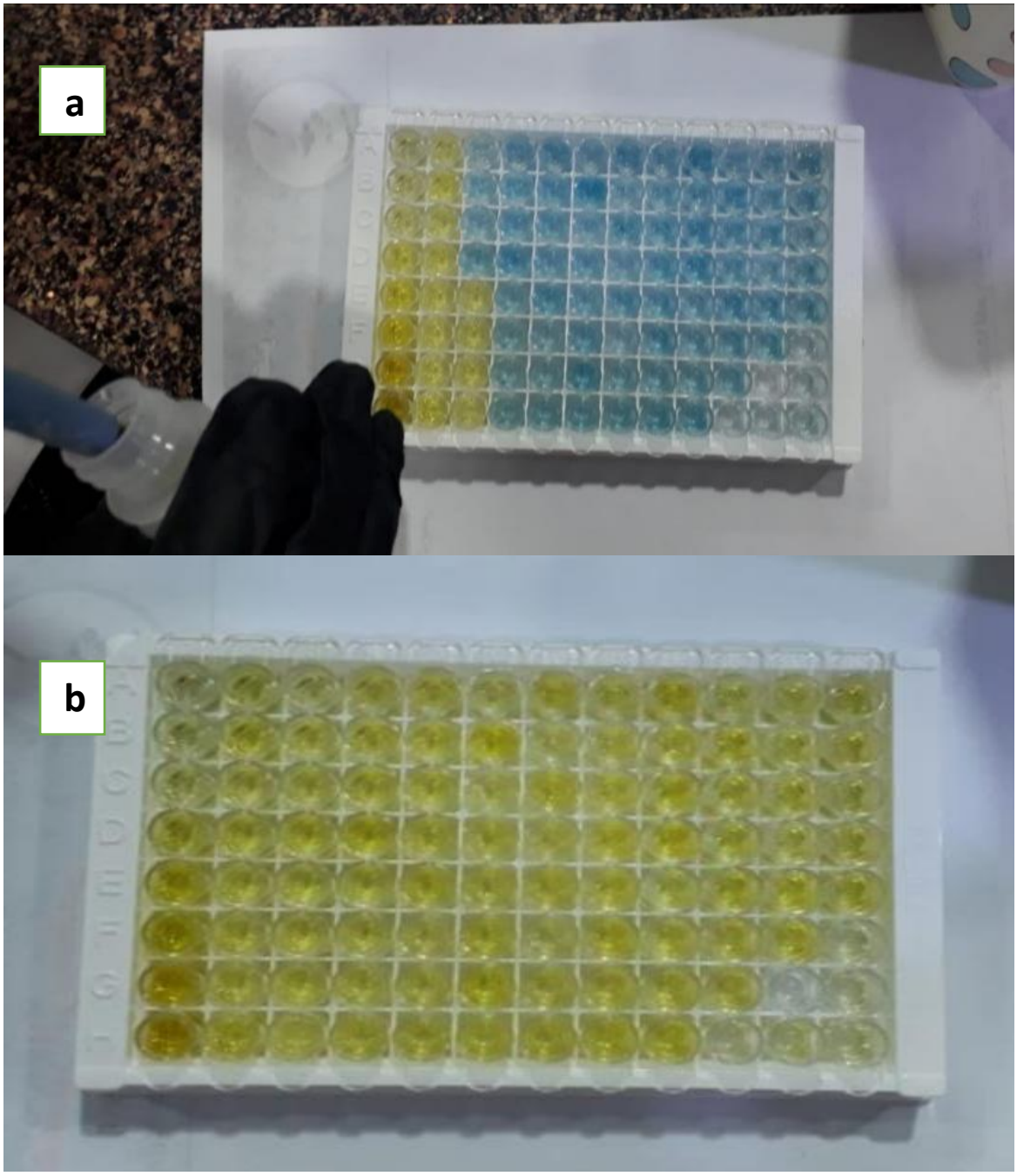


Figure 2.4 Plate of visfatin ELISA kit. a: adding stop solution to substrate and turned of color blue to yellow. b: after adding stop solution to all wells and turned entire color to yellow.

2.3.2 Adiponectin concentration

Kit Components

Item	Specifications(48T/96T)	Storage
ELISA Microplate (Dismountable)	8×6 /8×12	4°C/-20°C
Lyophilized Standard	1 vial/2 vial	4°C/-20°C
Sample / Standard Dilution Buffer	10ml/20ml	4°C
Biotin-labeled Antibody (Concentrated)	60ul/120ul	4°C
Antibody Dilution Buffer	5ml/10ml	4°C
HRP-Streptavidin Conjugate (SABC)	60ul/120ul	4°C (shading light)
SABC Dilution Buffer	5ml/10ml	4°C
TMB Substrate	5ml/10ml	4°C (shading light)
Stop Solution	5ml/10ml	4°C
Wash Buffer (25X)	15ml/30ml	4°C
Plate Sealer	3/5pieces	
Product Description	1 copy	

Principle of the Assay

This kit was based on ELISA technology. Anti-adiponectin antibody was pre-coated onto 96-well plates. The biotin conjugated anti-adiponectin antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the adiponectin amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of adiponectin can be calculated.

Reagent Preparation and Storage

The kit was put at room temperature for 20 minutes before use.

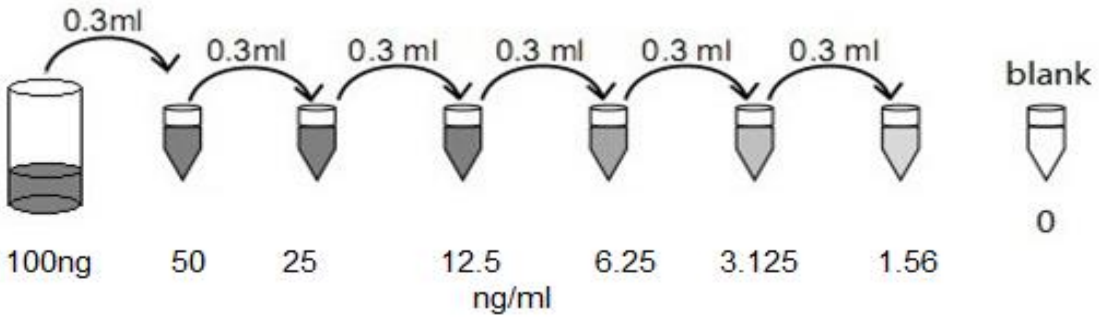
1, Wash Buffer:

30mL of Concentrated Wash Buffer was added into 750 mL Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

2. Standard:

1) 100ng/ml of standard solution: 1 ml of Sample / Standard dilution buffer was added into one Standard tube, keep the tube at room temperature for 10 minutes and mix them thoroughly.

2) 50ng/ml→1.56ng/ml of standard solutions: Label 6 Eppendorf tubes with 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.125ng/ml, 1.56ng/ml, respectively. Added 0.3 ml of the Sample/Standard dilution buffer into each tube. Added 0.3 ml of the above 100ng/ml standard solution into 1st tube and mix them thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix them thoroughly, and so on.



3. Preparation of Biotin-labeled Antibody Working Solution

It was prepared within 1 hour before experiment.

1) Calculating required total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

2) Diluting the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl Biotin-labeled antibody into 99μl Antibody Dilution Buffer).

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

It was prepared within 30 minutes before experiment.

1) Calculating required total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

2) Diluting the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl of SABC into 99μl of SABC Dilution Buffer).

5. Sample preparation

The sample was diluted with the provided dilution buffer. Dilution: 1:10. (i.e. Add 10μl of sample into 90μl of Sample/Standard Dilution Buffer). The test sample must be well mixed with the dilution buffer.

Assay Procedure

Before adding reagents into wells, equilibrate TMB Substrate for 30 min at 37 °C.

1. The plate washed 2 times before adding standard, sample and control (zero) wells.

2. Aliquot 0.1ml of 100ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.125ng/ml, 1.56ng/ml, standard solutions into the standard wells.

3. A 0.1 ml of Sample/Standard Dilution Buffer was added into the control (zero) well.

4. A 0.1 ml of properly diluted sample (serum) was added into test sample wells.

5. The plate sealed with a cover and incubated at 37 °C for 90 minutes.

6. The cover removed and discard the plate content, and plate washed 2 times with Wash Buffer.

7. A 0.1 ml of Biotin-labeled antibody working solution was added into above wells (standard, test sample and zero wells).

8. The plate sealed with a cover and incubated at 37°C for 60 min.

9. The cover removed, and plate washed 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time.

10. A 0.1 ml of SABC Working Solution was added into each well, cover the plate and incubate at 37°C for 30 minutes.

11. The cover removed and plate washed 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minute each time.

12. 90µl of TMB Substrate was added into each well, cover the plate and incubate at 37°C in dark within 15-30 minutes. It turns blue in the first 3-4 wells (with most concentrated adiponectin standard solutions), the other wells may not display obvious color.

13. 50µl of stop solution was added into each well and mix them thoroughly. The color changes to yellow immediately.

14. The O.D. absorbance was read at 450 nm in Microplate Reader immediately after adding the stop solution.

Regarding calculation: All data were calculated according to a standard curve in comparison to an optical density of treated samples to evaluate the concentration, and expressed as (ng/ml). (Appendix 3)

2.3.3 Leptin concentration

Principle of the Assay

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for leptin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of leptin is conjugated to biotin. During the first step, leptin presents in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin-HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin-HRP is removed by a washing step. Next, the enzyme substrate is added (TMB), forming a blue colored product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue color to a yellow color. The absorbance is measured on a microtiter plate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of leptin in patient samples and controls can be directly read.

Reagents provided

1. AA E-0030 Wash Buffer Concentrate – X10

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 ml/bottle

Storage: Refrigerate at 2-8oC

Stability: 12 months or as indicated on label

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

2.AA E-0055 **TMB Substrate** - Ready to use.

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 16 ml/bottle

Storage: Refrigerate at 2-8oC

Stability: Unopened at 2-8°C until expiration date on label.

3. AA E-0080 **Stopping Solution** - Ready to Use.

Contents: One vial containing 1M sulfuric acid.

Volume: 6 ml/bottle

Storage: Refrigerate at 2-8oC

Stability: Unopened at 2-8°C until expiration date on label.

4. Standards and **Controls**- Ready to use.

Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

Cat. No.	Standards	Concentration	Volume/vial
ME E-0301	Standard A	0 ng/ml	0.5 mL
ME E-0302	Standard B	1 ng/ml	0.5 ml
ME E-0303	Standard C	5 ng/ml	0.5 ml
ME E-0304	Standard D	10 ng/ml	0.5 ml
ME E-0305	Standard E	20 ng/ml	0.5 ml
ME E-0306	Standard F	50 ng/ml	0.5 ml
ME E-0307	Standard G	100 ng/ml	0.5 ml
ME E-0351	Control 1	Refer to vial labels for expected value and acceptable range	0.5 ml
ME E-0352	Control 2		0.5 ml

Contents: Leptin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of leptin.

Storage: Refrigerate at 2-8oC

Stability: Unopened at 2-8°C until expiration date on label.

5.ME E-0313 **Assay Buffer** - Ready to use.

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 20 ml/bottle

Storage: Refrigerate at 2-8oC

Stability: Unopened at 2-8°C until expiration date on label.

6.ME E-0331 **Anti-Leptin Monoclonal Antibody-Coated Break Apart Well Microplate**- Ready to use.

Contents: One 96 well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8oC

Stability: 12 months or as indicated on label.

7.ME E-0341 **Monoclonal Anti-Leptin-Biotin Conjugate**

Contents: One bottle containing a monoclonal anti-leptin antibody conjugated to biotin in a protein- based buffer with a non-mercury preservative.

Volume: 10 ml/bottle

Storage: Refrigerate at 2-8oC

Stability: 12 months or as indicated on label.

8. ME E-0340 **Streptavidin-HRP Conjugate Concentrate – X50**

Contents: One vial containing streptavidin conjugated to horseradish peroxidase in a protein-based buffer with a non-mercury preservative.

Volume: 0.4 ml/vial

Storage: Refrigerate at 2-8oC

Stability: Unopened at 2-8°C until expiration date on label.

Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µl of concentrate in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of concentrate in 12 ml of assay buffer. Discard any that is left over.

Assay procedure

All reagents must reach room temperature before use. Standards, controls and samples.

1. Working solutions prepared of the streptavidin-HRP - conjugate and wash buffer.

2. Pipetted 20 µl of each standard and samples into correspondingly labelled wells.

3. Pipetted 80 µl of the monoclonal anti-leptin-biotin conjugate into each well.

4. Plate incubated on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.

5. The wells washed 3 times with prepared wash buffer (300 µl/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry.

6. Pipetted 100 µl of prepared streptavidin-HRP - conjugate into each well.

7. The plate incubated on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.

8. The wells washed 3 times with prepared wash buffer (300 µl/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry.

9. Pipetted 100 µl of TMB substrate into each well at timed intervals.

10. Incubated on a plate shaker for 10-15 minutes at room temperature.

11. Pipetted 50 µl of stopping solution into each well at the same timed intervals as in step 9.

12. The plate was read on a microwell plate reader at 450 nm within 20 minutes after addition of the stopping.

Calculation

All data were calculated according to a standard curve in comparison to an optical density of treated samples to evaluate the concentration, and expressed as (ng/ml). (Appendix 4)

2.3.4 Insulin concentration

Principle of the assay

The Insulin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the insulin molecule. An aliquot of patient sample containing endogenous Insulin is incubated in the coated well with enzyme conjugate,

which is an anti-Insulin antibody conjugated with Biotin. After incubation the unbound conjugate is washed off. During the second incubation step Streptavidin Peroxidase Enzyme Complex binds to the biotin-anti-Insulin antibody. The amount of bound HRP complex is proportional to the concentration of Insulin in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of Insulin in the patient sample.

Reagents provided

ME E-0931 Microtiter wells

12x8 (break apart) strips, 96 wells; Wells coated with anti-Insulin antibody (monoclonal)

Standards

Cat. no.	Standard	Concentration	Volume/Vial
ME E-0901	Standard A (0)	0 µIU/ml	3 ml
ME E-0902	Standard B (1)	6.25 µIU/ml	1 ml
ME E-0903	Standard C (2)	12.5 µIU/ml	1 ml
ME E-0904	Standard D (3)	25 µIU/ml	1 ml
ME E-0905	Standard E (4)	50 µIU/ml	1 ml
ME E-0906	Standard F (5)	100 µIU/ml	1 ml

Conversion: µIU/mL x 0.0433 = ng/mL

ng/mL x 23.09 = µIU/mL

ME E-0940 Enzyme Conjugate

1 vial, 5 ml, ready to use, mouse monoclonal anti-Insulin conjugated to biotin, contain non-mercury preservative.

ME E-0915 Enzyme Complex

1 vial, 7 ml, ready to use, Streptavidin-HRP Complex, contain non-mercury preservative

FR E-0055 Substrate Solution

1 vial, 14 ml, ready to use, Tetramethylbenzidine (TMB).

FR E-0080 Stop Solution

1 vial, 14 ml, ready to use, contains 0.5 M H2SO4.

FR E-0030 Wash Solution

1 vial, 30 ml (40X concentrated) wash solution

Reagent Preparation

Wash Solution

Deionized water added to the 40X concentrated Wash Solution. 30 mL of concentrated wash solution was diluted with 1170 ml deionized water to a final volume of 1200 ml.

Assay procedure

1. Secured the desired number of Microtiter wells in the frame holder.

2. Dispensed 25 µl of each Standard and samples with new disposable tips into appropriate wells.

3. Dispensed 25 µl Enzyme Conjugate into each well. Thoroughly mixed for 10 seconds.

4. The plate incubated for 30 minutes at room temperature.

5. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (400 µl per well). Striked the wells sharply on absorbent paper to remove residual droplets.

6. Added 50 µl of Enzyme Complex to each well.

7. The plate incubated for 30 minutes at room temperature.

8. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.

9. Added 50 µl of Substrate Solution to each well.

10. The plate incubated for 15 minutes at room temperature.

11. The enzymatic reaction was stopped by adding 50 µl of Stop Solution to each well.

12. Determining the absorbance (OD) of each well at 450 $\pm$ 10 nm with a microtiter plate reader.

13. The wells was read within 10 minutes after adding the Stop Solution.

Calculation

All data were calculated according to a standard curve in comparison to an optical density of treated samples to evaluate the concentration, and expressed as (ng/ml). (Appendix 6).

Figure 2.5 Huma Reader device.

2.3.5 Determination of Glucose concentration

Test principle

The serum Glucose measured by Enzymatic reference method with hexokinase, by using commercial Kit (COBAS INTEGRA 400 plus, Catalogue no.04773365001).

Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP.

Glucose + ATP $\xrightarrow{HK}$ Glucose-6-phosphate + ADP

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically at 340 nm.

Glucose-6-phosphate + $NADP^+$ $\xrightarrow{G6PDH}$ Gluconate-6-P + NADPH + H^+

Reagents - working solutions

R1 TRIS buffer: 100 mmol/L, pH 7.8; Mg2+: 4 mmol/L; ATP: ≥ 1.7 mmol/L; NADP: ≥ 1.0 mmol/L; preservative

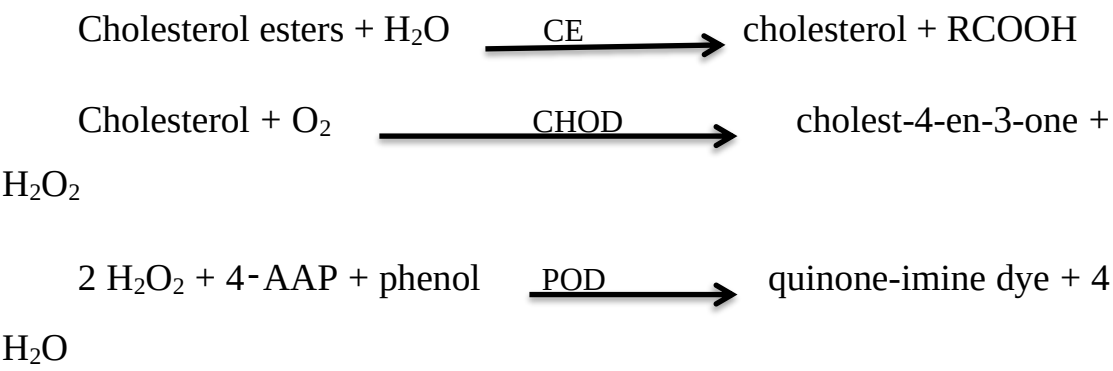
SR HEPES buffer: 30 mmol/L, pH 7.0; Mg2+: 4 mmol/L; HK (yeast): ≥ 130 µkat/L; G-6-PDH (E. coli): ≥ 250 µkat/L; preservative

2.3.6 Determination of Cholesterol concentration

Test principle

The serum Cholesterol measured by Enzymatic, colorimetric method, by using commercial Kit (COBAS INTEGRA 400 plus, Catalogue no. 0005168538190).

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinone-imine dye.



The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm.

Reagents - working solutions

R1 PIPES buffer: 225 mmol/L, pH 6.8; Mg^{2+}: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminoantipyrine: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %; cholesterol esterase (Pseudomonas spec.): ≥ 25 μkat/L (≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 μkat/L (≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 μkat/L (≥ 0.75 U/mL); stabilizers; preservative.

2.3.7 Determination of Triglyceride concentration

Test principle

The serum Triglyceride measured by Enzymatic colorimetric test by using commercial Kit (COBAS INTEGRA 400 plus, Catalog no. 0008058687190).

$$\text{Triglycerides} + 3\ H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + 3\ \text{RCOOH}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{MG++}]{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2\ H_2O + HCl$$

Reagents - working solutions

R1 PIPES buffer: 50 mmol/L, pH 6.8; Mg2+: 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥ 1.4 mmol/L; 4-aminophenazone: ≥ 0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; lipoprotein lipase (Pseudomonas spec.): ≥ 83 µkat/L; glycerol kinase (Bacillus stearothermophilus): ≥ 3

μkat/L; glycerol phosphate oxidase (E. coli): ≥ 41 μkat/L; peroxidase (horseradish): ≥ 1.6 μkat/L; preservative, stabilizers

2.3.8 Determination of HDL- C concentration

Test principle

The serum HDL-Cholesterol measured by Homogeneous enzymatic colorimetric assay, by using commercial Kit (COBAS INTEGRA 400 plus, Catalog no. 07529031001).

Non-HDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of cholesterol esterase and cholesterol oxidase towards non-HDL lipoproteins are blocked.

Finally only HDL-particles can react with cholesterol esterase and cholesterol oxidase. The concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase. Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{HDL-C esters} + H_2O \xrightarrow{\text{PEG-cholesterol esterase}} \text{HDL-C} + \text{RCOOH}$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to

Δ4-cholestenone and hydrogen peroxide.

HDL-cholesterol + O_2 $\xrightarrow{\text{PEG-cholesterol oxidase}}$ Δ 4-cholestenone + H_2O_2

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and EMSEa) to form a dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically at 583 nm.

2 H_2O_2 + 4-aminoantipyrine + EMSE + H^+ + H_2O $\xrightarrow{\text{peroxidase}}$ colored pigment + 5 H_2O

a) N-ethyl-N-(3-methylphenyl)-N'-succinylethylenediamine

Reagents - working solutions

R1 TAPSO b) buffer: 62.1 mmol/L, pH 7.77; polyanion: 1.25 g/L; EMSE: 1.08 mmol/L; ascorbate oxidase (cucurbita): ≥ 50 µkat/L; peroxidase (horseradish): ≥ 166.7 µkat/L; detergent; BSA: 2.0 g/L; preservative

SR Bis-Tris c) buffer: 20.1 mmol/L, pH 6.70; cholesterol esterase (microorganism): ≥ 7.5 µkat/L; cholesterol oxidase (recombinant E. coli): ≥ 7.17 µkat/L; cholesterol oxidase (microorganism): ≥ 76.7 µkat/L; peroxidase (horseradish): ≥ 333 µkat/L; 4-amino-antipyrine: 1.48 mmol/L; BSA: 3.0 g/L; detergents; preservative.

b) 2-Hydroxy-N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid

c) Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane

2.3.9 Determination of LDL-C concentration

Test principle

The serum LDL-Cholesterol measured by Homogeneous enzymatic colorimetric assay, by using commercial Kit (COBAS INTEGRA 400 plus, Catalog no. 0107005717190). Cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilize only LDL. The enzyme reacts to the lipoproteins other than LDL are inhibited by surfactants and a sugar compound. Cholesterol in HDL, VLDL and chylomicron is not determined.

$$\text{LDL-cholesterol esters} + H_2O \xrightarrow[\text{cholesterol esterase}]{\text{Detergent}}$$

cholesterol + free fatty acids (selective micellary solubilization)

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{LDL-cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \Delta 4\text{-cholestenone} + H_2O_2$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to $\Delta 4$-cholestenone and hydrogen peroxide.

$$2\ H_2O_2 + 4\text{-aminoantipyrine} + \text{EMSE}\ ^{a)} + H_2O + H^+ \xrightarrow{\text{peroxidase}} \text{red purple pigment} + 5H_2O$$

a) N-ethyl-N-(3-methylphenyl)-N-succinylethylenediamine

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and EMSE to form a red purple dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically at (583/659 nm).

Reagents - working solutions

R1 Bis-tris b) buffer: 20.1 mmol/L, pH 7.0; 4-aminoantipyrine: 0.98 mmol/L; ascorbate oxidase (AOD, Acremonium spec.): ≥ 66.7 μkat/L; peroxidase (recombinant from Basidiomycetes): ≥ 166.7 μkat/L; BSA: 4.0 g/L; preservative

SR MOPS c) buffer: 20.1 mmol/L, pH 7.0; EMSE: 2.16 mmol/L; cholesterol esterase (Pseudomonas spec.): ≥ 33.3 μkat/L; cholesterol oxidase (recombinant from E. coli) : ≥ 31.7 μkat/L; peroxidase (recombinant from Basidiomycetes): ≥ 333.3 μkat/L; BSA: 4.0 g/L; detergents; preservative

b) bis(2-hydroxyethyl)-amino-tris-(hydroxymethyl)-methane

c) 3-morpholinopropane-1-sulfonic acid

2.3.10 Determination of VLDL concentration

To determine the concentrations of VLDL, it was calculated by dividing the concentrations of T.G by five.

Figure 2.6 Cobas integra 400 plus device.

2.4 Statical analysis

The data were statistically analyzed using SSPS software and the significance of the observed differences, associations, or calculations was determined at p-value <0.05. Chi^2 statistical test was used to investigate the significance of associations, Kruskal-Wallis and Mann-Whitney tests were used for differences between the groups of non-parametric data, and Spearman's test to examine nonparametric correlations.

Chapter three

Results

3. Results

3.1 Distribution of samples population

As shown in table 3.1, the results were appeared no significant differences between the three groups according to age group.

Table (3.1) Distribution between control, obese and diabetic groups according to age group

		Category			Total	P-value
		Group I Lean	Group II Obesity	Group III Diabetic		
Age group	25-35	10	10	8	28	0.99 NS
		33.3%	33.3%	28.6%	31.8%	
	36-45	10	10	10	30	
		33.3%	33.3%	35.7%	34.1%	
	46-55	10	10	10	30	
		33.3%	33.3%	35.7%	34.1%	
Total		30	30	28	88	
		100.0%	100.0%	100.0%	100.0%	

NS; not significant

3.2 Biochemical results

The results were revealed a significant increase of visfatin hormone (p=0.003) in diabetic and obese group than control group. Moreover, the study appeared that leptin was highly significant increase in diabetic group (p=0.0001) in comparison with obese and control group. On the other hand, no significant differences of adiponectin and insulin levels between all groups (p=0.398, p=0.126) respectively (Table 3.2).

Table (3.2) Comparison between different groups according to hormones (visfatin, adiponectin, leptin and insulin) regardless age group.

Variables	Group I Lean n=30		Group II Obese healthy n=30		Group III Obese diabetic n=28		P-value
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	
Visfatin (ng/ml)	6.083 ± 1.7046	5.950	8.797±9.4173	7.050	7.754±2.4479	8.400	0.003*
Adiponectin (ng/ml)	56.047± 10.1805	54.350	54.603± 8.179	54.450	53.911± 12.5575	51.600	0.398 NS
Leptin (ng/ml)	8.080 ± 8.864	4.350	46.327± 23.22	42.450	115.700 ±30.210	118.000	0.0001*
Insulin (μIU/ml)	14.897± 5.1885	13.400	18.003± 10.120	15.050	20.550± 13.9523	14.400	0.126 NS

* Significant; P-value <0.05 NS; not significant

As shows in table 3.3, that BMI and WHR were highly significant increase (p=0.000) in obese than control group. However, the results appeared that T.C, LDL, T.G and VLDL had significant increase in obese group than control (p=0.010, p=0.002, p=0.017 and p=0.016) respectively. Moreover, significant decrease of HDL in obese than control group (p=0.012). While, there were no significant differences of glucose concentrations (p=0.929) between two groups.

Table (3.3) Comparison between group I and group II regarding BMI, WHR, glucose and lipid profile regardless age group.

Variables	Group I Lean n=30		Group II Obese healthy n=30		P-value
	Mean ±SD	Median	Mean ±SD	Median	
BMI (kg/m^2)	23.380 ± 1.6658	24.050	34.140± 3.1399	33.200	0.000*
WHR	0.7857 ± 0.0606	0.8000	0.8663± 0.07761	0.8850	0.000*
Glucose (mmol/l)	93.963 ± 6.2184	92.800	95.617± 11.8287	93.550	0.929 NS
Total cholesterol (mmol/l)	180.063± 31.7482	182.000	206.202± 35.7653	204.150	0.010*
Triglycerides (mmol/l)	82.157± 37.2205	78.800	107.493± 50.3963	88.700	0.017*
HDL-C (mmol/l)	48.210± 8.8651	48.050	41.580± 10.4861	40.750	0.012*
LDL-C (mmol/l)	92.967± 23.5520	95.450	115.647± 27.0835	120.100	0.002*
VLDL (mmol/l)	16.433± 7.4358	15.750	21.493± 10.0788	17.700	0.016*

* Significant; P-value <0.05 NS; not significant

In table 3.4 visfatin level had significant increase in obese group than control (p=0.011). Furthermore, the data was appeared that leptin had highly significant increase (p=0.000) in obese than control group. In contrast, there were no significant differences of adiponectin (p=0.647) and insulin (p=0.160) between two groups.

Table (3.4) Comparison between group I and group II according to hormones (visfatin, adiponectin, leptin and insulin) regardless age group.

Variables	Group I Lean n=30		Group II Obese healthy n=30		P-value
	Mean ±SD	Median	Mean ±SD	Median	
Visfatin (ng/ml)	6.083 ± 1.7046	5.950	8.797± 9.4173	7.050	0.011*
Adiponectin (ng/ml)	56.047± 10.180	54.350	54.603± 8.1799	54.450	0.647 NS
Leptin (ng/ml)	8.080± 8.8648	4.350	46.327± 23.2231	42.450	0.000*
Insulin (µIU/ml)	14.897± 5.1885	13.400	18.003± 10.1209	15.050	0.160 NS

* Significant; P-value <0.05 NS; not significant

According to age group in table 3.5 between obese and control group, the result revealed that although of presence of simple differences in the mean of (BMI, WHR, glucose, HDL-C, LDL-C, T.G and VLDL), statistically there were no significant differences among age groups. However, significant increase of T.C was notice between age group (p=0.015). Furthermore, table 3.6 referred that visfatin, adiponectin, leptin and insulin had no significant effect among age group.

Table (3.5) Comparison between group I and group II regarding BMI, WHR, glucose and lipid profile according to age group.

Variables	Group I Lean n=30						Group II Obese healthy n=30						P-value
	25-35 N=10		36-45 N=10		46-55 N=10		25-35 N=10		36-45 N=10		46-55 N=10		
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	
BMI (kg/m^2)	22.850± 1.0783	22.950	22.940± 2.4568	24.450	24.350± 0.4116	24.500	34.490± 4.1725	32.600	33.040± 2.4432	32.250	34.890± 2.5066	33.850	0.369 NS
WHR	0.7650± 0.06115	0.7850	0.7880± 0.0502	0.7900	0.8040± 0.0686	0.8000	0.8590± 0.06657	0.8750	0.8430± 0.09604	0.8900	0.8970± 0.06360	0.9000	0.215 NS
Glucose (mmol/l)	88.980± 5.4377	90.500	96.520± 5.6202	97.000	96.390± 4.6953	96.450	94.930± 8.8447	97.900	100.18± 17.3591	93.550	91.740± 5.5604	92.100	0.439 NS
Total cholesterol (mmol/l)	171.56± 27.0745	165.70	192.12± 18.8206	190.25	176.51± 43.6429	180.150	180.83± 30.9140	173.500	209.78± 204.150	204.150	227.99± 27.0198	225.900	0.015*
Triglycerides (mmol/l)	76.960± 29.3584	72.500	92.420± 41.5618	79.750	77.090± 41.2199	60.500	101.40± 36.8497	90.950	102.73± 52.6846	87.150	118.35± 62.0707	84.850	0.889 NS
HDL-C (mmol/l)	44.020± 4.9130	44.950	46.900± 10.5622	50.050	53.710± 7.9065	52.800	36.220± 6.4197	35.800	42.170± 13.1517	41.200	46.350± 9.0852	46.050	0.072 NS
LDL-C (mmol/l)	90.360± 21.2651	89.150	100.90± 14.3605	101.150	87.550± 31.8943	84.250	102.650± 28.0701	100.000	116.27± 26.7856	121.600	128.02± 22.3591	128.200	0.111 NS
VLDL (mmol/l)	15.390± 5.8603	14.500	18.480± 8.3110	15.950	15.430± 8.2317	12.100	20.270± 7.3627	18.150	20.530± 10.5398	17.400	23.680± 12.4051	17.000	0.889 NS

* Significant; P-value <0.05 NS; not significant

Table (3.6) Comparison between group I and group II regarding hormones (visfatin, adiponectin, leptin and insulin) according to age group.

Variables	Group I Lean n=30						Group II Obese healthy n=30						P-value
	25-35 N=10		36-45 N=10		46-55 N=10		25-35 N=10		36-45 N=10		46-55 N=10		
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	
Visfatin (ng/ml)	6.430± 1.9213	5.900	5.730± 1.9551	5.400	6.090± 1.2556	6.300	11.970± 16.0411	6.800	7.090± 2.8211	7.000	7.330± 1.9166	7.500	0.932 NS
Adiponectin (ng/ml)	58.030± 9.8102	56.450	54.850± 9.6661	52.800	55.260± 11.7318	53.600	54.980± 7.4775	55.250	53.440± 6.4298	54.050	55.390± 10.7703	54.850	0.848 NS
Leptin (ng/ml)	5.430± 6.2638	3.500	7.880± 6.6325	5.400	10.930± 12.3742	4.400	52.960± 27.6467	52.350	48.110± 24.3524	44.700	37.910± 15.7880	38.150	0.318 NS
Insulin (µIU/ml)	15.130± 5.7856	12.600	14.940± 6.9870	11.900	14.620± 2.0746	13.400	15.650± 3.9059	15.800	21.770± 14.4450	15.150	16.590± 9.0561	13.800	0.503 NS

* Significant; P-value <0.05 NS; not significant

Table 3.7 was recorded that BMI, WHR, glucose, T.G and VLDL had higher significant increase (p=0.000) in diabetic group than control. However, the level of T.C (p=0.036) and LDL (p=0.016) had significantly increase in diabetic than control. In addition, HDL concentration had significant decrease in diabetic group than control (p=0.001).

Table (3.7) Comparison between group I and group III regarding BMI, WHR, glucose and lipid profile regardless age group.

Variables	Group I Lean n=30		Group III Obese diabetic n=28		P-value
	Mean ± SD	Median	Mean ± SD	Median	
BMI (kg/m²)	23.38 ± 1.6658	24.050	35.104± 4.3485	33.350	0.000*
WHR	0.7857± 0.0606	0.8000	0.9639± 0.08478	0.9500	0.000*
Glucose (mmol/l)	93.963± 6.2184	92.800	201.075± 118.07	164.300	0.000*
Total cholesterol (mmol/l)	180.063± 31.748	182.000	208.032± 49.467	201.000	0.036*
Triglycerides (mmol/l)	82.157± 37.2205	78.800	172.614± 69.189	149.500	0.000*
HDL-C (mmol/l)	48.210± 8.8651	48.050	39.615± 10.5173	38.200	0.001*
LDL-C (mmol/l)	92.967± 23.552	95.450	111.046± 28.583	112.150	0.016*
VLDL (mmol/l)	16.433± 7.4358	15.750	34.521± 13.8346	29.900	0.000*

* Significant; P-value <0.05 NS; not significant

The study results as illustrated in table 3.8, that visfatin (p=0.002) was significantly increase in diabetic than control. Furthermore, the leptin level

was high significant increase (p=0.000) in diabetic group than control. On other hand, adiponectin (p=0.243) and insulin (p=0.050) were showed no significant difference between two groups.

Table (3.8) Comparison between group I and group III according to hormones (visfatin, adiponectin, leptin and insulin) regardless age group.

Variables	Group I Lean n=30		Group III Obese diabetic n=28		P-value
	Mean ± SD	Median	Mean ± SD	Median	
Visfatin (ng/ml)	6.083 ± 1.7046	5.950	7.754± 2.4479	8.400	0.002*
Adiponectin (ng/ml)	56.047± 10.1805	54.350	53.911± 12.5575	51.600	0.243 NS
Leptin (ng/ml)	8.080 ± 8.864	4.350	115.700± 30.210	118.000	0.000*
Insulin (µIU/ml)	14.897± 5.1885	13.400	20.550± 13.9523	14.400	0.050 NS

* Significant; P-value <0.05 NS; not significant

However, when comparing between group I and group III based on age group (table 3.9), the data detected that in spite of existence whether little of decrease or increase in the mean of (BMI, WHR, glucose, T.C, HDL-C, LDL-C, T.G and VLDL), statistically there are no significant variation among age groups.

Moreover, table 3.10 was referred that visfatin, adiponectin and insulin had no significant differences between age group. Whereas, significant increase was recorded of the levels of leptin among all age group (p=0.043).

Table (3.9) Comparison between group I and group III regarding BMI, WHR, glucose and lipid profile according to age group.

Variables	Group I Lean n=30						Group III Obese diabetic n=28						P-value
	25-35 N=10		36-45 N=10		46-55 N=10		25-35 N=8		36-45 N=10		46-55 N=10		
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ±SD	Median	Mean ±SD	Median	Mean ±SD	Median	
BMI (kg/m^2)	22.850± 1.0783	22.950	22.940± 2.4568	24.450	24.350± 0.4116	24.500	35.063± 2.8339	35.200	34.830± 4.5765	33.350	35.410± 5.4173	32.800	0.946 NS
WHR	0.7650± 0.06115	0.7850	0.7880± 0.0502	0.7900	0.8040± 0.0686	0.8000	0.9225± 0.03991	0.9250	0.9890± 0.08888	0.9750	0.9720± 0.9950	0.9950	0.263 NS
Glucose (mmol/l)	88.980± 5.4377	90.500	96.520± 5.6202	97.000	96.390± 4.6953	96.450	192.638± 85.2727	205.950	166.840± 78.6351	147.600	242.06± 163.568	172.050	0.635 NS
Total cholesterol (mmol/l)	171.56± 27.0745	165.70	192.12± 18.8206	190.25	176.51± 43.6429	180.150	198.275± 47.3394	187.850	187.340± 20.5755	194.500	236.53± 60.9266	226.750	0.082 NS
Triglycerides (mmol/l)	76.960± 29.3584	72.500	92.420± 41.5618	79.750	77.090± 41.2199	60.500	159.388± 78.2634	141.100	183.880± 64.5418	214.400	171.930± 71.6569	149.500	0.907 NS
HDL-C (mmol/l)	44.020± 4.9130	44.950	46.900± 10.5622	50.050	53.710± 7.9065	52.800	42.954± 13.0282	39.850	37.180± 9.5409	36.800	39.380± 9.6039	37.150	0.581 NS
LDL-C (mmol/l)	90.360± 21.2651	89.150	100.90± 14.3605	101.150	87.550± 31.8943	84.250	102.788± 32.8222	107.550	103.150± 18.7986	98.150	125.550± 29.9340	127.800	0.222 NS
VLDL (mmol/l)	15.390± 5.8603	14.500	18.480± 8.3110	15.950	15.430± 8.2317	12.100	31.888± 15.6548	28.250	36.770± 12.9087	42.900	34.380± 14.3225	29.900	0.907 NS

* Significant; P-value <0.05 NS; not significant

Table (3.10) Comparison between group I and group III regarding hormones (visfatin, adiponectin, leptin and insulin) according to age group.

Variables	Group I Lean n=30						Group III Obese diabetic n=28						P-value
	25-35 N=10		36-45 N=10		46-55 N=10		25-35 N=8		36-45 N=10		46-55 N=10		
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ±SD	Median	Mean ±SD	Median	Mean ±SD	Median	
Visfatin (ng/ml)	6.430± 1.9213	5.900	5.730± 1.9551	5.400	6.090± 1.2556	6.300	8.613± 3.2202	9.100	7.190± 2.5427	7.200	7.630± 1.5557	7.800	0.196 NS
Adiponectin (ng/ml)	58.030± 9.8102	56.450	54.850± 9.6661	52.800	55.260± 11.7318	53.600	58.550± 18.9382	49.950	51.090± 4.8748	51.600	53.020± 11.8954	51.650	0.911 NS
Leptin (ng/ml)	5.430± 6.2638	3.500	7.880± 6.6325	5.400	10.930± 12.3742	4.400	125.113± 28.4334	120.500	127.790± 17.7557	125.400	96.080± 33.5425	92.300	0.043*
Insulin (µIU/ml)	15.130± 5.7856	12.600	14.940± 6.9870	11.900	14.620± 2.0746	13.400	27.263± 22.0625	16.550	16.400± 5.7289	13.300	19.330± 10.5711	14.450	0.331 NS

* Significant; P-value <0.05 NS; not significant

The statical analysis in table 3.11 indicated that BMI (p=0.597), HDL (p=0.418), T.C (p=0.821) and LDL (p=0.586) had no significant differences between two group. Conversely, WHR, glucose, T.G and VLDL shown significantly higher increase in diabetic group than control (p=0.000).

Table (3.11) Comparison between group II and group III regarding BMI, WHR, glucose and lipid profile regardless age group.

Variables	Group II Obese healthy n=30		Group III Obese diabetic n=28		P-value
	Mean ±SD	Median	Mean ± SD	Median	
BMI (kg/m^2)	34.140± 3.1399	33.200	35.104± 4.3485	33.350	0.597 NS
WHR	0.8663± 0.07761	0.8850	0.9639± 0.08478	0.9500	0.000*
Glucose (mmol/l)	95.617± 11.8287	93.550	201.075± 118.07	164.300	0.000*
Total cholesterol (mmol/l)	206.202± 35.7653	204.150	208.032± 49.467	201.000	0.821 NS
Triglycerides (mmol/l)	107.493± 50.3963	88.700	172.614± 69.189	149.500	0.000*
HDL-C (mmol/l)	41.580± 10.4861	40.750	39.615± 10.5173	38.200	0.418 NS
LDL-C (mmol/l)	115.647± 27.0835	120.100	111.046± 28.583	112.150	0.586 NS
VLDL (mmol/l)	21.493± 10.0788	17.700	34.521± 13.8346	29.900	0.000*

* Significant; P-value <0.05 NS; not significant

The results revealed that visfatin (p=0.262), adiponectin (p=0.263) and insulin (p=0.549), had no significant differences between two group. On the other hand, leptin level shown significantly higher increase in diabetic group than control (p=0.000). (Table 3.12)

Table (3.12) Comparison between group II and group III according to hormones (visfatin, adiponectin, leptin and insulin) regardless age group.

Variables	Group II Obese healthy n=30		Group III Obese diabetic n=28		P-value
	Mean ±SD	Median	Mean ± SD	Median	
Visfatin (ng/ml)	8.797± 9.4173	7.050	7.754± 2.4479	8.400	0.262 NS
Adiponectin (ng/ml)	54.603± 8.1799	54.450	53.911± 12.5575	51.600	0.263 NS
Leptin (ng/ml)	46.327± 23.2231	42.450	115.700± 30.210	118.000	0.000*
Insulin (µIU/ml)	18.003± 10.1209	15.050	20.550± 13.9523	14.400	0.549 NS

* Significant; P-value <0.05 NS; not significant

The table 3.13 between obese and diabetic group according to age group shown that there were no significant variations in BMI, HDL and LDL (p=0.706), (p=0.271) and (p=0.150) respectively. In contrast, significant differences were observed in WHR (p=0.004), glucose (p=0.0001), T.C (p=0.025), T.G (p=0.011) and VLDL (p=0.012) between age group.

Moreover, the results in table 3.14 shown there were no significant differences in visfatin, adiponectin and insulin (p=0.483), (p=0.861), (p=0.631) respectively. Except leptin level was revealed highly significant differences between age group (p=0.0001).

Table (3.13) Comparison between group II and group III regarding BMI, WHR, glucose and lipid profile according to age group.

Variables	Group II Obese healthy n=30						Group III Obese diabetic n=28						P-value
	25-35 N=10		36-45 N=10		46-55 N=10		25-35 N=8		36-45 N=10		46-55 N=10		
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ±SD	Median	Mean ±SD	Median	Mean ±SD	Median	
BMI (kg/m^2)	34.490± 4.1725	32.600	33.040± 2.4432	32.250	34.890± 2.5066	33.850	35.063± 2.8339	35.200	34.830± 4.5765	33.350	35.410± 5.4173	32.800	0.706 NS
WHR	0.8590± 0.06657	0.8750	0.8430± 0.09604	0.8900	0.8970± 0.06360	0.9000	0.9225± 0.03991	0.9250	0.9890± 0.08888	0.9750	0.9720± 0.9950	0.9950	0.004*
Glucose (mmol/l)	94.930± 8.8447	97.900	100.18± 17.3591	93.550	91.740± 5.5604	92.100	192.638± 85.2727	205.950	166.840± 78.6351	147.600	242.06± 163.568	172.050	0.0001*
Total cholesterol (mmol/l)	180.83± 30.9140	173.500	209.78± 204.150	204.150	227.99± 27.0198	225.900	198.275± 47.3394	187.850	187.340± 20.5755	194.500	236.53± 60.9266	226.750	0.025*
Triglycerides (mmol/l)	101.40± 36.8497	90.950	102.73± 52.6846	87.150	118.35± 62.0707	84.850	159.388± 78.2634	141.100	183.880± 64.5418	214.400	171.930± 71.6569	149.500	0.011*
HDL-C (mmol/l)	36.220± 6.4197	35.800	42.170± 13.1517	41.200	46.350± 9.0852	46.050	42.954± 13.0282	39.850	37.180± 9.5409	36.800	39.380± 9.6039	37.150	0.271 NS
LDL-C (mmol/l)	102.650± 28.0701	100.000	116.27± 26.7856	121.600	128.02± 22.3591	128.200	102.788± 32.8222	107.550	103.150± 18.7986	98.150	125.550± 29.9340	127.800	0.150 NS
VLDL (mmol/l)	20.270± 7.3627	18.150	20.530± 10.5398	17.400	23.680± 12.4051	17.000	31.888± 15.6548	28.250	36.770± 12.9087	42.900	34.380± 14.3225	29.900	0.012*

* Significant; P-value <0.05 NS; not significant

Table (3.14) Comparison between group II and group III regarding hormones (visfatin, adiponectin, leptin and insulin) according age group.

Variables	Group II Obese healthy n=30						Group III Obese diabetic n=28						P-value
	25-35 N=10		36-45 N=10		46-55 N=10		25-35 N=8		36-45 N=10		46-55 N=10		
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ±SD	Median	Mean ±SD	Median	Mean ±SD	Median	
Visfatin (ng/ml)	11.970± 16.0411	6.800	7.090± 2.8211	7.000	7.330± 1.9166	7.500	8.613± 3.2202	9.100	7.190± 2.5427	7.200	7.630± 1.5557	7.800	0.483 NS
Adiponectin (ng/ml)	54.980± 7.4775	55.250	53.440± 6.4298	54.050	55.390± 10.7703	54.850	58.550± 18.9382	49.950	51.090± 4.8748	51.600	53.020± 11.8954	51.650	0.861 NS
Leptin (ng/ml)	52.960± 27.6467	52.350	48.110± 24.3524	44.700	37.910± 15.7880	38.150	125.113± 28.4334	120.500	127.790± 17.7557	125.400	96.080± 33.5425	92.300	0.0001*
Insulin (µIU/ml)	15.650± 3.9059	15.800	21.770± 14.4450	15.150	16.590± 9.0561	13.800	27.263± 22.0625	16.550	16.400± 5.7289	13.300	19.330± 10.5711	14.450	0.631 NS

* Significant; P-value <0.05 NS; not significant

3.3 Correlation between different parameters

In the present study, no correlation between visfatin and other parameters. However, there were negative correlation between glucose and BMI (r=-0.395, p=0.4). Conversely, positive correlation was observed between LDL-C and T.C (r=0.884, p=0.0001) in diabetic group (Appendix 6).

On the other hand, in obese group there were positive correlation between visfatin and BMI (r=0.404, p=0.03), and no correlation was showed between visfatin and other parameters. Moreover, a positive correlation was found between LDL-C and T.C (r=0.948, p=0.0001). In contrast, the data were showed a negative correlation between HDL-C and T.G (r=-0.567, p=0.001). Additionally, a negative correlation between VLDL and HDL-C (r=-0.567, p=0.001) was observed (Appendix 7).

Chapter four
Discussion

4. Discussion

One of the most frequent medical disorders is obesity. Obesity is characterized by low-grade inflammatory responses and increased oxidative stress (Wnuk *et al.*,2020). Obesity has developed into a hazardous condition that involves a variety of interventions, treatments, and preventions. Adipokines are small polypeptide growth factors released primarily by white and brown adipose tissue adipocytes such as visfatin, adiponectin, leptin and resistin (Gui *et al.*,2017).

Furthermore, adipokines are hormones that can influence a variety of physiological and pathological processes, particularly those linked to immune and inflammatory activities. Skeletal muscle, kidney, pancreas and immune systems, can all benefit from adipokines. Adipokines' extensive impacts may explain (at least in some part) the systemic issues that are commonly linked with obesity (Saeidi *et al.*, 2021).

As far as the authors are aware, this is the first study that aimed to evaluate the relationship between visfatin and obesity in basrah women. It also aimed to evaluate the relation between visfatin, adiponectin, leptin and insulin.

4.1 Hormones

The results of this study have shown that there was a significant increase in visfatin in diabetic and obese group in comparison with control group. Several studies agreed with this study results, its clarified that obesity causes increase the release of visfatin from adipocytes. Mabrouk was found significantly higher levels of visfatin in obese diabetics patients compared to healthy normal weight group and no differences between obese and

diabetic group (Mabrouk *et al.*,2013). Similarity, no differences of visfatin level among obese and diabetic group (Kamińska *et al.,* 2010).

Moreover, another experimental study was showed that serum visfatin concentration was significantly raised in obese and diabetic mice than control (Naz *et al.*,2017).

Alnowihi *et al.*, (2020) and Berndt *et al.,* (2005) were recorded that the obese women showed significantly higher visfatin than lean women. Furthermore, another study illustrated that serum level of visfatin was significantly higher in obese women when compared to controls (Zahorska-Markiewicz *et al*,2007; de Luis *et al.*,2008; Kamińska *et al.*,2010). Haider *et al.,* (2006) and Garcia-Fuentes *et al.*, (2007) also found significantly higher visfatin levels in patients with morbid obesity in comparison with lean individuals.

Previously published report was appeared that plasma visfatin was increased in patients with diabitic than control group. The elevated in visfatin level in individuals with DM might indicate impaired visfatin signalling in target tissues, biosynthetic dysregulation, or a response to hyperglycemia or hyperinsulinemia in a diabetic condition (Chen *et al.*,2006; Li *et al.*,2006; El-Shafey *et al.*,2012).

Similar results were also recorded in rats, that high levels of fasting serum visfatin was observed in diabetic rat when comparison with control (Kang *et al,* 2010; Abed and Ali, 2018). Hetta *et al.,* (2018) shown there were significant increase in the mean of visfatin serum in diabetics group than obese and the control group.

Moreover, another research reported that T2D patients showed a significant high levels of serum visfatin than healthy subjects (Eid *et al,*

2012; Kocot *et al.*,2017). This may be due to that increase in adipocytes will result in increase in visfatin levels (Arner, 2006). In addition, high level of visfatin was showed in T2D group than control, and the lowest level of visfatin was recorded in T1D group (Abed and Ali, 2018).

The hypothesis that this resulted from a compensatory mechanism developed in response to impaired insulin action, which confirms insulin mimetic effect of visfatin. This theory seems to be confirmed by other studies, which demonstrated that plasma visfatin concentration was dependent on the degree of insulin resistance. However, it should be noted that the relationship between serum visfatin level and insulin resistance remains unclear and studies revealed conflicting results (Shaker *et al.*,2011; Cheng *et al.*,2011; Picu *et al.*, 2017).

In contrast, several studies demonstrated that obese subjects had significant lower visfatin levels compared to subjects with normal body weight (Pagano *et al.*,2006; Jian *et al.*,2006). Another research was reported no significant differences of visfatin concentration between obese and lean subjects (Hofsø *et al.*,2009).

The reasons of these differences are not clear yet, however it may be caused by some factors:

1. Ethnic diversity can influence visfatin levels and sensitivity.

2. Because the inclusion criteria for the various research differed, confounding factors like age, T2DM duration, medication therapy, and lifestyle may influence visfatin concentrations and other features of relevance, obscuring the results (Jian *et al.*,2006).

On the other hand, in spite of the study results were showed presence of simple decrease or increase of adiponectin level between obese, diabetic and control subjects, statistically there were no significant differences between all groups. Many studies revealed similar results, since, no significant differences were observed between obese healthy and obese diabetic subjects in the level of adiponectin (Vasilescu *et al.*,2011).

Furthermore, a study was reported that adiponectin concentration was similar in both obese and control subjects (Martínez Larrad *et al.*,2016). Moreover, no significant differences had shown in the level of adiponectin between obese and diabetic group.The majority of adiponectin release is found in subcutaneous adipose tissue. This finding might be explained by the fact that adiponectin reduces obesity by increasing energy expenditure and impairing adipocyte differentiation (Ayman *et al.*,2019). Another possible cause to obesity related declines in adiponectin may be dysfunctional of subcutaneous adipose tissue (Meyer *et al.*, 2013).

Other studies were noticed that adiponectin level was decreased in diabetic group in compared to obese and control (Chen *et al.*,2006; Susilowati *et al.*,2016; Kocot *et al*.,2017). In addition, obese individual showed lower concentration of adiponectin in compared with lean persons (Larsen *et al*.,2019).

Other workers were observed that the level of adiponectin was lower in obese diabetic rats and increased after weight loss after gastric sleeve surgery (Stoica *et al*., 2021). Moreover, (Gariballa *et al.*, 2019) was observed decreased of total adiponectin level in obese group than control.

Furthermore, the data of this study was found significantly highly increase in leptin level in diabetic group than obese and control. Several studies agreement with this study result, Hofsø *et al.* (2009) found that the level of leptin consistently higher in obese subjects than lean group (Larsen *et al.*,2019). Furthermore, higher leptin concentration was found in diabetes obese subjects mor than healthy group (Kocot *et al.*,2017; Adami *et al.*, 2017). Das was reported significantly increased leptin concentration in T2D patients compared to healthy controls (Das *et al.*,2013).

Liu *et al.*, (2020) was demonstrated that there was significant increase in the level of leptin concentration in obese patients with newly diagnosed T2D in comparison to patients with newly diagnosed T2D who had normal BMI and control group. Similarly, previous study was noticed significantly higher leptin level in diabetes obese subjects than non-diabetic group (Esteghamati *et al.*,2011).

Conversely, Rajković had shown no differences of leptin between diabetics and healthy group (Rajkovic *et al.*,2014). Vasilescu was reported no differences of leptin level between overweight diabetics and overweight healthy controls. The same observations were notice in obese subjects when compared to control group (Vasilescu *et al.*,2011).

Although this adipokine has been known for many years, its roles in the pathophysiology of DM remain controversial (Lee *et al.*,2019). The findings of previous studies have suggested that leptin may represent a predictor of obesity and T2D (Ghadge and Khaire, 2019). However, the relationship between serum leptin level and many other clinical indicators in individuals with T2D is still a matter of discussion (Liu *et al.*, 2020). In addition, comparisons of leptin concentrations between non-obese patients with diabetes and healthy controls have yielded inconsistent results

perhaps due to differences in the methods used for the selection of study participants (Vasilescu and Ionescu-Tirgoviste,2011; Das *et al.*,2013).

In addition, although of the statistical analysis of present study had shown no significant differences in insulin level between all group, the data was recorded elevated in mean of insulin in diabetic and obese more than control group (20.550, 18.003 and 14.897) respectively. This perhaps due to the fact that the type of diabetes was not determined by authors when the participants samples were collected.

This fact has been proven in several studies. Since, the results referred that insulin concentration had significant increase in T2D than T1D and control group (Abed and Ali, 2018; Barbosa-da-Silva *et al.*, 2014). These elevated insulin levels may be due to increased insulin resistance due to insulin over secretion to overcome the tissue resistance (Schultz *et al*, 2013). Moreover, in the T1D group the insulin levels were the lowest when compared to the T2D and control group, these findings due to that alloxan treated rats undergone destruction of pancreatic β-cells (Udia *et al*, 2016).

Furthermore, it was conducted that no significant difference was observed of insulin level between obese and obese diabetic group (Mabrouk *et al.*,2013).

4.2 BMI, WHR, glucose and lipid profile

Nevertheless, the results of this study were appeared a significant increase in the anthropometric measurements (BMI and WHR) in obese and diabetic group than control. In addition, fasting glucose, T.C, LDL, T.G and VLDL were highly significant increase in diabetic and obese

group in comparison with control group. Additionally, the results revealed a significant decrease of HDL in obese and diabetic group than control.

Many previously studies revealed similar results. BMI and WHR had significant increase in obese subjects and obese diabetics in compared with control group, the levels of T.C, T.G and LDL-C were significant increase in diabetic and obese group than control while HDL-C degrease in diabetic and obese than control group (Ayman *et al.*,2019).

Alnowihi *et al.*, (2020) and Berndt *et al.*, (2005) were recorded that those obese women showed significantly higher lipid profile than lean women. In contrast, obese women had significant lower HDL-C than lean women. Moreover, HDL shown significant decrease in obese group than control (Zahorska-Markiewicz *et al*,2007).

Moreover, (Baltacı *et al.*, 2016) was conducted that the variables BMI, WHR, T.C, LDL-C, T.G) were significant increase in obese group in comparison with control. Conversely, HDL-C level was significantly decrease in obese subjects than lean group.

Furthermore, previous study on obese children was revealed that BMI, T.C, LDL-C, T.G) were significantly higher in obese group than control, HDL-C level was significantly decrease in obese subjects than normal weight group (Li *et al.*, 2013).

In addition, the results of this study were showed there no significant differences between obese and obese diabetic subjects for T.C, HDL-C and LDL-C, these results agreed with previous research (Vasilescu *et al.*,2011).

However, the data results showed that WHR had significant increase in diabetic group than obese and lean group, this result is agreed with several studies (Chen *et al.*,2006).

On the other hand, several studies were appeared different result. There were no significant differences of T.C, LDL-C and T.C concentrations between obese and control group (Zahorska-Markiewicz *et al*,2007).

Obesity is global epidemic was associated with dyslipidemia, that is mainly caused by insulin resistance and pro-inflammatory adipocytokines (Vekic *et al.*,2019). The typical dyslipidemia of obesity consists of increased triglycerides and FFA, decreased HDL-C with HDL dysfunction and normal or slightly increased LDL-C with increased small dense LDL (Wang and Peng,2011). Plasma FFA levels are known to be higher in obese individuals as a result of increased fatty acid production from adipose tissue and a decrease in plasma FFA clearance (Klop *et al.*, 2013). The increase in FFA and obesity-induced inflammation play a crucial role in the development of insulin resistance (Capurso and Capurso, 2012).

The results of current study also revealed no significant differences of glucose concentrations between obese and control group and significant increase in the level of glucose in diabetic subjects than obese and control. This conducted was agreed with several studies. However, there were no significant differences of fasting glucose concentrations between obese and control group (Zahorska-Markiewicz *et al*,2007).

Similarity, no significant variation of fasting glucose between obese and control group and high significant increase of glucose in diabetic subjects than obese and control (Mabrouk *et al.*,2013).

4.3 Correlation between parameters

The results of this study were agreed with many researches, no correlation was found between visfatin and anthropometric measurements (BMI and WHR) in diabetic group (Esteghamati *at al.,* 2011). Furthermore, no correlation was conducted between visfatin and insulin, and glucose in diabetic group (Ashoori *et al.*, 2018).

Moreover, in obese group the results were agreed with (Li *et al.*, 2013; Baltacı *et al.*,2016), since, there was positive correlation between visfatin and BMI. In addition, no correlation was observed between visfatin and insulin, and glucose (Taşkesen *et al.,* 2012; Li *et al.,* 2013).

Chapter five
Conclusions and recommendation

5.1 Conclusions

1. Visfatin levels were higher in obese and diabetic women than lean women.
2. An increase of visfatin in obesity may be another criterion in addition to an increase in glucose determining diabetes.
3. Adiponectin levels were decreased in diabetic and obese women compared to control group.
4. Leptin concentration was increased in diabetic compared to obese and control group.
5. Virtually, the occurrence of DM seems to be correlated with an increase in visfatin level and the presence of obesity appears to increase this effect.

5.2 Recommendations

1. Conducting more extensive studies on diabetes and its types (T1D and T2D) and its relationship to obesity and role of visfatin in development and diagnosis of obesity DM.

2. Making further investigations are being carried out to determine the levels of visfatin hormone in both gender and at various ages. Their relationship to obesity and role of visfatin in development and diagnosis of obesity DM.

3. Further researches are needed to see whether the regulating of adipokines concentrations, especially visfatin, leptin and adiponectin, in DM and obese patients could be a viable new technique for controlling metabolic disorders.

4. Conducting other studies on the relationship of visfatin with other hormones such as thyroid hormones or other glands.

5. We prefer to conducting genetic studies on visfatin in large population samples of obese individuals and functional characterization of the genetic variations.

References

Achari, A. E., & Jain, S. K. (2017). Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. International journal of molecular sciences, 18(6), 1321.

Abed, S. S., & Ali, H. O. A. Assessment of visfatin hormone role in induced type-1 and type-2 diabetes in an experimental rat model. Biochem. Cell, 18(2), 2413-2417,

Adami, G. F., Gradaschi, R., Andraghetti, G., Scopinaro, N., & Cordera, R. (2016). Serum leptin and adiponectin concentration in type 2 diabetes patients in the short and long term following biliopancreatic diversion. Obesity surgery, 26(10), 2442-2448.

Adeghate, E. (2008). Visfatin: structure, function and relation to diabetes mellitus and other dysfunctions. Current medicinal chemistry, 15(18), 1851-1862.

Ahmed, S. E., Maher, F. T., & Naji, N. A. (2016). Effect of leptin and oxidative stress in the blood of obese individuals. Biochem Anal Biochem, 5(288), 2161-1009.

Ali, I. M. M., Yenzeel, J. H., & Al-ansari, H. M. S. (2020). Evaluation of Oxidative Stress and LeptinLevel in Samples of Iraqi Obese Women. Iraqi Journal of Science, 1565-1570.

Alipoor, E., Hosseinzadeh, F. M., & Hosseinzadeh-Attar, M. J. (2018). Adipokines in critical illness: a review of the evidence and knowledge gaps. Biomedicine & Pharmacotherapy, 108, 1739-1750.

Al-Kaseer, E. A., Hussein, H. M. A., & Jasim, H. M. (2018). Obesity among females in Al-Sader City Baghdad, Iraq, 2017. Journal of the Faculty of Medicine, 60(2), 105-107.

Alnowihi, S. M., Al Doghaither, H. A., & Osman, N. N. (2020). Serum visfatin concentration and its relationship with sex hormones in obese Saudi women. International Journal of Health Sciences, 14(3), 9.

Alqarni,S.S.M. (2016). A review of prevalence of obesity in Saudi Arabia. J Obes Eat Disord, 2(2), 25.

Ambroszkiewicz, J., Chełchowska, M., Rowicka, G., Klemarczyk, W., Strucińska, M., & Gajewska, J. (2018). Anti-inflammatory and pro-inflammatory adipokine profiles in children on vegetarian and omnivorous diets. Nutrients, 10(9), 1241.

Amjad, S., Baig, M., Zahid, N., Tariq, S., & Rehman, R. (2019). Association between leptin, obesity, hormonal interplay and male infertility. Andrologia, 51(1), e13147.

Apostolopoulos, V., de Courten, M. P., Stojanovska, L., Blatch, G. L., Tangalakis, K., & de Courten, B. (2016). The complex immunological and inflammatory network of adipose tissue in obesity. Molecular nutrition & food research, 60(1), 43-57.

Argolo, D. F., Hudis, C. A., & Iyengar, N. M. (2018). The impact of obesity on breast cancer. Current Oncology Reports, 20(6), 47.

Arner P (2006) Editorial: Visfatin-A True or False Trail to Type 2 Diabetes Mellitus. The Journal of Clinical Endocrinology & Metabolism 91, 28–30. doi:10.1210/jc.2005-2391.

Ashoori, M., Nezhadali, M., & Shiehmorteza, M. (2018). The relationship between visfatin levels and Anthropometric parameters, and insulin resistance in women with prediabetes and type 2 diabetes. Yafteh, 20(3), 9-18.

Askarpour, M., Alizadeh, S., Hadi, A., Symonds, M. E., Miraghajani, M., Sheikhi, A., & Ghaedi, E. (2020). Effect of bariatric surgery on the circulating level of adiponectin, chemerin, plasminogen activator inhibitor-1, leptin, resistin, and visfatin: a systematic review and meta-analysis. Hormone and Metabolic Research, 52(04), 207-215.

Atkinson, M. A., Eisenbarth, G. S., & Michels, A. W. (2014). Type 1 diabetes. The Lancet, 383(9911), 69-82.

Ayman, S. S., Mohamed, T., Amira, M. J., Wafaa, I. R., & Abd-Elreheem, M. D. (2019). The Association between Resistin, Leptin and Adiponectin with Obesity and Type 2 Diabetes Mellitus. The Medical Journal of Cairo University, 87(December), 4227-4237.

Balistreri, C. R., Caruso, C., & Candore, G. (2010). The role of adipose tissue and adipokines in obesity-related inflammatory diseases. Mediators of inflammation, 2010.

Baltacı, D., Tuncel, M. C., Cetinkaya, M., Gunduz, M. T., Ozbey, Z., Admis, O., ... & Ankarali, H. (2016). Evaluation of visfatin in patients with obesity, metabolic syndrome, insulin resistance and impaired glucose tolerance; case-control study. Evaluation, 4(2), 61-67.

Barazzoni, R., Cappellari, G. G., Ragni, M., & Nisoli, E. (2018). Insulin resistance in obesity: an overview of fundamental alterations. Eating and weight disorders-studies on anorexia, bulimia and obesity, 23(2), 149-157.

Barber, T. M., Hanson, P., Weickert, M. O., & Franks, S. (2019). Obesity and polycystic ovary syndrome: implications for pathogenesis and novel management strategies. Clinical Medicine Insights: Reproductive Health, 13, 1179558119874042.

Barbosa-da-Silva S, Sarmento I B, Bargut T C L, Souza-Mello V, Aguila M B and Mandarim-DE-Lacerda C A (2014) Animal models of nutritional induction of type 2 diabetes mellitus. Int. J. Morphol. 32(1), 279-2

Bascones-Martínez, A., Matesanz Perez, P., Escribano-Bermejo, M., González Moles, M. A., Bascones Ilundain, J., & Meurman, J. H. (2011). Periodontal disease and diabetes: review of the literature.

Bayani, M., Pourali, M., & Keivan, M. (2017). Possible interaction between visfatin, periodontal infection, and other systemic diseases: A brief review of literature. European journal of dentistry, 11(3), 407.

Behboudi-Gandevani, S., Tehrani, F. R., Yarandi, R. B., Noroozzadeh, M., Hedayati, M., & Azizi, F. (2017). The association between polycystic ovary syndrome, obesity, and the serum concentration of adipokines. Journal of endocrinological investigation, 40(8), 859-866.

Berndt, J., Klöting, N., Kralisch, S., Kovacs, P., Fasshauer, M., Schön, M. R., & Blüher, M. (2005). Plasma visfatin concentrations and fat depot–specific mRNA expression in humans. Diabetes, 54(10), 2911-2916.

Berthoud, H. R., & Klein, S. (2017). Advances in obesity: Causes, consequences, and therapy. Gastroenterology, 152(7), 1635-1637.

Blüher, M. (2020). Metabolically healthy obesity. Endocrine reviews, 41(3), 405-420.

Brinton, L. A., & Trabert, B. (2018). Role of estrogen and progesterone in obesity associated gynecologic cancers. In Focus on Gynecologic Malignancies (pp. 41-61). Springer, Cham.

Capurso, C., & Capurso, A. (2012). From excess adiposity to insulin resistance: the role of free fatty acids. Vascular pharmacology, 57(2-4), 91-97.

Carbone, F., Liberale, L., Bonaventura, A., Vecchiè, A., Casula, M., Cea, M., & Nencioni, A. (2011). Regulation and function of extracellular nicotinamide phosphoribosyltransferase/visfatin. Comprehensive Physiology, 7(2), 603-621.

Chang, Y. H., Chang, D. M., Lin, K. C., Shin, S. J., & Lee, Y. J. (2011). Visfatin in overweight/obesity, type 2 diabetes mellitus, insulin resistance, metabolic syndrome and cardiovascular diseases: a meta-analysis and systemic review. Diabetes/metabolism research and reviews, 27(6), 515-527.

Chen, M. P., Chung, F. M., Chang, D. M., Tsai, J. C. R., Huang, H. F., Shin, S. J., & Lee, Y. J. (2006). Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. The Journal of Clinical Endocrinology & Metabolism, 91(1), 295-299.

Cheng, L., Wang, J., Dai, H., Duan, Y., An, Y., Shi, L., & Zhao, B. (2021). Brown and beige adipose tissue: a novel therapeutic strategy for obesity and type 2 diabetes mellitus. Adipocyte, 10(1), 48-65.

Cheng, Q., Dong, W., Qian, L., Wu, J., & Peng, Y. (2011). Visfatin inhibits apoptosis of pancreatic b-cell line, MIN6, via the mitogen-activated protein kinase/phosphoinositide 3-kinase pathway. J Mol Endocrinol, 47(1), 13-21.

Colditz, G. A., & Peterson, L. L. (2018). Obesity and cancer: evidence, impact, and future directions. Clinical chemistry, 64(1), 154-162.

Czech, M. P. (2017). Insulin action and resistance in obesity and type 2 diabetes. Nature medicine, 23(7), 804.

Dağ, Z. Ö., & Dilbaz, B. (2015). Impact of obesity on infertility in women. Journal of the Turkish German Gynecological Association, 16(2), 111.

Das, P., Bhattacharjee, D., Bandyopadhyay, S. K., Bhattacharya, G., & Singh, R. (2013). Association of obesity and leptin with insulin resistance in type 2 diabetes mellitus in Indian population. Indian J Physiol Pharmacol, 57(1), 45-50.

De Farias Lelis, D., de Freitas, D. F., Machado, A. S., Crespo, T. S., & Santos, S. H. S. (2019). Angiotensin-(1-7), adipokines and inflammation. Metabolism, 95, 36-45.

de Luis, D. A., Sagrado, M. G., Conde, R., Aller, R., Izaola, O., & Romero, E. (2008). Effect of a hypocaloric diet on serum visfatin in obese non-diabetic patients. Nutrition, 24(6), 517-521.

De Meyts, P. (2004). Insulin and its receptor: structure, function and evolution. Bioessays, 26(12), 1351-1362.

DeNicola, E., Aburizaiza, O. S., Siddique, A., Khwaja, H., & Carpenter, D. O. (2015). Obesity and public health in the Kingdom of Saudi Arabia. Reviews on environmental health, 30(3), 191-205.

De Oliveira, L. F., de Azevedo, L. G., da Mota Santana, J., de Sales, L. P. C., & Pereira-Santos, M. (2019). Obesity and overweight decreases the effect of vitamin D supplementation in adults: systematic review and meta-analysis of randomized controlled trials. Reviews in Endocrine and Metabolic Disorders, 1-10.

Divella, R., De Luca, R., Abbate, I., Naglieri, E., & Daniele, A. (2016). Obesity and cancer: the role of adipose tissue and adipo-cytokines-induced chronic inflammation. Journal of Cancer, 7(15), 2346.

Donoyama, N., Suoh, S., & Ohkoshi, N. (2018). Adiponectin Increase in Mildly Obese Women After Massage Treatment. The Journal of Alternative and Complementary Medicine, 24(7), 741-742.

Doustjalali, S. R., Sabet, N. S., AA, A., Thw, I., ZZ, N., AHA, Z., & OO, K. T. (2020). Correlation between body mass index (BMI) & waist to hip ratio (WHR) among primary school students. International Journal of Pharmaceutical Research, 12(3).

Duan, D. M., Jhang, J. Y., Wu, S., Teng, M. S., Hsu, L. A., & Ko, Y. L. (2020). Modification effect of sex and obesity on the correlation of LEP polymorphisms with leptin levels in Taiwanese obese women. Molecular genetics & genomic medicine, 8(3), e1113.

Eid M S, Gamal F N, Sorogy E and M M A-B H (2012) Is There a Relationship Between Visfatin Level and Type 2 Diabetes Mellitus in Obese and Non Obese Patients? Journal of Diabetes & Metabolism doi:10.4172/2155-6156.s11-001.

El-Shafey, E. M., El-Naggar, G. F., Al-Bedewy, M. M., & El-Sorogy, H. (2012). Is there a relationship between visfatin level and type 2 diabetes mellitus in obese and non obese patients. J Diabetes Metab S, 11, 2.

Esteghamati, A., Alamdari, A., Zandieh, A., Elahi, S., Khalilzadeh, O., Nakhjavani, M., & Meysamie, A. (2011). Serum visfatin is associated with type 2 diabetes mellitus independent of insulin resistance and obesity. Diabetes research and clinical practice, 91(2), 154-158.

Fedewa, M. V., Hathaway, E. D., Ward-Ritacco, C. L., Williams, T. D., & Dobbs, W. C. (2018). The effect of chronic exercise training on leptin: a systematic review and meta-analysis of randomized controlled trials. Sports medicine, 48(6), 1437-1450.

Feingold, K. R., & Grunfeld, C. (2018). Obesity and dyslipidemia. South Dartmouth: MDText.com.Inc.

Fukuhara, A., Matsuda, M., Nishizawa, M., Segawa, K., Tanaka, M., Kishimoto, K., & Shimomura, I. (2005). Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science, 307(5708), 426-430.

Forny-Germano, L., De Felice, F. G., & Vieira, M. N. D. N. (2019). The role of leptin and adiponectin in obesity-associated cognitive decline and Alzheimer's disease. Frontiers in neuroscience, 12, 1027.

García-Fuentes, E., García-Almeida, J. M., García-Arnés, J., García-Serrano, S., Rivas-Marín, J., Gallego-Perales, J. L., & Soriguer, F. (2007). Plasma visfatin concentrations in severely obese subjects are increased after intestinal bypass. Obesity, 15(10), 2391-2395.

Gariballa, S., Alkaabi, J., Yasin, J., & Al Essa, A. (2019). Total adiponectin in overweight and obese subjects and its response to visceral fat loss. BMC endocrine disorders, 19(1), 1-6.

Ghadge, A. A., & Khaire, A. A. (2019). Leptin as a predictive marker for metabolic syndrome. Cytokine, 121, 154735.

Ghanbarzadeh, M., & Omidi, M. (2017). The effects of physical activity on serum visfatin level: A literature review. International Journal of Basic Science in Medicine, 2(2), 83-89.

Gomez-de-Regil, L., Avila-Nava, A., Gutierrez-Solis, A. L., & Lugo, R. (2020). Mobile Apps for the Management of Comorbid Overweight/Obesity and Depression/Anxiety: A Systematic Review. Journal of healthcare engineering.

Gong, Z., Zhang, X., Su, K., Jiang, R., Sun, Z., Chen, W., & Dutta, P. (2019). Deficiency in AIM2 induces inflammation and adipogenesis in white

adipose tissue leading to obesity and insulin resistance. Diabetologia, 62(12), 2325-2339.

Goossens, G. H. (2017). The metabolic phenotype in obesity: fat mass, body fat distribution, and adipose tissue function. Obesity facts, 10(3), 207-215.

Gui, Y., Pan, Q., Chen, X., Xu, S., Luo, X., & Chen, L. (2017). The association between obesity related adipokines and risk of breast cancer: a meta-analysis. Oncotarget, 8(43), 75389.

Gunaid, A. A. (2012). Obesity, overweight and underweight among adults in an urban community in Yemen. EMHJ-Eastern Mediterranean Health Journal, 18 (12), 1187-1193, 2012.

Gupta, G., Wadhwa, R., Pandey, P., Singh, S. K., Gulati, M., Sajita, S., & Dua, K. (2020). Obesity and diabetes: pathophysiology of obesity-induced hyperglycemia and insulin resistance. In Pathophysiology of obesity-induced health complications (pp. 81-97). Springer, Cham.

Haider, D. G., Schindler, K., Schaller, G., Prager, G., Wolzt, M., & Ludvik, B. (2006). Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. The Journal of Clinical Endocrinology & Metabolism, 91(4), 1578-1581.

Hales, C. M., Carroll, M. D., Fryar, C. D., & Ogden, C. L. (2020). Prevalence of obesity and severe obesity among adults: United States, 2017–2018.

Harding, J. L., Pavkov, M. E., Magliano, D. J., Shaw, J. E., & Gregg, E. W. (2019). Global trends in diabetes complications: a review of current evidence. Diabetologia, 62(1), 3-16.

Heo, Y. J., Choi, S. E., Jeon, J. Y., Han, S. J., Kim, D. J., Kang, Y., & Kim, H. J. (2019). Visfatin induces inflammation and insulin resistance via the

NF-κB and STAT3 signaling pathways in hepatocytes. Journal of diabetes research, 2019.

Hetta, H. F., Ez-Eldeen, M. E., Mohamed, G. A., Gaber, M. A., ElBadre, H. M., Ahmed, E. A., & Ahmad, M. (2018). Visfatin serum levels in obese type 2 diabetic patients: relation to proinflammatory cytokines and insulin resistance. Egypt J Immunol, 25(2), 141-151.

Hofsø, D., Ueland, T., Hager, H., Jenssen, T., Bollerslev, J., Godang, K., & Hjelmesæth, J. (2009). Inflammatory mediators in morbidly obese subjects: associations with glucose abnormalities and changes after oral glucose. European journal of endocrinology, 161(3), 451-458.

Hug, C., & Lodish, H. F. (2005). Visfatin: a new adipokine. Science, 307(5708), 366-367.

Jian, W. X., Luo, T. H., Gu, Y. Y., Zhang, H. L., Zheng, S., Dai, M., & Luo, M. (2006). The visfatin gene is associated with glucose and lipid metabolism in a Chinese population. Diabetic Medicine, 23(9), 967-973.

Jiménez, J. M., Carbajo, M. A., López, M., Cao, M. J., Rúiz-Tovar, J., García, S., & Castro, M. J. (2020). Changes in Lipid Profile, Body Weight Variables and Cardiovascular Risk in Obese Patients Undergoing One-Anastomosis Gastric Bypass. International Journal of Environmental Research and Public Health, 17(16), 5858.

Jung, U. J., & Choi, M. S. (2014). Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. International journal of molecular sciences, 15(4), 6184-6223.

Kamińska, A., Kopczyńska, E., Bronisz, A., Żmudzińska, M., Bieliński, M., Borkowska, A., & Junik, R. (2010). An evaluation of visfatin levels in obese subjects. Endokrynologia Polska, 61(2), 169-173.

Kang Y S, Song H K, Lee M H, Ko G J, Han J Y and Han S Y (2010) Visfatin is upregulated in type-2 diabetic rats and targets renal cells. Kidney International 78, 170–81. doi:10.1038/ki.2010.98.

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B. J., & Lernmark, Å. (2017). Type 1 diabetes mellitus. Nature reviews Disease primers, 3(1), 1-17.

Khoramipour, K., Chamari, K., Hekmatikar, A. A., Ziyaiyan, A., Taherkhani, S., Elguindy, N. M., & Bragazzi, N. L. (2021). Adiponectin: Structure, physiological functions, role in diseases, and effects of nutrition. Nutrients, 13(4), 1180.

Kim, B. M., Lee, B. E., Park, H. S., Kim, Y. J., Suh, Y. J., Kim, J. Y., & Ha, E. H. (2016). Long working hours and overweight and obesity in working adults. Annals of Occupational and Environmental Medicine, 28(1), 36.

Kim, M. K., Lee, J. H., Kim, H., Park, S. J., Kim, S. H., Kang, G. B., & Eom, S. H. (2006). Crystal structure of visfatin/pre-B cell colony-enhancing factor 1/nicotinamide phosphoribosyltransferase, free and in complex with the anti-cancer agent FK-866. Journal of molecular biology, 362(1), 66-77.

Kim, S. H., & Park, M. J. (2017). Effects of growth hormone on glucose metabolism and insulin resistance in human. Annals of pediatric endocrinology & metabolism, 22(3), 145.

Klop, B., Elte, J. W. F., & Cabezas, M. C. (2013). Dyslipidemia in obesity: mechanisms and potential targets. Nutrients, 5(4), 1218-1240.

Koch, A., Weiskirchen, R., Krusch, A., Bruensing, J., Buendgens, L., Herbers, U., & Tacke, F. (2018). Visfatin serum levels predict mortality in critically ill patients. Disease Markers, 2018.

Kocot, J., Dziemidok, P., Kiełczykowska, M., Hordyjewska, A., Szcześniak, G., & Musik, I. (2017). Adipokine profile in patients with type 2 diabetes depends on degree of obesity. Medical science monitor: international medical journal of experimental and clinical research, 23, 4995.

Koka, S., Xia, M., Zhang, C., Zhang, Y., Li, P. L., & Boini, K. M. (2019). Podocyte NLRP3 inflammasome activation and formation by adipokine visfatin. Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology, 53(2), 355.

Kong, Y., Zhang, S., Wu, R., Su, X., Peng, D., Zhao, M., & Su, Y. (2019). New insights into different adipokines in linking the pathophysiology of obesity and psoriasis. Lipids in health and disease, 18(1), 171.

Kumari, R., Kumar, S., & Kant, R. (2019). An update on metabolic syndrome: metabolic risk markers and adipokines in the development of metabolic syndrome. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 13(4), 2409-2417.

Łagowska, K., & Jeszka, J. (2011). Adipose tissue as an endocrine organ. Medicina Sportiva, 15(3), 140-6.

Landecho, M. F., Tuero, C., Valentí, V., Bilbao, I., de la Higuera, M., & Frühbeck, G. (2019). Relevance of leptin and other adipokines in obesity-associated cardiovascular risk. Nutrients, 11(11), 2664.

Larsen, M. A., Isaksen, V. T., Paulssen, E. J., Goll, R., & Florholmen, J. R. (2019). Postprandial leptin and adiponectin in response to sugar and fat in obese and normal weight individuals. Endocrine, 66(3), 517-525.

Lee, M. J., Wu, Y., & Fried, S. K. (2013). Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Molecular aspects of medicine, 34(1), 1-11.

Lee, M. W., Lee, M., & Oh, K. J. (2019). Adipose tissue-derived signatures for obesity and type 2 diabetes: adipokines, batokines and microRNAs. Journal of clinical medicine, 8(6), 854.

Li, L., Yang, G., Li, Q., Tang, Y., Yang, M., Yang, H., & Li, K. (2006). Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. Experimental and clinical endocrinology & diabetes, 114(10), 544-548.

Li, R. Z., Ma, X. Y., Hu, X. F., Kang, S. X., Chen, S. K., Cianflone, K., & Lu, H. L. (2013). Elevated visfatin levels in obese children are related to proinflammatory factors. Journal of Pediatric Endocrinology and Metabolism, 26(1-2), 111-118.

Lima, R. P. A., Do Nascimento, R. A. F., Luna, R. C. P., Persuhn, D. C., da Silva, A. S., Gonçalves, M. D. C. R., & Vidal, H. (2017). Effect of a diet containing folate and hazelnut oil capsule on the methylation level of the ADRB3 gene, lipid profile and oxidative stress in overweight or obese women. Clinical epigenetics, 9(1), 110.

Liu, W., Zhou, X., Li, Y., Zhang, S., Cai, X., Zhang, R., & Ji, L. (2020). Serum leptin, resistin, and adiponectin levels in obese and non-obese patients with newly diagnosed type 2 diabetes mellitus: a population-based study. Medicine, 99(6).

Mabrouk, R., Ghareeb, H., Shehab, A., Omar, K., El-Kabarity, R. H., Soliman, D. A., & Mohamed, N. A. (2013). Serum visfatin, resistin and IL-18 in a group of Egyptian obese diabetic and non diabetic individuals. Egypt J Immunol, 20(1), 1-11.

Makhdoumi, P., Zarif-Yeganeh, M., & Hedayati, M. (2014). Physical Activity and Obesity Related Hormones. Zahedan J Res Med Sci. 2014;16(8):6-11.

Mancuso, P., & Bouchard, B. (2019). The impact of aging on adipose function and adipokine synthesis. Frontiers in endocrinology, 10, 137.

Mansour, A. A., Al-Maliky, A. A., & Salih, M. (2012). Population overweight and obesity trends of eight years in Basrah, Iraq. Epidemiol, 2(110), 2161-1165.

Martínez Larrad, M. T., Corbatón Anchuelo, A., Fernández Pérez, C., Pérez Barba, M., Lazcano Redondo, Y., Serrano Ríos, M., & Segovia Insulin Resistance Study Group (SIRSG). (2016). Obesity and cardiovascular risk: Variations in visfatin gene can modify the obesity associated cardiovascular risk. Results from the Segovia population based-study. Spain. PloS one, 11(5), e0153976.

Meyer, L. K., Ciaraldi, T. P., Henry, R. R., Wittgrove, A. C., & Phillips, S. A. (2013). Adipose tissue depot and cell size dependency of adiponectin synthesis and secretion in human obesity. Adipocyte, 2(4), 217-226.

Mir, E., & Fathi, M. (2018). Changes in Plasma Visfatin and Insulin Resistance Index in Obese Women with Type 2 Diabetes after Pilates Exercise. Journal of Health and Care, 20(1), 30-39.

Moravveji, A., Sayyah, M., Shamsnia, E., & Vakili, Z. (2019). Comparing the prolonged effect of interval versus continuous aerobic exercise on blood

inflammatory marker of Visfatin level and body mass index of sedentary overweigh/fat female college students. AIMS public health, 6(4), 568.

Nadulska, A., Szwajgier, D., & Opielak, G. (2017). Obesity and metabolic syndrome. An innovative treatment of endometriosis with the use of Plasma Technology-case report, 35.

Naz, R., Tauqeer, S., Bibi, Y., & Ayub, M. (2017). Level of visfatin in obese and diabetic Balb/c mice. Pak J Physiol, 13(3), 36-8.

Niu, G., Li, J., Wang, H., Ren, Y., & Bai, J. (2016). Associations of A-FABP with anthropometric and metabolic indices and inflammatory cytokines in obese patients with newly diagnosed type 2 diabetes. BioMed research international, 2016.

Nurdiantami, Y., Watanabe, K., Tanaka, E., Pradono, J., & Anme, T. (2018). Association of general and central obesity with hypertension. Clinical nutrition, 37(4), 1259-1263.

Ogurtsova, K., da Rocha Fernandes, J. D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N. H., & Makaroff, L. E. (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes research and clinical practice, 128, 40-50.

Olokoba, A. B., Obateru, O. A., & Olokoba, L. B. (2012). Type 2 diabetes mellitus: a review of current trends. Oman medical journal, 27(4), 269.

Olszanecka-Glinianowicz, M., Kocełak, P., Nylec, M., Chudek, J., & Zahorska-Markiewicz, B. (2012). Circulating visfatin level and visfatin/insulin ratio in obese women with metabolic syndrome. Archives of medical science: AMS, 8(2), 214.

Omer, T. (2020). The causes of obesity: an in-depth review. Adv Obes Weight Manag Control, 10(4), 90-94.

Ormazabal, V., Nair, S., Elfeky, O., Aguayo, C., Salomon, C., & Zuñiga, F. A. (2018). Association between insulin resistance and the development of cardiovascular disease. Cardiovascular diabetology, 17(1), 1-14.

Ouchi, N., Parker, J. L., Lugus, J. J., & Walsh, K. (2011). Adipokines in inflammation and metabolic disease. Nature reviews immunology, 11(2), 85-97.

Pagano, C., Pilon, C., Olivieri, M., Mason, P., Fabris, R., Serra, R., & Vettor, R. (2006). Reduced plasma visfatin/pre-B cell colony-enhancing factor in obesity is not related to insulin resistance in humans. The Journal of Clinical Endocrinology & Metabolism, 91(8), 3165-3170.

Parida, S., Siddharth, S., & Sharma, D. (2019). Adiponectin, obesity, and cancer: clash of the bigwigs in health and disease. International journal of molecular sciences, 20(10), 2519.

Pereira, S. S., & Alvarez-Leite, J. I. (2014). Adipokines: biological functions and metabolically healthy obese profile. Journal of Receptor, Ligand and Channel Research, 7, 15-25.

Peterson, C. M., Thomas, D. M., Blackburn, G. L., & Heymsfield, S. B. (2016). Universal equation for estimating ideal body weight and body weight at any BMI. The American journal of clinical nutrition, 103(5), 1197-1203.

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. Physiological reviews, 98(4), 2133-2223.

Pham, D. V., & Park, P. H. (2020). Recent insights on modulation of inflammasomes by adipokines: a critical event for the pathogenesis of obesity and metabolism-associated diseases. Archives of Pharmacal Research, 1-20.

Picu, A., Petcu, L., Ştefan, S., Mitu, M., Lixandru, D., Ionescu-Tîrgovişte, C., & Chifiriuc, M. C. (2017). Markers of oxidative stress and antioxidant defense in Romanian patients with type 2 diabetes mellitus and obesity. Molecules, 22(5), 714.

Rajkovic, N., Zamaklar, M., Lalic, K., Jotic, A., Lukic, L., Milicic, T., & Lalic, N. M. (2014). Relationship between obesity, adipocytokines and inflammatory markers in type 2 diabetes: relevance for cardiovascular risk prevention. International journal of environmental research and public health, 11(4), 4049-4065.

Rashad, N. M., Abd-Elrahman, M. A., Amal, S., & Amin, A. I. (2018). Serum visfatin as predictive marker of cardiometabolic risk in women with polycystic ovary syndrome. Middle East Fertility Society Journal, 23(4), 335-341.

Rathnayake, N., Alwis, G., Lenora, J., & Lekamwasam, S. (2020). Optimal Cutoff Values for Anthropometric Adiposity Measures of Sri Lankan Adult Women. Journal of Obesity, 2020.

Revollo, J. R., Grimm, A. A., & Imai, S. I. (2007). The regulation of nicotinamide adenine dinucleotide biosynthesis by Nampt/PBEF/visfatin in mammals. Current opinion in gastroenterology, 23(2), 164-170.

Rodríguez, A., Becerril, S., Hernández-Pardos, A. W., & Frühbeck, G. (2020). Adipose tissue depot differences in adipokines and effects on skeletal and cardiac muscle. Current opinion in pharmacology, 52, 1-8.

Saeidi, A., Haghighi, M. M., Kolahdouzi, S., Daraei, A., Abderrahmane, A. B., Essop, M. F., & Zouhal, H. (2021). The effects of physical activity on

adipokines in individuals with overweight/obesity across the lifespan: A narrative review. Obesity reviews, 22(1), e13090.

Saltiel, A. R. (2021). Insulin signaling in health and disease. The Journal of Clinical Investigation, 131(1).

Schetz, M., De Jong, A., Deane, A. M., Druml, W., Hemelaar, P., Pelosi, P., ... & Jaber, S. (2019). Obesity in the critically ill: a narrative review. Intensive care medicine, 1-13.

Schultz A, Neil D, Aguila M B and Mandarim-de-Lacerda C A (2013) Hepatic adverse effects of fructose consumption independent of overweight/obesity. Int. J. Mol. Sci. 14(11), 21873-21886.

Seth, M., Biswas, R., Ganguly, S., Chakrabarti, N., & Chaudhuri, A. G. (2021). Leptin and obesity. Physiology International, 107(4), 455-468.

Shaker, O., El-Shehaby, A., Zakaria, A., Mostafa, N., Talaat, S., Katsiki, N., & Mikhailidis, D. P. (2011). Plasma visfatin and retinol binding protein-4 levels in patients with type 2 diabetes mellitus and their relationship to adiposity and fatty liver. Clinical biochemistry, 44(17-18), 1457-1463.

Shimobayashi, M., Albert, V., Woelnerhanssen, B., Frei, I. C., Weissenberger, D., Meyer-Gerspach, A. C., & Hall, M. N. (2018). Insulin resistance causes inflammation in adipose tissue. The Journal of clinical investigation, 128(4), 1538-1550.

Shpilman, M., Hollander-Cohen, L., Ventura, T., Gertler, A., & Levavi-Sivan, B. (2014). Production, gene structure and characterization of two orthologs of leptin and a leptin receptor in tilapia. General and comparative endocrinology, 207, 74-85.

Slattery, D., Amiel, S. A., & Choudhary, P. (2018). Optimal prandial timing of bolus insulin in diabetes management: a review. Diabetic Medicine, 35(3), 306-316.

Sommer, G., Garten, A., Petzold, S., Beck-Sickinger, A. G., Blüher, M., Stumvoll, M., & Fasshauer, M. (2008). Visfatin/PBEF/Nampt: structure, regulation and potential function of a novel adipokine. Clinical Science, 115(1), 13-23.

Sonoli, S. S., Shivprasad, S., Prasad, C. V., Patil, A. B., Desai, P. B., & Somannavar, M. S. (2011). Visfatin-a review. Eur Rev Med Pharmacol Sci, 15(1), 9-14.

Stastny, J., Bienertova-Vasku, J., & Vasku, A. (2012). Visfatin and its role in obesity development. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 6(2), 120-124.

Stoica, L., Gadea, R., Navolan, D. B., Lazar, F., Duta, C., Stoian, D., ... & Dobrescu, A. (2021). Plasma ghrelin, adiponectin and leptin levels in obese rats with type 2 diabetes mellitus after sleeve gastrectomy and gastric plication. Experimental and Therapeutic Medicine, 21(3), 1-1.

Straub, L. G., & Scherer, P. E. (2019). Metabolic messengers: adiponectin. Nature Metabolism, 1(3), 334-339.

Su, X., & Peng, D. (2020). Emerging functions of adipokines in linking the development of obesity and cardiovascular diseases. Molecular Biology Reports, 1-16.

Susilowati, R., Sulistyoningrum, D. C., Witari, N. P., Huriyati, E., Luglio, H. F., & Julia, M. (2016). Sexual dimorphism in interleukin 17A and adipocytokines and their association with insulin resistance among

obese adolescents in Yogyakarta, Indonesia. Asia Pacific journal of clinical nutrition, 25(Suppl 1), S93-S101.

Taşkesen, D., Kirel, B., & Us, T. (2012). Serum visfatin levels, adiposity and glucose metabolism in obese adolescents. Journal of Clinical Research in Pediatric Endocrinology, 4(2), 76.

Thomas, D., & Apovian, C. (2017). Macrophage functions in lean and obese adipose tissue. Metabolism, 72, 120-143.

Torres, S., Fabersani, E., Marquez, A., & Gauffin-Cano, P. (2019). Adipose tissue inflammation and metabolic syndrome. The proactive role of probiotics. European journal of nutrition, 58(1), 27-43.

Turki, S. G., AL-Naqeeb, A. A., Muhammed, Z. J., & Ahmed, W. H. (2020). Evaluation of Serum Leptin, Interlukein–6 and some Biochemical Parameters in Iraq obese Adult Patients. Medico Legal Update, 20(3), 996-1002.

Udia M P, Takem L P, Ufot U F, Antai A B and Owu D U (2016) Insulin and alpha amylase levels in alloxan-induced diabetic rats and the effect of Rothmannia hispida (K. Schum) Fagerl leaf extract. The Journal of Phytopharmacology 5(1), 1-5.

Unamuno, X., Gómez-Ambrosi, J., Rodríguez, A., Becerril, S., Frühbeck, G., & Catalán, V. (2018). Adipokine dysregulation and adipose tissue inflammation in human obesity. European journal of clinical investigation, 48(9), e12997.

Upadhyay, J., Farr, O., Perakakis, N., Ghaly, W., & Mantzoros, C. (2018). Obesity as a disease. Medical Clinics, 102(1), 13-33.

Urbanavičius, V., Abalikšta, T., Brimas, G., Abraitienė, A., Gogelienė, L., & Strupas, K. (2013). Comparison of changes in blood glucose, insulin

resistance indices, and adipokine levels in diabetic and nondiabetic subjects with morbid obesity after laparoscopic adjustable gastric banding. Medicina, 49(1), 2.

Vakilian, M., Tahamtani, Y., & Ghaedi, K. (2019). A review on insulin trafficking and exocytosis. Gene, 706, 52-61.

Vasilescu, R., Ifrim, S., & Ionescu-Tirgoviste, C. (2011). Relationship between plasma adipokines, inflammation, insulin resistance and subclinical atherosclerosis in newly diagnosed type 2 diabetes. J Diab Mell, 1(2), 17-25.

Vekic, J., Zeljkovic, A., Stefanovic, A., Jelic-Ivanovic, Z., & Spasojevic-Kalimanovska, V. (2019). Obesity and dyslipidemia. Metabolism, 92, 71-81.

Vicente, S. E. D. C. F., Corgosinho, F. C., da Silveira Campos, R. M., Masquio, D. C. L., e Silva, L. O., Kravchychyn, A. C. P., & Dâmaso, A. R. (2017). The impact of adiponectin levels on biomarkers of inflammation among adolescents with obesity. Obesity Medicine, 5, 4-10.

Walker, B. B., Shashank, A., Gasevic, D., Schuurman, N., Poirier, P., Teo, K. ,& Lear, S. A. (2020). The local food environment and obesity: evidence from three cities. Obesity, 28(1), 40-45.

Wang, H., & Peng, D. Q. (2011). New insights into the mechanism of low high-density lipoprotein cholesterol in obesity. Lipids in health and disease, 10(1), 1-10.

Wang, Z. V., & Scherer, P. E. (2016). Adiponectin, the past two decades. Journal of molecular cell biology, 8(2), 93-100.

Wharton, S., Raiber, L., Serodio, K. J., Lee, J., & Christensen, R. A. (2018). Medications that cause weight gain and alternatives in Canada: a

narrative review. Diabetes, metabolic syndrome and obesity: targets and therapy, 11, 427.

Wnuk, A., Stangret, A., Wątroba, M., Płatek, A. E., Skoda, M., Cendrowski, K., & Szukiewicz, D. (2020). Can adipokine visfatin be a novel marker of pregnancy-related disorders in women with obesity? Obesity Reviews, 21(7), e13022.

Wu, M. H., Tsai, C. H., Huang, Y. L., Fong, Y. C., & Tang, C. H. (2018). Visfatin Promotes IL-6 and TNF-α Production in Human Synovial Fibroblasts by Repressing miR-199a-5p through ERK, p38 and JNK Signaling Pathways. International journal of molecular sciences, 19(1), 190.

Yamauchi, T., Iwabu, M., Okada-Iwabu, M., & Kadowaki, T. (2014). Adiponectin receptors: a review of their structure, function and how they work. Best practice & research Clinical endocrinology & metabolism, 28(1), 15-23.

Yanai, H., & Yoshida, H. (2019). Beneficial effects of adiponectin on glucose and lipid metabolism and atherosclerotic progression: mechanisms and perspectives. International journal of molecular sciences, 20(5), 1190.

Yu, H., Chhabra, K. H., Thompson, Z., Jones, G. L., Kiran, S., Shangguan, G., & Low, M. J. (2020). Hypothalamic POMC deficiency increases circulating adiponectin despite obesity. Molecular metabolism, 35, 100957.

Yu, F., Li, J., Huang, Q., & Cai, H. (2018). Increased peripheral blood visfatin concentrations may be a risk marker of coronary artery disease: a meta-analysis of observational studies. Angiology, 69(9), 825-834.

Zamora, M. (2019). Elucidating the Potential Mechanism of Visfatin, an Adipocytokine with Insulin-like Properties.

Zahorska-Markiewicz, B., Olszanecka-Glinianowicz, M., Janowska, J., Kocełak, P., Semik-Grabarczyk, E., Holecki, M., & Skorupa, A. (2007). Serum concentration of visfatin in obese women. Metabolism, 56(8), 1131-1134.

Zatterale, F., Longo, M., Naderi, J., Raciti, G. A., Desiderio, A., Miele, C., & Beguinot, F. (2020). Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes. Frontiers in physiology, 10, 1607.

Zhang, H., & Sairam, M. R. (2014). Sex hormone imbalances and adipose tissue dysfunction impacting on metabolic syndrome; a paradigm for the discovery of novel adipokines. Hormone molecular biology and clinical investigation, 17(2), 89-97.

Zhang, Y., Huo, Y., He, W., Liu, S., Li, H., & Li, L. (2019). Visfatin is regulated by interleukin-6 and affected by the PPAR-γ pathway in BeWo cells. Molecular medicine reports, 19(1), 400-406.

Appendixes

Appendix 1 Questionary of study.

A questionnaire for study

No: Date:

Name: Age:

Address: Mobile:

Education level: Marital status:

Measurements:

Body mass	Waist circumference
Height	Hip circumference
BMI	WHR

Health state:

Pregnancy:
Medication:
Chronic diseases:
Endocrine diseases:
Irregular menstrual cycle:
Others:

Hormones:

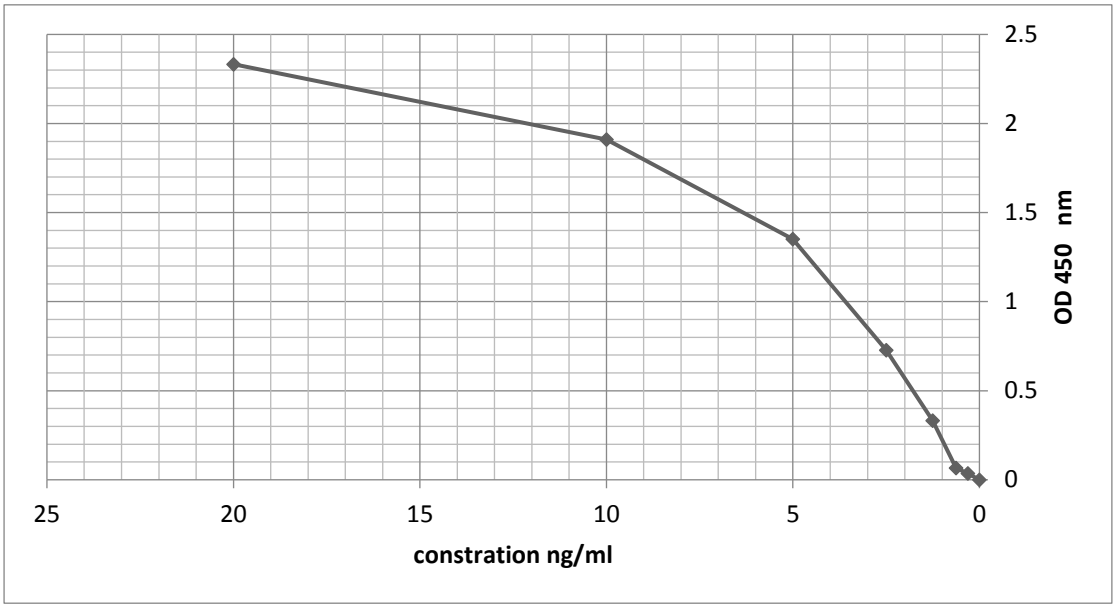
Visfatin
Adiponectin
Leptin
Insulin

Biochemical:

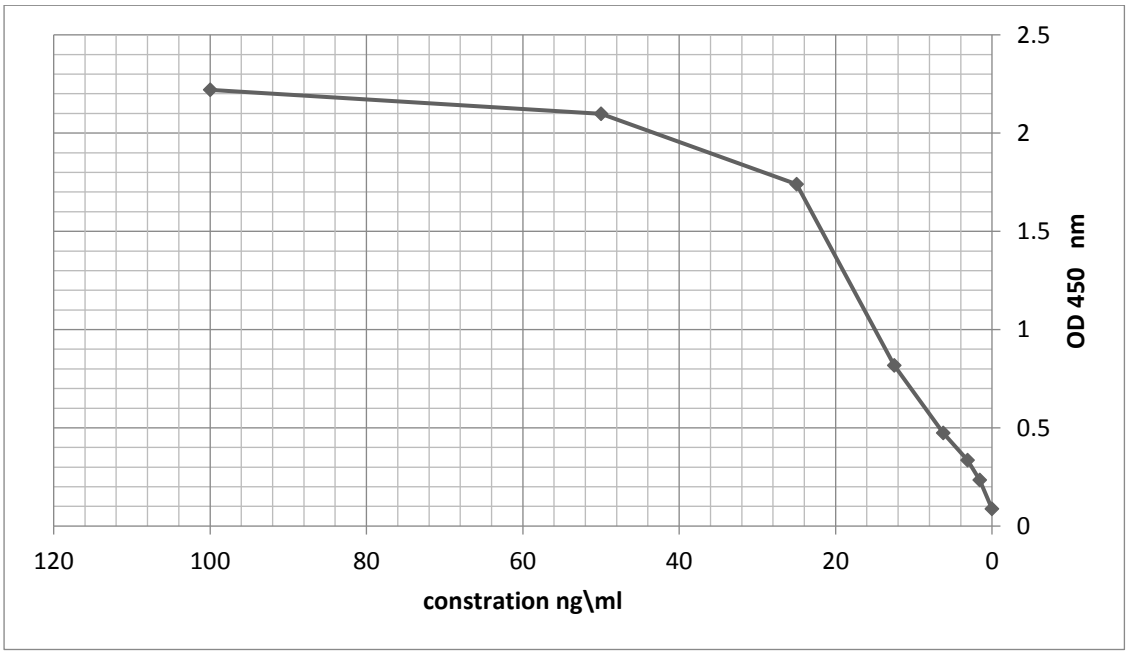
Cholesterol	LDL
Triglycerides	HDL
Glucose	VLDL

NOTICE Signature

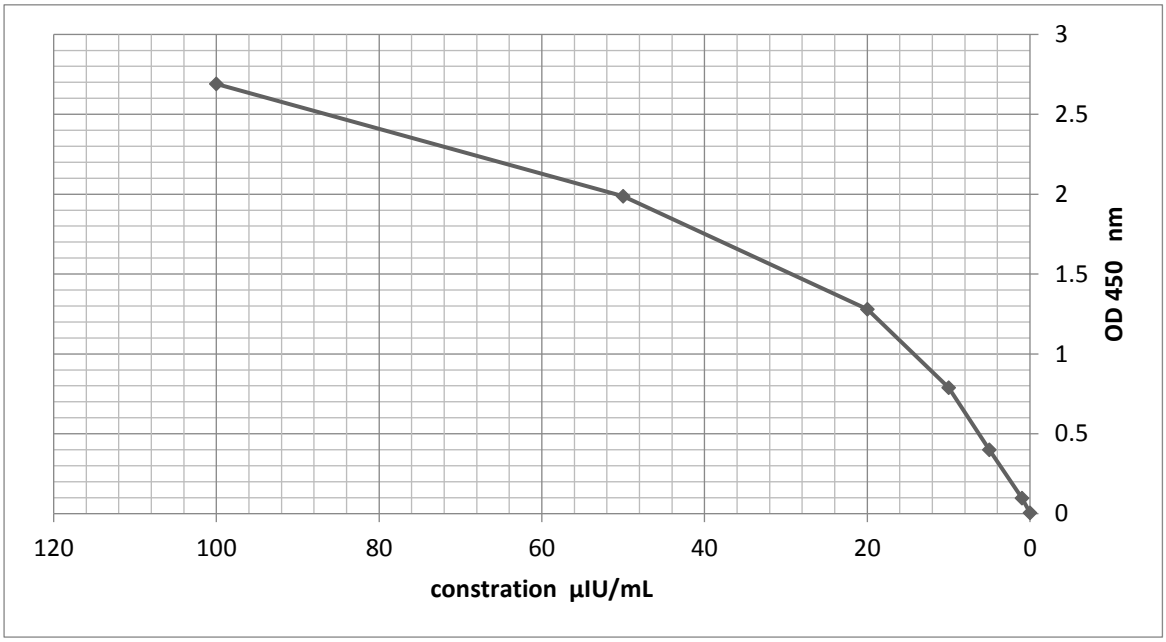
Appendix 2 Elisa visfatin standard Curve.



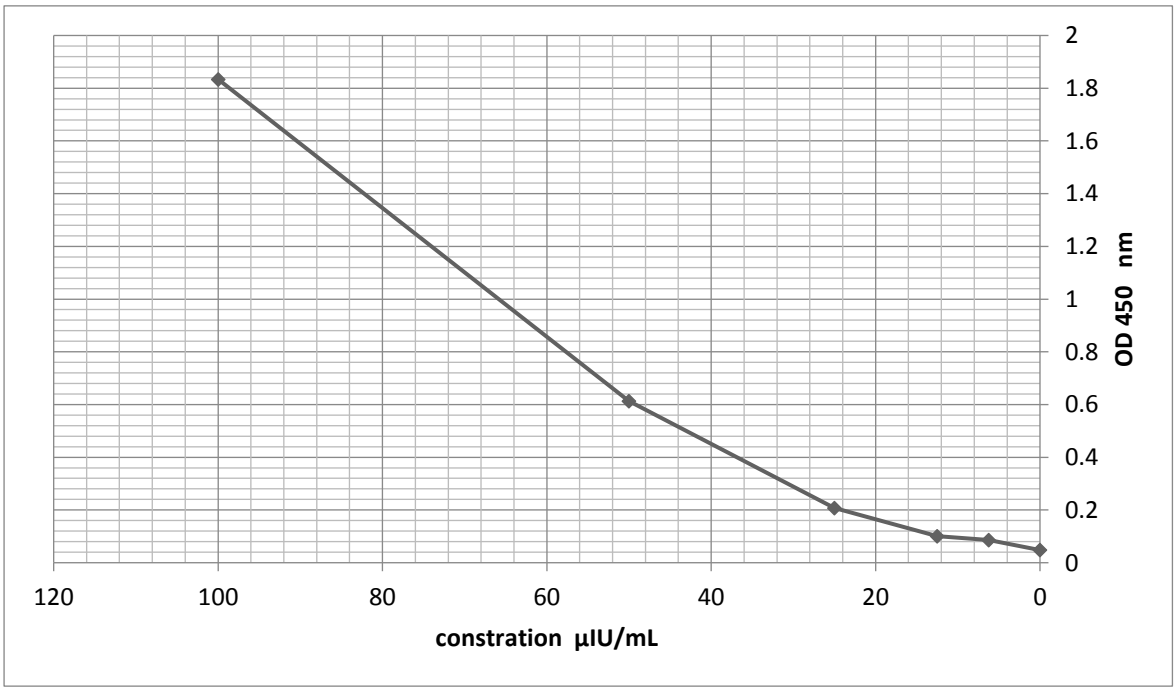
Appendix 3 Elisa adiponectin standard Curve.



Appendix 4 Elisa leptin standard Curve.



Appendix 5 Elisa insulin standard Curve.



Appendix 6 The correlation between parameters in diabetic group.

		BMI	WHR	Vis.	Adipo.	Lep.	Insulin	Glucose	HDL	T.C	LDL	T.G	VLDL
BMI	R	1.00	-.01	.08	-.13	-.08	.17	-.395	.18	-.15	-.20	-.18	-.18
	Sig.	.	.95	.68	.51	.67	.38	.04	.35	.45	.30	.37	.37
WHR	R	-.01	1.00	-.20	.168	.14	-.17	.29	-.07	.21	.18	.27	.27
	Sig.	.945	.	.301	.391	.480	.372	.131	.732	.277	.363	.167	.167
Vis.	R	.082	-.20	1.00	.154	-.165	.296	-.012	.342	.016	-.155	-.01	-.01
	Sig.	.679	.301	.	.433	.401	.126	.954	.075	.937	.430	.974	.974
Adipo.	R	-.130	.168	.154	1.000	.239	.273	-.111	-.145	.093	.173	.152	.152
	Sig.	.511	.391	.433	.	.221	.159	.575	.461	.638	.378	.441	.441
Lep.	R	-.084	.139	-.16	.239	1.000	-.017	.003	-.302	.047	.088	.182	.182
	Sig.	.671	.480	.401	.221	.	.932	.987	.118	.812	.657	.355	.355
Insulin	R	.171	-.17	.296	.273	-.017	1.000	-.125	.256	.120	.119	.005	.005
	Sig.	.383	.372	.126	.159	.932	.	.525	.189	.543	.548	.978	.978
Glucose	R	-.395	.292	-.01	-.111	.003	-.125	1.000	-.276	.199	.079	.237	.237
	Sig.	.04	.131	.954	.575	.987	.525	.	.155	.311	.690	.224	.224
HDL	R	.185	-.07	.342	-.145	-.302	.256	-.276	1.000	.282	.319	-.29	-.29
	Sig.	.347	.732	.075	.461	.118	.189	.155	.	.146	.098	.138	.138
T.C	R	-.150	.213	.016	.093	.047	.120	.199	.282	1.00	.884	.363	.363
	Sig.	.446	.277	.937	.638	.812	.543	.311	.146	.	0.0001	.058	.058
LDL	R	-.202	.179	-.15	.173	.088	.119	.079	.319	.884	1.000	.257	.257
	Sig.	.303	.363	.430	.378	.657	.548	.690	.098	0.0001	.	.186	.186
T.G	R	-.176	.269	-.01	.152	.182	.005	.237	-.287	.363	.257	1.00	1.00
	Sig.	.371	.167	.974	.441	.355	.978	.224	.138	.058	.186	.	.
VLDL	R	-.176	.269	-.01	.152	.182	.005	.237	-.287	.363	.257	1.00	1.00
	Sig.	.371	.167	.974	.441	.355	.978	.224	.138	.058	.186	.	.

Appendix 7 The correlation between parameters in obese group.

		BMI	WHR	Vis.	Adipo.	Lep.	Insulin	Glucose	HDL	T.C.	LDL	T.G	VLDL
BMI	R	1.000	.117	.404	-.067	.274	.010	.043	-.219	.115	.147	.062	.062
	Sig.	.	.540	.03	.726	.142	.958	.824	.245	.544	.439	.745	.745
WHR	R	.117	1.00	-.01	.003	.331	-.186	-.126	-.066	.251	.247	.169	.169
	Sig.	.540	.	.939	.985	.074	.325	.508	.729	.180	.188	.373	.373
Vis.	R	.404	-.01	1.00	.076	.226	.111	.053	-.174	-.02	.004	.093	.093
	Sig.	.03	.939	.	.691	.230	.559	.779	.357	.904	.985	.626	.626
Adipo.	R	-.067	.003	.076	1.000	-.059	.175	-.081	-.098	-.05	.020	-.02	-.02
	Sig.	.726	.985	.691	.	.755	.354	.671	.607	.795	.914	.933	.933
Lep.	R	.274	.331	.226	-.059	1.000	.057	.120	-.316	-.03	.098	-.16	-.16
	Sig.	.142	.074	.230	.755	.	.764	.526	.089	.887	.606	.385	.385
Insulin	R	.010	-.19	.111	.175	.057	1.000	-.019	-.322	.029	.137	.029	.029
	Sig.	.958	.325	.559	.354	.764	.	.920	.082	.878	.471	.878	.878
Glucose	R	.043	-.13	.053	-.081	.120	-.019	1.000	-.061	-.21	-.19	-.15	-.15
	Sig.	.824	.508	.779	.671	.526	.920	.	.750	.263	.320	.403	.403
HDL	R	-.219	-.07	-.17	-.098	-.316	-.322	-.061	1.000	.128	.013	-.56	-.56
	Sig.	.245	.729	.357	.607	.089	.082	.750	.	.501	.946	.001	.001
T.C	R	.115	.251	-.02	-.050	-.027	.029	-.211	.128	1.00	.948	.109	.109
	Sig.	.544	.180	.904	.795	.887	.878	.263	.501	.	0.0001	.566	.566
LDL	R	.147	.247	.004	.020	.098	.137	-.188	.013	.948	1.000	.051	.051
	Sig.	.439	.188	.985	.914	.606	.471	.320	.946	0.0001	.	.788	.788
T.G	R	.062	.169	.093	-.016	-.164	.029	-.158	-.567	.109	.051	1.00	1.00
	Sig.	.745	.373	.626	.933	.385	.878	.403	.001	.566	.788	.	.
	N	30	30	30	30	30	30	30	30	30	30	30	30
VLDL	R	.062	.169	.093	-.016	-.164	.029	-.158	-.567	.109	.051	1.00	1.00
	Sig.	.745	.373	.626	.933	.385	.878	.403	.001	.566	.788	.	.

الخلاصة

الفزفاتين هو عبارة عن اديبوكين يفرز بشكل رئيسي عن طريق الأنسجة الدهنية الحشوية وقد ارتبط بالسمنة. يمتلك الفزفاتين خصائص تحاكي عمل هرمون الانسولين. كذلك يقوم الفزفاتين بدور مهم في تطور العديد من الامراض المزمنة والالتهابات.

كان الهدف من الدراسة هو تقييم تراكيز مصل الفزفاتين والأديبونكتين واللبتين في نساء البصرة من أوزان مختلفة لتحديد العلاقة مع السمنة وداء السكري لدى النساء في مدينة البصرة.

في هذه الدراسة ، تم اختيار 88 امرأة من مختلف الأوزان بين تشرين الثاني وشباط ، من أعضاء الهيئة التدريسية و الموظفين والطلاب من جامعة البصرة ، ومن مركز الغدد الصماء التابع لمستشفى الموانىء التعليمي اعمارهم تتراوح من 25 الى 55 سنة. تم تقسيم المشاركين حسب أعمارهم إلى ثلاث فئات عمرية. تراوحت الفئة العمرية الأولى ما بين 25-35 سنة ، والفئة الثانية تراوحت بين 36-45 سنة ، والفئة الثالثة تراوحت بين 46-55 سنة..

تم تسجيل القياسات الأنثروبومترية لجميع المشاركين ، وتم تقسيم المشاركين إلى مجموعتين رئيسيتين وفقًا لمؤشر كتلة الجسم. المجموعة الأولى ضمت 30 امرأة نحيفة تمتلك مؤشر كتلة الجسم بين (18-24.9) ، المجموعة الثانية ضمت 58 امرأة بدينة مع مؤشر كتلة الجسم أكثر من 30 ، والتي تم تقسيمها بدورها إلى مجموعتين فرعيتين. تضم المجموعة الثانية 30 امرأة بدينة تتمتع بصحة جيدة والمجموعة الثالثة التي تضم 28 امرأة بدينة مصابة بداء السكري.

تم جمع عينات الدم لفحص المعايير الكيميائية الحيوية ، بما في ذلك مستويات فيزفاتين ، أديبونكتين ، لبتين ، إنسولين ، جلوكوز ، الكوليسترول الكلي ، البروتين الدهني منخفض الكثافة ، الدهون الثلاثية ، البروتين الدهني عالي الكثافة والبروتين الدهني منخفض الكثافة جدا.

أظهرت النتائج أن النساء المصابات بداء السكري والسمنة تمتلك مستويات أعلى من الفزفاتين (p = 0.003) واللبتين (p = 0.0001) مقارنة بالنساء النحيفات. علاوة على ذلك ، أظهرت النساء المصابات بداء السكري والسمنة زيادة معنوية في الكوليسترول الكلي (p = 0.024)، والدهون الثلاثية (p = 0.0001) والبروتين المنخفض الكثافة (p = 0.005) مقارنة بالنساء النحيفات. وكذلك كان لدى النساء المصابات بداء السكري والسمنة انخفاض كبير في البروتين الدهني عالي الكثافة (p = 0.003) مقارنة بالنساء النحيفات. كذلك لم يتم العثور على فروقات ذات دلالة إحصائية في مستويات الأديبونكتين والأنسولين بين المجموعات.

استنتجت هذه الدراسة وجود ارتفاع في مستويات الفزفاتين عند النساء المصابات بداء السكري والسمنة. وهذا يشير إلى أن مستويات الفيزفاتين مرتبطة بشدة بالسمنة ومرض السكري ، وبالتالي يمكن استخدامه كعلامة لتشخيص وعلاج مرض السكري.

وزارة التعليم العالي والبحث العلمي
جامعة البصرة

كلية العلوم
قسم علوم الحياة

الدور الفسيولوجي لهرمون الفزفاتين على مستويات الدهون والكلوكوز للنساء البدينات المصابات بالسكري

رسالة مقدمة الى
مجلس كلية العلوم - جامعة البصرة
وهي جزء من متطلبات نيل شهادة الماجستير
علوم الحياة / الفسلجة / علم الدم

مقدمة من

زهراء محمود حسين الحجاج

بكالوريوس علوم – علوم الحياة
2010

باشراف

الأستاذ المساعد الدكتور

هناء سلمان كاظم

1443 2021