**Ministry of Higher Education**
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# Estimation of Irisin , Chemerin and some biomarkers in obesity , diabetic and sub-fertile men

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

**{وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلَّا قَلِيلًا }**

**صدق الله العلي العظيم**

سورة الاسراء (٨٥)

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We certify that this thesis entitled "**Estimation of Irisin , Chemerin and some biomarkers in obesity , diabetic and sub-fertile men** " has been prepared under our supervision at the college of science , university of misan ; as a partial fulfillment of the requirements for the degree of master of biology .

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# Dedication

To everyone who taught me a letter in this mortal world

To the example of devotion and sincerity... my beloved father

To whom I gave my happiness and comfort to her happiness...

my virtuous mother

To those who have never been stingy in helping me... my dear

husband

To the beats of my heart... my dear's children (Jafar and Sara)

To the one who gave me advice and guidance... my dears

brother and sisters

To everyone who supported me even with a smile... my husband's family

To everyone who called me well...

I dedicate this humble work to you

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***Raya Najim***

# SUMMARY

# *Summary*

The current study aimed to investigate the role of irisin , chemerin and some hormonal and biochemical parameters in obese , diabetic (type 2) and sub-fertile men in Maysan province , conducted in each of the Al-Sadr Teaching Hospital , Center for Diabetes and Endocrine , the main blood bank , Alsajad laboratory and Aleiman laboratory during the period from December 2020 to July 2021 .

The whole sample is about 80 men (aged 35 - 45 years) , divided into four main groups (20 men / group ) , as a following :

- Control group (healthy men) .
- Obesity group (have a BMI over than 30 kg / $m^2$ ) .
- Diabetic group (Type 2 diabetes mellitus) .
- Sub-fertile group (Hyperprolactineamia).

The present results revealed that :

1- Body mass index (BMI) increased significantly ($p \leq 0.05$) in different groups in comparison with the control .

2- Irisin increased significantly ($p \leq 0.01$) in the obesity group and decreased significantly ($p \leq 0.01$) in the diabetic and sub-fertility groups in comparison with the control .

3- Chemerin increased significantly ($p \leq 0.01$) in different groups in comparison withthe control .

4- Follicle Stimulating Hormone (FSH) , luteinizing Hormone (LH) and testosterone (T) decreased significantly ($p \leq 0.05$) (except testosterone in diabetic group) indifferent groups in comparison with the control .

5- Prolactin (PRL) increased significantly ($p \leq 0.01$) in different groups in comparison with the control .

6- Triiodothyronine (T3) and thyroxine (T4) increased significantly ($p \leq 0.01$) in obesity group and decreased not significantly (except T4 in diabetic group) in diabetic and sub-fertility groups in comparison with the control .

7- Insulin , insulin resistance , glucose and glycated hemoglobinA1c (HbA1c) increased significantly ($p \leq 0.01$) (except HbA1c in obesity and sub-fertility groups)in different groups in comparison with the control .

8- Interleukin-6 (IL-6) increased significantly ($p \leq 0.05$) (except diabetic group) in different groups , C-reactive protein (CRP) increased significantly ($p \leq 0.05$) (exceptobesity and sub-fertility groups) in comparison with the control .

9- Nitric oxide synthase (NOS) decreased significantly ($p \leq 0.01$) (except sub-fertilitygroup) in different groups in comparison with the control .

10- Total cholesterol (TC) , triglyceride (TG) and low density lipoprotein (LDL) increased significantly ($p \leq 0.01$) (except LDL in diabetic and sub-fertility groups) in different groups , high density lipoprotein (HDL) decreased significantly ($p \leq 0.05$) in different groups in comparison with the control .

The physiological impact of these results be discussed according to the influence of the fat mass (indicated by BMI ) , insulin resistance and the high level of prolactin hormone on all the studied parameters in obesity , diabetic and sub-fertility groups.

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# *List of Abbreviations*

| Abbreviations | |
|---|---|
| Abs. | Absorbance |
| ADA | American Diabetes Association |
| ANOVA | One-way Analysis Of Variance |
| BAT | Brown adipose tissue |
| BMI | Body mass index |
| CCRL2 | CC chemokine receptor-like 2 |
| CETP | Cholesterol ester transfer protein |
| CMKLR1 | G-protein coupled receptor chemokine like receptor 1 |
| CRP | C-Reactive Protein |
| CVD | Cardiovascular diseases |
| DBP | Diastolic blood pressure |
| DM | Diabetes mellitus |
| DMRT | Duncan's new multiple range test |
| ED | Erectile dysfunction |
| ELISA | Enzyme-linked Immunosorbent Assays |
| eNOS | endothelial NOS |
| F.B.G. | Fasting Blood Glucose |
| FNDC5 | Fibronectin domain-containing protein 5 |
| FSH | Follicular stimulating hormone |
| GDM | Gestational Diabetes Mellitus |
| GH | Growth hormone |
| GK | Glycerol kinase |
| GLU | Glucose |
| GnRH | Gonadotropin releasing hormone |
| GOD | Glucose oxidase |
| GPO | Glycerol phosphate oxidase |
| GPR1 | G protein-coupled receptor 1 |
| HbA1c | Glycated Hemoglobin A1c |
| HCR | Human chemokine receptor |
| HDL | High density lipoprotein |
| HOMA-IR | Homeostatic Model Assessment for Insulin Resistance |
| HPG | Hypothalamus-pituitary-gonadal axis |
| HPL | Hyperprolactinemia |
| IDDM | Insulin Dependent Diabetes Mellitus |
| IGR | Impaired glucose regulation |
| IL-6 | Interleukin - 6 |
| iNOS | inducible NOS |
| IR | Insulin Resistance |
| LDL | Low-density lipoprotein |
| LH | Luteinizing hormone |
| LPS | Lipopolysaccharide |
| MAGI | Male accessory gland infection |

| | |
|---|---|
| NAFLD | Non-alcoholic fatty liver disease |
| NCEP | Centers for Environmental Prediction |
| NIDDK | National Institute of Diabetes and Digestive and Kidney Diseases |
| NIDDM | Non-Insulin Dependent Diabetes Mellitus |
| nNOS | neuronal NOS |
| NO | Nitric Oxide |
| NOS | Nitric Oxide Synthase |
| O.D. | Optical density |
| PCOS | Polycystic ovarian syndrome |
| PGC- α | Peroxisome proliferator-activated receptor-gamma coactivator-1 |
| PL | Placental lactogen |
| PRL | Prolactin |
| PRLR | Prolactin receptor |
| R1 | Reagent 1 |
| R2 | Reagent 2 |
| RARRES2 | Retinoic Acid Receptor Responder 2 |
| SAT | Subcutaneous adipose tissue |
| SD | Standard Deviation |
| SHBG | Sex hormone binding globulin |
| T | Testosterone |
| T2D | Type 2 diabetes |
| T2DM | Type 2 diabetes mellitus |
| T3 | Triiodothyronine |
| T4 | Thyroxine |
| TC | Total cholesterol |
| TG | Triglycerides |
| TIG2 | Tazarotene-induced gene 2 |
| TNF- α | Tumor necrosis factor-α |
| TSH | Thyroid stimulating hormone |
| UCP-1 | Uncoupling protein 1 |
| WAT | White adipose tissue |
| WHO | World Health Organization |

# CHAPTER ONE

# INTRODUCTION

# 1. Introduction

Adipose tissue is a metabolically active organ that serves not only as a primary storage place for excess energy but also as an endocrine organ capable of producing a variety of biologically active substances that help maintain metabolic homeostasis and to store energy in the body (surrounding organs) (Coelho *et al.*, 2013) . Adipocytes are present in this dynamic tissue , as well as the vascular stroma , that contains blood cells , endothelial cells , granulosa cells and adipose precursor cells , among other cell types (Saely *et al.*, 2012) . Adipocytes secretion , cellular structure and phenotype , the quantity and number of fat cells , vascular stromal cells and immune system cells varies depending on where in the body adipose tissue is found (Trzeciak-Ryczek *et al.*, 2011) .

In mammals , there are two types of adipose tissue : brown and white , both of them have different shapes and functions ,

Brown adipose tissue (BAT) specialized in producing heat (thermogenesis) , is almost absent in adult humans but is present at birth , brown adipocytes are smaller than white adipose tissue adipocytes with a medium diameter , BAT improves metabolic processes and increases overall energy expenditure , resulting in weight loss (Lidell and Enerback , 2010) . However , BAT promotes metabolism without causing weight loss by increasing glucose and lipid uptake into the bloodstream (Stanford *et al.*, 2013) .

White adipose tissue (WAT) is not merely a fuel storage organ , but also a key component of metabolic homoeostatic mechanisms , the two major types of WAT are visceral fat, localized within the abdominal cavity and mediastinum, and subcutaneous fat in the hypodermis . Visceral obesity correlates with increased risk of insulin resistance and cardiovascular diseases, while increase of subcutaneous fat is associated with favourable plasma lipid profiles (Wronska and Kmiec , 2012). Both adipose and muscle tissues release cytokines and other peptides

known as adipokines and myokines , which help maintain metabolic homeostasis by contributing to tissue communication (Patel *et al.*, 2012) . Many adipokines , including leptin , adiponectin , resistin , visfatin , irisin (Gesta *et al.*, 2007) and chemerin (Zhou and Rui , 2013) , are released from adipose tissue , these hormones may have local effects depending on fat distribution and type of fat , mediating the link between fat metabolism and increased metabolic and physiologic functions .

Irisin is a polypeptide hormone with 112 amino acid residues that is primarily generated in muscle tissue after proteolytic cleavage of its precursor , fibronectin domain-containing protein 5 (FNDC5) , it has a molecular weight of about 12 kDa (Vitali *et al.* , 2012) . It belongs to the adipomyokine class since it operates in both adipose and muscle tissue (adipokine and myokine) and is a thermogenic protein that stimulates energy expenditure via WAT browning , although skeletal muscle is the primary source of irisin , current research has found irisin and (FNDC5) mRNA expression in a variety of human tissues (Rodriguez *et al.*, 2017) . Irisin is primarily released by white subcutaneous adipose tissue (SAT) and plays a key regulatory role in the conversion of white fat to brown fat , implying a potential role in reducing fat accumulation and obesity while also improving metabolic status (Gesta *et al.*, 2007) .

Obesity increases circulating irisin levels in comparison to non-obese people , the increase in circulating irisin levels in obesity is thought to be an accommodating compensatory response to obesity-induced metabolic dysfunction , such as a decrease in insulin levels (Perakakis *et al.*, 2017) .

Irisin has been found to improve insulin resistance and diabetes type 2 (T2D) by increasing insulin receptor sensitization in skeletal muscle and the heart , as well as improving hepatic glucose and lipid metabolism , promoting pancreatic cell functions and converting white adipose tissue to brown adipose tissue , moreover , serum irisin levels were significantly lower in the new-onset T2D patient group

(Park *et al.,* 2013) . Muscle FNDC5/irisin appears to have a positive relation with insulin resistance , and there has been a link between irisin and insulin sensitivity ( Roca-Rivada *et al.*, 2013) .

Treatment with irisin in obese male rats enhanced the FSH , LH and testosterone hormone levels in blood resulting in promote spermatogenesis and sperm properties such as sperm count and motility (Nanees and Reham , 2018). In addition, higher irisin expression in sertoli cells and undifferentiated spermatogonia transcripts in organotypic primate testicular tissue culture suggested that irisin may play a role in spermatogenesis in *vitro* investigation (Wahab *et al.,* 2020). The testis bears the highest expression of irisin among the male reproductive tissues followed by the prostate gland, furthermore , irisin is predominantly expressed in developing germ cells , surrounding tubules and leydig cells within the testis (The Human Protein Atlas , 2021) .

On the other side chemerin is a protein encoded by the Retinoic Acid Receptor Responder 2 (RARRES2) gene in humans , first discovered as a retinoid responsive gene found in psoriatic skin lesions (Nagpal *et al.*, 1997) . Chemerin was first identified as an adipokine in 2007 (Goralski *et al.*, 2007) . It is also highly expressed in the liver (Krautbauer *et al.,* 2013) . Its presence in other tissues , such as the placenta and the ovary , has also been confirmed (Tsiotra *et al.,* 2018) . Chemerin receptors (CMKLR1 , GRP1 and CCRL2) are present in human and rodent testes , and are located specifically on leydig cells and partially on germ cells (Dupont *et al.,* 2015). Chemerin is thought to play a role in adipogenesis , adipocyte metabolism and glucose homeostasis according to Goralski and his colleagues (2007) , in addition , to be a chemoattract macrophages and dendritic cells during the immunological response (Wittamer *et al.,* 2003) .

Furthermore , positive associations between serum chemerin and body mass index (BMI) , blood triglyceride , waist-to-hip ratio and blood pressure , however

, an inverse correlation be detected with HDL-cholesterol (Bozaoglu *et al.,* 2007) . Moreover , chemerin have also been linked to fatty liver diseases indicators. (Sell *et al.,* 2010) .

On the other hand , the association between chemerin and insulin sensitivity appears to be significant , Ouwens and his colleagues (2012) found a strong positive connection between systemic chemerin and insulin resistance . Furthermore , data on the association between chemerin expression in adipose tissue and insulin sensitivity is scarce and inconcelusive (Tan *et al.,* 2009) .

A negatively association appeared between this adipokine (chemerin) with spermatic motility and positively relation with sperm concentration due to a local secretion of chemerin in human male genital tract , particularly at the testicular region , furthermore , many physiological properties linked with chemerin including the steroidogenesis and sperm properties (Thomas *et al.,* 2013) . Surprisingly , investigations on male rats in *vitro* revealed that chemerin inhibited steroidogenesis (Dupont *et al.,* 2015) .

In view of these controversy , the present study represents an attempt to shed some light about the irisin and chemerin hormones and their relationship with other hormonal , biochemical and pro-inflammatory parameters in obese , diabetic and sub-fertile men in Maysan province .

# CHAPTER TWO

# LITERATURE REVIEW

# 2.1 Irisin

## An Overview and its Related with Obesity, Diabetes and Fertility

In Harvard University, Bostrom and his colleagues (2012) identified a new peptide secreted from muscle tissue, was discovered during a study that looked for factors secreted by skeletal muscles in response to peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α), which they named irisin, to highlight its role as a messenger that comes from skeletal muscle to other body regions. The name irisin originated from the Greek Goddess Iris, who was the daughter of Thaumas and Electra and was the goddess messenger of good news from Gods to humans in Greek mythology (Arhire *et al.,* 2019 ; Grimal, 1996). This name proved to be very appropriate so it relates to the main function of irisin, which is to transmit the beneficial effects of physical exercise to adipose tissue (browning and thermogenesis) and other organs involved in metabolism (Arhire *et al.*, 2019).

Irisin was represented as an exercise-induced myokine with a 112-amino-acid peptide structure (Hecksteden *et al.,* 2013). Irisin is a new myokine produced in mice and humans by proteolytic cleavage of fibronectin type III domain-containing 5 (FNDC5), and it can also be secreted in small amounts by adipose tissue and the liver (Polyzos *et al.,* 2014). The FNDC5 structure is made up of a 29-amino-acid signaling peptide, 94-amino-acid domain, and C-terminal that acts as a lysis site before being secreted as irisin, this molecule has been observed in other mammals, and it may have very similar activities and structure to humans ; for example, mice and humans have a 100% similar structure (Aydin, 2014).

Exercise has been shown to increase the expression of PGC-1α in muscles (Pilegaard *et al.*, 2003), which increases thermogenesis in brown adipose tissue by regulating mitochondrial biogenesis, and produces a lot of uncoupling protein 1 (UCP-1), a BAT biomarker (Boström *et al.,* 2012). PGC-1α is involved in the

physiological effects of exercise, including white-to-brown fat conversion (Handschin and Spiegelman, 2008), improvement of insulin sensitivity, and insulin signaling (Wenz *et al.*, 2009). Irisin is largely released in reaction to exercise (Boström *et al.*, 2012; Huh *et al.*, 2012).

According to Bostrom and his colleagues (2012) PGC-1α activation is thought to increase the expression of its downstream target FNDC5, FNDC5 is a membrane protein found in the brain and skeletal muscle that is severed by unknown proteolytic enzymes after exercise, resulting in the release of a new protein (called irisin) that contains the most of the fibronectin III domain, as a result, they hypothesized that circulating irisin levels are higher in individuals who participated in exercise-induced activities and decreases gradually in individuals who are less active and sedentary. Furthermore, Kang and his colleagues (2019) found a significant increase in serum irisin levels after swimming exercise and a reduction in body fat mass in mice.

Irisin is a strong messenger that sends signals to specific cells such as skeletal muscle, liver, fat, pancreas, heart and the brain, irisin's action on various targeted tissues or organs in humans has shown its physiological activities for promoting health or regulating a number of metabolic disorders (Gizaw *et al.*, 2017).

Vacek (2015) showed that after exercise, the irisin hormone transfers from muscle to fat tissue to signal fat cells to begin burning energy instead of storing it, the finding lighted hope and press coverage that irisin might hold the key to fighting diabetes and obesity, maybe one day taking the form of a pill that might soften absent the pounds without the bother of a workout.

Muscle tissue has been recognized as an endocrine organ that releases an assortment of cytokines, named myokines, which direct numerous physiological and metabolic pathways (Pedersen and Febbraio, 2012).

Numerous studies have focused on the association between irisin and metabolic diseases.

Obesity is associated with a significant imbalance in cytokine secretion, which is a strong predictor of insulin resistances and T2D development (Fantuzzi *et al.*, 2005). Although FNDC5 / irisin levels have been correlated to glucose metabolism, there is no evidence of an association between inflammatory markers and irisin levels, however, it is well known that visceral adiposity is correlated with high levels of C-reactive protein (CRP) and interleukin-6 (IL-6), and that individuals with high CRP but not IL-6 levels have low irisin levels (Leung *et al.*, 2018). Furthermore, increased FNDC5 / irisin levels were associated to an improved metabolic profile and a lower risk of T2D in middle-aged males with grade 1 obesity (Bonfante *et al.*, 2017).

Bostrom and his colleagues (2012) found that irisin level improved the obesity and glucose homeostasis, and it could be used as a therapeutic protein for human metabolic disorders and other diseases that improve with exercise. Moreno-Navarrete and his colleagues (2013) suggested that circulating irisin levels in plasma or serum are related to overweight / obesity in various groups of people. Obese individuals had a higher circulating irisin level than controls, according to a clinical trial comprising 94 obese patients who participated in a weight management program (Crujeiras *et al.*, 2014 ; Tibana *et al.*, 2017). Surprisingly, while most researches confirm irisin's significance in obesity prediction, many investigations have found that circulating irisin levels are lower in obese people than in healthy people (Belviranli *et al.*, 2016). Furthermore, results of circulating irisin were incompatible in obese vs healthy children, boys or girls (Nigro *et al.*, 2017).

The attention be attracted for the irisin as a potential new target for combating type 2 diabetes and insulin resistance, irisin improves insulin resistance and type 2

diabetes by increasing insulin receptor sensitivity in skeletal muscle and the heart, improving hepatic glucose and lipid metabolism, promoting pancreatic cell activities and converting white to brown adipose tissue (Liu and Ding, 2017; Gizaw *et al.,* 2017). Irisin's ability to reprogram WAT cells to take on the phenotype of BAT via yet undetermined receptors has been proposed as a possible therapeutic target for metabolic disorders such as obesity and T2D (Hofmann *et al.,* 2014).

Furthermore, Liu and his colleagues (2014) found that T2D patients had decreased circulating irisin levels than healthy individuals. However, some of whom attect with T2D had elevated levels of irisin (Rodrigues *et al.,* 2016).

Irisin levels have been found to be decreased in overweight, obese, and T2D patients in various investigations (Moreno-Navarrete *et al.,* 2013). Some researches found a positive correlation between irisin levels and body mass index (BMI) (Huh *et al.,* 2012), while others found a null (Timmons *et al.,* 2012) or even a negative correlation (Choi *et al.,* 2013).

Irisin has been shown to have a detrimental effect on the hypothalamus-pituitary-gonadal (HPG) axis in several investigations, some of irisin's effects on sex hormones are recognized, but its reproductive effects are mostly unknown, the amount of systemic irisin in human increases about the time of puberty (Reinehr *et al.,* 2015) and continues to increase significantly throughout pregnancy (Hernandez-Trejo *et al.,* 2016). These results support the theory that irisin has an effect on the hypothalamus-pituitary-gonadal axis and reproductive function control (Reinehr *et al.,* 2015). besides that, Jiang and his colleagues (2017) found that irisin stimulates both FSH and LH mRNA expression in primary cultures of tilapia pituitary cells *in vivo* and *in vitro.*

Irisin contains antioxidative, anti-inflammatory, and antiapoptotic properties, suggesting that it could be used to treat obesity, diabetes, nonalcoholic fatty liver

disease, osteoporosis, and potentially cancer (Askari *et al.,* 2018). Furthermore, irisin was found to be associated with age, BMI, lipid profile [total cholesterol (TC), triglycerides (TG)], fasting blood glucose (FBG), and diastolic blood pressure (DBP) in non-diabetic persons (Liu *et al.*, 2013).

On the other hand, Sorisky and his colleagues (2000) found a high stimulation of thyroid stimulating hormone (TSH) activates adipocyte receptors leading to adipogenesis via pre-adipocyte differentiation. Furthermore, Lapa and his colleagues (2015) found a relationship between BAT production with higher TSH in hypothyroid individuals.

Moreover, hypothyroidism patients have low serum irisin levels than hyperthyroid patients (Ruchala *et al.*, 2014). Also Zybek-Kocik and his colleagues (2016) found that the patients with long-term hypothyroidism have less irisin than those with short-term hypothyroidism, on the other hand, a negative correlation between irisin and TSH and a positive correlation with free-T4.

Nevertheless, Ateş and his colleagues (2016) found a positive correlation between irisin with TSH and a negative correlation with free-T4. Panagiotou and his colleagues (2016) concluded that irisin's proposed metabolic effects are the most likely independently of thyroid axis hormones.

In human adipocytes, triiodothyronine (T3) and irisin both increased UCP-1 expression, decreased lipid accumulation and prevented DNA damage (Oliveira *et al.*, 2018).

Nevertheless, some studies found no correlation detected between serum irisin and metabolic disorders in thyroid dysfunctions (samy *et al.*, 2015).

## 2.2 Chemerin

## An Overview and its Related with Obesity, Diabetes and Fertility

The chemerin molecule was first identified in (1997) as a retinoid responsive gene found in psoriatic skin lesions, and rediscovered in 2003 as an adipocyte signaling molecular (chemoattractant protein) that plays a role in adipogenesis and later described as an adipokine in 2007. (Goralski *et al.*, 2007 ; Helfer and Wu, 2018). Chemerin, known as a retinoic acid receptor responder protein 2 (RARRES2) or RAR-responsive protein TIG2 (Tazarotene-induced gene 2), is an adipokine that has autocrine, paracrine, and even endocrine functions in the body (Rourke *et al.*, 2013 ; Nagpal *et al.*, 1997).

Its a multifunctional protein that is encoded by the RARRES2 gene in humans, it was named due to its ability to stimulate leukocyte chemotaxis, it was considered as a secretory protein with multiple biological effects , it was an important adipocytokine that has been confirmed to control adipocyte differentiation, acts as a proinflammatoryadipocytokine that increases the cellular production of inflammatory cytokines (Yu *et al.*, 2012) and associated significantly to white blood cell count, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) (Landgraf *et al.*, 2012 ; Rowicka *et al.*, 2020).

Chemerin includes 163 amino acids that are released as an inactive 18-KDa propeptide before being cleaved into a 16-KDa active protein 137 amino acids that bind to the G-protein coupled receptor chemokine like receptor 1 (CMKLR1) (Wittamer *et al.*, 2003). It is initially secreted as prochemerin by the liver and white adipose tissue (Krautbauer *et al.*, 2013). It has also been confirmed that it is expressed at a high level in the liver and other tissues such as the placenta and ovary (Tsiotra *et al.*, 2018). It's primary function during the immune response to chemoattract macrophages and dendritic cells (Wittamer *et al.*, 2003 ; Jacenik and

Fichna, 2020). In addition chemerin plays a role in adipogenesis, adipocyte metabolism and glucose homeostasis (Goralski *et al.*, 2007 ; Helfer and Wu, 2018). It shares in both adaptive and innate immunity by acting as a chemoattractant protein that ligand for the G-protein coupled receptor CMKLR-1 (also known as ChemR23 in humans) (Nagpal *et al.*, 1997).

Many studies pointed a number of receptors that linked to chemerin.

G protein-coupled receptor 1 (GPR1) was the first receptors that be discovered in the human hippocampus (Marchese *et al.*, 1994), G-protein coupled receptor chemokine like receptor 1 (CMKLR1) was a another receptors be discovered in 1996 (Gantz *et al.*, 1996) and the CC chemokine receptor-like 2 (CCRL2) which was initially found in humans known as the human chemokine receptor (HCR) (Fan *et al.*, 1998 ; Zabel *et al.*, 2008).

In human and mouse adipocytes, chemerin and CMKLR1 are highly expressed, both chemerin and its receptor CMKLR1 (chemokine-like receptor1) were highly expressed in human white adipose tissue, indicating that it is a primary source of chemerin, chemerin also stimulates lipolysis in mature white adipose cells by directly activating hormone-sensitive lipase (Goralski *et al.*, 2007 ; Roh *et al.*, 2007).

Furthermore, chemerin expression increases in obesity, insulin resistance, metabolic syndrome (Corona-Meraz *et al.*, 2016) and type 2 diabetes (Chakaroun *et al.*, 2012). Obese individuals had significantly higher chemerin levels in comparison with healthy weight (Bozaoglu *et al.*, 2007 ; Ernst and Sinal, 2010). Chemerin levels are lower in obese patients who have lost weight by diet or bariatric surgery (Sell *et al.*, 2010). Saremi and his colleagues (2010) found that a 12-week aerobic exercise program reduced serum chemerin levels in overweight and obese people. Weight loss programs (such as 12 weeks of exercise, a 6 month

diet with a calorie restriction, or 12 months following an overweight surgery) reduced serum chemerin levels in obese persons (Chakaroun *et al.*, 2012) and in rats (Lin *et al.*, 2019). Chemerin levels are associated to obesity indices in a direct positive association, it has been related to visceral obesity and cardiovascular diseases as a result (Shin *et al.*, 2012 ; Zdanowicz *et al.*, 2022). Its plays a role in adipose tissue development, inflammation and glucose homeostasis and it may have a role in the metabolic dysfunction that is associated with obesity and obesity-related diseases (Bozaoglu *et al.*, 2007).

Chemerin adipokine has been correlated with body fat (Alfadda *et al.*, 2012) and be a higher serum levels in obese than in lean (Landgraf *et al.*, 2012 ; Yoo *et al.*, 2012). As a result, chemerin appeared to play a role in solving problems and complications in patients' cases (obesity and leanness) when searching for the physiological correlations of chemerin in abnormal body fats (Subramanian *et al.*, 2012).

Alfadda and his colleagues (2012) hypothesized that subcutaneous fat depots are the major source of circulating chemerin in humans, and demonstrated significantly higher chemerin mRNA expression in subcutaneous than in visceral adipose tissue, in addition, circulating levels of chemerin were negatively correlated with chemerin mRNA expression in subcutaneous adipose tissue, however, this correlation did not exist in visceral adipose tissue, the negative correlation observed specifically in subcutaneous adipose tissue results from a negative feedback loop, whereby elevated circulating chemerin levels inhibit chemerin mRNA expression.

El-Mesallamy and his colleagues (2011) discovered that serum chemerin levels were higher in patients with type 2 diabetes than in healthy, and concluded that chemerin plays an important role in obesity and related disorders like diabetes. Chemerin in T2D, it was similar in male and female patients and increased in

patients with high CRP (Weigert *et al.*, 2010). There is a positive connection between chemerin and insulin resistance (Jialal *et al.*, 2013 ; Habib *et al.*, 2017). Despite its role in the progression of insulin resistance, chemerin is thought to be associated in elevated cancer risk and mortality as one of the new adipokines (Weigert *et al.*, 2010).

Circulating chemerin have a positive relationship with BMI, systolic blood pressure, insulin resistance index and triglyceride in diabetes patients (Lin *et al.*, 2012).

Chemerin and its receptor CMKLR1 are both expressed in pancreatic cells, showing that they play a role in insulin secretion regulation, moreover, chemerin and CMKLR1 deletion mice had lower glucose-stimulated insulin release, while chemerin transgenic mice have higher insulin secretion in glucose tolerance tests (Takahashi *et al.*, 2011).

Chemerin inhibited leydig cells steroidogenesis in an experimental setting, as a result, chemerin could be a promising new candidate in the relationship between sex steroid regulation, obesity and metabolic diseases (Li *et al.*, 2014).

The RARRES2 and CMKLR1 genes are also expressed in males' reproductive tracts, specifically in rat and human leydig cells (Gao *et al.*, 2014). Chemerin suppresses gonadal steroidogenesis *in vitro* (Li *et al.*, 2014) and CMKLR1 deficiency in mice decreases leydig cell size and testosterone production *in vivo* (Zhao *et al.*, 2019). As a result, the molecular mechanism underlying CMKLR1's effect on testosterone production remains uncertain.

Campos and his colleagues (2008) found that chemerin level are lower in sub-fertile males than in control, and they are negatively associated with plasma luteinizing hormone (LH) and *sex hormone binding globulin* (SHBG) concentrations in humans. Thus, understanding the chemerin's role in male

fertility is crucial, its concentration in seminal fluid was found to be negatively correlated with spermatic motility and positively correlated with sperm concentration (Thomas *et al.,* 2013), while Bobjer and his colleagues (2018), discovered a negative correlation between plasma chemerin and LH, estradiol, and SHBG levels. Moreover, even after correcting for BMI, they discovered that serum chemerin concentration was lower in subfertile men compared to controls, which means that despite its positive association with BMI, this adipokine was associated with reproductive function independently (Bobjer *et al.,* 2018). Vasectomy patients had lower serum chemerin levels than healthy men (Thomas *et al.,* 2013), implying that chemerin is secreted locally in the male genital canal, despite the fact that chemerin levels in seminal fluid have been reported to be much lower than in blood plasma (Bongrani *et al.,* 2019). Chemerin level have also been associated to fatty liver disease markers (Sell *et al.,* 2010).

Nevertheless, Huh and his colleagues (2011) found no link between chemerin levels and BMI and waist circumference.

Serum chemerin levels are significantly higher in the hypothyroid group and significantly lower in the hyperthyroid group, furthermore, serum chemerin levels were found to have a significant negative correlation with serum levels of T3 and T4 and a significant positive correlation with serum levels of TSH in both studied groups (Gong *et al.,* 2018).

## 2.3 Obesity and Fertility

Obesity is derived from latin word obesus, which means "one who has gained weight from eating", it may have originally appeared in Thomas Venner's writings in 1620 (Barnett *et al.,* 2005). However, the deleterious impact of obesity on one's health has long been recognized, as shown by Hippocrates, Galen and Avicenna's

works, in his encyclopedic medical treatise ElKanun fi't-Tb (The Cannon of Medicine) (Abdel-Halim, 2005). Obesity, defined as an abnormal accumulation of fat in adipose tissue, is a metabolic condition caused by behavioral, environmental and heritable factors (Liu and Ding, 2017).

Since 1980, the global prevalence of obesity and overweight has risen, with roughly a third of the global population being categorized as overweight or obese, obesity rates have risen in people of all ages and genders, regardless of geographic location, ethnicity or socioeconomic level, however obesity is more common in older people and women, although absolute prevalence rates of overweight and obesity varied greatly between areas and countries, this tendency was consistent, obesity prevalence rates appear to have leveled off in various wealthy countries over the last few years, in epidemiological investigations (Chooi *et al.*, 2019).

Obesity and overweight are characterized by an excess of fat mass and are most typically represented by the body mass index (BMI), and the waist circumference with waist / hip ratio (Fejes *et al.*, 2005). Overweight people have a BMI a range between 25.0 – 29.9 $kg/m^2$ and obese people have a BMI of over 30 $kg/m^2$ (WHO, 2020) (Table 2.1).

**Table (2.1) : Classification of Adults according to BMI. (WHO, 2020)**

| Classification | BMI | Risk of comorbidities |
|---|---|---|
| Underweight | < 18.5 | |
| Normal range | 18.5–24.9 | |
| Overweight | Classification | Average |
| Pre-obese | 25.0–29.9 | Increased |
| Obese I | 30.0–34.9 | Moderate |
| Obese II | 35.0–39.9 | Severe |
| Obese III | ≥ 40.0 | Very severe |

Obesity defined as an increase in fat mass, and also defined as a gain in body weight of more than 20% of an individual's optimal body weight, obesity and overweight may result in serious health risks linked to an increase of chronic diseases such as T2DM, CVDs, cancer, hypertension, gallbladder disease, musculoskeletal disorders and lower health-related quality of life (van *et al.*, 2015 ; Britannica, 2021).

Food patterns, sedentary lifestyle, socioeconomic level and genes have been contributed to the tremendous global rise in obesity, in addition, modern lifestyle that encourages increasing caloric consumption which is discouraging physical activity is the root cause of obesity (Hruby *et al.*, 2015).

Obesity is a risk factor for hypertension, dyslipidemias, non-alcoholic fatty liver disease (NAFLD), cardiovascular diseases (CVD) and cancer, as well as the underlying cause of metabolic abnormalities that lead to type 2 diabetes mellitus (T2DM) (WHO, 2014).

Aras and his colleagues (2021) showed that the 5% loss or more in total body weight improves glycemic control, lowers the requirement for diabetic medications and improves quality of life.

Baumgartner and his colleagues (2017) monitored a link between obesity and to an increased risk of hypothyroidism, including overt and subclinical hypothyroidism, people with thyroid disorders are more likely to acquire diseases other than thyroid disease, such as cardiovascular diseases, cancer, obesity and unfavorable pregnancy outcomes.

Insulin resistance is often related with increased adiposity, it’s a precipitating factor for the development of CVDs, type 2 diabetes and altered metabolism (Wali *et al.*, 2021). Obesity is a risk factor for diabetes that is linked to insulin resistance, obese people have increased levels of non-esterified fatty acids,

glycerol, hormones and pro-inflammatory cytokines released by adipose tissue, which may contribute to the development of insulin resistance (Wondmkun, 2020).

Infertility is a common medical condition described as a couple's inability to achieve a natural pregnancy after a year or more of unprotected sex, there are two types of infertility primary infertility, which affects couples who have never fathered a child and secondary infertility, which affects couples who have already had a natural pregnancy but unable to conceive again (Zegers-Hochschild *et al.,* 2017). Infertility in men is most usually caused by some impairment in semen ejection, low or absence sperm, abnormal morphology and motility of the sperm, while, in female reproductive system, infertility can be occurred due to abnormal ovaries, uterus, fallopian tubes and endocrine system (WHO, 2018 ). Furthermore, the mating partners be infertiled by influenced via their age, surgical history, medications, exposure to environmental toxins, systemic diseases and genetic problems (Leslie *et al.,* 2021).

Obesity has been identified as an important modulator of reproductive hormones, the hormonal dysregulations in obese males may be connected with an increased risk of sperm quality changes (Hammoud *et al.*, 2008). Obesity can affect with the GnRH-FSH / LH pulse and the functioning of sertoli and leydig cells, affecting the secretion of sex hormones, sperm production and maturation ( Leisegang *et al.,* 2020). Serum testosterone, gonadotropin and sex hormone-binding globulin (SHBG) have all been shown to decrease with increasing body mass index (BMI), whereas estradiol has been shown to increase with increasing BMI (Keihani *et al.,* 2020).

Obesity may be associated with a prolactinoma and can lead to negative effects such as insulin resistance and metabolic syndrome, hyperprolactinemia lead to abnormal lipid profile, weight gain and cardiovascular diseases, moreover,

increased prolactin levels reduce testosterone production by disrupting with 17- β -estradiol synthesis (Ali and Mirza, 2021).

Obesity has a harmful negative effects on health in general and on the reproductive function in both male and female (Giviziez *et al.*, 2016). In human male, obesity can lead to infertility, primarily owing to disturbance of the hypothalamus-pituitary-gonadal (HPG) axis, increased testicular temperature, impairment of sperm physical and molecular structure, lower sperm quality and erectile dysfunction related to peripheral vascular disease (Amiri and Ramezani, 2020).

Obesity's negative impact on male reproductive is rapidly becoming recognized, obesity is associated with decreased sperm concentration and motility, increased sperm DNA damage and alterations in reproductive hormones in both humans and animals (Liu and Ding, 2017).

The correlation between obesity and erectile dysfunction can be explained by the fact that obese people have lower testosterone levels and higher levels of various proinflammatory cytokines (Fui *et al.*, 2014). Patients with prolactinomas have a higher BMI and low-density lipoprotein (LDL) regardless of dopamine agonists, and hypercholesterolemia is essentially a consequence of obesity rather than being directly associated to hyperprolactinemia, this can be inferred from the fact that the relationship between hypercholesterolemia and hyperprolactinemia was removed following BMI modifications ( Božidar *et al.*, 2016).

Obesity and T2DM are associated with a low testosterone levels and negatively affect on sperm parameters in men with reduction in semen volume, sperm count, sperm concentration and progressive motility (Zhong *et al.*, 2021). Obese men with T2DM might develop secondary hypogonadism, because of peripheral and central insulin resistance, as well as the action of proinflammatory cytokines

(TNF-α and IL-6) on the hypothalamic pituitary gonadal (HPG) axis (Bhasin *et al.,* 2010). Increased circulating insulin levels, which are linked to obesity-related insulin resistance, low testosterone levels are revealed to be connected with insulin resistance and obesity, indicating that insulin resistance has an independent effect on testosterone production (Tsai *et al.,* 2004). Obesity not only raises the risk of insulin resistance and type 2 diabetes, but it's also a crucial factor in the diagnosis of metabolic syndrome (Kahn *et al.,* 2006). Low testosterone and SHBG levels, independent of insulin resistance are associated with abdominal obesity and high triglyceride levels, both of which are components of metabolic syndrome (Li *et al.,* 2010).

## 2.4 Diabetes and Fertility

Diabetes be derived from the Greek term "diabaino ", which also means "to go or run through" (Laios *et al.,* 2012).

Diabetes was declared as a global threat by the United Nations in December 2006, and a world diabetes day was established, 14 November the Frederick Banting's birthday (one of the four scientists who discovered the insulin) is the day to be remembered every year since 2007 (Das *et al.,* 2021).

Diabetes mellitus is a collection of carbohydrate metabolic disorders characterized by chronic hyperglycemia caused by abnormalities in insulin action, insulin secretion or both (Poznyak *et al.,* 2020), or its a chronic disease in which the body is unable to make enough insulin or use it effectively, patients with type 2 diabetes mellitus have a significantly higher risk of cardiovascular morbidity and mortality than individuals without diabetes and they are disproportionately impacted by cardiovascular diseases (Martín-Timón *et al.,* 2014).

Diabetes be classified for main clinical types (WHO, 2019) :

• Insulin Dependent Diabetes Mellitus (IDDM) :

Accounts for only 5–10% of all diabetes, known as type 1 diabetes or insulin-dependent diabetes or juvenile-onset diabetes, caused by a cellular-mediated autoimmune destruction of pancreatic β-cells, the rate of β-cell destruction in this type of diabetes is quite variable, being rapid in some individuals (mostly infants and children) and slow in others (mainly adults) and such individuals become dependent on insulin for survival (ADA, 2009).

• Non-Insulin Dependent Diabetes Mellitus (NIDDM) :

Accounts about 90–95 % of all diabetes cases, known as type 2 diabetes, non-insulin-dependent diabetes or adult-onset diabetes, characterized by insulin resistance (in most cases) and relative insulin deficit, these people do not require insulin treatment to survive and frequently throughout their lives (ADA , 2009).

• Gestational Diabetes Mellitus (GDM) :

A condition in which a non-diabetic woman develops high blood sugar levels during pregnancy, it can occur during pregnancy due to insulin resistance or decreased insulin production, babies born to mothers with gestational diabetes experience low blood sugar after birth, jaundice and have a higher risk of being overweight and developed type 2 diabetes, untreated mothers with GDM may leading to stillbirth (NIDDK, 2016).

• Other types of diabetes mellitus linked to specific illnesses.

On the other hand, hyperglycemia (an increase in blood glucose level caused by a relative or absolute insulin deficit), lipoprotein abnormalities and oxidative stress are all symptoms of diabetes mellitus (DM) (Scoppola *et al.,* 2001). Chronic hyperglycemia is linked to long-term diabetic complications including a damage in functioning of the cardiovascular system, kidneys, eyes and nerves (wild *et al.,* 2004). Polyuria, polydipsia and weight loss despite polyphagia, hyperglycemia, ketosis, glycosuria, acidosis and coma are all symptoms of diabetes mellitus, additionally, feeling tired or ill, urinating more than usual, being abnormally thirsty or hungry, unexplained weight loss, blurred vision, headache, fever and dry itchy skin are all possible diabetes symptoms (Young *et al.,* 2000).

Physical activity is useful in preventing diabetes and reducing plasma insulin levels, as well as enhancing insulin sensitivity and glucose intolerance in T2DM and healthy people (Yang *et al.,* 2019). It was proposed that exercise and physical fitness protect against glucose intolerance, whereas inactivity causes glucose tolerance to deteriorate (Schranz *et al.,* 1991), glucose intolerance was shown to increase from 8% in highly active subjects to 30% in moderately active subjects and 62% in individuals with low activity levels (Naway, 2011). Furthermore, Park and his colleagues (2022) suggest that the moderate activity may be more effective for improving glucose control in comparison with the light activity.

Moreover, diabetic men have been demonstrated to have a significant higher risk of erectile dysfunction (ED), diabetes has been linked to both male and female sexual dysfunction, diabetes is a known risk factor for male sexual dysfunction, a threefold greater risk of erectile dysfunction when compared with nondiabetic men (Maiorino *et al.,* 2014). Beyond the effects of comorbidities like advanced age, antihypertensive medication, a smoking and high BMI, the severity and duration of diabetes, as well as its vascular and neurological problems, which cause abnormalities in the endothelium or nitric oxide-related mechanisms in the

corpora cavernosa have been strongly connected to the development of sexual dysfunction in men (Getie *et al.,* 2021).

Testosterone concentrations are positively correlated to T2D in women but negatively associated in men (Ding *et al.,* 2020 ; 2006) high testosterone levels have a potential protective role in reducing the risk of T2D in elderly men (Salminen *et al.,* 2015). Low testosterone levels are associated with an increase risk of T2D in men (Schipf *et al.,* 2011). Moreover, T2D patients with low testosterone levels had poor glycemic control and increased insulin resistance, which may contribute to the longer duration of diabetes (Oh *et al.,* 2002). In addition, mortality increased in men with T2D, especially those with low testosterone concentrations (Malipatil *et al.*, 2019). Furthermore, Ottarsdottir and his colleagues (2018) found a relationship between low testosterone levels in men with the insulin resistance, while, the short-term testosterone supplementation trial improved insulin sensitivity in obese men.

Although the prevalence of sub-infertility in diabetic patients of childbearing age is well understood, the mechanisms through which diabetes mellitus (DM) induces male infertility are yet unknown, male infertility may be caused by diabetes mellitus in variety of mechanisms pre-testicular, testicular and post-testicular, in the pre-testicular hypogonadism develops in DM patients due to central (hyperleptinemia or changes in hypothalamic GnRH pulsatile release in overweight or obese individuals) and peripheral (alteration of leydig cell function) mechanisms resulting in lower serum gonadotropin and testosterone levels (Condorelli *et al.,* 2018).

In testicular mechanism DM considered as the main causes of highe oxidative stress, as evidenced by increased reactive oxygen species (ROS) production in seminal fluid and lipoperoxidation (Amaral *et al.,* 2008) or sperm DNA

fragmentation or sperm mitochondrial bioenergy alteration (Agbaje *et al.*, 2007) or enzymatic glycation end products (Mallidis *et al.*, 2008).

In post-testicular mechanism the sperm damage and / or prevent seminal fluid release might be attributed to male accessory gland infection / inflammation (MAGI) (Condorelli *et al.*, 2013), where the DM-MAGI association amplifies the inflammatory response in semen, erectile and / or ejaculatory dysfunction, both of which are well-known DM side effects (Guest *et al.*, 2008).

In men and women, testosterone has a different effect on the risk of T2DM, reduced testosterone levels in men are linked to an increased risk of T2DM, whereas increased testosterone levels and lower SHBG levels in women are linked to an increased risk of T2DM (Gyawali *et al.*, 2018). Interestingly, a study on men found that the inverse relationship between testosterone and the risk of diabetes is largely dependent on the phenotype of abdominal obesity (Grossmann, 2011).

## 2.5 Hyperprolactinemia

Hyperprolactinemia (HPL), the most common endocrine disorder of the hypothalamic pituitary axis and prolactinomas (most common secretory tumors of the pituitary gland accounting for up to 40% of total pituitary adenomas ), one of the most commonly metabolic disorders among both men and women and is the persistent increase of serum prolactin levels (Glezer and Bronstein, 2018).

In men, HPL characterized as a high serum prolactin level, and when this prolactin level exceeds the upper limit (15 to 20 ng/ml) that called it hyperprolactinemia which caused by either physiological or pathological factors, stress and exercise can produce small increase in prolactin levels and they

are considers as a physiological hyperprolactinemia factors (Majumdar and Mangal, 2013 ; Thapa and Bhusal, 2021).

Pituitary tumors that result from high levels of prolactin are called prolactinomas that caused a wide variety of symptoms either due to mass effect of the tumor or due to hypersecretion of prolactin according to size of the tumors, prolactinomas can be classified as microprolactinoma (smaller than 10 mm), macroprolactinoma (larger than 10 mm) or giant prolactinoma (larger than 4 cm) (Pekic *et al.*, 2019 ; Cooper and Greenman, 2018).

Pereira-Lima and her colleagues (2013) found in their study, 50% of patients with macroadenomas were obese and 20% were overweight, 30% of patients with microadenomas were obese and 30% were overweight, and 32.1% of patients with other causes of hyperprolactinemia were obese and 37.9% were overweight, When compared to microprolactinoma and other types of hyperprolactinemia, patients with macroprolactinoma had a higher prevalence of obesity.

The prolactin is a polypeptide hormone that regulates lactation, breast development and many other physiological functions, its chemical structure is similar to that of growth hormone and placental lactogen hormone, they compose a "prolactin / growth hormone / placental lactogen" family, which is distinguished by the presence of a conserved helix bundle protein composition, all hormones in this family are descended from a common ancestral gene (Al-Chalabi *et al.*, 2021). The PRL gene, which is found in human chromosome 6 as a single copy, related to both growth hormone (GH) and placental lactogen (PL) in terms of evolution and function (Forsyth and Wallis, 2002).

The prolactin receptor (PRLR) expressed in most tissues, the liver, mammary gland, adrenal gland and hypothalamus are the highest sites of expression, this expression varies in response to changes in circulating PRL and steroid hormones,

while human GH, PL and PRL bind to the PRLR and mimic some of PRL's functions (Clevenger *et al.*, 2003).

Korkmaz and his colleagues (2021) mentioned that HPL caused by pituitary tumors, primary hypothyroidism, medicines, renal failure, polycystic ovarian syndrome (PCOS) and other physiological factors such as pregnancy and lactation.

The effect of PRL on glucose metabolism and insulin resistance is dependent on the amount of PRL in the blood, improves glucose homeostasis by increasing β -cell mass under certain conditions, such as pregnancy, whereas excessively high PRL levels in serum indicate a high risk of obesity and dysmetabolism, such as decreased insulin sensitivity, abnormal glucose tolerance or insulin resistance (Tuzcu *et al.*, 2009). High levels of PRL increase insulin resistance and impair insulin-secretory capacity in diabetic mice, in contrast to the normal adaptive increases in glucose-stimulated insulin secretion via expanded β-cell mass and insulin sensitivity observed with moderately elevated PRL levels (Park *et al.*, 2011). Patients with HPL have insulin resistance and glucose intolerance in comparison with normal individuals and in comparison with age, sex and weight (Pala et al., 2015).

Wang and his colleagues (2013) found that the role of prolactin on glucose metabolism and insulin resistance depends on its circulating concentration, prolactin levels decreased from normal glucose regulation to impaired glucose regulation (IGR) to diabetes, high circulating prolactin associates with lower prevalence of diabetes and IGR, and it was significantly associated with a lower risk of prevalent diabetes and IGR in men and postmenopausal women . In patients with T2DM, a higher PRL level is linked to diabetes complications, diabetic medication primarily metformin, lowered PRL serum levels in T2DM patients by improving insulin resistance, as a result, a high PRL level in patients

with T2DM is seen to be a positive occurrence in overcoming IR and diabetic complications (Al-Nami *et al.*, 2019).

Hyperprolactinemia causes metabolic changes, dopamine agonists must control PRL excess to promote weight loss and improve metabolic profile (Auriemma *et al.*, 2018). Dopamine agonists are commonly used to treat prolactinomas pharmacologically, bromocriptine and cabergoline are the most frequently treated dopamine agonists. cabergoline has greater response rate and fewer side effects, becoming the preferred medicine for treating prolactinomas, bromocriptine was approved to help people with diabetes mellitus improve their glycemic control (Bolyakov and Paduch , 2011).

On the other hand, infertility is caused by HPL in about 11% of oligospermic males, it inhibits GnRH pulsatile secretion, leading to lower pulsatile release of FSH, LH and testosterone, resulting in spermatogenic arrest, impaired sperm motility and sperm quality, it leads to secondary hypogonadism and infertility later, it also causes primary hypogonadism and infertility by acting on prolactin receptors in sertoli cells and leydig cells in the testes, which influence spermatogenesis and steroidogenesis, on the other hand, patients with oligospermia or azoospermia who have normal levels of gonadotrophins have higher levels of prolactin, indicating that prolactin plays a role in gametogenesis that is independent of gonadotrophins (Masud *et al.*, 2007).

The HPL can inhibit testosterone production and male fertility by causing adrenal corticoid hypersecretion or inhibiting GnRH secretion via prolactin receptors on hypothalamic dopaminergic neurons (Dabbous and Stephen, 2017).

The HPL impairs the pulsatile LH release, which results in a decrease of serum testosterone secretion and generally believed that this hypogonadism is the main cause of ED, serum testosterone is in the normal range in nearly half of the ED

patients with marked HPL, in addition, serum SHBG is low in HPL males (Vermeulen *et al.,* 1982 ; Redman *et al.,* 2012), which attenuates the biological impact of low total testosterone by increasing its unbound proportion, during treatment of hyperprolactinemic men with the prolactin-lowering agent bromocriptin, sexual improvement correlates better with serum PRL's decrease than with testosterone's increase, also erection can return prior to any increase in testosterone (Buvat, 2003).

Green and Amadi (2020) showed that a various degrees of sexual dysfunction among hyperprolactinemia patients as erectile dysfunction which decreased libido, gynecomastia and galactorrhea in these patients.

Nevertheless, PRL improved insulin sensitivity and healthy growth of adipose tissue in both rodents and humans, and these effects are shown during obesity and insulin resistance, both of which are linked to low circulating PRL levels (Ruiz-Herrera *et al.*, 2017).

## 2.6 Nitric Oxide Synthase (NOS) and Fertility

NOS a family of enzymes that produced nitric oxide (NO) by the oxidation of L-arginine to L-citrulline (Cinelli *et al.,* 2020). There are three isoforms of NOS, two of these, neuronal NOS (nNOS) and endothelial NOS (eNOS), are permanently expressed, nNOS is largely present in the nervous system and is required for neuronal signaling, eNOS is found in the endothelium and is required for vasodilation and control of blood pressure (Alderton *et al.,* 2001). These two isoforms create nanomolar amounts of NO for short time periods (seconds to minutes), whereas the third iNOS is inducible, iNOS is not present in cells all the time and is only expressed when the cell is induced or stimulated, generally by pro-inflammatory cytokines and / or bacterial lipopolysaccharide (LPS) (Kone *et*

*al.,* 2003). iNOS synthesizes a higher amount of NO in chronic inflammatory conditions, therefore, iNOS is primarily responsible for the increased production of NO (Nathan and Xie, 2004).

NOS appears to play an important role in skeletal muscle to the regulation of many muscle functions including blood flow, contraction, metabolism and in particular skeletal muscle glucose uptake (Lau *et al.,* 2000 ; McConell *et al.,* 2006).

Krause and his colleagues (2012) observed that NOS levels were lower in the obese T2DM group compared to control, T2DM non-obese patients had higher NOS concentration than controls, as a result, the presence of diabetic comorbidities should be considered when evaluating NOS levels in diabetic patients. However, Foroumandi and his colleagues (2019) found NO levels were to be positively associated with BMI in both male and female peoples, increased NO levels in obese people may be due to increase NOS production, furthermore, in animal investigation have revealed that plasma and aorta NO levels in obese animals were significantly greater than in normal weight animals (Valerio *et al.,* 2006). In human study it also found that obese and overweight individuals had higher NO levels than normal weight people (Fujita *et al.*, 2011).

In obese and diabetic states, the bioavailability of NO is decreased in both adult and adolescent humans, because the availability of NO is dependent upon its generation and degradation, lower levels observed in obese states may be due to downregulation of NOS, diminished NOS activity, or by reaction of NO with reactive oxygen species (ROS) such as superoxide, in particular, eNOS abundance and activity are reported to decrease remarkably in obese and diabetic states and are likely a central feature regulating body composition (Gruber *et al.,* 2008 ; Sansbury *et al.,* 2014).

Kurohane and Ishikawa (2013) found the evidence to support the physiological role of eNOS and nNOS in β-cells, such as regulation of insulin secretion and protection against apoptosis, which is involved in the development of both T1DM and T2DM.

Insulin resistance in T2DM is not only a metabolic disorder characterized by decreased insulin mediated insulin-stimulated glucose disposal but also a vascular disorder associated with impaired NOS activity and elevated circulating risk factors for atherosclerotic cardiovascular disease (Sangeeta *et al.*, 2005).

The effect of diabetes on NOS isoforms in comparison with the control group, there was a significant decrease in nNOS in the cerebellum of the diabetic group, iNOS increased significantly in diabetic group in comparison with control group, and the third isoform, eNOS, there was no significant changes in its level in the diabetic cerebellum (Elhessy *et al.*, 2020).

On the other hand, NOS is found in sertoli cells, leydig cells, spermatocytes, neuronal plexus in the adventitia of arterioles, vascular endothelial cells, immature sperm head and smooth muscle cells, implying that NO / NOS can maintain testicular arteriole tension, regulate testosterone secretion and influence sperm development (Luo *et al.*, 2021). Furthermore, NOS was expressed in interstitial cells and blood vessels *in vitro* culture of interstitial cells or seminiferous tubules, indicating that the testis can create NO (O'Bryan *et al.*, 2000).

The NO is important role in the biology of the penis, specifically in penile erection, NO is shown in studies to cause smooth muscle relaxation, which is the base of an erection, NO synthase subtypes play different roles in this process, nNOS initiates the erectile response, eNOS promotes the maximum erectile response and iNOS inhibition may result in penile oxidative stress, implying that iNOS may actually promote the protective mechanism of fibrosis and aberrant wound healing (Burnett and Musicki, 2005).

Endothelial nitric oxide synthase (eNOS) is important in erectile functioning, as well as, testosterone deficiency decreases eNOS activity by upregulating reactive oxygen species production, and the decreased eNOS activity decreases cGMP levels in the penis (Li *et al.*, 2016).

The PRL interferes penile erection by directly inhibiting the relaxation of the corpus cavernosum penis (Vega *et al.*, 2010), NO generated by nNOS predominates in the nervous system and mediates many biological functions, making it a likely messenger for the actions of PRL in the brain but also peripherally, and NO produced within the erectile tissue by nNOS is a main promotor of penile erection, opposite effects of PRL on nNOS activity are not unexpected since the regulation of this enzyme is cell specific (Bugajski *et al.*, 2004 ; Wu *et al.*, 2007).

# CHAPTER THREE

# MATERIALS AND METHODS

# 3.1 THE MATERIALS

## 3.1.1 Subjects

The current study was conducted in Al.Sadr Teaching Hospital., some official and private centers and laboratories in Maysan province, during December 2020 to July 2021. The whole sample included 80 men aged 35 – 45 years, divided in four groups (20 men / groups) as following :

- Control group (healthy men).
- Obesity group (have a BMI over than 30 kg / $m^2$ ).
- Diabetic group (Type 2 diabetes mellitus).
- Sub-fertile group (Hyperprolactineamia).

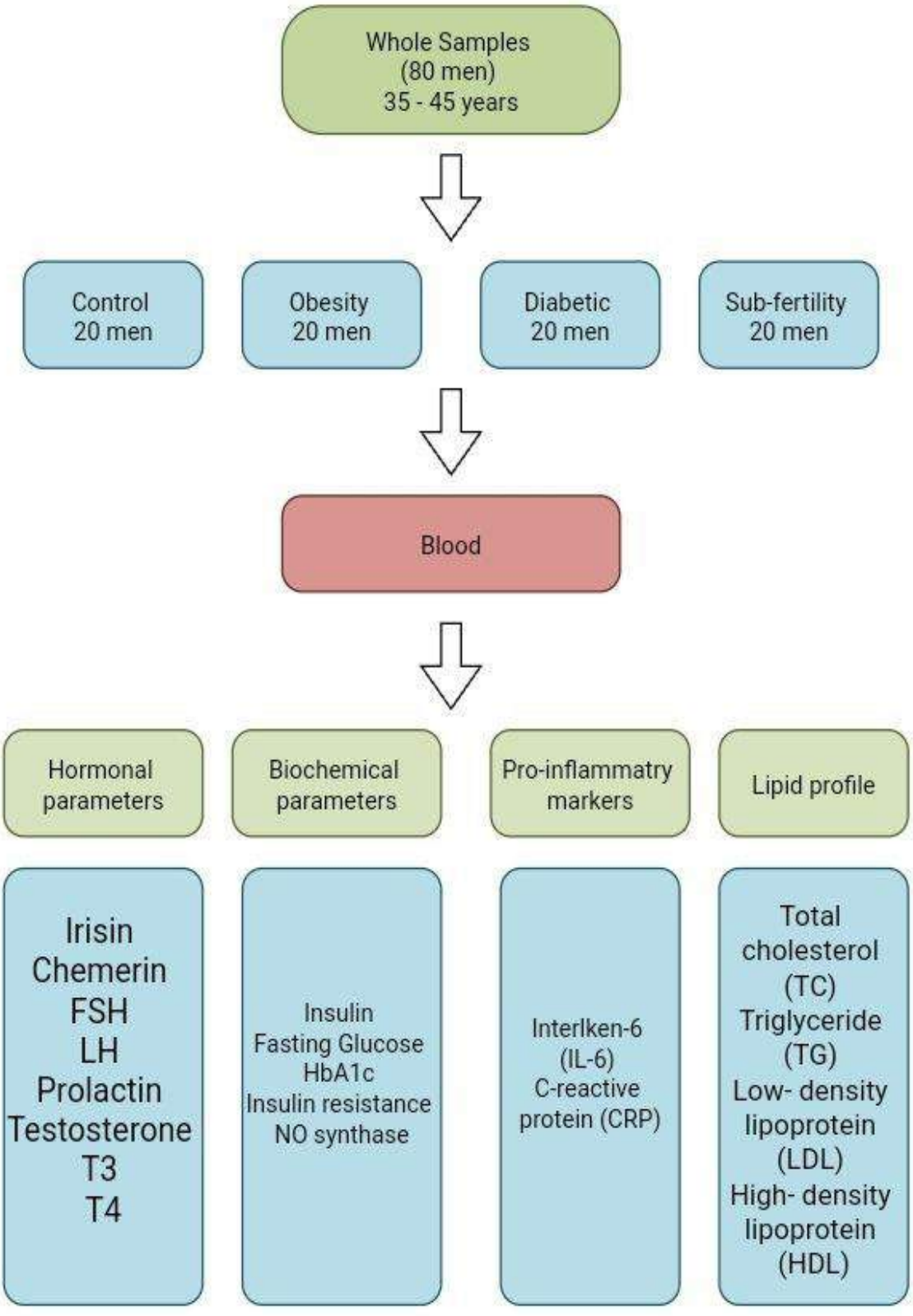
The sample has been checked medically by a specialist physicians and has been diagnosed with obesity, diabetic and sub-fertile (hyperprolactinemia) according to (BMI, HbA1c and prolactin levels respectively). Men with chronic diseases, tumors and those whom treatment with hormonal drug has been excluded.

A questionnaire has been designed to obtain the actual information about the sample individuals in Maysan province.

## 3.1.2 Blood Samples

Eight to ten milliliters of venous blood samples were drawn at 8 - 11 am, using the disposable needle, and plastic syringe for each man. The blood was left at room temperature for (15) minutes for coagulation, centrifuged at (3000) rpm for (5) minutes, then the serum and plasma separated and transferred for storage (-20°**C**)**.**

### 3.1.3 Experimental Design



## 3.1.4 Instruments and Equipment

The tools and equipment that used in this study together with their countries of the origin were shown in Table: (3.1).

**Table (3.1) : The instruments and equipment used in this study.**

| No. | Instrument | Origin |
|---|---|---|
| 1 | Alcohol | UAE |
| 2 | Centrifuge | Japan |
| 3 | Cold box | China |
| 4 | Cotton | Turkey |
| 5 | EDTA tubes | China |
| 6 | Electronic scales | China |
| 7 | Enzyme-linked Immunosorbent Assays (ELISA) | USA |
| 8 | Eppendorf tubes (1.5 ml) | UK |
| 9 | Frozen deep freeze | Germany |
| 10 | Gel tubes | China |
| 11 | Gloves | Turkey |
| 12 | Micro pipetes (10ml, 20ml, 100ml and 200 ml) | Germany |
| 13 | Mindray bs_200 | China |
| 14 | Plain tubes | England |
| 15 | Staining rakes | China |
| 16 | Stature meter | China |
| 17 | Syringe | China |
| 18 | Test tubes for dilution | China |
| 19 | Tips (10ml, 20ml, 100ml and 200 ml) | China |
| 20 | Vidas | Italy |

## 3.1.5 Laboratory Kits

The laboratory kits, used in this study are show in Table (3.2).

**Table (3.2) : The laboratory kits used in this study.**

| No. | Material (Kits) | Origin |
|---|---|---|
| 1 | Irisin | Sunlong biotech / China |
| 2 | Chemerin | Sunlong biotech / China |
| 3 | Interleukin - 6 | Sunlong biotech / China |
| 4 | Nitric oxide synthase | Sunlong biotech / China |
| 5 | Insulin | Sunlong biotech / China |
| 6 | FSH | BioMeriux / France |
| 7 | LH | BioMeriux / France |
| 8 | Prolactin | BioMeriux / France |
| 9 | Testosterone | BioMeriux / France |
| 10 | T3 | BioMeriux / France |
| 11 | T4 | BioMeriux / France |
| 12 | Glucose manual | Spinreact /Spain |
| 13 | HbA1c | Bio Lab / France |
| 14 | Cholesterol | Bio Lab / France |
| 15 | Triglyceride | Bio Lab / France |
| 16 | High density lipoprotein (HDL) | Bio Lab / France |
| 17 | Low density lipoprotein (LDL) | Bio Lab / France |
| 18 | C-Reactive Protein | Spinreact /Spain |

### 3.1.6 Diagnostic Kit

#### 3.1.6.1 ELISA Kit

The contents of ELISA kits are shown in the following:

**Table (3.3) : (Irisin, chemerin, Insulin, IL-6 and NOS) ELISA kit Components.**

| No. | Item | Specifications |
|---|---|---|
| 1 | User-manual. | 1.0 |
| 2 | Closure-plate membrane. | 2.0 |
| 3 | Sealed-bag. | 1.0 |
| 4 | Micro Elisa strip plate | 1.0 |
| 5 | Standard : 45 ng/ml | 0.5 ml × 1 bottle |
| 6 | Standard_diluent. | 1.50 ml ×1 bottle |
| 7 | HRP- Conjugate-reagent. | 6.0 ml × 1 bottle |
| 8 | Sample_diluent. | 6.0 ml × 1 bottle |
| 9 | Chromogen_ Solution-A. | 6.0 ml × 1 bottle |
| 10 | Chromogen _Solution-B. | 6.0 ml × 1 bottle |
| 11 | Stoped-Solution. | 6.0 ml × 1 bottle |
| 12 | Washed-Solution. | 20 ml (30 X) × 1 bottle |

#### 3.1.6.2 Vidas Automated Kits

The contents of Vidas kits for FSH, LH, Prolactin, Testosterone, T3 and T4 are listed as the following :

Materials required. (but, not provid):

− Pipette, with disposable tips that will dispense : 3ml, 2ml and 200µl.

− Disposable glove.

− Instrument of the VIDAS family.

### 3.1.6.3 Mindary Automated Kits

1. Lipid profile (TC, TC, HDL-C and LDL-C).
2. CRP.
3. HbA1c

**Table (3.4) : Mindary kit Components.**

| No. | Item | Specifications |
|---|---|---|
| 1 | reagent | 2vial |
| 2 | Disposable stirrers | 2 × 50 |
| 3 | Negative control. | 1 × 1 ml |
| 4 | Positive control. | 1 × 1 ml |
| 5 | Test cads | 3 |

# 3.2 METHODS

## 3.2.1 Body Mass Index (BMI)

calculate by dividing weight with (kg) by, the square of height with ($m^2$) according to (WHO, 2020), obese individual has an over 30 $kg/m^2$ (BMI).

## 3.2.2 Determination of Hormones Assay

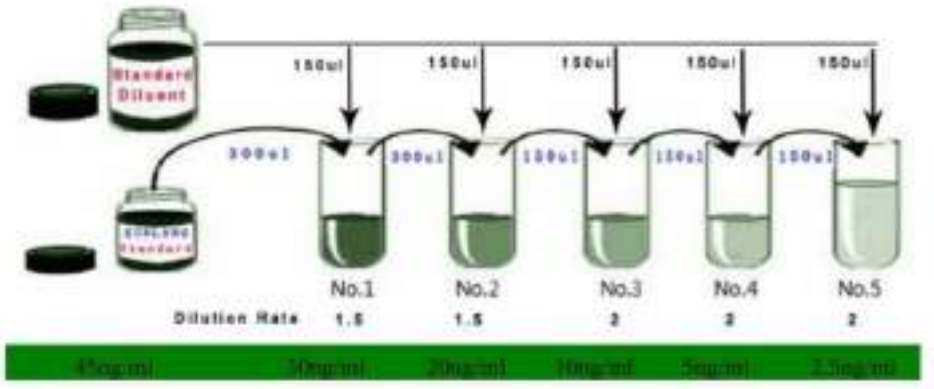
### 3.2.2.1 Determination of Irisin Hormone

Irisin is evaluate by using enzyme-linked-immunosorbent-assay (ELISA) system, with human irisin kit.

**Principle of Assay**

1 – Standards-dilution: Dilute the standard, first in tiny-tubes, then pipette the 50ul from each tube, into a microplate wells (each tube, used two wells).

| | | |
|---|---|---|
| 30ng/ml | Standard No.1 | 300µl Original Standard + 150µl Standard diluents |
| 20ng/ml | Standard No.2 | 300µl Standard No.1 + 150µl Standard diluents |
| 10ng/ml | Standard No.3 | 150µl Standard No.2 + 150µl Standard diluent |
| 5ng/ml | Standard No.4 | 150µl Standard No.3 + 150µl Standard diluent |
| 2.5ng/ml | Standard No.5 | 150µl Standard No.4 + 150µl Standard diluent |




2 - As a blank control, leave the well vacant in a Micro Elisa strip-plate. (40 µl) Sample-dilution buffer, and (10 µl) sample is introduce to sample wells, (dilution-factor is 5). Without hitting the well wall, sample shall be load on to the bottom. Mix thoroughly, with the light hand.

3 – Incubation. : after the sealing with a closure-plate membrane, incubate for (30) minutes at (37°C).

4 – Dilution,: dilute a concentrate washing-buffer (30-times, for 96 T and 20-times for 48 T) with distilled water.

5 – Washing, : remove the Closure-plate membrane with a care, aspirate, and refill with wash-solution. After (30) seconds of the resting discard the wash-solution. repeat the washing process 5-times more.

6 – Addition: 50 µL of Horseradish Peroxidase HRP-Conjugate-reagent, to the each well, excepts for the empty-control well,.

7 – Incubation: as a described in Step(3).

8 – Washing: as a described in Step (5).

9 – Coloring: in each-well, pour (50 µl) Chromogen Solution-A and (50 µl) Chromogen Solution-B, gently mixing, and incubate at (37°C) for (15) minutes.

10 - Termination: to a halt the reaction, pour (50 µl) stop-solution into each well,. The well's hue should be shift, from (blue- to yellow).

11 - Using: a Microtiter-Plate reader., read an absorbance optical density O.D. at (450 nm). The blank-control well's O.D. value is set to (0). After adding the stop-solution, (the assay should be completed, within (15) minutes).

**Assay Range** : 0.5 ng/ml - 30 ng/ml.

### 3.2.2.2 Determination of Chemerin Hormone

Chemerin is evaluate by using enzyme-linked-immunosorbent-assay (ELISA) system, with human chemerin kit, as described in Irisin determination method .

### 3.2.2.3 Determination of fertility hormones (FSH, LH, T and PRL):

Determinate by using Vidas system, with human FSH, LH, T and PRL kits,

**Principle of Assay**

1 - Remove the necessary components from kit, and store the remain components at (2 – 8 °C ).

2 - Allow time for the components, to warm up to room temperature (approximately 30 minutes).

3 - For each sample:control or calibrator, to be tested, use one " FSH, LH, T and PRL " strip, and one " FSH, LH, T and PRL " SPR. After an appropriate SPRs have been withdrawn, making sure the storage pouch, has been carefully resealed.

4 - The test was identified by the (FSH, LH, T and PRL) code, on an instrument. The calibrator must be identified by S1, and tested in duplicate, if the control tested, it should be to identified by C1.

5 - Label the (FSH, LH, T and PRL) Reagent Strips with the appropriate sample ID numbers if necessary.

6 - Using a vortex type mixer, combine the calibrator, control, and sample.

7 - For this test; the calibrator, control and sample tests portion is (200 µl).

8 - Place the (FSH, LH, T and PRL) Reagent-Strips and SPRs in an instrument's appropriate positions. Check that the color-labels on the SPRs and the Reagent-Strips match assay code.

9 - As recommended in the Operator's-Manual of vidas of biomerieux, begin the assay processing. The equipment performs all of the assay processes automatically.

10 - After pipetting, re-close the vials, and bring them to a necessary temperature.

11 - The assay will take about (40) minutes to complete. Removed the SPRs, and strips from an instrument once an assay is finished.

**Assay range (FSH) :** 1.7 - 12 mlU/ml.

**Assay range (LH) :** 1.7 – 7 mlU/m.

**Assay range (T) :** 2.80 – 8 mlU/m.

**Assay range (PRL) :** 4.6 – 21.4 mlU/m.

### 3.2.2.4 Determination of Thyroid Hormones (T3 and T4) :

Determinate by using Vidas system, with **T3 and T4** human kits, as described in Fertility hormones determination method.

**Assay range (T3) :** 1.3 – 3.1 mlU/m.

**Assay range (T4) :** 66 – 181 mlU/m.

## 3.2.3 Determination of Biochemical Assay

### 3.2.3.1 Determination of Insulin Hormone

Insulin was evaluated by using enzyme-linked immunosorbent assay (ELISA) system, with insulin human kit, as described in Irisin determination method

**Assay Range :** 0.3 mU/L -20 mU/L.

### 3.2.3.2 Determination of Glucose Assay

Glucose (GLU) was evaluated by using Mindray system, with human GLU kit. The serum glucose was determined by enzymatic colorimetric (GOD-PAP) method, using kit supplied by Spinreact, Spain (Trinder, 1969).

**Assay procedure:**

| Solutious | Sample | Standard | Blank |
|---|---|---|---|
| Workin greagent | (lml) | (lml) | (lml) |
| Standard | | 10µL | |
| Sample | 10µL | | |

Mixed and incubate for (10 min) at (37 ºC) or (20 min) at the room-temperature (15-25. ºC) reading of the absorbance A of the samples and the standard-against the blank at 505 nm. The colour is stable for (30 min).

**Calculations:**

Glucose mg/dl= $\boldsymbol{Asample}$ /$\boldsymbol{Astandard}$× **100**

**Assay Range :** 70 – 120 mg / dl.

### 3.2.3.3 Determination of Homeostatic-Model-Assessment for Insulin-Resistance ( HOMA – IR ) Assay:

Is calculate by dividing:

(Fasting-insulin) (μU/mL) x (fasting-glucose) (mg/dl) ÷ 405 (Onishi *et al.*, 2010)

### 3.2.3.4 Determination of HbA1c Assay :

HbA1c was evaluated by using Mindray system, with human HbA1c kit.

**Principle of Assay**

1 - The HbA1c kit put out of refrigerator for 10 min.

2 - The reagents are placed in their place on the side of the external device.

3 - The two milliliter (2ml) of blood was taken by medical syringe and place it in EDTA tube and then,the sample mixed gently by inverting the tube.

4 - The sample tubes allowed to reach the room temperature (25 c) before performing assy.

5 - The sample tube is loaded into the D-10sample rack and put it in the place known inside the device D-10

6 - Patient QC ID was appearing on the screen after they have been acanned by the barcode reader.

7 - The DONE button was press after you have entered each patient ID

8 - The START button was press to begin the analysis.

9 - The steps for the device followed to start the calibration process automatically.

**Assay range :** 4.2 – 6.4.

## 3.2.4 Determination of Pro-inflammatory Assay

### 3.2.4.1 Determination of Interleukin-6 Assay

IL-6 was evaluated by using enzyme-linked immunosorbent assay (ELISA) system, with human IL-6 kit, as described in Irisin determination method.

**Assay Range :** 2 ng/L -80 ng/L.

### 3.2.4.2. Determination of CRP Assay

CRP is evaluate by using mindray automate, with human CRP kit (Osmand *et al.*, 1977).

**Assay Procedure**

The reagent and sample allowed to reach to room temperature. The (50 µL ) of the samples placed and one drop of each, positive and negative controls into the separate circles, on the slide test. The CRP-latex reagent, mixed vigorous or on the vortex mixer before used and added 1 drop (50 µL) next to the samples to be test. The drops. mixed with the stirrers, spreaded them over the entire-surface of the circle. different stirrers, were used for each samples. The slide placed on a mechanical rotator at 80-100 r.p.m for 2 minutes.

**Calculation of Results**

Calculations C-RP level was determined by the automated analyzer used the prepared calibration-curve, it was recommend that the multi-point calibration curve be developed used the C-RP Multi-standard set. It was recommend that user determined calibration frequencies as this would depend on an instrument, and type / number of other assay be running. Initially, calibration, should be a perform each day.

**Assay Range :** 0 – 5 mg / L.

## 3.2.5. Determination of Nitric Oxide Synthase (NOS) Assay

NOs was evaluated by using enzyme-linked immunosorbent assay (ELISA) system, with human NOS kit, as described in Irisin determination method

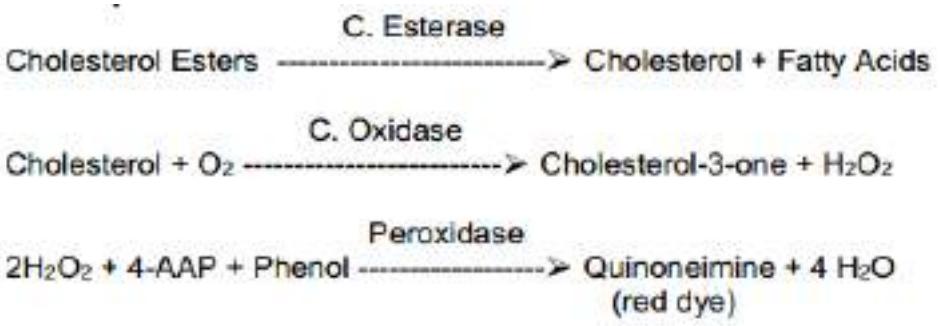
**Assay Range :** 0.8 μmol/L - 30 μmol/L.

## 3.2.6 Lipid Profile Assay

### 3.2.6.1 Total Cholesterol

The TC was evaluated by using mindray automated, with human TC kit (Richmond, l972).

**Principle of Assay**




The intensity of red color was produce directly proportional to the TC in the samples when read at (500 nm).

**Reagent Preparation**

The 4-Aminoantipyrine 0.25 mM, Cholesterol Esterase > 150u/L, TC Oxidase >150 u/L, Peroxidase > 1500u/L, Phenol > 15mM, Phosphate Buffer, pH 6.8, non-reactive stabilizer and preservative. Reagent Preparations, The reagent was ready to used.

**Calculation of Results**

Abs: Absorbance

Abs. (Patient) x Concentration of Std. = Cholesterol (mg/dl) Abs. (Standard) (mg/dl) Example: Abs(Patient) = 0.40, Abs(Standard) = 0.32

**Assay Range :** 135 – 200 mg / dl.

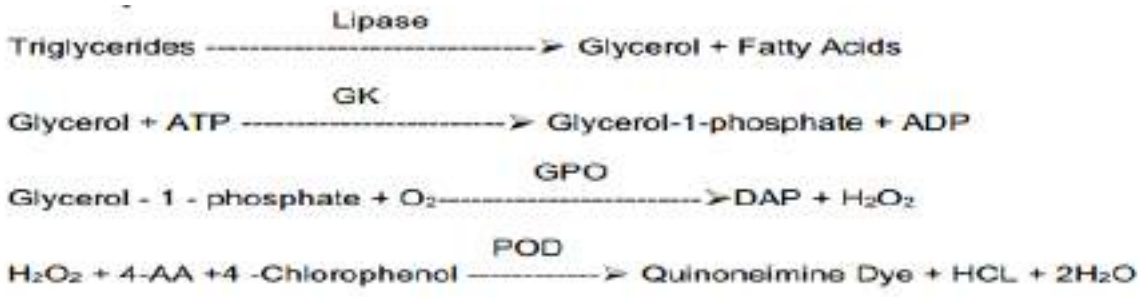
### 3.2.6.2 Measurement of Serum Triglyceride (TG) Assay

TG was evaluated by using mindray automated, with human TG kit (Villanova,1992).Very Low Density Lipoprotein estimated by dividing TG by five (Friedewald *et al.*,1972).

**Principle of Assay**

Serum TG are hydrolyzed to the glycerol and free fatty-acids by the lipase. In the presence of (ATP) and a glycerol kinase, the glycerol was converted to glycerol-1-phosphate,. The glycerol-1-phosphate was then oxidized by glycerol phosphate oxidase to yield hydrogen peroxide.

The condensation of the hydrogen peroxide with (4-chlorophenol) and (4-aminophenazone) in the presence of peroxidase produced a red color quinonimine dye which absorbed at, or near (500 nm). The intensity of the color complex formed was directly proportional to the TG concentration of samples.



**Assay Procedure**

The procedures are linear to, 1000 mg/dl (11.3 mmol/L). Specimens above this limited must be diluted (1:1 ) with saline and re –assayed. Multiply the results by ( *2* ) to compensated, for the dilution.

**Calculation of Results**

TG results were expressed as a mg/dl or mmol/L.

TG = Abs. ( Unk) x (Conc.) Std Abs. Std

Example: Abs. Unk = 0.2430 Abs. Std. = 0.3100 Conc.

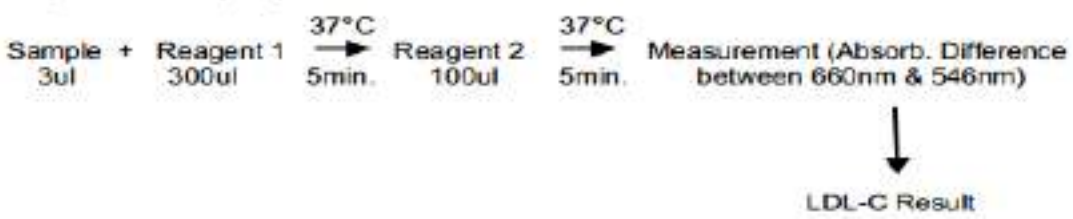
**Assay Range :** 90 – 200 mg / dl.

### 3.2.6.3 Low Density Lipoprotein

LDL was evaluated by using mindray automated, with human LDL-C kit (Badimon *et al.*,1990).

**Assay Procedure**

Below was a general example of the auto LDL TM. tested procedure for the automated analyzer. All analyzer application should be a validated in accordance with (NCEP), and (CLIA) recommendations.10 For assistance with the applications, on an automated analyzers.




**Calculation of Result**

To converted from conventional units to (S.I. units), multiplied the conventional units by (0.02586.)

Example: mg / dL x 0.02586 = mmol / L (LDL).

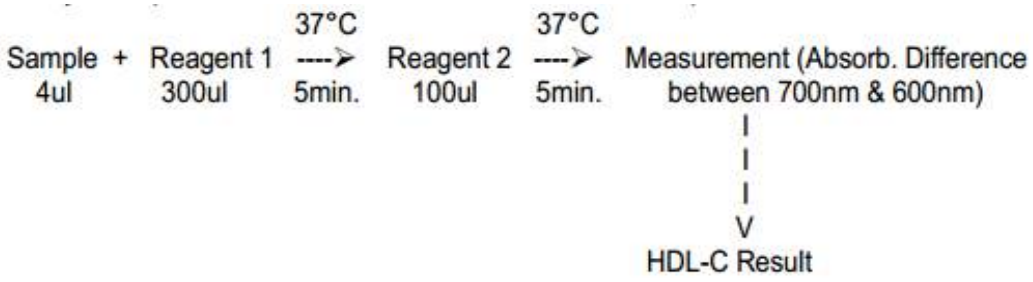
**Assay range :** 0 – 135 mg / dl.

### 3.2.6.4 High Density Lipoprotein

HDL-C was evaluated by using mindray automated,with human HDL-C kit (Gotto, 1988).

**Assay Procedure**

Below was the generals example of the auto HDL test procedures for the automated analyzer, all the analyzed applications should be, validated in an accordance with (NCEP), and (CLIA) recommendations. For assistant with applications, on automated. analyzers, please contacted the technical service department (*37*°C).




**Calculation of Results**

To convert from conventional units to (SI Units), multiply the conventional units by (0.02586. mg/dl x 0.02586 = mmol /L) HDL.

**Assay range :** 40 – 60 mg / dl.

## 3.3 Statistically Analysis

Statistically analysis was performed by IBM (SPSS) statistics, version 23 (IBM Co., Armonk., NY., USA). The statistically analysis was performed by the one-way-Analysis-Of-Variance (ANOVA), followed by (*Duncan's* ) a new multiple range test (DMRT) at a ($p \leq 0.05$) and ($p \leq 0.01$) significant level (Steel *et al.*, 1997).

# CHAPTER FOUR

# RESULTS

# 4.1 Hormonal Parameters

## 4.1.1 Irisin

The results revealed that :

Irisin levels in sub-fertility group decreased significantly ($p \leq 0.01$) in comparison with the control, obesity group and not significantly with diabetic group.

Irisin levels in diabetic group decreased significantly ($p \leq 0.01$) in comparison with the control and obesity group, whereas irisin in obesity group increased significantly ($p \leq 0.01$) in comparison with the control. (Table (4.1) ; Figure (4.1)).

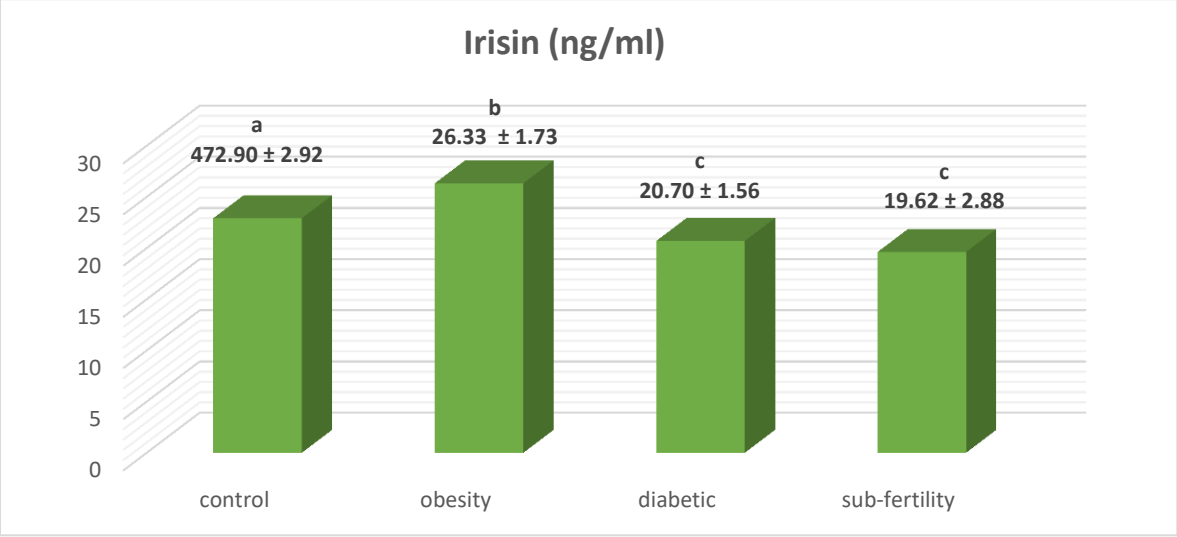



**Figure (4-1) : Levels of Irisin hormone in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference.

## 4.1.2 Chemerin

The results revealed that :

Chemerin levels in sub-fertility group increased significantly ($p \leq 0.01$) in comparison with the control, obesity group and diabetic group.

Chemerin levels in diabetic group increased significantly ($p \leq 0.01$) in comparison with the control and decreased significantly ($p \leq 0.01$) with the obesity group, whereas in obesity group increased significantly ($p \leq 0.01$) with control. (Table (4.1) ; Figure (4.2)).

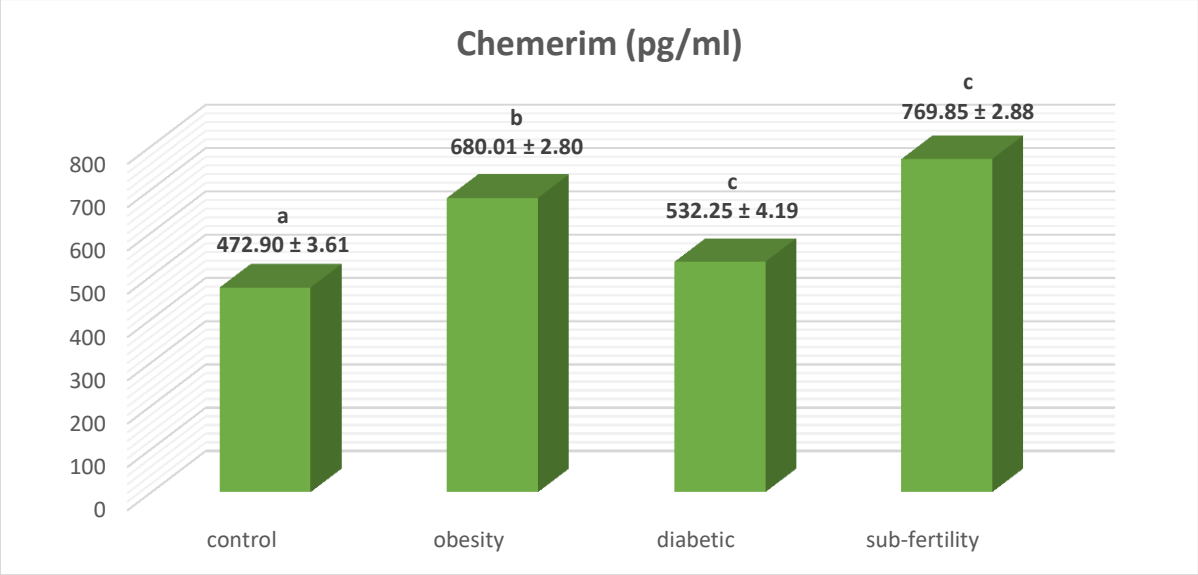



**Figure (4-2) : Levels of Chemerin hormone in different groups.**

*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \geq 0.01$) between groups .
*Similar small letters represent no significant difference .

### 4.1.3 Follicle Stimulating Hormone (FSH)

The results revealed that :

The levels of FSH in sub-fertility group decreased significantly ($p \leq 0.05$) in comparison with the control, obesity group and diabetic group.

The FSH levels in diabetic group decreased significantly ($p \leq 0.05$) in comparison with the control and increased not significantly with obesity group, whereas in obesity group decreased significantly ($p \leq 0.05$) in comparison with the control. (Table (4.1) ; Figure (4.3)).

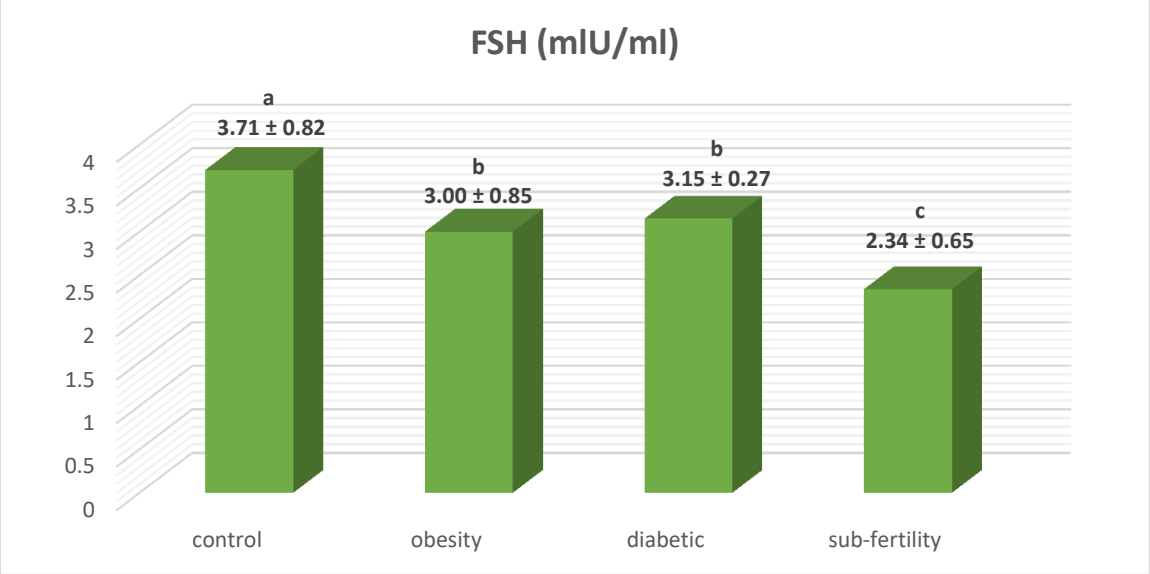



**Figure (4-3) : Levels of FSH in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.05$) between groups .
*Similar small letters represent no significant difference .

### 4.1.4 Luteinizing Hormone (LH)

The results revealed that :

The LH levels in sub-fertility group decreased significantly ($p \leq 0.05$) in comparison with the control, obesity group and diabetic group.

The LH levels in diabetic group decreased significantly ($p \leq 0.05$) in comparison with the control and increased not significantly with obesity group, whereas in obesity group decreased significantly ($p \leq 0.05$) in comparison with the control. (Table (4.1) ; Figure (4.4)).

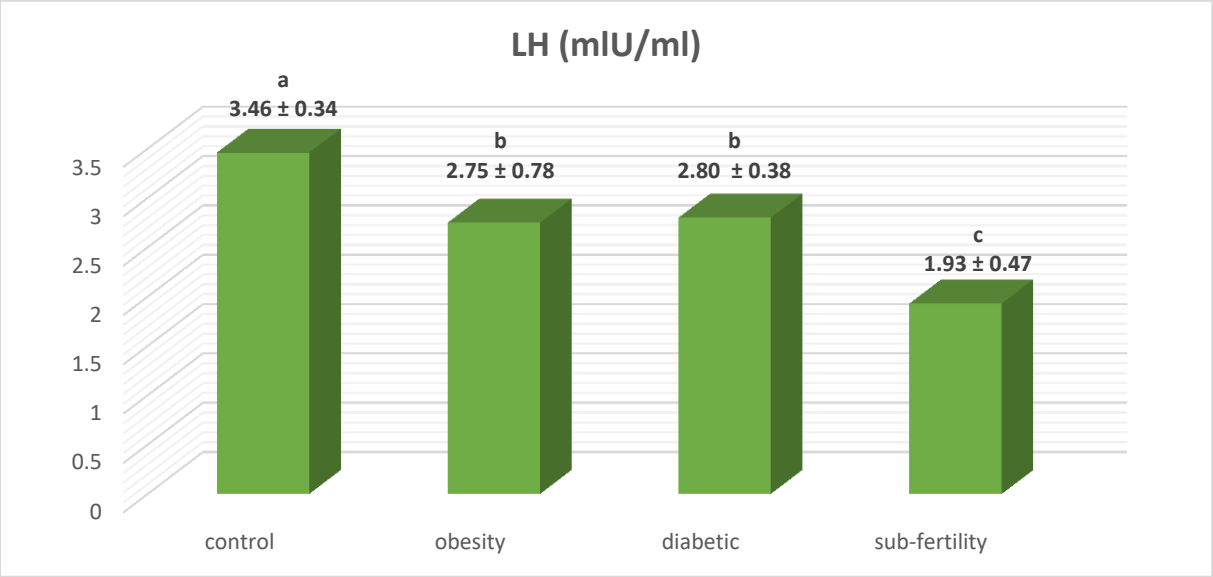



**Figure (4-4) : Levels of LH in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.05$) between groups .
*Similar small letters represent no significant difference .

### 4.1.5 Testosterone (T)

The results revealed that :

The T levels in sub-fertility group decreased significantly ($p \leq 0.05$) in comparison with control and diabetic group and not significant with obesity group.

The T levels in diabetic group decreased not significantly in comparison with the control and increased significantly ($p \leq 0.05$) with the obesity group, whereas in obesity group decreased significantly ($p \leq 0.05$) in comparison with control. (Table (4.1) ; Figure (4.5)).

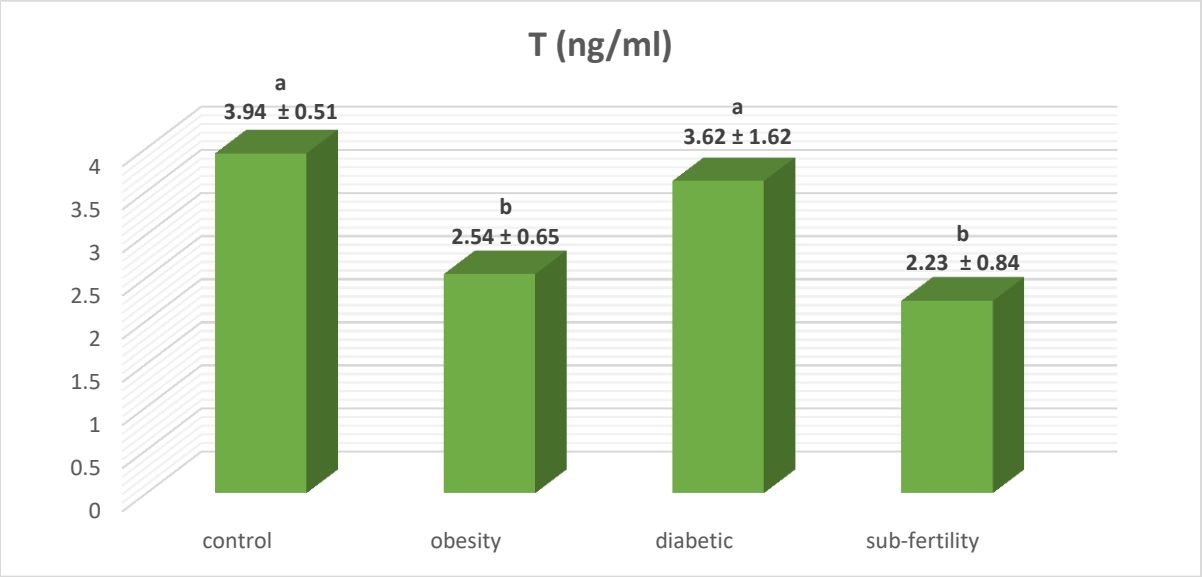



**Figure (4-5) : Levels of T in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.05$) between groups .
*Similar small letters represent no significant difference .

## 4.1.6 Prolactin (PRL)

The result revealed that :

The levels of PRL in sub-fertility group increased significantly ($p \leq 0.01$) in comparison with the control, obesity group and diabetic group.

The PRL levels in diabetic group increased significantly ($p \leq 0.01$) in comparison with the control and decreased not significantly with the obesity group, whereas in obesity group increased significantly ($p \leq 0.01$) in comparison with the control. (Table (4.1) ; Figure (4.6)).

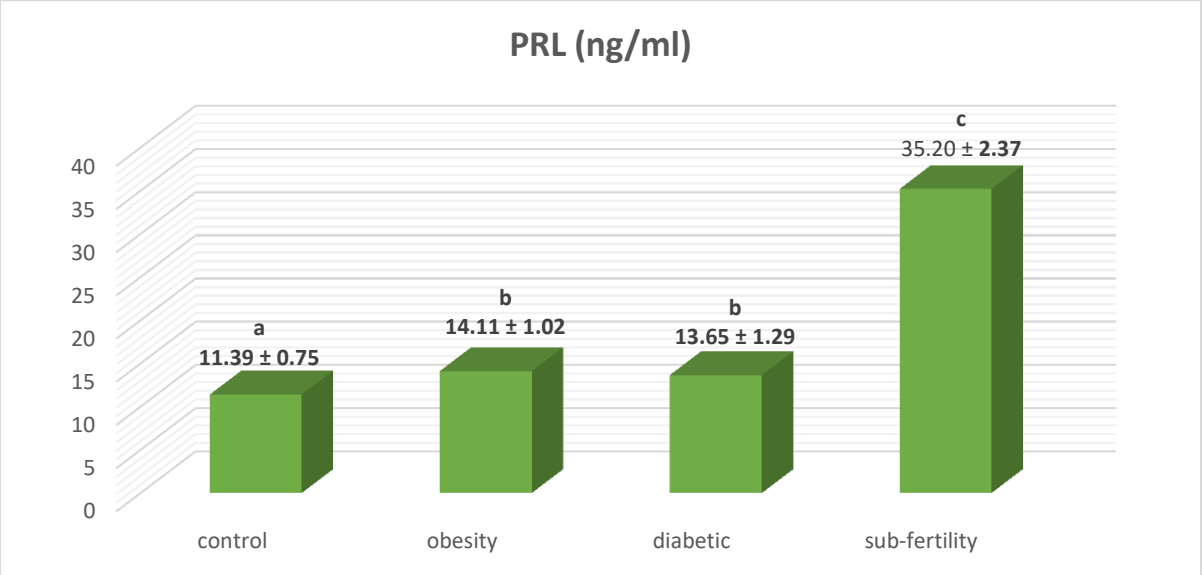



**Figure (4-6) : Levels of PRL in different groups.**

*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

### 4.1.7 Triiodothyronine (T3)

The results revealed that :

The levels of T3 in sub-fertility decreased not significantly in comparison with the control and diabetic groups and decreased significantly ($p \leq 0.01$) in comparison with the obesity group.

The T3 levels in diabetic group decreased not significantly in comparison with the control and obesity groups, and in obesity group increased not significantly in comparison with control. (Table (4.1) ; Figure (4.7)).

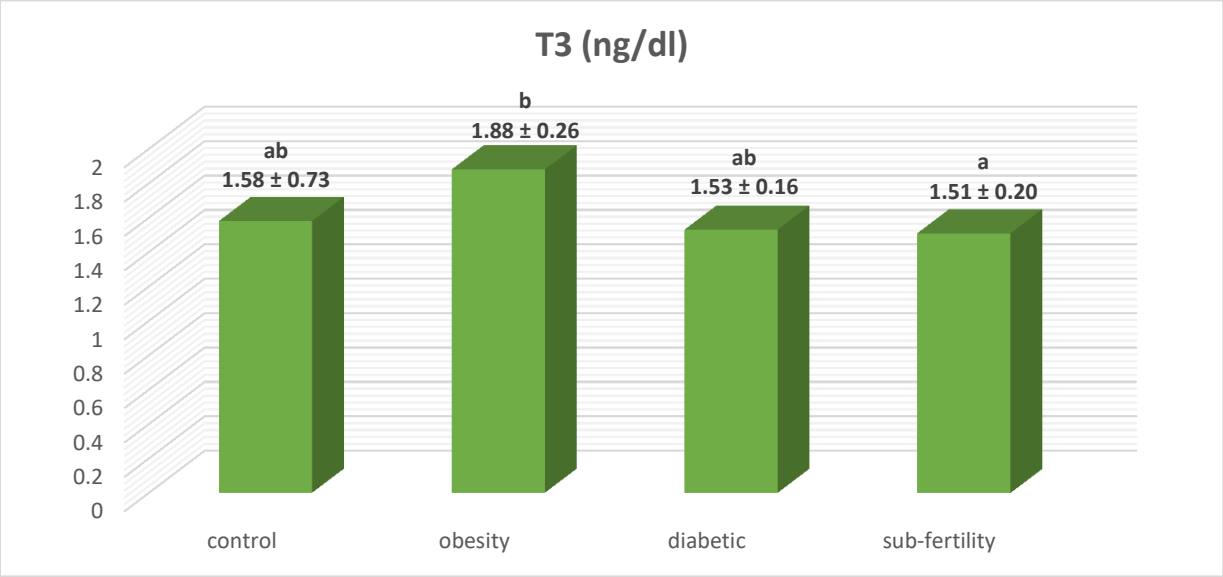



**Figure (4-7) : Levels of T3 in different groups**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

### 4.1.8 Thyroxine (T4)

The results revealed that :

The levels of T4 in sub-fertility group decreased not significantly in comparison with the control and significantly ($p \leq 0.01$) with the obesity group, and increased not significantly with diabetic group.

The T4 levels in diabetic group decreased significantly ($p \leq 0.01$) in comparison with the control and obesity groups, and in obesity group increased significantly ($p \leq 0.01$) in comparison with the control. (Table (4.1) ; Figure (4.8)).

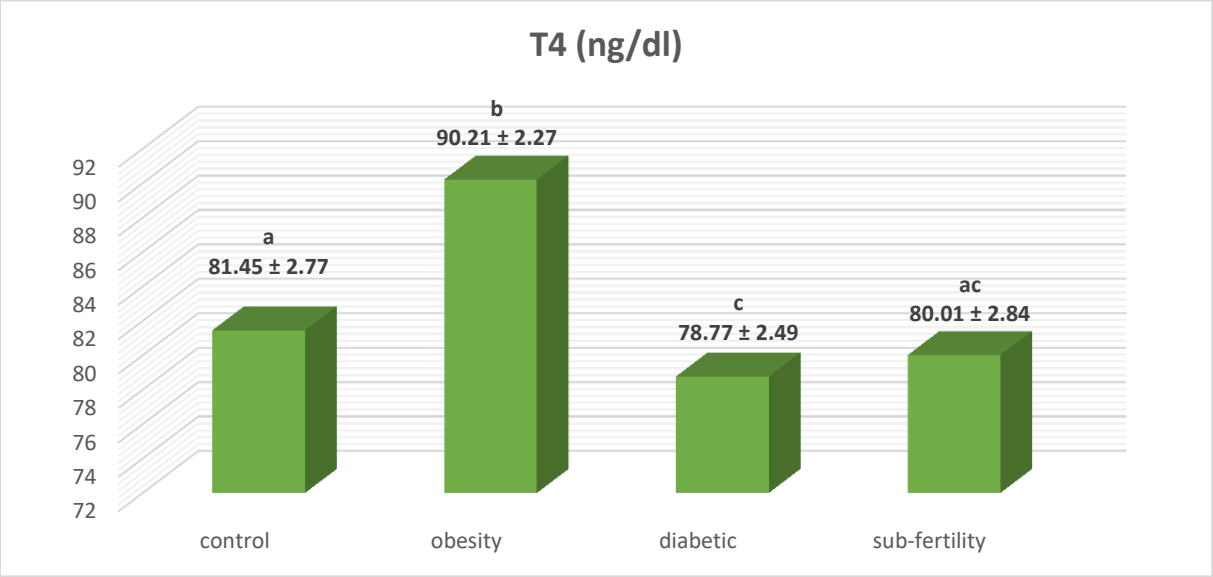



**Figure (4-8): Levels of T4 in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference.

# 4.2 Biochemical Parameters

## 4.2.1 Insulin

The results revealed that :

Insulin levels in sub-fertility group increased significantly ($p \leq 0.01$) in comparison with the control, diabetic group and decreased significantly ($p \leq 0.01$) in the obesity group.

Insulin levels in diabetic group increased not significantly in comparison with the control and decreased significantly ($p \leq 0.01$) with obesity group, and in obesity group increased significantly ($p \leq 0.01$) in comparison with the control. (Table (4.2) ; Figure (4.9)).

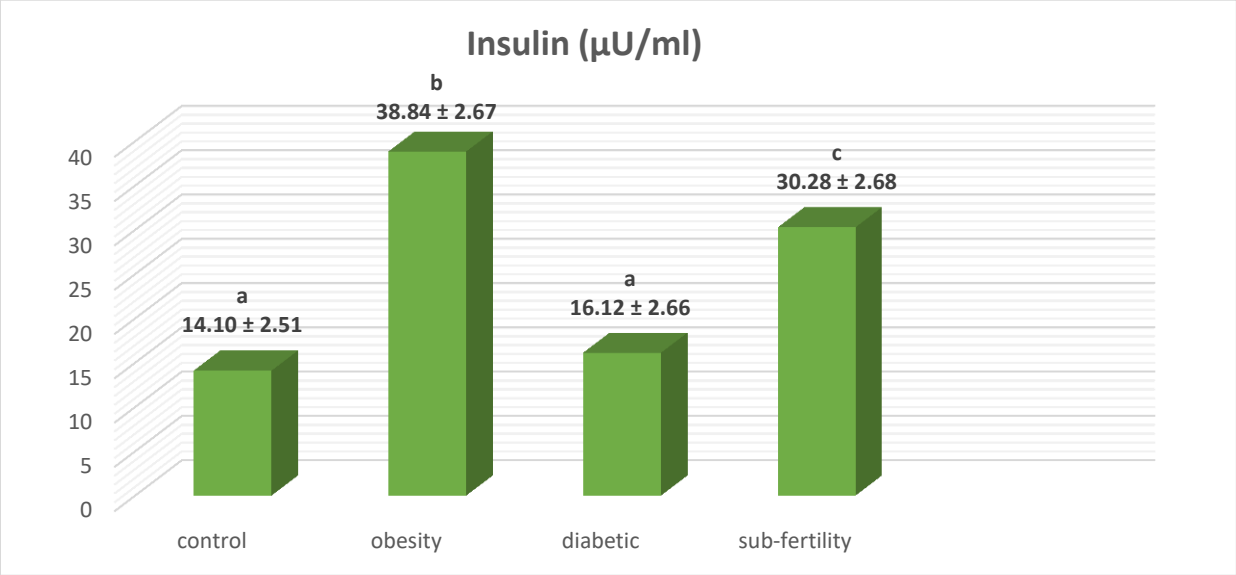



**Figure (4-9) : Levels of Insulin in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

## 4.2.2 Fasting Blood Glucose (F.B.G.)

The results revealed that :

Glucose levels in sub-fertility group increased significantly ($p \leq 0.01$) in comparison with the control and degreased significantly ($p \leq 0.01$) with the obesity group and diabetic group.

Glucose levels in diabetic group increased significantly ($p \leq 0.01$) in comparison with the control and obesity groups, and in obesity group increased significantly ($p \leq 0.01$) in comparison with the control. (Table (4.2) ; Figure (4.10)).

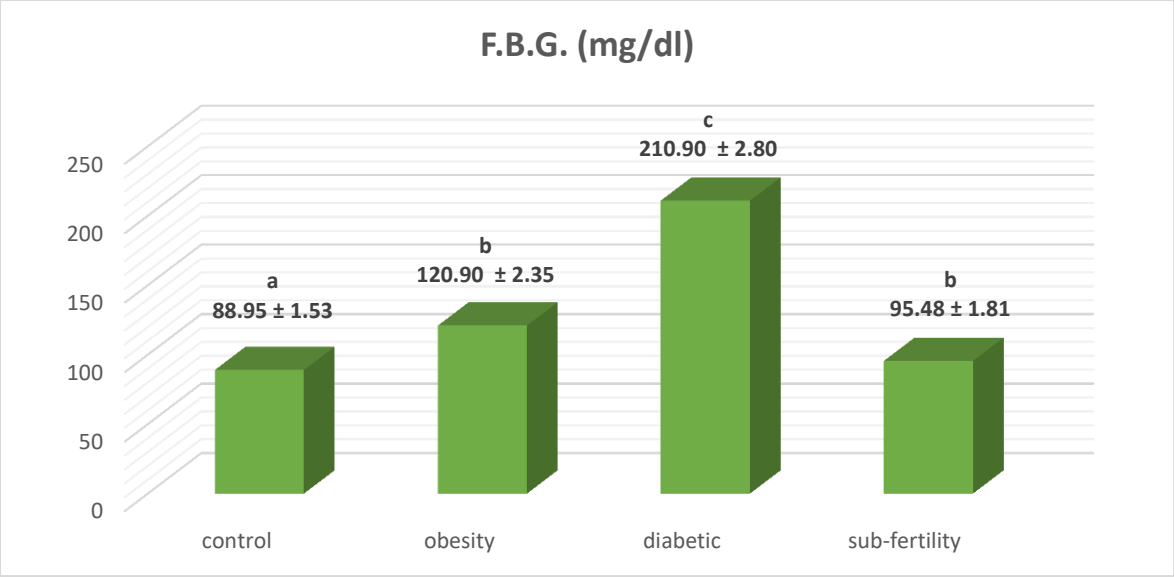



**Figure (4-10) : Levels of F.B.G. in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

### 4.2.3 Insulin Resistance (IR)

The results revealed that :

The levels of IR in sub-fertility group increased significantly ($p \leq 0.01$) in comparison with the control and decreased significantly ($p \leq 0.01$) with the obesity group and decreased significantly ($p \leq 0.01$) with the diabetic group.

The IR levels in diabetic group increased significantly ($p \leq 0.01$) in comparison with the control and obesity group, whereas in obesity group increased significantly ($p \leq 0.01$) in comparison with the control. (Table (4.2) ; Figure (4.11)).

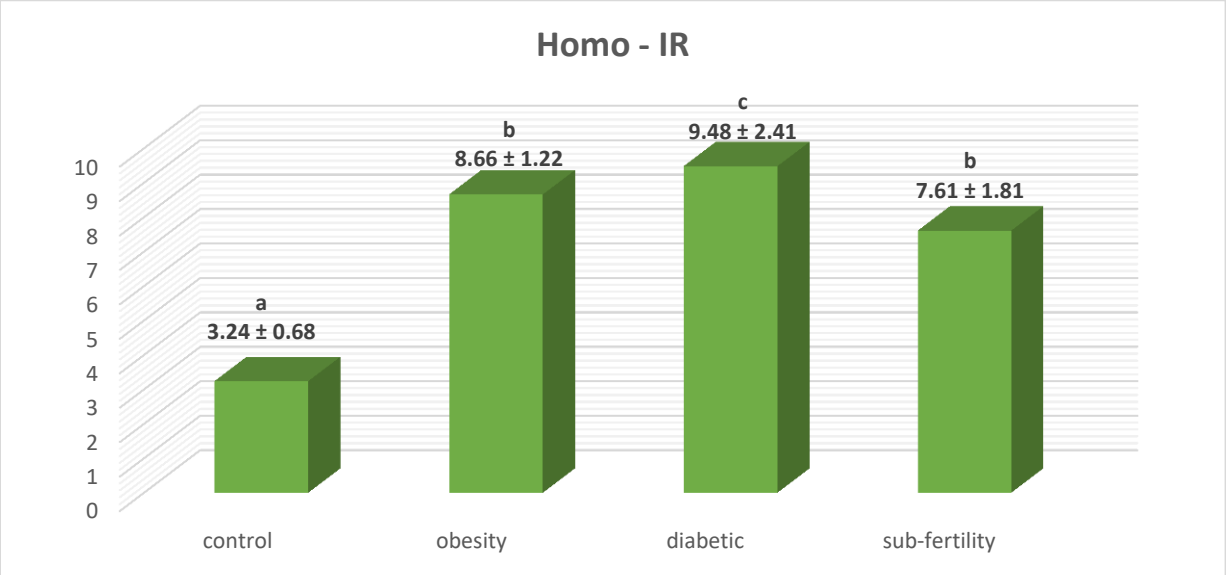



**Figure (4-11) : Levels of HOMA-IR in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

## 4.2.4 Glycated Hemoglobin A1c (HbA1c)

The results revealed that :

The levels of HbA1c in sub-fertility group increased not significantly in comparison with the control and obesity group and decreased significantly ($p \leq 0.01$) with the diabetic group.

The HbA1c levels in diabetic group increased significantly ($p \leq 0.01$) in comparison with the control and obesity group, whereas in obesity group increased not significantly in comparison with the control. (Table (4.2) ; Figure (4.12)).

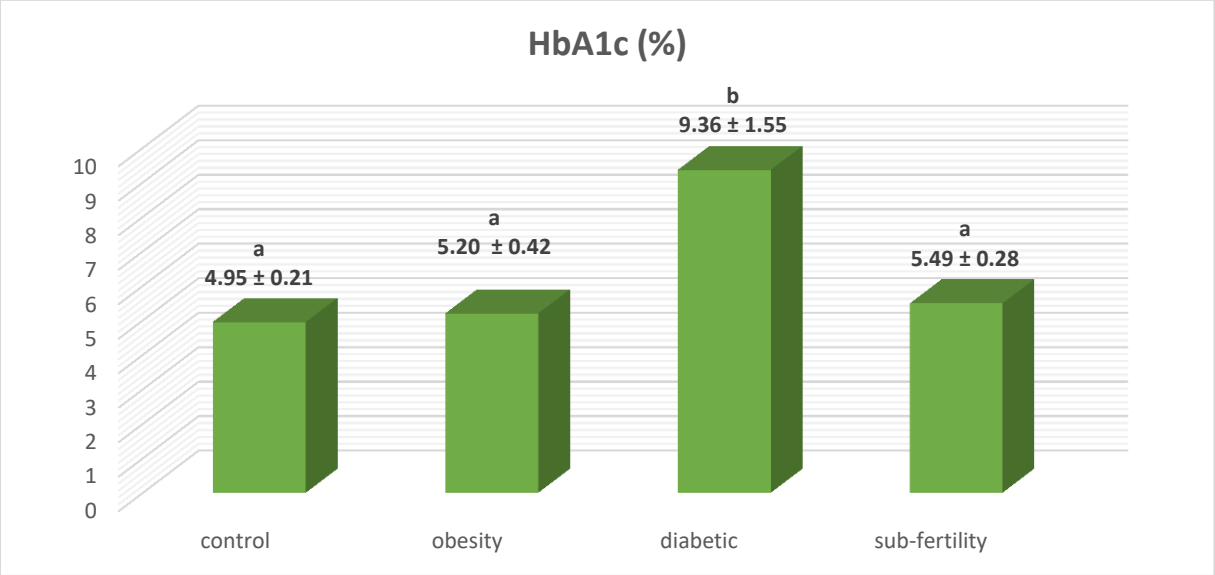



**Figure (4-12) : Levels of HbA1c in different groups.**

*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

## 4.2.5 Pro-inflammatory markers

### 4.2.5.1 Interleukin - 6 (IL-6)

The results revealed that :

The levels of IL-6 in sub-fertility group increased significantly ($p \leq 0.05$) in comparison with the control, obesity group and diabetic group.

The IL-6 levels in diabetic group increased not significantly in comparison with the control and decreased not significantly with obesity group, and in obesity group increased significantly ($p \leq 0.05$) in comparison with the control. (Table (4.3) ; Figure (4.13)).

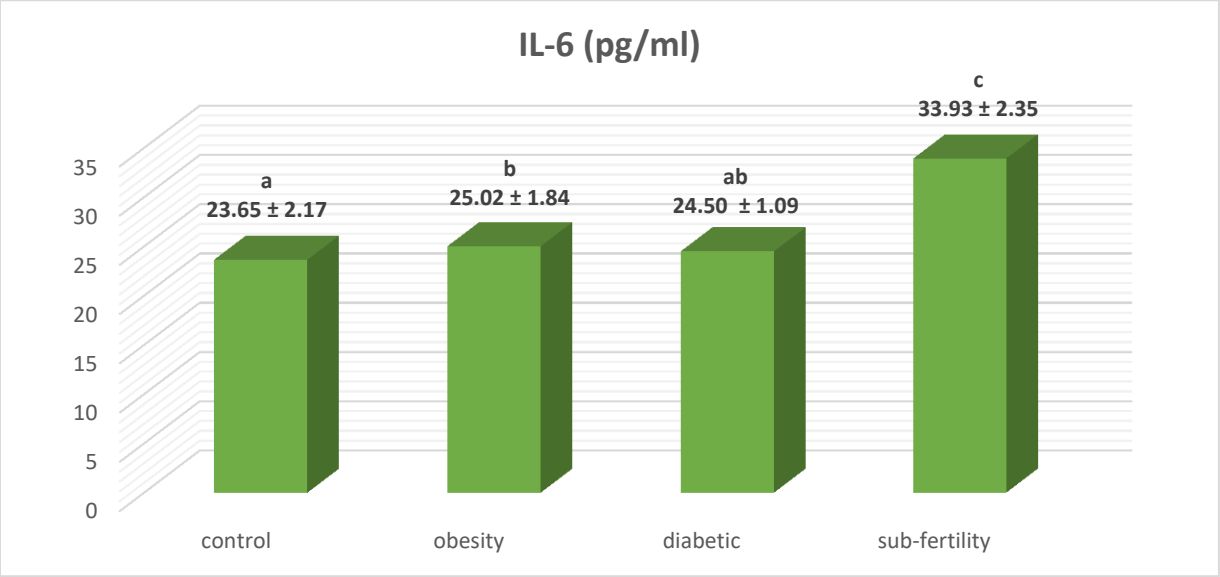



**Figure (4-13) : Levels of IL-6 in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

### 4.2.5.2 C – Reactive Protein (CRP)

The results revealed that :

The levels of CRP in sub-fertility group increased significantly ($p \leq 0.05$) in comparison with the control and increased not significantly with obesity group and decreased not significantly with diabetic group.

The CRP levels in diabetic group increased significantly ($p \leq 0.05$) in comparison with the control and not significantly with obesity group, and in obesity group increased not significantly in comparison with the control. (Table (4.3) ; Figure (4.14)).

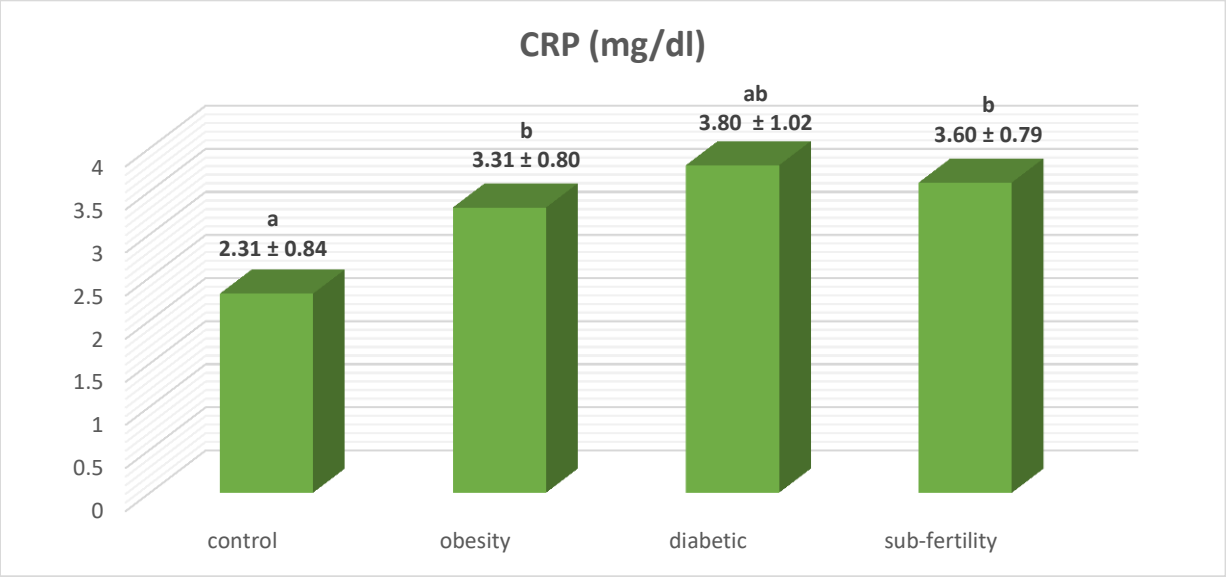



**Figure (4-14) : Levels of CRP in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

## 4.2.6 Nitric Oxide Synthase (NOS)

The results revealed that :

The levels of NOS in sub-fertility group decreased not significantly in comparison with the control and increased not significantly with the obesity group and diabetic group.

The NOS levels in diabetic group decreased significantly ($p \leq 0.05$) in comparison with the control and not significantly with the obesity group, whereas the obesity group decreased significantly ($p \leq 0.05$) in comparison with the control. (Table (4.3) ; Figure (4.15)).

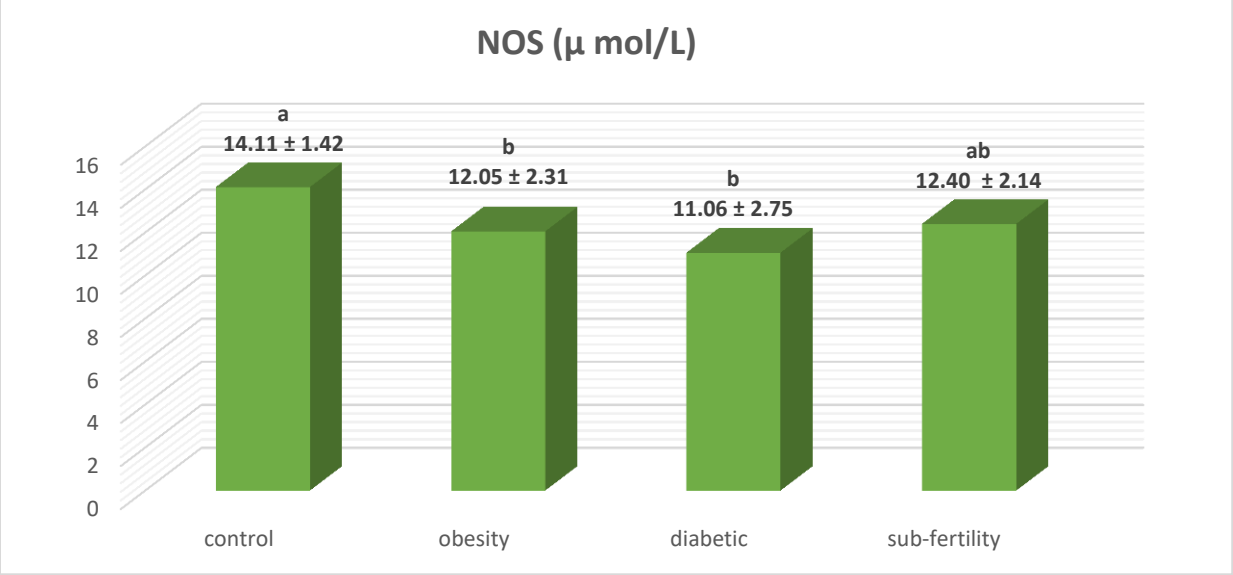



**Figure (4-15) : Levels of NOS in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference.

## 4.2.7 Lipid Profile

### 4.2.7.1 Total Cholesterol (TC)

The results revealed that :

The levels of TC in sub-fertility group increased significantly ($p \leq 0.01$) in comparison with the control and decreased significantly ($p \leq 0.01$) in the obesity group and diabetic group.

The TC levels in diabetic group increased significantly ($p \leq 0.01$) in comparison with the control and decreased significantly ($p \leq 0.01$) with the obesity group, whereas in the obesity group increased significantly ($p \leq 0.01$) in comparison with the control. (Table (4.4) ; Figure (4.16)).

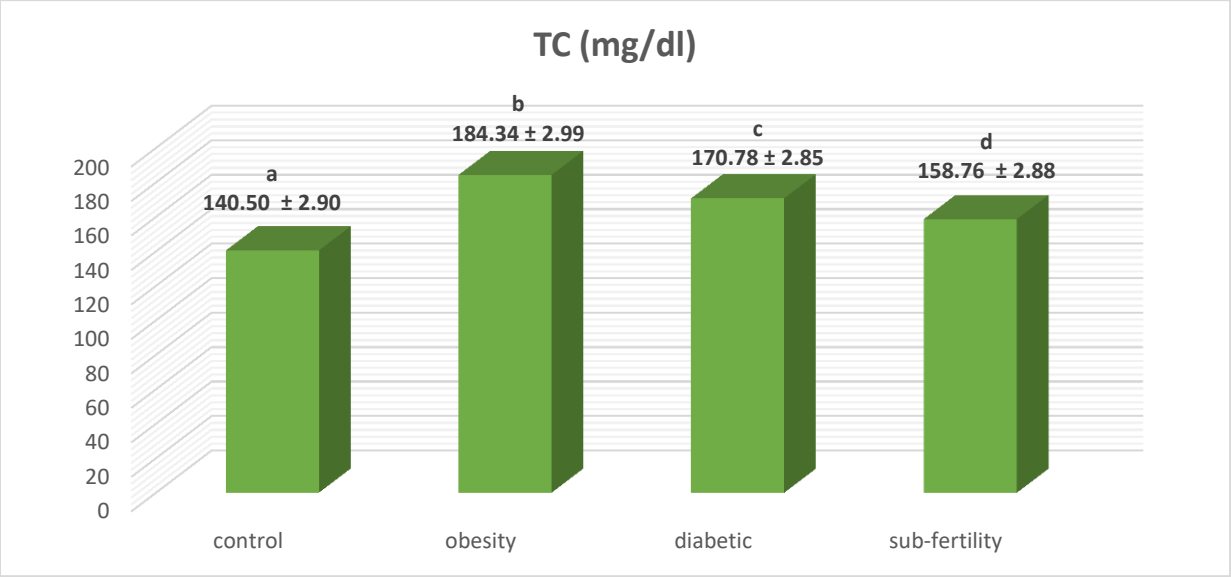



**Figure (4-16) : Levels of TC in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

### 4.2.7.2 Triglyceride (TG)

The results revealed that :

The levels of TG in sub-fertility group increased significantly ($p \leq 0.01$) in comparison with the control and decreased significantly ($p \leq 0.01$) with the obesity and not significantly with the diabetic group.

The TG levels in diabetic group increased significantly ($p \leq 0.01$) in comparison with the control and decreased significantly ($p \leq 0.01$) with the obesity group, whereas in obesity group increased significantly ($p \leq 0.01$) in comparison with the control. (Table (4.4) ; Figure (4.17)).

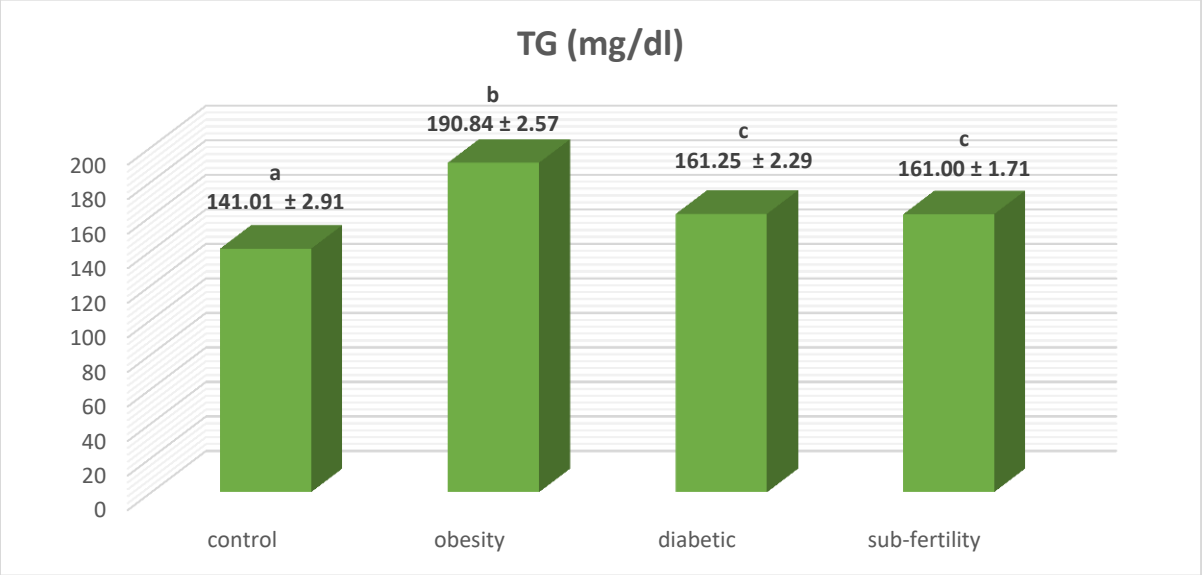



**Figure (4-17) : Levels of TG in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

### 4.2.7.3 Low Density Lipoprotein (LDL)

The results revealed that :

The levels of LDL in sub-fertility group increased not significantly in comparison with the control, and decreased significantly ($p \leq 0.01$) with obesity group and not significantly with diabetic group.

The LDL levels in diabetic group increased not significantly in comparison with the control and decreased significantly ($p \leq 0.01$) with the obesity group, whereas in obesity group increased significantly ($p \leq 0.01$) in comparison with the control. (Table (4.4) ; Figure (4.19)).

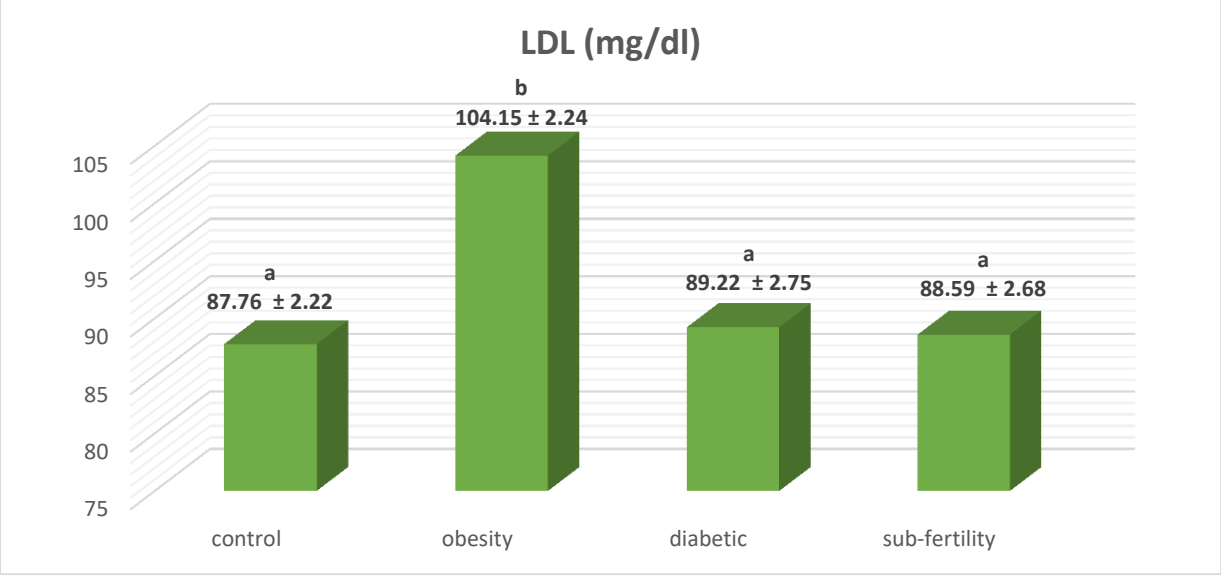



**Figure (4-18) : Levels of LDL in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

### 4.2.7.4 High Density Lipoprotein (HDL)

The results revealed that :

The levels of HDL in sub-fertility group decreased significantly ($p \leq 0.05$) in comparison with the control, obesity group and diabetic group.

The HDL levels in diabetic group decreased significantly ($p \leq 0.05$) in comparison with the control and increased significantly ($p \leq 0.05$) with obesity group, whereas in obesity group decreased significantly ($p \leq 0.05$) in comparison with the control. (Table (4.4) ; Figure (4.18)).

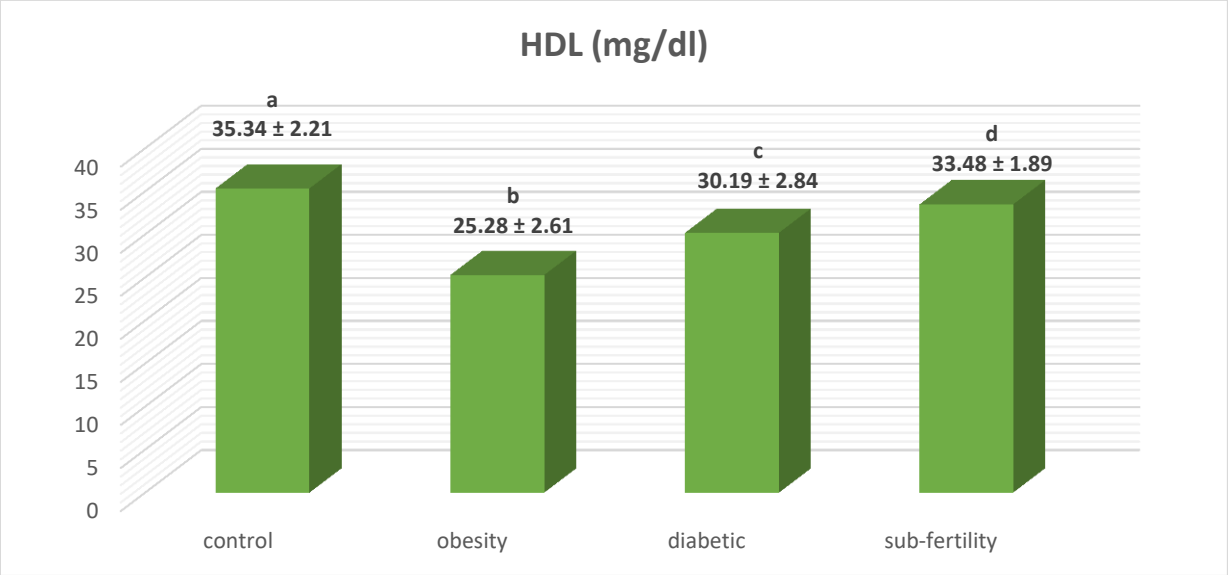



**Figure (4-19) : Levels of HDL in different groups**.
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

## 4.2.8 Body Mass Index (BMI)

The results revealed that :

The levels of BMI in sub-fertility group increased significantly ($p \leq 0.05$) in comparison with the control and not significantly with diabetic group and decreased significantly ($p \leq 0.05$) with the obesity group.

The BMI levels in diabetic group increased significantly ($p \leq 0.05$) in comparison with the control and decreased significantly ($p \leq 0.05$) with the obesity group, whereas obesity group increased significantly ($p \leq 0.05$) in comparison with the control. (Table (4.4) ; Figure (4.20)).

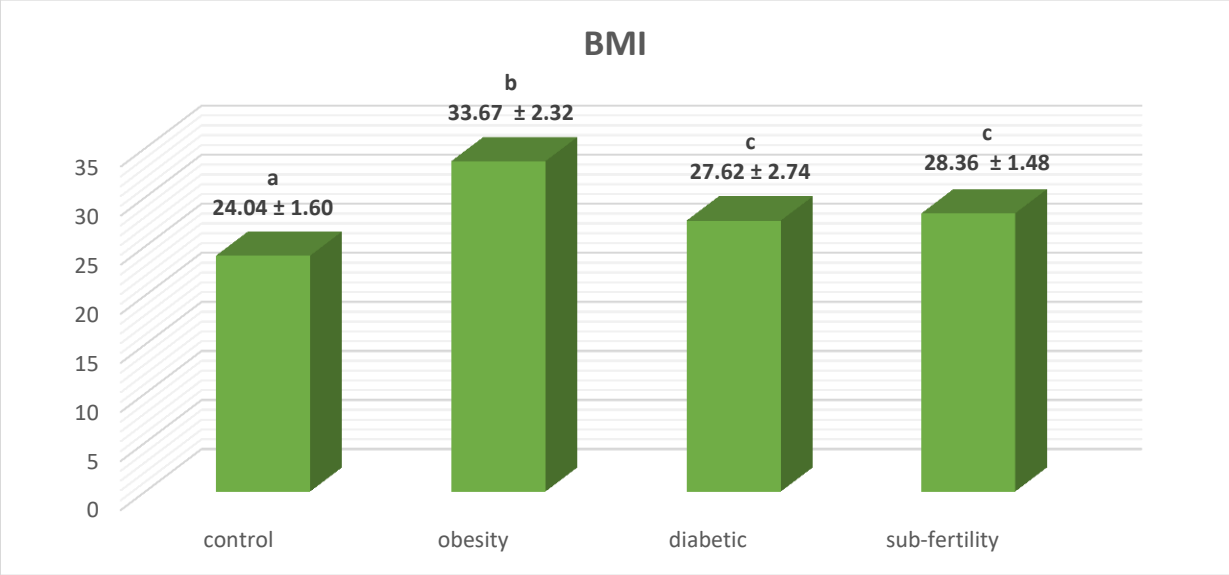



**Figure (4-20) : Levels of BMI in different groups.**
*The values represent mean ± SD.
*Different small letters represent significant difference in ($p \leq 0.01$) between groups .
*Similar small letters represent no significant difference .

# CHAPTER FIVE

# DISCUSSION

## 5.1. Irisin

The present results showed that irisin level increased significantly ($p \leq 0.01$) in obesity group and decreased significantly ($p \leq 0.01$) in diabetic and sub-fertility groups in comparison with the control. (Table (4.1) ; Figure (4.1)).

The high level of irisin in obesity group might be attributed to lipoprotein irisin's secretion mainly by adipose tissue and by the adipocytes cells in obese individuals whom have a high BMI. In diabetic group, irisin level decreased according to influence of insulin resistance, which has a negative association with insulin resistance, irisin level decreased significantly due to the less amount of testosterone production via the inhibition of gonadotropin (FSH and LH) by the high levels of prolactin in sub-fertile men.

Jia and his colleagues (2019) found that circulating irisin level was higher in obese than in healthy people, this high irisin secretion be considered as accommodative compensatory response to obesity-induced metabolic dysfunction via the irisin resistance.

Shoukry and his colleagues (2016) represented that the high level of irisin in obese people might be indicated as increased adipose and muscular tissue in these people and / or a compensatory process to combat obesity.

Bostrom and his colleagues (2012) suggested that disrupted irisin signaling in adipose tissue might be associated with the development of T2DM. In addition, irisin levels were significantly lower in patients with T2DM (Shelbaya *et al.*, 2018).

Chen and his colleagues (2015) suggested that irisin receptor (PGC-1α) expression and activity are low in type 2 diabetes patients, that PGC-1α is important for mitochondrial homeostasis for it regulates mitochondrial biogenesis

and oxidative metabolism, and mitochondrial function also plays a role in insulin resistance.

The findings of Naelitz and his colleagues (2021) are in consistence with the presents results in sub-fertile men, they found that highest prolactin inhibits the pulsatile release of FSH and LH, reducing gonadal testosterone synthesis and possibly leading to hypogonadotropic hypogonadism.

Bostrom and his colleagues (2012) mentioned that when irisin and FNDC5 activated, this might downregulate the expression of estrogen receptors in the hypothalamus, ensuring optimal pulsatile GnRH release and consequent FSH and LH release into the circulatory, resulting in leydig cell dependent testosterone production, re-establishment of energy balance by irisin may also inhibit the conversion of testosterone to estrogen via repression of aromatase activity.

Treatment with irisin in obese male rats enhanced the FSH, LH and testosterone hormone levels in blood resulting in promoten spermatogenesis and sperm properties such as sperm count and motility (Nanees and Reham, 2018).

In addition, a low levels of irisin in sub-fertile men in comparison with the healthy men (Saleh *et al.,* 2018), and a positive correlation between circulating irisin and testosterone levels (Tekin *et al.*, 2019).

## 5.2. Chemerin

The current results showed that chemerin increased significantly ($p \leq 0.01$) in different groups in comparison with the control**.** (Table (4.1) ; Figure (4.2)).

The high level of chemerin might be attributed to lipoprotein chemerin's secretion mainly by adipose tissue and by the adipocytes cells according to a high BMI and / or a high insulin resistance and / or the inhibition of prolactin hormone

on gonadotropin ( FSH, LH ) thereby testosterone production, leading to an increase in chemerin secretion in obesity, diabetic and sub-fertility groups. In addition, these three groups classified as a low-grade inflammation therefore, chemerin as a pro-inflammatory cytokine attracts and activates immune cells and might be played a physiological role in these different groups.

The relationship between chemerin and BMI and body fat is related to this important finding that fat tissue is the main source of chemerin secretion in humans (Ernst and Sinal, 2010), therefore, its expected that with the increase of fat levels in humans, the production and release of chemerin also increases, in fact, chemerin plays a role in adipogenesis and adipocyte metabolism (Goralski *et al.*, 2007).

Kiymet and his colleagues (2007) found that chemerin mRNA highly expressed in mature adipocytes and was increased in adipose tissue of obese animals, these observations suggest that chemerin expression may reflect the state of differentiation of adipocytes, adipocyte cell size or total body fat mass.

Shan and his colleagues (2012) mentioned that a high levels of chemerin are the results of IR or an attributable factor for IR, hyperinsulinemia associated with IR that could up-regulate its expression in adipose tissue.

Cheon and his colleagues (2017) found that chemerin levels were significantly associated with obesity and the visceral adipose tissue was a major site of chemerin secretion in newly diagnosed T2DM patients, moreover, the abdominal visceral fat was identified as an independent predictor of serum chemerin levels in subjects newly diagnosed with T2DM, as well as, chemerin may be a mediator linking visceral obesity and T2DM, furthermore, the chemerin a significantly associated with BMI.

Akgul and his colleagues (2019) observed that chemerin levels were significantly higher in patients newly diagnosed with T2DM than in the control

group and thought that the chemerin have an impact on insulin-mediated glucose regulation.

Habib and his colleagues (2017) found that serum chemerin levels are elevated in patients with type 2 diabetes compared to control, and are positively correlated with adiposity and insulin resistance in patients with type 2 diabetes.

Estienne and his colleagues (2020) demonstrated that chemerin seminal plasma concentration but not chemerin blood plasma was negatively correlated with spermatozoa concentration and motility in chicken sperm, seminal chemerin levels are negatively associated with the rooster fertility, and chemerin produced locally by the testis or male tract could negatively affect *in vivo* sperm quality and testosterone production through CMKLR1. In human, Bobjer and his colleagues (2018) demonstrated that chemerin concentration in serum was negatively correlated with male fertility parameters such as plasma LH, SHBG and E2.

Obesity, diabetes and hyperprolactinemia linked to low-grade inflammation (Wittamer *et al.*, 2003 ; Sharif *et al.*, 2021 ; García-Rizo *et al.*, 2021).

Obesity is also associated with an increase in macrophage infiltration in white adipose tissue, with this cell type constituting up to 60% of all cells detected in obese adipose tissue (Wittamer *et al.,* 2003). These infiltrating macrophages secrete proinflammatory cytokines (chemerin), which can exacerbate insulin resistance by interacting with insulin signaling (Trayhurn and Wood, 2005) and had the ability to enhance chemotaxis of immature DCs and macrophages via activation of CMKLR1 (Caspar-Bauguil *et al.*, 2009), insulin resistance leading not only to hyperglycemia but also to low-grade inflammation and when adipose tissue become dysfunctional, it produces pro-inflammatory cytokines (Sharif *et al.*, 2021), the high levels of PRL correlations with high level of inflammatory and hepatic parameters while lower levels of immune parameters highlights its effect in the pro-inflammatory state ( García-Rizo *et al.*, 2021).

Weigert and his colleagues (2010) found that chemerin was elevated in T2DM subjects with a higher CRP levels and positively correlated with CRP in normal weight, overweight and T2DM subjects after adjusting for BMI.

Ernst and Sinal (2010) found that the role of chemerin in inflammation and metabolism might provide a link between chronic inflammation and obesity, as well as obesity-related disorders such as T2DM and cardiovascular diseases.

## 5.3. FSH, LH and T

The present results showed that FSH, LH and testosterone decreased significantly ($p \leq 0.05$) (except testosterone in diabetic group) in different groups. (Table (4.1) ; Figures (4.3), (4.4) and (4.5)).

The low level of FSH and LH in the groups of the current study indicated low testosterone and that effect was caused either by the aromatase activity in the obese group or by the influence of insulin resistance and / or hyperprolactinemia or both of them.

Chambers and Richard (2015) found that obese individuals exhibited a decrease in testosterone levels, based on the fact that in obese subjects there is more aromatase activity that converts testosterone to estradiol, thus hypoandrogenemia and increased estrogen levels that change the negative feedback mechanism in hypothalamic and pituitary area.

Pauli and his colleagues (2008) suggested that a partial hypogonadotropic hypogonadism based upon decreased FSH and low normal LH levels in obese men.

Hussein and Al-Qaisi (2012) showed a significant reduction of serum FSH and LH in diabetic men in comparison with non-diabetics.

Testosterone decreased significantly as a BMI increased that indicated an occurrence of hypogonadism in patients with type 2 diabetes and overweight or obesity, due to the high conversion of testosterone into estrogen by the high activity of aromatase enzyme leading to reduction the level of testosterone (Langer *et al.*, 2002).

Dabbous and Atkin (2017) showed that elevated levels of serum prolactin have a detrimental effect on male reproduction through inhibition of the pulsatile release of gonadotrophins from the anterior pituitary gland, and a direct effect on spermatogenesis.

Tsutsumi and Webster (2009) mentioned that prolactin inhibits pulsatile GnRH secretion and consequently inhibits the pulsatile release of FSH, LH and testosterone, this results in marked effects on spermatogenesis ranging from alteration in sperm quality to complete spermatogenic arrest.

Pitteloud and his colleagues (2005) found that the low testosterone levels associated with insulin resistance result in part from an alteration in leydig cells function.

Moreover, Benjamin and his colleagues (2014) reported that reduction of FSH, LH and testosterone among Nigerian infertile males presenting with hyperprolactinemia associated with hypogonadotropic hypogonadism compared to the normal controls.

Zeitlin and Rajfer (2000) mentioned that hyperprolactinemia induced hypogonadism by interfering with the secretion of GnRH from the hypothalamus, the resulting decrease in serum testosterone believed to be the cause of the erectile dysfunction.

Nevertheless, Natah and his colleagues (2013) showed that diabetic patients had a higher FSH and LH than control, that mean when a fewer or poor-quality leydig

cells, more LH needed to produced more testosterone levels, furthermore, in these diabetic people, leydig cells and follicle cells become resistant to gonadotropin hormones, resulting in higher amounts of FSH, LH and a lower normal range of estradiol and testosterone.

## 5.4. Prolactin

The present results showed that prolactin level increased significantly ($p \leq 0.01$) in different groups in comparison with the control. (Table (4.1) ; Figure (4.6)).

The high secretion of prolactin hormone occurred might be due to the increased of fat mass and BMI in the obesity group and the development of insulin resistance in diabetic group, besides, the positive relationship between prolactin and insulin resistance.

Human adipose tissue produced PRL as well as expressed the PRL receptor (PRLR) highlighted an action of PRL as a cytokine involved in adipose tissue function, biologically active PRL secreted by all adipose tissues such as breast, visceral and subcutaneous ( Brandebourg *et al.*, 2007).

Altered prolactin secretion in obesity appears to be a marker of hypothalamic-pituitary dysfunction that may be explained by alterations in central dopaminergic tone (Kopelman, 2000).

Liu and his colleagues (2021) found that increased PRL level might be an adaptive response for protecting against metabolic disorders in obesity.

Daimon and his colleagues (2017) showed that higher serum PRL levels within the physiological range is associated with insulin resistance in non-diabetic men.

Park and his colleagues (2012) found that physiologically elevated prolactin levels induced normal adaptive increases in glucose-stimulated insulin secretion through expanding β-cell mass and improving hepatic insulin sensitivity.

High PRL serum levels in patients with T2DM, that confirms a positive association between high PRL levels and IR, this high PRL serum level is to overcome IR and development of T2DM (Al-Nami *et al.,* 2019).

Wang and his colleagues (2013) found that a high circulating prolactin level was significantly associated with a lower risk of prevalent diabetes and impaired glucose regulation in men and postmenopausal women and this is the first study to investigate the association between circulating prolactin and glucose regulation in a large sample of community-based men and women.

High prolactin levels in patients with T2DM are linked with diabetic complications, elevated prolactin levels in T2DM may be a compensatory mechanism against hyperglycemia, since prolactin plays a vital role in the enhancement of pancreatic β-cell function to overcome IR (Rasheed *et al.,* 2019).

Park and his colleagues (2011) mentioned that the effect of a physiological high prolactin level and pathological hyperprolactinemia on glucose metabolism could be different, excessive high levels of prolactin exacerbate whole-body and hepatic insulin resistance and impair the insulin secretory capacity in diabetic mice.

Furthermore, prolactin stimulated the development of adipose tissue via prolactin receptors found in adipose tissue, additionally, hyperprolactinaemia induced hypogonadism could contribute to an adverse body composition especially in men, as androgen deficiency itself increases body fat and facilitates lean mass reduction (Posawetz *et al.,* 2021).

## 5.5. T3 and T4

The present results showed an increase tendency in obesity group and a significant decrease in diabetic and sub-fertility groups. (Table (4.1) ; Figures (4.7) and (4.8)).

The increment of T3 and T4 in obesity group might be attributed to influence of the high level of irisin hormone in this group (Table (4.1) ; Figure (4.1)) and a positive relationship with the thyroid hormones. However, T3 and T4 reduced significantly due to the impact of insulin resistance and / or high level of prolactin via the hypothalamic - pituitary – thyroid gland axis in diabetic and sub-fertility groups respectively.

Irisin levels associated positively with thyroid hormones, FT4 was a significant predictor of serum irisin level, as well as, irisin levels decreased in patients with hypothyroidism, this decrease was directly associated with the reduction of thyroid hormones (Yang *et al.*, 2019).

Vyakaranam and his colleagues (2014) found that the higher insulin levels and insulin resistance which correlates negatively with FT3 and FT4, hence, an increased insulin resistance associated with T2D.

Insulin resistance have an association with the decrease of thyroid hormones, indicating higher levels of insulin resistance leading to lower levels of thyroid hormones (Binobead *et al.*, 2021).

During the sixth and twelfth months after the hypothyroidism presentation, insulin resistance developed relatively to type 2 diabetes (Chen *et al.*, 2019).

Rai and his colleagues (2013) found that the levels of T3, T4, FT3 and FT4 decreased significantly in diabetic individuals and this decrease occurred by the alterations in the hypothalamus-pituitary-thyroid gland axis.

Patients with hyperprolactinemia showed a high insulin resistance than normal individuals, higher PRL levels within the physiological range is associated with insulin resistance in men (Yang *et al.*, 2021 ; Daimon *et al.*, 2017).

Bahar and his colleagues (2011) showed that a negative association between prolactin and T3, T4 and the increase in prolactin levels ( TRH response ) associated with a decrease in T3 and T4 levels, moreover, when the prolactin decreased via the TRH response T3 and T4 were above the normal ranges.

Nevertheless, Udiong and his colleagues (2007) showed that the T4 in diabetic individuals be higher than the non-diabetic and no significant difference in T3 hormone between diabetics and non-diabetic individuals.

## 5.6. Insulin, insulin resistance, glucose and HbA1c

The present results increased significantly (except HbA1c in obesity and sub-fertility groups) in different groups. (Table (4.2) ; Figures (4.9), (4.10), (4. 11) and (4.12)).

These results are in consistence with their obese, diabetic and hyperprolactinemic samples and they considered as a real indicators for those type of individuals.

In obese individuals insulin resistance developed as a compensatory increase in endogenous insulin production, thus, elevated levels of endogenous insulin associated with insulin resistance and lead to weight gain, which in turn exacerbated insulin resistance, as this continues, β-cells activity became unable to meet the demand for insulin caused by insulin resistance, resulting in hyperglycemia, in which a mismatch between insulin demand and insulin production thereby blood sugar raised to levels consistent with T2D (Freeman and Pennings, 2021).

Obesity considered as a triggering factor for diabetes associated with insulin resistance and in obese individuals pro-inflammatory cytokines including the adipose tissue hormones participated in the development of insulin resistance (Wondmkun, 2020 ; Wang *et al.,* 2013).

Furthermore, the glycemic control decreased in overweight or obesity people, thereby many pre- and / or diabetic indicators appeared (Boye *et al.,* 2021).

Moreover, HbA1c correlated positively with glucose and be more common in obese individuals (Onal *et al.,* 2014).

In hyperprolactinemia more insulin resistance, hyperglycemia and glucose intolerance would be recognized (Yang *et al.*, 2021 ; Wang *et al.*, 2013).

Hyperprolactinemia exacerbated with whole-body and hepatic insulin resistance and impaired insulin secretory capability in diabetes individuals with hyperprolactinemia (Berinder *et al.,* 2001).

On the other hand, Park and his colleagues (2012) found that the physiological elevation in prolactin levels induced normal adaptive increases in glucose-stimulated insulin secretion through expanding β-cell mass and improving hepatic insulin sensitivity.

## 5.7. Pro-inflammatory markers ( IL-6 and CRP )

The present results showed that IL-6 increased significantly (except the diabetic group) in different groups. The CRP increased significantly in different groups (Table (4.3) ; Figures (4.13) and (4.14)).

Many directions beyond these current results such as the high values of pro-inflammatory cytokines (IL-6 and CRP) associated with adipocyte hypertrophy, these cytokines promote strongly the development of insulin resistance and

pathogenesis of T2DM and these cytokines positively correlated with hyperprolactinemia and with high levels of prolactin secretion, in addition, these different groups (obesity, diabetic and sub-fertility) be considered as a low-grade inflammation, thus, these high cytokine release leading to decreased the testosterone production in the current study, whereas, CRP as a prime inflammatory marker correlated negatively with the testosterone.

Bowker and his colleagues (2020) showed IL-6 mediates in small part the links between obesity, insulin resistance and cardio-metabolic diseases.

El-Mikkawy and his colleagues (2020) mentioned significantly higher levels of IL-6 in subjects with overweight and obesity as compared to those of healthy control, moreover, a significantly positive correlation was found between circulating levels of IL6 and BMI only in subjects with very severe (grade III) obesity.

Amit and his colleagues (2009) found that the increasing appreciation that adipose tissue is an active endocrine organ producing a variety of hormones and cytokines that may affect CRP levels, of these, IL-6 is thought to be the principal cytokine involved in CRP release from the liver and up to one third of circulating IL-6 is derived from adipose tissue.

Mahwati and Nurrika (2020) found an association between obesity indicators and CRP levels, there is a positive correlation among BMI, waist circumference (WC) and CRP.

Khaodhiar and his colleagues (2004) found that subjects with obesity had significantly higher serum levels of IL-6 and CRP compared to control, and that serum levels of IL-6 and CRP were only positively correlated with BMI in morbid obese subjects, suggesting that IL-6 could be secreted in an endocrine manner in proportion to fat mass expansion, with an associated increase in CRP hepatic production.

Obese patients, as well as those with chronic inflammatory diseases and abnormal serum lipid concentrations, had higher serum IL-6 levels (Galcheva *et al.,* 2011).

Brunn and his colleagues (2003) found that the levels of IL-6 were increased and correlated with measures of insulin resistance in male subjects with abdominal obesity.

The positive association between CRP and T2DM is dependent of insulin resistance and BMI, the mechanism of the association between CRP and T2DM is still not known in detail (Kanmani *et al.,* 2019).

Increased IL-6 levels in obese people may raise the risk of insulin resistance and T2DM (Takumansang *et al.,* 2013).

Elevated circulating IL-6 levels were an independent predictor of T2DM and were thought to played a role in the development of inflammation, insulin resistance, and cell dysfunction (Akbari and Hassan-Zadeh, 2018).

IL-6 levels were more strongly related with HbA1c, which indicates average glycemic levels in the three months preceding measurement, than with other glycemic traits, infections and other inflammatory challenges are related with reactive hyperglycemia, which may be aggravated in patients with greater IL-6 levels (Dungan *et al.,* 2009 ; Nakamura *et al.*, 2012).

Phosat and his colleagues (2017) mentioned that the role of CRP as an initiated marker for T2DM, where they found that the pre-diabetic and T2DM groups had markedly higher CRP levels than the control, subjects with high CRP levels had an elevated risk of pre-diabetes and T2DM, even after adjusting for confounders (i.e. age, BMI and gender) this strong association remained.

Liu and his colleagues (2019) found in study of 202 patients with hyperprolactinemia have a higher levels of IL-6 compared to healthy controls.

Li and his colleagues (2019) observed that elevated levels of hs-CRP were significantly associated with an increased risk of ED after adjustment for conventional ED risk factors, including BMI, age, testosterone, alcohol, smoking, physical activity , hypertension, diabetes and dyslipidemia.

Tremellen and his colleagues (2017) found a low testosterone levels in men were significantly associated with high level of inflammatory markers (CRP) in different clinical conditions such as obesity.

## 5.8. Nitric Oxide Synthase (NOS)

The present results showed that NOS decreased significantly (except the sub-fertility group) in different groups (Table (4.3) ; Figure (4.15)).

The current decreased might be attributed to the high adipose tissue as indicator by BMI measurement and / or influence of insulin resistance and / or influence of chemerin secretion that correlated negatively with NOS and / or high level of prolactin secretion.

Chemerin was positively correlated with body mass index and serum insulin and was negatively correlated with eNOS (Wang *et al.*, 2014).

Chemerin reduces NO production, enhances NO breakdown and also decreases NO-dependent cGMP signalling, thereby reducing vascular relaxation, the chemerin leads to destabilization of the eNOS dimer, impairing the catalytic function of the enzyme (Neves *et al.*, 2014).

Kraus and his colleagues (2012) mentioned that the skeletal muscle eNOS protein was significantly higher in the non-obese compared with the obese subjects.

Insulin resistance may be the cause of the reduced nitric oxide production in type 2 diabetes, insulin insufficiently stimulated NOS activity in skeletal muscle of type 2 diabetic subjects (Tessari *et al.*, 2010 ).

In humans, insulin resistance is often associated elevated fasting plasma levels of cholesterol and triglycerides, eNOS deficiency might be directly altered lipid metabolism (Duplain *et al.,* 2001).

Kashyap and his colleagues (2005) found that the basal and insulin-stimulated muscle NOS activity was impaired in T2DM subjects, paralleling the severity of insulin resistance.

The inhibition of expressed neuronal NO synthase decreased NO levels and increased basal PRL release, expression of inducible NO synthase also increased NO and inhibited PRL basal, whereas inhibition of this enzyme decreased NO production and recovered PRL release (Andric *et al.,* 2003).

Duvilanski and his colleaguse (1995) found that the actions of NO in controlling prolactin release are complex by effects mediated both by the hypothalamus and also by the pituitary gland itself, the actions of NO within the hypothalamus stimulate, whereas the action at the pituitary level inhibits prolactin secretion.

Slightly high PRL caused decrease in blood pressure (BP) caused by increased nitric oxide (NO) production, whereas higher PRL lead to increased BP due to decreased NO production (Dourado *et al.*, 2020).

## 5.9. Lipid Profile ( TC, TG, HDL and LDL )

The present results showed that TC and TG increased significantly in different group, LDL increased significantly in obesity group and not significantly in diabetic and sub-fertility groups and HDL decreased significantly in different groups (Table (4.4) ; Figures (4.16), (4.17), (4.18) and (4. 19)).

These results might be attributed to the influence of high BMI and / or the influence of insulin resistance and / or the influence of high prolactin in different groups.

Abnormalities in the lipid profile, particularly hypertriglyceridemia and the decreased of HDL-C, have been shown to be correlated with several diseases, including obesity, diabetes and cardiovascular disease (Shabana *et al.,* 2020).

Milyani and Al-Agha (2019) found that the high BMI associated with an increase level of LDL and a decrease level of HDL.

Furthermore, in hyperglycemic individuals the increment of TG regularly accompanied by the low HDL, hepatic lipase converted the HDL into smaller particles that rapidly cleared from plasma resulting in a further decrease in HDL content (Goldberg, 2001 ; Sutter *et al.*, 2014).

The presence of an abnormal lipid profile related to the presence of insulin resistance in addition to T2DM, insulin resistance has been linked to high levels of TG and low levels of HDL, as a result, the lipid profile is highlighted in practically all T2DM follow-up programs and is a significant risk factor (Li *et al.*, 2014).

In diabetic patients lower HDL cholesterol level is attributed to triglyceride enrichment by cholesterol ester transfer protein and increased hepatic triglyceride lipase activity, although HDL particles are produced by the liver and a significant portion of them are formed from remnant particles of TG-rich lipoproteins as they are metabolized, this metabolism is often defective in diabetes, reducing the production of HDL from this source, a protein called cholesterol ester transport protein (CETP) transports cholesterol ester away from HDL particles in exchange for TG from VLDL particles, this transport lowers HDL in the blood, which also promotes for small, dense LDL particles ( Brunzell and Hokanson, 1999 ; Hu and Willett, 2002 ; Naway, 2011).

Diabetic individuals have a significantly higher amount of LDL caused by high insulin effect on the number of HDL receptors, therefore, chronic insulin insufficiency might be linked to a lower level of LDL receptor (Suryawanshi *et al.*, 2006).

Erem and his colleagues (2010) found that hyperprolactinemia has been associated with hypercholesterolemia and hypertriglyceridemia.

Pala and his colleagues (2015) found that increased levels of LDL-cholesterol and TG in patients with prolactinoma, as well as a high total cholesterol and low HDL in patients with prolactinoma compared to controls.

The TC and LDL decreased significantly after PRL was reduced by DA agonist treatment in the investigation of women and men with hyperprolactinemia, furthermore, insulin sensitivity improved, and there was a link between improved insulin sensitivity and lower PRL levels (Berinder *et al.*, 2011).

In men hypogonadism, hyperprolactinemia might be associated with elevation of TC, TG and LDL and the risk of TC elevation paralleled with LDL elevation and HDL reduction (Oppenheim *et al.*, 1989).

Nevertheless, Milyani and Al-Agha (2019) showed that no association between BMI and TG level.

Serri and his colleagues (2006) found in hyperprolactinemic patients, HDL did not change in these individuals and also after treatment with cabergoline (DA agonist).

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENDATIONS

# 6.1. Conclusions

The results of the present study included the following conclusions :

1. Irisin level increased significantly in obesity group and decreased significantly in diabetic and sub-fertility groups , thereby metabolic dysfunction might be indicated in these groups.
2. Chemerin level increased significantly in different groups via it is pro-inflammatory action in these groups .
3. Prolactin level increased in accompanied with FSH , LH , testosterone and NOS reduction may be reflect the low fertility in these different groups .
4. T3 and T4 levels increased significantly (except T3) in obesity group and decreased not significantly (except T4 in diabetic group) in diabetic and sub-fertility groups , through the high secretion of irisin hormone and the disrabting of hypothalamic - pituitary – thyroid gland axis .
5. Insulin , insulin resistance , glucose and HbA1c levels increased significantly (except HbA1c) in different groups , reflecting the physiological impact of these groups .
6. TC , TG and LDL levels increased and HDL level decreased in different groups , that represented a metabolic deterioration in these groups .
7. The excessive values of pro-inflammatory markers (IL-6 and CRP) in different groups might be pointed an inflammation condition specifically related with these groups .

## 6.2. Recommendations

Further studies would be recommended and including the following :

1. Studying the role of another adipose hormones and their impact on additional physiological and pathological conditions .
2. Studying the molecular and genetical bases for some metabolic syndrome diseases such as obesity , diabetes ... etc.

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# APPENDIXES

# 1. Questionnaire

| **Case No.** | |
|---|---|
| Name | |
| Age | |
| Social status | |
| Number of year marriage | |
| Number of children | |
| Duration of diabetes | |
| Are you suffer from(cardiac disease , hypertension, acute and chronic infections , renal disease, hepatic dysfunction , prostate surgery and dysthyroidism ) | |
| Does he have problem in fertility ? | |
| Length | |
| Weight | |
| BMI | |
| Smoking | |
| **Plasma examination** | |
| HbA1c | |
| **Serum examination** | |
| Irisin | |
| Chemerin | |
| FSH | |
| LH | |
| PRL | |
| T | |
| T3 | |
| T4 | |
| Insulin | |
| IR | |
| F.B.G. | |
| NOS | |
| IL-6 | |
| CRP | |
| TC | |
| TG | |
| HDL | |
| LDL | |

## 2. Results

**Table (4.1) : The levels Irisin , Chemerin , FSH , LH , Prolactin , Testosterone , T3 and T4 hormones in different groups .**

| Parameters | Control | Obesity | Diabetes | Sub-fertility |
|---|---|---|---|---|
| Irisin (ng/ml) | $22.91 \pm 2.92^{a}$ | $26.33 \pm 1.73^{b}$ | $20.70 \pm 1.56^{c}$ | $19.62 \pm 2.88^{c}$ |
| Chemerim (pg/ml) | $472.90 \pm 3.61^{a}$ | $680.01 \pm 2.80^{b}$ | $532.25 \pm 4.19^{c}$ | $769.85 \pm 2.20^{d}$ |
| FSH (mlU/ml) | $^{**}3.71 \pm 0.82^{a}$ | $^{**}3.00 \pm 0.85^{b}$ | $^{**}3.15 \pm 0.27^{b}$ | $^{**}2.34 \pm 0.65^{c}$ |
| LH (mlU/ml) | $^{**}3.46 \pm 0.34^{a}$ | $^{**}2.75 \pm 0.78^{b}$ | $^{**}2.80 \pm 0.38^{b}$ | $^{**}1.93 \pm 0.47^{c}$ |
| Testosterone (ng/ml) | $^{**}3.94 \pm 0.51^{a}$ | $^{**}2.54 \pm 0.65^{b}$ | $^{**}3.62 \pm 1.62^{a}$ | $^{**}2.23 \pm 0.84^{b}$ |
| PRL (ng/ml) | $11.39 \pm 0.75^{a}$ | $14.11 \pm 1.02^{b}$ | $13.65 \pm 1.29^{b}$ | $35.20 \pm 2.37^{c}$ |
| T3 (ng/dl) | $1.58 \pm 0.73^{ab}$ | $1.88 \pm 0.26^{b}$ | $1.53 \pm 0.16^{ab}$ | $1.51 \pm 0.20^{a}$ |
| T4 (ng/dl) | $81.45 \pm 2.77^{a}$ | $90.21 \pm 2.27^{b}$ | $78.77 \pm 2.49^{c}$ | $80.01 \pm 2.84^{ac}$ |

* The values represent mean ± SD.

* Different small letters represent significant difference between groups .

* Similar small letters represent no significant difference .

* The significant difference is under the levels of ($p \leq 0.01$) .

** The significant difference is under the levels of ( $p \leq 0.05$ ) .

**Table (4.2) : Levels of Insulin , Glucose , Insulin resistance and HbA1c in different groups.**

| Parameters | Control | Obesity | Diabetes | Sub-fertility |
|---|---|---|---|---|
| Insulin (µU/ml) | $14.10 \pm 2.51^{a}$ | $38.84 \pm 2.67^{b}$ | $16.12 \pm 2.66^{a}$ | $30.28 \pm 2.68^{c}$ |
| Glu (mg/dl) | $88.95 \pm 1.53^{a}$ | $120.90 \pm 2.35^{b}$ | $210.90 \pm 2.80^{c}$ | $95.48 \pm 1.74^{d}$ |
| Homo - IR | $3.24 \pm 0.68^{a}$ | $8.66 \pm 1.22^{b}$ | $9.48 \pm 2.41^{c}$ | $7.61 \pm 1.81^{b}$ |
| HbA1c (%) | $4.95 \pm 0.21^{a}$ | $5.20 \pm 0.42^{a}$ | $9.36 \pm 1.55^{b}$ | $5.49 \pm 0.28^{a}$ |

* The values represent mean ± SD.

* Different small letters represent significant difference between groups .

* Similar small letters represent no significant difference .

* The significant difference is under the levels of ($p \leq 0.01$) .

**Table (4.3) : The levels of IL-6 , CRP and NO Synthase in different groups .**

| Parameters | Control | Obesity | Diabetes | Sub-fertility |
|---|---|---|---|---|
| IL-6 (pg/ml) | **23.65 ± 2.17$^{a}$ | **25.02 ± 1.84$^{b}$ | **24.50 ± 1.09$^{ab}$ | **33.93 ±2.35$^{c}$ |
| CRP (mg/dl) | **2.31 ± 0.84$^{a}$ | **3.31 ± 0.80$^{b}$ | **3.80 ± 1.02$^{b}$ | **3.60 ± 0.79$^{b}$ |
| NOS (µ mol/L) | 14.11 ± 1.42$^{a}$ | 12.05 ± 2.31$^{b}$ | 11.06 ± 2.75$^{b}$ | 12.40 ± 2.14$^{ab}$ |

* The values represent mean ± SD.

* Different small letters represent significant difference between groups .

* Similar small letters represent no significant difference .

* The significant difference is under the levels of ($p \leq 0.01$) .

** The significant difference is under the levels of ( $p \leq 0.05$ ) .

**Table (4.4) : Lipid profile and BMI in different groups .**

| Parameters | Control | Obesity | Diabetes | Sub-fertility |
|---|---|---|---|---|
| TC (mg/dl) | 140.50 ± 2.90$^{a}$ | 184.34 ± 2.99$^{b}$ | 170.78 ± 2.85$^{c}$ | 158.76 ±2.88$^{d}$ |
| TG (mg/dl) | 141.01 ± 2.91$^{a}$ | 190.84 ± 2.57$^{b}$ | 161.25 ± 2.29$^{c}$ | 161.00 ±1.71$^{c}$ |
| LDL (mg/dl) | 87.76 ± 2.22$^{a}$ | 104.15 ± 2.24$^{b}$ | 89.22 ± 2.57$^{a}$ | 88.59 ± 2.68$^{a}$ |
| HDL (mg/dl) | **35.34 ± 2.21$^{a}$ | **25.28 ± 2.61$^{b}$ | **30.19 ± 2.84$^{c}$ | **33.48 ±1.89$^{d}$ |
| BMI | **24.04 ±1.60$^{a}$ | 33.67 ± 2.32$^{b}$ | 27.62 ± 2.74$^{c}$ | 28.36 ± 1.48$^{c}$ |

* The values represent mean ± SD.

* Different small letters represent significant difference between groups .

* Similar small letters represent no significant difference .

* The significant difference is under the levels of ($p \leq 0.01$) .

** The significant difference is under the levels of ( $p \leq 0.05$ ) .

# الخلاصـــة

هدفت الدراسة الحاليه الى التعرف على دور كل من الايريسين والكيمرين وبعض مستويات المعايير الهورمونية والكيموحيويه في الرجال المصابين بالبدانة , السكري ( النوع الثاني ) والخصوبة المنخفضة في محافظة ميسان , والتي اجريت في كل من مستشفى الصدر التعليمي , مركز السكري والغدد الصم , مصرف الدم الرئيسي بالاضافة الى مختبر السجاد ومختبر الايمان الاهليين خلال الفترة من كانون الاول ٢٠٢٠ الى تموز ٢٠٢١ .

بلغت عينة الدراسة الكلية (٨٠) رجل ( تتراوح اعمارهم بين ٣٥ – ٤٥ سنة ) حيث تم تقسيم العينه الى اربعة مجاميع وكالاتي :

- مجموعة السيطرة (الرجال الاصحاء) .
- مجموعة البدانة (يكون مؤشر كتلة الجسم اعلى من ٣٠ كغم \ م$^{٢}$ ) .
- مجموعة السكري ( النوع الثاني) .
- مجموعة الخصوبة المنخفضة (فرط بروﻻكتين الدم) .

أظهرت النتائج الحالية ما يلي:

١- ارتفع مؤشر كتلة الجسم ( BMI ) معنويا (أ ≤ ٠,٠٥) في المجاميع المدروسة كافة مقارنة مع مجموعة السيطرة .
٢- ارتفع مستوى الايريسين في مجموعة البدانة معنويا ( أ ≤ ٠,٠١) وانخفض معنويا ( أ ≤ ٠,٠١) في كل من مجموعتي السكري والخصوبة المنخفضة عند مقارنتهم مع مجموعة السيطرة .
٣- ارتفع مستوى الكيميرين معنويا ( أ ≤ ٠,٠١) في المجاميع المدروسة كافة مقارنة مع مجموعة السيطرة .
٤- انخفضت مستويات الهرمون المنبه للجريب (FSH) , الهورمون اللوتيني ( LH ) و هورمون الشحمون الخصوي معنويا (أ ≤ ٥ ٠,٠) (عدا الشحمون الخصوي في مجموعة السكري) في المجاميع المدروسة كافة مقارنة مع مجموعة السيطرة .

٥- ارتفع مستوى هورمون الحليب (البرولاكتين) معنويا (أ ≤ ٠,٠١) في المجاميع المدروسة كافة مقارنة مع مجموعة السيطرة .

٦- ارتفعت مستويات ثلاثي ايويد الثيرونين ( T٣ ) والثيروكسين ( T٤ ) معنويا (أ ≤ ٠,٠١) في مجموعة البدانة وانخفضت بشكل غير معنوي (عدا T٤ في مجموعة السكري ) في مجموعتي السكري والخصوبة المنخفضة مقارنة مع مجموعة السيطرة .

٧- ارتفعت مستويات هورمون الانسولين , مقاومة الانسولين , الكلوكوز والهيموغلوبين السكري A١c ( HbA١c) معنويا (أ ≤ ٠,٠١) ( عدا HbA١c في مجموعتي البدانة والخصوبة المنخفضة ) في المجاميع المدروسة كافة مقارنة مع مجموعة السيطرة .

٨- ارتفع مستوى الانترلوكين – ٦ معنويا (أ ≤ ٠,٠٥) (عدا مجموعة السكري ) في المجاميع الدراسية المختلفة , وارتفع مستوى البروتين الفعال – C بشكل معنوي تحت مستوى (أ ≤ ٠,٠٥) (عدا مجموعتي البدانة والخصوبة المنخفضة) مقارنة مع مجموعة السيطرة .

٩- انخفض مستوى انزيم مخلقة اكسيد النتريك NOS معنويا (أ ≤ ٠,٠١) (عدا مجموعة الخصوبة المنخفضة ) في المجاميع المدروسة كافة مقارنة مع مجموعة السيطرة .

١٠- ارتفعت مستويات الكوليسترول الكلي ( TC ) والدهون الثلاثية ( TG ) و البروتين الدهني منخفض الكثافة ( LDL ) معنويا (أ ≤ ٠,٠١) ( عدا LDL في مجموعتي السكري و الخصوبة المنخفضة ) في المجاميع الدراسية المختلفة كما انخفض مستوى البروتين الدهني عالي الكثافة ( HDL ) معنويا (أ ≤ ٠,٠٥ ) في المجاميع المدروسة كافة مقارنة مع مجموعة السيطرة .

تمت مناقشة الابعاد الفسيولوجية لهذة النتائج استنادا الى كتلة الدهون ( حسب مقياس كتلة الجسم BMI ) , مقاومة الانسولين وارتفاع مستوى هورمون البرولاكتين للمعايير المدروسة كافة في مجاميع البدانة , السكري والخصوبة المنخفضة .

# الخلاصة

**وزارة التعليم العالي والبحث العلمي**
**جامعة ميسان**
**كلية العلوم**
**قسم علوم الحياة**

# تقدير الايريسين والكيميرين وبعض المؤشرات الحيوية في الرجال البدينين , المصابين بالسكري وقلة الخصوبة

رسالة مقدمة

الى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

**ريا نجم رسول**

بكالوريوس تربية / علوم حياة (٢٠١٠)

بإشراف

**أ . د . أحمد عبود خليفة**

اذار ٢٠٢٢ م

شعبان ١٤٤٣ هـ