T.C.

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INVESTIGATION OF SERUM IRISIN,ADROPIN AND PREPTIN VALUES IN OBESE AND NON-OBESE INDIVIDUALS

MASTER THESİS

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ETHICS CONTRACT

According to the thesis writing guide of Tokat Gaziosmanpaşa University Graduate Education Institute, I prepared under the consultancy of (Assoc.Prof.Dr. Muzaffer KATAR) I declare that the Master's thesis named INVESTIGATION OF SERUM IRISIN, ADROPIN AND PREPTIN VALUES IN OBESE AND NON OBESE INDIVIDUALS" is an original work in accordance with scientific ethical values and rules, and I will accept all kinds of legal sanctions if the opposite is determined. I declare.

2022/09/01

Shilan Rashad Ali

JURY ACCEPTANCE AND APPROVAL PAGE

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ÖZET

Obez ve obez olmayan kişilerde serum irisin, adropin ve preptin değerlerinin araştırılması

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Obezite, tüm dünyada hızla yayılan ve birçok hastalığın altında yatan temel etkenlerden biridir. Günümüzde periferik dokuların endokrin fonksiyonları, kuvvet homeostazının rehberliğinde önem kazanmaktadır. İrisin, adropin sonra preptin, kuvvet homeostazının kılavuzunda yer alan yeni keşfedilen endokrin faktörlerdir. İrisin, kas kaynaklı bir proteindir ve beyaz yağ dokusunu kahverengi yağ dokusuna dönüştürerek enerji harcamasına neden olur. Adropin, güç homeostazını düzenlemede ve insülin yanıtını korumada rol oynayan ENHO geni aracılığıyla kodlanan bir proteindir. Preptin, pankreasın β hücrelerinden insülin yoluyla birlikte emilen bir peptit hormonudur. Bu enerji düzenleyici peptitlerin T2DM ve T2DM ile mücadelede hücresel iletişimde rol oynayarak klinik fayda sağlayabileceği düşünülmektedir. Çalışmamız 18-50 yaş arası, fazla kilolu veya obez (n= 59), sağlıklı (kontrol, n=59) ve T2DM (n= 59) bireylerden oluşmaktadır. Tüm kan örnekleri jel tüpe alındı, 4°C'de 3500 rpm'de 10 dakika santrifüj edildi. Serum kısmı incelenmek üzere bir proteaz inhibitörü olan aprotinin içeren Eppendorf tüplerine ayrılmış ve çalışma gününe kadar -20°C'de derin dondurucuda saklanmıştır. Kan alımı tamamlandıktan sonra, ticari olarak temin edilebilen ELISA kitleri kullanılarak kan serumu örneklerinde irisin, adropin ve preptin seviyeleri analiz edilecektir. Ticari olarak temin edilebilen bir ELISA kiti kullanılarak hazırlanan serum örneklerinde irisin, adropin ve preptin miktarı incelenmiştir. İRİSİN (NG/ML), sağlıklı, obezite ve 1 tip 2 diyabet mellituslu obezite hastalarında, obezite ve T2DM gruplarında İRİSİN düzeyinin kontrol grubuna göre anlamlı derecede azaldığını göstermiştir ($p < 0.05$, $F = 19.617$). ADROPİN (PG/ML), tip 2 diyabet mellituslu sağlıklı, obezite ve obezite hastalarında elde edilen sonuçlar, obezite ve T2DM gruplarında ADROPİN düzeyinin kontrol grubuna göre önemli ölçüde azaldığını göstermiştir. ($p < 0.05$, $F = 3.632$). PREPTİN (NG/L), kontrol, obezite ve T2DM olmak üzere üç grupta, PREPTİN düzeyinin obezitede anlamlı olarak arttığını ve Type2 diabetes melituslu hastaların kontrol grubuna göre arttığını gösterdi ($p<0.05$, $F = 6.846$). Bu çalışma ile; Obez, T2DM

ve obez olmayan gruplarda irisin, adropin ve preptin konsantrasyonları karşılaştırılarak bu üç endokrin faktörün enerji homeostazı ile ilişkisinin araştırılması amaçlandı. Bakılması planlanan her üç parametrenin de enerji metabolizması ile ilgili olması ve obezitenin önemi giderek artan bir sağlık sorunu olması bu çalışmanın planlanmasında etkili olmuştur. Bu parametreleri karşılaştırdıktan sonra, serum,irisin, adropin ve preptini bir biyobelirteç olarak T2DM ve control obezite geliştirme riskinin değer endirilmesi olarak kullanılması gerektiğini bulduk.

Anahtar Kelimeler : İrisin , Adropin, Preptin, Obezite

ABSTRACT

INVESTIGATION OF SERUM IRISIN,ADROPIN AND PREPTIN VALUES IN OBESE AND NON-OBESE INDIVIDUALS

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Obesity is one of the main factors spreading rapidly all over the world and underlying many diseases. Today, the endocrine functions of peripheral tissues gain importance in the guideline of strength homeostasis. Irisin, adropin then preptin are newly discovered endocrine factors involved in the guideline of strength homeostasis. Irisin is a muscle-derived protein and reasons energy spending by altering white adipose tissue to brown adipose tissue. Adropin is a protein encoded via the ENHO gene, which acting a role in regulating strength homeostasis and keeping insulin response. Preptin is a peptide hormone absorbed together through insulin from the β cells of the pancreas. It is thought that these energy regulatory peptides may provide clinical benefit by playing a role in cellular communication in the fight against and T2DM. Our study consists of individuals aged 18-50 years, who are overweight or obese (n= 59) , healthy (control, n=59) and T2DM (n= 59) . All blood samples were taken in to gel tube, centrifuged for 10 minutes at 3500 rpm by the side of 4°C. The serum portion stayed separated into Eppendorf tubes containing aprotinin, a protease inhibitor, to be studied, and stored in a deep freezer at -20°C until the study day. After the blood collection was completed, Irisin, adropin and preptin levels will be analysed in blood serum samples, using commercially obtainable ELISA kits. The amount of irisin, adropin and preptin was examined in serum samples prepared using a commercially available ELISA kit. İRİSİN (NG/ML) in healthy, obesity and obesity patients with type 2 diabetes mellitus were result show that level of İRİSİN in the obesity groups and T2DM groups are significantly decrease than the control group ($p < 0.05$, $F = 19.617$). ADROPİN (PG/ML) in healthy, obesity and obesity patients with type 2 diabetes mellitus were result show that level of ADROPİN in the obesity groups and T2DM groups are significantly decrease than the control group. ($p < 0.05$, $F = 3.632$). PREPTİN (NG/L) in the three groups of control, obesity and T2DM were result show that level of PREPTİN significantly increase in obesity and patients with

Type2 diabetes mellitus compered to the control group ($p < 0.05$, $F = 6.846$). With this work; It was aimed to investigate the relationship of these three endocrine factors with energy homeostasis by comparing the concentrations of irisin, adropin and preptin in obese ,T2DM and non-obese groups. The fact that all three of the parameters planned to be looked at are related to energy metabolism and that obesity is an increasingly important health problem has been effective in the planning of this study. After comparing these parameter we found that serum irisin , adropin and preptin should be utilized as a biomarker for assessing the risk of developing T2DM and control obesity.

Keywords : Irisin , Adropin, Preptin, Obesity

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LIST OF ABBREVIATIONS

ADP:Adenosine diphosphate

AgRP:agouti-related peptide

AMPK:Adenosine monophosphate-activated protein kinase

ATP: adenosine triphosphate

BALB/C:Binaural Alternate Loudness Balance Test

BMI:Body mass index

cAMP:cyclic adenosine monophosphate

cDNA:Copy Deoxyribonucleic Acid

DASH: Dietary Approaches to Stop Hypertension

DCCT:Diabetes Control and Complications Trial

DKA:diabetic ketoacidosis

DM: diabetes mellitus

ELISA:enzyme-linked immunoassay

ENCHO: energy homeostasis associated gene

Enho:energy homeostasis associated

eNOS:endothelial nitric oxide synthetase

ER stress:Endoplasmic reticulum stress

ERK1/2:extracellular signal-regulated kinase ½

EP: Eppendorf

FNDC5:fibronectin type III domain containing 5

GABA:Gamma-aminobutyric acid

GDM: gestational diabetes mellitus

GLUT4:Glucose transporter type 4

HBA1C:hemoglobin A1c

HDL:high -denisty lipoprotein

HHS:hyperglycemic hyperosmolar state

HK2:hexokinase 2

HOMA-IR :model assessment-estimated insulin resistance

HT:hypertension

IGF-II:Insulin-like growth factor 2

kg: kilogram

LDL: low-density lipoprotein

LPL:lipoprotein lipase

M:meter

MSH:Melanocyte-Stimulating Hormone

NPY:neuropeptide

OGTT:oral glucose tolerance test

PCK1:Phosphoenolpyruvate carboxykinas

PGC1α:proliferator-activated receptor gamma coactivator 1-alpha

PI3K-Akt:Phosphatidylinositol 3-kinase

PKA:protein kinase A

PKC:protein kinase C

PPARA:Peroxisome Proliferator Activated Receptor Alpha

Pro IGF II :pro-insulin-like-growth factor II

ProIGF–IIE:proinsulin-like growth factor IIE

PVN:paraventricular nucleus

PYGM:Muscle glycogen phosphorylase

ROS:reactive oxygen species

T1DM: type 1 diabetes mellitus

T2DM: type 2 diabetes mellitus

TCH :total cholesterol

TGs: triglyceride

TURDEP:Turkey Diabetes Epidemiology

UCP1:ncoupling protein-1

VEGFR2:vascular endothelial growth factor receptor 2

WHO:World Health Organization.

CHAPTER ONE
1. Introduction

1.1. Definition of Obesity

Although obesity is generally known as being overweight, the explanation of obesity by the WHO [World Health Organization] is: "Irregular or excessive fat growth in adipose tissues to the level that it damages health." made in the form. In other words, obesity is a compound, multifactorial illness considered by a rise in body fat and consequent behaviour, endocrine, and metabolic changes. Obesity happens after the sum of strength taken from nourishment overdoes the sum of energy expended by digestion system and physical action, so that the fat mass in the body increases compared to the lean body mass. Excess fat in obese individuals paves the way for many important diseases that affect body systems such as the circulatory system, respiratory system, hormonal system, and gastrointestinal system. Obesity has shown to growth the risk of developed HT, type 2 DM, dyslipidaemia, cardiovascular diseases, and certain types of cancer (colon, breast, gallbladder) (WHO.,2013).

According to the results of the TURDEP-I (Turkey Diabetes Epidemiology Survey) study conducted in our country in 1998, the prevalence of obesity was found to be 30% in women, 13% in men, and generally 22.3% in epidemiological data (Satman et al.,2002). According to the results of the TURDEP-II study completed in 2010, the prevalence of obesity in Turkey is 32%, and according to the TURDEP-I conducted in 1998, the prevalence of obesity has increased by 44% in 12 years. (Satman I et al.,2002).

1.1.1. Classification of Obesity

Excess fat consumption is not only a cause of the development of obesity, it is also important that the excess calories are taken together with the proteins and carbohydrates stored as adipose tissue.

Today, an unhealthy diet, caused by too fatty and high-calorie foods and drinks, is the core purpose for the growth in obesity.

Another important factor is the reduction in the amount of energy consumed in daily activities due to the convenience of technological developments.

The BMI or “Quetelet index” is the most accepted and practical method used to assess obesity.

This index, determined by Quetelet in 1835, is the relation of measured weight (kg) to height (m) squared. Defines obesity as a BMI of 30 or more by WHO. The WHO BMI categorisation of obesity is presented in Table 1.1.1(WHO., 2004.).

Table 1.1.1. WHO Categorisation of Obesity According to BMI

Group	BMI
Under weight	**<18.5**
Normal range	**≥18.5 and <25**
Over weight	**≥25 and <30**
Obesity	**≥30**
Obesity**1**	**≥30 and <35**
Obesity **II**	**≥35 and <40**
Obesity **III**	**≥40**

Although secondary causes of obesity are less common, leptin deficiency, hypothalamic, Cushing's syndrome, growth hormone deficiency and hypothyroidism, damage must be considered. Drugs that can cause obesity include antipsychotics, antidepressants, lithium, antiepileptic drugs, insulin, sulfonylureas, oral contraceptives, and corticosteroids (Bray et al.,2006).

1.1.2. Epidemiology of Obesity

The predominance of overweight and weight is expanding at an disturbing rate around the world. For case, within the Joined together States (USA), the predominance of obesity expanded from 15% to 30.9% between 1980 and 2000, and comparative discoveries have been detailed in developing countries. Obesity may be a worldwide issue influencing roughly 300 million individuals around the world (Racette et al., 2003). In the WHO 2002 report, the highest average BMI value among all regions belongs to the European region, with approximately 26.5 kg/m2. According to the latest data from different countries in the region, the prevalence of obesity in adult males has increased from 5% to 20% and up to 30% in adult females. Unfortunately, the highest prevalence internationally of obese adult women has been reported from Turkey (about 30%) (WHO.,2005).

1.1.3. Obesity Etiology

The etiopathogenesis of obesity is extremely complex. Because there are many mechanisms that affect energy intake or expenditure in the human organism. Obesity is not only a simple weakness of will or a personal control problem, but also a complex disorder associated with energy metabolism diseases and appetite regulation (Lyznicki et al.,2001). We can collect the factors that affect the development of obesity under the following headings:

1. **Genetic Factors:**
2. **Nutritional Regulatory Disorder**
3. **Age**
4. **Gender**
5. **Socioeconomic Factors**
6. **Psychogenic Factors**
7. **Hormonal Causes**

1.1.4. Obesity and Tissue

Obesity is a chronic, metabolic illness considered via an increased number or size of fat cells. The total number of fat cells in the normal human body is about 5x1010, and this number increases in obese individuals. Many diseases that appear with obesity are thought to be associated with this increased adipose tissue function (Wisse et al.,2007). Adipose tissue is the key fat storage site in our body, so it acting an significant part in buffering the daily circulating dietary fat flow (Goossens et al.,2008 and Frayn et al .,2001).

Here are two sorts of adipose tissue in warm blooded animals: white adipose and brown adipose. These two sorts of adipocytes show different morphology and function. Brown adipocytes convert energy for heat production (thermogenesis), while white adipocytes store and release energy according near the metabolic requirements of the animal (Young et al.,1976). Brown adipose tissue, which specializes in heat production, is almost absent in adult humans. Approximately 2–3% of a new-born's weight is brown adipose tissue, but this adipose tissue turns in to white adipose tissue by the advancement of the thermoregulation mechanism (Wisse et al.,2007). Brown adipocytes get their colour from dense vascular formation and the presence of abundant packed mitochondria. These blood vessels assistance provide energy for storing, oxidation and distribute the heat produced by the myriad mitochondria to different parts of the body (Kiess et al.,2008 and Fonseca-Alaniz et al.,2006). The insides of the mitochondrial membranes of brown adipose tissue contains a protein called uncoupling protein-1 (UCP1) that pushes protons from the interprotein space into the mitochondrial matrix (Jastroch.,2010). When UCP1 is stimulated, it does not reason adenosine triphosphate (ATP) creating, because it is an undissociated protein1, but instead reasons a high temperature release (Jastroch et al.,2010 and Affourtit et al.,2012).

In humans, white adipose tissue consists of a central intra-abdominal component associated with metabolic risk and a peripheral subcutaneous adipose tissue depot associated with protective effects on energy homeostasis (Jensen et al.,2008 and Kwok et al.,2016). Adipose tissue constitutes 10–20% of body weight (Wisse et al.,2007). Its pleiotropic structure is based on the capacity of adipose cells to secrete various hormones, development variables, proteins, cytokines, complement components, and network

proteins. Fat tissue too expresses receptors for numerous of these factors included within the regulation of numerous processes, such as nourishment ingestion, energy consumption, metabolic homeostasis, immunity, and blood pressure homeostasis (Costa et al.,2006 and Matsuzawa et al.,2006).

Apart from these 2 types, an intermediate brown kind of fat cell called "beige" (Ishibashi et al.,2015) or else "brite" (brown-in-white) (Petrovic et al.,2010) adipocytes was defined recently. Beige/brite adipocytes mostly develop in response to exposure to cold or other adrenergic stimulation mechanisms of white adipose tissue depots (Montanari et al.,2017 and Nedergaard et al.,2014).A female-specific type of adipocyte called pink adipocyte arises with the transformation of white adipose tissue into pink adipocytes during pregnancy (Cinti et al.,2017). It produces and secretes milk and plays a important part in female metabolism during the early phases of brood feeding (Giordano et al.,2014).

1.2. Diabetes Mellitus

1.2.1. Definition of Diabetes Mellitus

Diabetes Mellitus World Health Organization (WHO) Diabetes MellitusInsulin effect, insulin secretion, or chronic hyperglycaemia cause disorders in carbohydrates, oil, and protein metabolism caused by flaws in insulin secretion or defects in both insulin and insulin effect. Hyperglycaemia plays a role in disease-related complications (Inzucchi et al.,2012). Various pathogenic processes production a part in the development of diabetes. These pathogenic processes constitute a range that ranges from the lack of insulin to resisting the insulin effect of the beta cells of the pancreas. The source of abnormalities in carbohydrate, oil, and protein breakdown in diabetes is the disorder in the impact of insulin on the target tissues. The effect of defective insulin is caused by disruption of insulin tissue response at one or more points of the impact of inadequate insulin and/or the effect of the hormone in the complex pathways. The defect in insulin secretion and the defect in insulin activity are often present in the same patient and are mostly unclear in which the main reason for hyperglycaemia is in question (Kahn et al.,2005). The symptoms of prominent hyperglycaemia consist of polyuria, polyidypsy, nocturia, unexplained weight loss, sometimes polyvasm, and visual impairment. Sensitivity and growth disorders to some disease may as well accompany chronic hyperglycaemia (Harvey et al.,2011).

1.2.2. Diabetes Mellitus Diagnosis

Diabetes is diagnosed by using both clinical and biochemical parameters. Diagnostic criteria for diabetes, which were revised and agreed upon, were published in the panels made by the American National Diabetes Data Group and the World Health Organization (WHO) expert committees. These new criteria reflect epidemiological and metabolic evidence (Burant et al.,2004). The criteria determined by the ADA, which were revised in 2010, for the diagnosis of diabetes are given in Table 1.2.2.

Table 1.2.2 shows the diagnostic criteria for diabetes mellitus *(*Diabetes Care., 2013).

Plasma glucose 200 mg/dl (11.1 mmol / l) at any period of day and regardless of the time since the last meal. (Diabetes symptoms are polyuria, polydipsia, and unexplained weight loss.)
Or
Fasting plasma glucose ≥ 126 mg/dl (≥7.0 mmol/l). (Hunger is definite as a minimum of 8 and a maximum of 14 hours without calories)
Or
In OGTT, 2nd hour plasma glucose was 200 mg/dl (11.1 mmol/l). (The OGTT should be performed in fasting conditions with 75 g of glucose melted in water after 3 days of adequate carbohydrate intake (150 g/day) as defined by WHO.)
Or
HbA1c value ≥ 6.5% (This test ought to be performed in laboratories by the NGSP (National Glycohemoglobin Standardization Program) approved technique standardized with DCCT (Diabetes Control and Complications Trial) assay.)

A diagnosis can be made with one of the above criteria, but the diagnosis should be confirmed with one of these criteria again the next day. Severe hyperglycemia, which occurs in acute situations such as infection, trauma, and stress, is not considered sufficient for the diagnosis of DM. Therefore, a definitive diagnosis should be made by performing confirmatory tests after the acute transient situation has resolved *(*Diabetes Care., 2003).

1.2.3. Classification of Diabetes Mellitus

Diabetes mellitus; It is evaluated in 4 categories: type 1 diabetes mellitus (T1 DM), type 2 diabetes mellitus (T2 DM), gestational diabetes mellitus (GDM) and specific DM associated with other factors.

1.2.3.1. Type 1 Diabetes Mellitus

Type I DM, which accounts for 5-10% of all diabetes patients; It was originally described as "young onset" or "insulin dependent". Type I DM; It occurs as a result of autoimmune destruction of pancreatic β cells *(*Diabetes Care., 2014*)*. Absolute or relative insulin deficiency is observed due to autoimmune β cell damage of pancreatic β cells. Although the cause of β-cell destruction is not known exactly, it is generally believed that environmental factors and genetic predisposition are the main triggers (You et al.,2016).

1.2.3.2. Type2 Diabetes Mellitus

Type II DM, which is the most common type of diabetes; It is also called "adult-onset diabetes " or " insulin independent diabetes". Type 2 diabetes mellitus includes all patients with relative peripheral insulin resistance and insulin deficiency (Diabetes Care., 2017). The prevalence of type II DM, which is usually detected in advanced ages, is constantly decreasing due to sedentary living conditions, unhealthy diet, and obesity (International Diabetes Federation.,2017).

1.2.3.3. Gestational Diabetes Mellitus

"It is defined as any degree of hyperglycemia that has not been previously detected and occurs for the first time in pregnancy. This explanation consists of undiagnosed cases of type 2 DM diagnosed early in pregnancy and true GDM that develops later". Gestational diabetes has a significant impact on the diabetes epidemic. It increases the risk of developing diabetes in the mother and new-born in the later period of life (Mirghani Dirar et al.,2017).

1.2.3.4. Specific DM Types Due to Other Factors

Except for Type 1 DM, Type 2 DM and GDM, some drugs and diseases may cause DM to develop. These factors are grouped into 8 main categories (Diabetes care.,2014)

- ✓ Endocrinopathies
- ✓ Genetic defect of β-cell functions (monogenic forms of diabetes)
- ✓ Exocrine tissue diseases of the pancreas
- ✓ Genetic defects under the influence of insulin
- ✓ Infections
- ✓ Pharmaceuticals or chemical agents
- ✓ Genetic syndromes associated with1diabetes
- ✓ Rare forms of immune-mediated diabetes

1.2.4. Complications of Diabetes Mellitus

Diabetes mellitus complications are the main cause of morbidity and mortality associated with this chronic metabolic disorder (Diabetes care.,2013). Diabetes mellitus complications are classified as acute and chronic.

1.2.4.1. Acute complications include hypoglycemia, diabetic ketoacidosis (DKA)h, hyperglycemic hyperosmolar state (HHS), hyperglycemic diabetic coma, stroke, loss of consciousness and infections (Ganesh Kumar et al.,2012).

1.2.4.2. Chronic complications are mostly related to the long-term effects of hyperglycemia on the vascular system and are divided into microvascular (retinopathy , nephropathy, and neuropathy) and macrovascular (coronary artery disease, peripheral vascular disease, atherosclerosis , myocardial infarction) (BresaterLE et al.,1996). In addition to its acute and chronic complications, diabetes has been associated with increased cancer rates, physical and cognitive disability, depression, and tuberculosis (Ganesh Kumar et al.,2012).

1.3. Irisin

1.3.1. Definition of Irisin

Irisin discovered in 2012 by Boström et al. These researchers derived the name of the irisin from the symbol of the rainbow, "Iris," the daughter of Thaumas and Elektra, who brought good news from the gods to humans in Greek mythology (Aydin et al.,2014 and Lovren et al.,2010). Irisin is a thermogenic protein that converts white adipose tissue to brown adipose tissu and provides energy expenditure (Lovren et al.,2010).

Irisin is defined as a myokine that protects individuals from metabolic diseases and is released from skeletal muscle when regular exercise is performed. This myokine has been discovered to be a membrane protein called fibronectin type III domain containing 5 (FNDC5) in muscles. The proteolytic product of the FNDC5 protein was named irisin (Butler et al.,2014).

1.3.2. Structure and Characterization of the Irisin

The irisin is thought to be a proteolytic derivative of fibronectin type III domain containing 5 (FNDC5), which is released into the bloodstream in response to some, but not all, types of exercise (Wrann et al.,2013).

FNDC5 was discovered simultaneously by two independent research groups in 2002 (Teufel et al.,2002 and Ferrer-Martínez et al.,2002) and was known as a protein that mediates browning of adipose tissues under the control of the muscle peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) (Boström et al.,2012). FNDC5 (Boström.,2012), also known as protein 2 (FRCP2) (Teufel et al.,2002), and PeP (Ferrer-Martínez et al.,2002) is a glycosylated type 1 membrane protein with 209 amino acids (aa). FNDC5 contains an N-terminal signal sequence of 29 amino acids (aa), a fibronectin type III region of 94 amino acids (aa), an unidentified region of 28 amino acids (aa), a transmembrane domain of 19 aa, and a C-terminal portions of 39 aa. (Wrann et al.,2015).

After the discovery that transmembrane FNDC5 is larger compared to its cellular counterpart, FNDC5, it has been suggested that FNDC5 should be cleaved from its C-

terminus, modified (glycosylated) and secreted by unknown proteases (Boström et al.,2012).

The C-terminal fragment of FNDC5 is found in the cytoplasm, while the extracellular N-terminal is proteolytically cleaved to produce irisin, which is released into the circulation (Panati et al.,2018 and , Mahgoub et al.,2018). After degradation of FNDC5 by yet unknown proteases, 112 aa protein, irisin, is released into the circulation (Wrann et al.,2015). Studies in both animal models and humans have also confirmed that irisin is the main source of FNDC5 gene expression (Lecker et al.,2012 , Timmons et al.,2012 and Huh et al.,2012).

According to Bostrom et al., the theoretical molecular weight of irisin is 12.6 kDa (Boström et al.,2012). In a later study, 32 kDa and 24 kDa glycosylated and deglycosylated FNDC5 / irisin forms were revealed, respectively, in human serum samples (Lee et al.,2014). Studies have revealed that the irisin band found in the experiments is around 22-24 kDa if it is irisin and not FNDC5 (Huh et al.,2012 and Roca-Rivadaet.,2013 and Lee et al.,2014). This indicates that further studies are needed to accurately determine the exact structure and cleavage site of FNDC5.

1.3.3. Irisin Expression and Regulation

Irisin is an adipo-myokine secreted by subcutaneous and visceral adipose tissue outside of skeletal muscle (Roca-Rivada et al.,2013). However, the expression of FNDC5 in adipose tissue was found to be 100–200 times lower than that in skeletal muscle (Perakakis et al.,2017). Various factors that alter the level of FNDC5 and indirectly the level of circulating irisin include cold (Lee et al.,2014) and physical activity (Moreno-Navarrete et al.,2013). According to Boström et al., circulating irisin levels increase after exercise. In their study, they reported that exercise-induced PGC1-α increased the expression of the FNDC5 gene (Boström et al.,2012). Confirming the study, another study found that circulating irisin levels increased in people participating in exercise-related activities and gradually decreased in those who were less active (Moreno et al.,2015).

However, some data have not confirmed the activation of the FNDC5 gene with exercise (Timmons et al.,2012)(Kurdiova et al.,2014), with people who train regularly generally showing a lower serum level of irisin (Qiu et al.,2015). Timmons et al., as a

result of their study, suggested that FNDC5 expression is increased only in highly active elderly individuals and that it is not associated with metabolic status, contrary to the statement that "physical exercise increases irisin level in people with metabolic disorders (Huh et al.,2015) (Timmons et al.,2012). Serum irisin levels were found to be significantly higher than urban residents, and this difference was associated with activities in the residential area (Moreno et al.,2015).

The type of physical activity is suspected to play a role in irisin levels, as an increase in irisin has been noted after high-intensity exercise and after resistance exercise (Tsuchiya et al.,2014), but not after endurance exercise.

Among the factors that change the level of the circulating irisin is temperature. Aydin et al. In a study conducted by Obese and healthy individuals, one of the two groups did a 45-minute jog, and the other group took a 45-minute shower in a Turkish bath at 50°C. Researchers have reported that irisin amounts increase after both exercise and shower, but higher amount of irisin is secreted and transferred to saliva after shower in Turkish bath. The increase was observed to be higher in obese individuals compared to control subjects (Aydin et al.,2013).

In another study investigating whether exposure to cold is an afferent signal for irisin secretion in humans, it was detailed that cold introduction expanded circulating irisin, and an acceptance of irisin secretion corresponding to the concentrated of shivering and comparable to exercise-induced emission (Lee et al.,2014). It is by and large accepted that circulating irisin levels increase in people taking part in exercise-related exercises and continuously decrease in those who are less active (Roca-Rivada et al.,2013). In this manner, given the generally advantageous effects of work out on corpulence, diabetes, cardiovascular, and other illnesses, it is thought that irisin may play a critical part in a variety of functions related with these infections and in other as however unexplored functions.

1.3.4. Irisin in Metabolic Diseases:

1.3.4.1.Irisin and Obesity:

Obesity is a chronic disease that affects both adults and children, is common in developed and developing countries, and is defined as the overgrowth of adipose tissue (WHO.,2000).

It is thought that irisin will have a role in promoting lipid mobilization and glucose uptake by increasing UCP1, enabling the differentiation of white adipocyte into beige adipocytes, and improving adipose tissue capacity (Moreno-Navarrete et al.,2013). Therefore, it is suggested that irisin levels may be associated with overweight or obesity in different groups of people. However, this relationship remains controversial due to conflicting results reported.

In a study conducted on 81 individuals, 40 of whom were healthy controls, it was reported that circulating irisin was significantly lower in obese individuals compared to healthy controls, and irisin may play a role in the regulation of endothelial function in obesity (Hou et al.,2015). In another study involving 75 metabolically healthy non-obese adults and 51 metabolically healthy obese adults, serum irisin levels were found to be significantly lower in the metabolically healthy obese group, and irisin was negatively correlated with BMI (Liu et al.,2017). Other studies confirm the negative relationship between BMI and irisin (Hou et al.,2015, Choi et al.,2014). A study involving 300 obese participants, revealed that serum irisin levels were not significantly higher than age- and sex-matched controls (Liu et al.,2013).

Contrary to these studies, most studies support the role of irisin in the prediction of obesity and the positive correlation between BMI and irisin (Liu et al.,2013and Stengel et al.,2013). In a clinical study involving 94 obese patients, obese subjects participating in a weight loss program were shown to have higher irisin levels than controls (Crujeiras et al.,2014). Likewise, in a study conducted on 40 patients, the irisin levels of healthy individuals were found to be higher than anorectic individuals, but lower than obese individuals. At the end of the study, a positive correlation was found between irisin, BMI and body weight (Stengel et al.,2013). Other clinical studies confirm these findings (Hee Park et al.,2013, Tibana et al .,2017 and Chen et al.,2013).

A meta-analysis of 18 studies (1005 cases, 1242 controls) in total provides evidence that circulating irisin levels in obese individuals are higher than in general

healthy controls, and that circulating irisin levels are affected by ethnicity and age (Jia et al.,2019). However, prospective studies are still needed to clearly understand the effects and relationship of ethnicity, lifestyle, and weight loss on irisin level.

1.3.4.2.Irisin and Diabetes:

It has been shown that irisin exhibits therapeutic potential in insulin resistance and T2DM by stimulating browning of white adipose tissue, promoting glucose uptake in skeletal muscle and heart, improving hepatic glucose, lipid metabolism, and improving pancreatic cell function (Hee Park et al.,2013 and Park et al.,2015). It is also argued that irisin may improve hepatic metabolism by reducing Endoplasmic reticulum stress (ER stress) and contribute to ß-islet cell survival and function (Chen et al.,2016). It has been proven that irisin stimulates beta cell proliferation, insulin biosynthesis and secretion by restricting apoptosis induced by high glucose (Liu et al.,2017) or high saturated fatty acids (Natalicchio et al.,2017) on pancreatic beta cells. In this way, irisin can increase glucose tolerance and reduce insulin resistance (Qiao et al.,2016 , Zhu et al.,2015, Ates et al.,2017). Animal studies have also confirmed that exogenous irisin administration significantly lowers blood glucose levels at 60, 90 and 120 minutes, increases insulin sensitivity (Boström et al.,2012).

In cross-sectional studies, irisin levels in the diabetic state seem to diverge between the two types of diabetes. While irisin levels have been reported to be higher in type 1 diabetes mellitus (T1DM) than in controls (Ates et al.,2017and Espes et al.,2015), in many studies including 2 meta-analyses (Du X-L et al.,2016, Zhang et al.,2016) patients with T2DM were found to be lower than controls (Shoukry et al.,2016, Shelbaya et al.,2018, Assyov et al.,2016). Liu et al. In his study on 156 individuals, 96 of whom had T2DM, he found that circulating irisin in T2DM patients was significantly lower than in non-diabetic controls. It is thought that this observed decrease may be caused by the decrease in muscle function and PGC-1α expression in T2DM (Liu et al.,2013).

Contrary to these studies, there are studies that have found that irisin levels are decreased in T1DM patients compared to healthy individuals (Tentolouriset al.,2018) and that serum irisin levels are higher in T2DM patients than in controls (García-Fontana et al 2016, Huh et al.,2016). In a case-control study involving 73 T2DM and 55 non-diabetic

controls, irisin levels were found to be higher than in the control group, and it was suggested that TG and glucose levels could be modulated by irisin as a compensatory mechanism to improve metabolic status (García-Fontana et al 2016).

Published studies have shown that irisin levels increase throughout pregnancy (Kuzmickiet al.,2014, Garcés et al.,2014), and increased irisin levels may be an adaptive response to compensate for increased insulin resistance during pregnancy (Kuzmickiet al.,2014, Ebert et al.,2014). A prospective study measured circulating irisin levels in the first and second trimesters and showed that circulating irisin levels in pregnant women who subsequently developed gestational diabetes mellitus (GDM) were significantly lower, which was interpreted as maternal irisin levels early in pregnancy could predict the development of GDM. (Erol et al.,2016). In a meta-analysis that included 22 studies that analysed irisin levels not only in blood but also in cord blood and breast milk, and investigating the relationship between irisin levels and prenatal and postnatal gestational diabetes, circulating irisin levels in the GDM group were controlled during pregnancy. It was found to be significantly lower than the levels in the group (Cui et al.,2020). Only a few studies (Ebert et al.,2014, Piya et al.,2014) have produced opposite results.

These findings and studies provide new insights into the contribution of irisin to glucose metabolism and potentially become the focus of future research in the treatment of diabetes, but more studies are needed to fully understand the relationship.

1.3.4.3.Irisin and Lipid Metabolism:

It is known that irisin increases fatty acid β-oxidation via AMPK and stimulates lipid metabolism by increasing the expression of PPARA (Perakakis et al.,2017). In addition, since it is a thermogenic protein, it provides energy expenditure by transforming white adipose tissue into brown adipose tissue (Boström et al.,2012). All these effects that the irisin influence the lipid parameters.

Although many studies have reported a positive correlation between irisin levels and an unfavourable lipid profile, the results are inconsistent. In the studies performed, fasting TGs in sedatives (Moreno et al.,2015) and people with metabolic syndrome (Hee Park et al.,2013), and total cholesterol (TCH), low-density lipoprotein (LDL) and

fasting fatty acids in Chinese patients (Tang et al.,2015), and LDL in Korean adults (Jang et al.,2017). A positive correlation was observed between TGs and circulating irisin level.

In contrast to these studies, a study of 430 men and 537 women found significant inverse relationships between irisin and circulating LDL and TG levels for men. Among women, those without lipid-lowering drugs an inverse relationship was seen between irisin and TCH in women (Oelmann et al.,2016). In a different study conducted among middle-aged healthy women, an inverse relationship was found between irisin and HDL and TCH (Perakakis et al.,2017). It is thought that the differences in the studied populations or the different ELISA kits used for irisin measurements may be responsible for the differences in the results. However, further studies are needed on the function of irisin and its biochemical pathways in humans.

1.3.4.4.The Effect of Exercise on the Irisin

Existing studies show that the changes in the irisin level with exercise vary depending on the type and intensity of the exercise, the physical activity level of the person doing the exercise, and whether there is any metabolic disorder (Villarroya.,2020, Vaughan et al.,2015, Polyzos et al.,2015). Although some of these studies have shown that acute exercise leads to a significant increase in the level of irisin in the circulation (Norheim et al.,2014, Kraemer et al.,2014), there are also studies showing that acute exercise does not affect the level of irisin (Pekkala et al.,2013). In addition, studies examining how exercise programs applied for 2 to 12 weeks affect the level of resting irisin have also revealed conflicting results, and it is understood that this effect differs according to the type of exercise (Kraemer et al.,2014, Tsuchiya et al.,2014, Bluher et al.,2014). The effects of exercise on metabolism do not occur in a single way or depending on a single factor, on the contrary, it changes according to both internal (intensity, duration, type of exercise) and external (age, gender, race, region, nutrition habits, body composition) factors. Depending on the irisin, it makes it difficult to determine the changes that occur (Egan et al.,2013). Meta-analysis studies examining how different types of exercise affect the irisin have shown that acute exercise and chronic strength training increase the resting

irisin level (Fox et al.,2018), while endurance training reduces it insignificantly (Qiu et al.,2016). Both meta-analyses concluded that the responses of the irisin to exercise are acute and that physiological adaptations due to chronic exercise are not sufficient to keep the resting irisin level high (Fox et al.,2018, Qiu et al.,2016). Studies examining the effects of acute exercise on the irisin are summarized below.

In studies examining the effect of acute exercise on the irisin, they reported an increase in irisin in general. In the first study examining the effect of acute exercise on the irisin (Huh et al.,2012) found an acute increase in the irisin and showed that the main reason for this increase was the decrease in the muscle ATP level, and the irisin returned to the resting level after the ATP returned to its normal level after exercise (Huh et al.,2012).

It has been shown that this increase in irisin level is due to the increase in PGC1-α concentration in skeletal muscle due to exercise, and the intensity of exercise has a significant effect on irisin concentration (Tsuchiya et al.,2014). It is thought that the level of irisin secreted from skeletal muscle, adipose tissue, cardiac muscle, and cerebellum purkinje cells and the interaction of this secreted irisin with other cytokines and hormones may explain this sharp decrease. In another study, Löflller et al. (Loffler et al.,2015) showed that irisin increased significantly in young and child participants after a single session of high-intensity and aerobic exercise (Loffler et al.,2015).

This increase in irisin observed immediately after acute exercise is thought to be independent of PGC1-α. (Sriwijitkamol et al.,2007) showed that the increase in exercise-induced PGC1-α level would not occur during or immediately after exercise, and that an increase could be observed in the 2nd and 3rd hours after exercise (Sriwijitkamol et al.,2007). Therefore, the increase in irisin level during exercise (Kraemer et al.,2014, Kraemer et al.,2014) or immediately after exercise (Huh et al.,2015, Loffler et al.,2015) should be induced by other stimuli, not by PGC1-α. (Polyzos et al.,2015). However, although these mechanisms are still unclear, there are some possible factors. First, the main factor affecting the irisin level; It is thought that the intensity of exercise (Kraemer et al.,2014, Daskalopoulou et al.,2014) and the increase in the concentration of ADP and inorganic phosphate in the environment as a result of the inability to produce the required amount of ATP during high-intensity exercise is an important stimulus for the formation and secretion of the irisin (Villarroya.,2012 , Vaughan et al.,2015, Sanchis-Gomar et al.,2012).

Studies examining the effects of exercise programs on the irisin have generally reported conflicting results. For example, Blüher et al. (Bluher et al.,2014) found that physical activity for 150 minutes per week and for one-year increased irisin levels in obese children by 18%, regardless of gender and age (Bluher et al.,2014). However, it was concluded that this change in the irisin may not have been caused by the exercise, but by the growth of the participants in the growth period (Bluher et al.,2014). Although the underlying causes have not been fully clarified, it is thought that the main reason for the decrease in the irisin after the exercise program is the increased sensitivity of the irisin receptor due to exercise (Tsuchiya et al.,2016).

1.3.4.5.Diet Quality and Irisin

In a study examining the effect of diet macronutrient composition on irisin, mice divided into four groups were given a standard, high-fat, high-carbohydrate, high-protein diet for 60 days. It has been reported that high-fat and high-protein diet suppresses the expression of FNDC5, while high-protein diet prevents the decrease in FNDC5 and irisin levels and contributes to the increase in brown adipose tissue (de Macedo et al.,2017). In a study by Ko et al. (Ko B-J et al.,2016) examining the effects of diet quality and dietary patterns on circulating cardiometabolic markers, a positive correlation was found between Dietary Approaches to Stop Hypertension (DASH: Dietary Approaches to Stop Hypertension) score and irisin level. Same In the study, it was determined that there was an increase in irisin concentrations with high fruit consumption, low meat consumption. In the study of Park et al. (Park et al.,2014), no relationship was found between irisin level and diet quality or quantity. In a study on mice, irisin levels were compared in groups given a high-fat diet, calorie restriction, and a normal diet for 3 months. While energy restriction and high-fat diet did not cause a significant change in serum irisin levels, a decrease in blood irisin levels was found with decreased FNDC5 expression in brown adipose tissue, visceral and epididymal adipose tissue and muscle after 48 hours of fasting (Varela-Rodriguez et al.,2016). Similarly, Anastasillaki et al.(Anastasillaki et al.,2014) also found no relationship between dietary habits, total energy, macronutrient intake or any food group with circulating irisin level. In

addition, no significant relationship was observed between the Mediterranean diet score and the irisin level (Anastasillaki et al.,2014).

1.6. Adropin

1.6.1. Definition of adropin

Adropin was first described by Kumar et al. It is a protein discovered in mice by the term adropin comes from the Latin words “adura” meaning “to set on fire” and “pinquis” meaning “fats or oils” (Kumar et al.,2008). Studies in mouse models to understand the regulation and mechanism of action of adropin have revealed that adropin is a peptide that plays a role in metabolic homeostasis (Ganesh Kumar et al .,2012). Although the source and mechanism of release is controversial, studies in mice show that gene expression and circulating adropin levels are affected by dietary macronutrients and energy balance (Ganesh Kumar et al .,2012, Gao et al., 2014, Partridge et al.,2014).

1.6.2. Structure and Characterization of Adropin

Adropin is encoded by the energy homeostasis-related gene symbolized as "Enho", which is mostly expressed in the liver and brain. Enho is located on the 9p13,3 chromosome and plays a role in the regulation of glucose homeostasis and lipid metabolism (Kumar et al.,2008).

Adropin consists of 76 amino acids and its molecular weight is 4.5 kDa (Kumar et al.,2008). Adropin1-33 is a secretory signal peptide, while adropin 34-76 is a peptide hormone. Adropin34-76 has been shown to be biologically active when administered to mice and cultured cells (Kumar et al.,2008, Gao et al.,2015). The N-terminus of amino acids 1-9 is localized in the cytoplasm. The 9-30 region of amino acids is the transmembrane domain (Li et al.,2016). The C terminal of amino acids 30-76 is localized outside the surface of the plasma membrane (Wong et al.,2014).

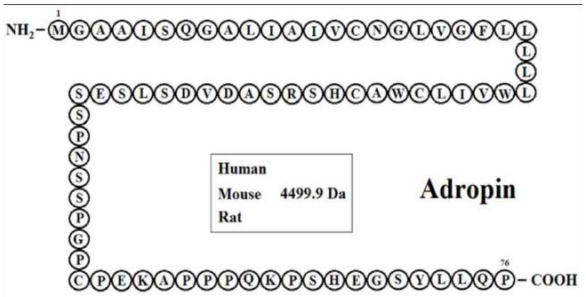


Figure 1.6.2. Amino acid sequence of adropin (Aydin et al.,2014).

The aa sequence of adropin is one-to-one in humans, mice, and rats (Kumar et al.,2008). The half-life of adropin has not yet been determined. However, its half-life is assumed to be only a few minutes (Aydin et al.,2014).

1.6.3. **Adropin Expression and Regulation:**

The expression of the Enho transcript was first described in the liver and brain (Kumar et al.,2008, Wong et al.,2014). When Adropin was discovered by Kumar and colleague, they observed that hepatic energy homeostasis associated (Enho) expression was affected by fasting and feeding state, leading to an increase in its expression when fed a fatty diet. However, a decrease in this expression was observed in chronic high-fat diet administration (Kumar et al.,2008). In another study conducted in mice, it was reported that high adropin levels were observed in mice fed a high-fat, low-carbohydrate diet, and low adropin levels were observed in mice fed a low-fat, high-carbohydrate diet (Ganesh Kumar et al .,2012). In human studies, it was stated that the level of adropin in the plasma was stable and did not show any significant changes due to fasting or food. It has been suggested that the level of adropin can be regulated only by acute signals in the nutritional state (Butler et al.,2015). But later, Butler et al. found that glucose intake decreased adropin level, while fructose intake increased plasma adropin concentration. Moreover, this increase was greater in individuals with hypertriglyceridemia (Butler et al.,2015). Plasma adropin levels have also been suggested to correlate with dietary fat intake (positive) and carbohydrate (negative) (St-Onge et al.,2014).

Recent studies show that adropin is expressed in a variety of rat tissues with potential tissue specificity . Adropin immunoreactivity has been observed in the heart, intestine, pancreas, kidney , umbilical veins, salivary glands, and some peripheral tissues (Lovren et al.,2010, Aydin et al.,2013). Adropin levels according to wet weight of tissues: Pancreas > liver > kidney > heart > brain > cerebellar tissue. Adropin has also been found in milk, whey, and plasma of dairy cows (Aydin et al.,2013).

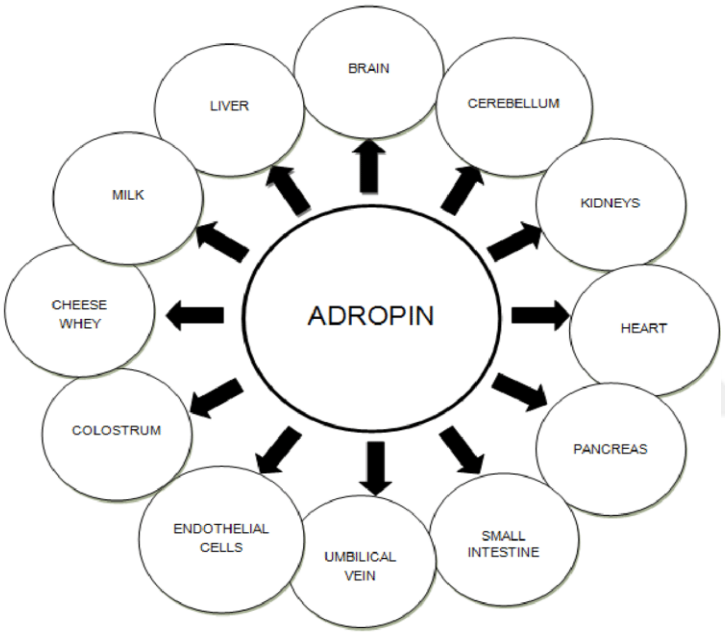


Figure 1.6.3 Adropin in tissues and body bodies (Kumar et al.,2008, Aydin et al.,2014)

The plasma adropin concentration is estimated to range from 1 to 10 ng/mL (Aydin et al.,2014). However, adropin levels vary in physiological and pathophysiological May change in different situations. Human serum adropin levels differ between men and women.

It has been shown that there is no relationship between adropin levels and age.Similarly, adropin levels in paediatric age groups , it was stated that there was no gender difference (Sayin et al.,2014). But Butler et al. In his study, plasma adropin levels in humans show a negative correlation with age. Adropin levels in individuals 30 years of age and younger, 40-50 or 50 and above Was found high (Butler et al.,2012).

1.6.4. Adropin and Metabolic Diseases

1.6.4.1. Adropin and Obesity

Kumar et al. in a study on mice, it was reported that low adropin levels induce adiposity, and dyslipidaemia (Ganesh Kumar et al .,2012).

In many studies, it was reported that adropin decreased in preobese and obese individuals and that adropin was negatively correlated with BMI (Butler et al.,2012, Chen et al.,2019, Kałużna et al.,2013), and it was suggested that adropin may be an independent marker in obesity (Chen et al.,2019).

In a study conducted with 64 obese 36 nonobese children aged 11 to 16 years, serum adropin levels of obese children were found to be significantly lower compared to nonobese children. Butler et al. In a study conducted by Adropin, serum adropin levels were found to be lower in obese patients (Butler et al.,2012). However, Gozal et al. Contrary to this relationship found between adropin and BMI in his study, no relationship was found between BMI and adropin concentration (Gozal et al.,2013).

1.6.4.2. Adropin and Diabetes

DM is an endocrinopathy associated with decreased insulin efficiency due to insufficient insulin production or secretion and/or decreased insulin sensitivity in liver, skeletal muscle, and adipose tissues.

In most human studies, serum adropin levels were decreased in individuals with T2DM (Zang et al.,2018, Wu et al.,2014), and adropin was inversely related to model assessment-estimated insulin resistance (HOMA-IR) values (Chen et al.,2019, Zang et al.,2018) . Zhang et al. They evaluated the serum adropin level in 116 T2DM patients and 60 healthy individuals with normal glucose tolerance. It has been reported that adropin with lower adropin level in the patient group showed negative correlation with BMI , TG, and fasting blood glucose, and HbA1c (Zang et al.,2018). In a study of 64 obese children, serum adropin levels were found to be lower in obese children (Sayin et al.,2014). Serum adropin levels were found to be low not only in T2DM, but also in children with T1DM and women with GDM (Yildirim et al.,2014, Beigi et al.,2015). Low adropin levels in GDM have been explained by carbohydrate intolerance (Yildirim et al.,2014).

Contrary to these studies, in experimental studies, adropin expression was found to be higher in all tissues of diabetic rats than in the control group (Aydin et al.,2013). In studies conducted on humans, the serum adropin level was found to be higher in diabetic individuals than in the control group (Palizban et al.,2019, Hosseini et al.,2016). In a study conducted on 93 healthy individuals with T2DM, serum adropin levels were found to be higher in the group with T2DM. It has been suggested that higher levels of this peptide may indicate an adaptive response to insulin resistance, endothelial dysfunction, dyslipidemia, and other conditions in the tissues of patients with T2DM (Palizban et al.,2019).

1.6.4.3. Adropin and Endothelial Dysfunction

Endothelium is an active tissue located in the cardiovascular system, surrounding the inner walls of the vessels, and playing a role in the regulation of various pathological and physiological events in the living body. The endothelium was initially regarded as a barrier between organs and blood, but later it was found that this tissue had a vasoactive role (Gibbons et al.,1997). Endothelial tissue performs its functions through mediators secreted in its cells. When there is any imbalance in the synthesis and metabolization of these molecules, a situation called endothelial dysfunction occurs (Cohn et al.,2001).

In a systematic review, low levels of adropin were reported to be an independent indicator of heart disease (Yosaee et al.,2016). In many studies, low adropin levels have been found to be associated with endothelial dysfunction (Ganesh Kumar et al .,2012, Lovren et al.,2010) and have become the main mechanism explaining the effect of adropin in heart patients. The effect of adropin on the endothelium was first reported in 2010 by Lovren et al. worked by. In a study using mice in vitro and in vivo Binaural Alternate Loudness Balance Test (BALB/C), the endothelial cells have increased higher proliferation of endothelial cell, increasing speed, and the capillary tube form is observed.

Based on these results, it has been suggested that adropin has a role in protecting and maintaining the endothelium (Lovren et al.,2010). It has been observed that adropin deficiency is associated with low nitric oxide levels in endothelial cells and that adropin induces the expression of endothelial nitric oxide synthetase (Lovren et

al.,2010) that endothelial functions are mainly dependent on nitric oxide synthetase.in human studies observed that the concentration of adropin decreased in children with sleep a pnea (Gozal et al.,2013).

1.6.4.4. Effect of Exercise on Adropin

Various studies have been conducted to examine how adropin levels are affected by exercise due to its effects on energy metabolism and body homeostasis (Zhang et al.,2017, Soori et al.,2019). All these studies examined the effect of aerobic exercise on adropin. In the study of Zhang et al., it was shown that a 12-week aerobic exercise program in obese adolescents significantly increased serum adropin levels regardless of weight gain or weight loss ((Zhang et al.,2017). In another study, Fujie et al. (Fujie et al.,2015) found that an 8-week aerobic exercise program significantly increased adropin levels in middle-aged and older adults, and this increase was positively correlated with an increase in maximal oxygen consumption (Fujie et al.,2015). In addition, increased adropin level due to exercise is associated with a decrease in arterial stiffness (Fujie et al.,2015). In another study conducted by the same research group, it was revealed that an 8-week aerobic exercise program also increased the resting adropin level in obese individuals, and there was a negative correlation between adropin level and abdominal visceral fat mass (Fujie et al.,2017). However, there are studies that did not find a change in adropin level after the exercise program (Özbay et al.,2020). For example, the findings of the study conducted by Özbay et al. (Özbay et al.,2020) showed that a 16-week aerobic exercise program performed in hot and cold conditions in young healthy men did not change the adropin level (Özbay et al.,2020). Similarly, Sanchis-Gomar et al. (Sanchis-Gomar et al.,2015) showed that regular 6-month soccer training did not affect the adropin level and was not associated with adropin and performance (Sanchis-Gomar et al.,2015).

Although the increase in adropin due to exercise has been demonstrated by different studies, the mechanisms underlying this increase are not yet fully known. On the other hand, the effect of adropin , which has been shown to increase due to aerobic exercise, on adropin, which improves substrate metabolism both at rest (Whyte et al.,2010) and during exercise (Talanian et al.,2007), reduces body fat (Gillen et al.,2013, Maillard et al.,2016) and increases insulin sensitivity (Little et al.,2011), is still being investigated.

1.7. Preptin

1.7.1. Definition of Preptin

Preptin, is a peptide hormone and recently isolated of 34 amino acid (3890 Da) contains 34 amino acids isolated. Preptin was first purified by Buchanan et al. in 2001 and isolated from βTC6-F7 β-cells by culturing from mice (Buchanan et al.,2001).

1.7.2. Structure and Characterization of Preptin

Preptin is found in the islet β-cell granules of the pancreas and is secreted together with insulin and amylin in response to glucose. Preptin is a derivative of the proinsulin-like growth factor IIE (ProIGF–IIE) peptide and increases insulin secretion. ProIGF–IIE is a preptin precursor. Insulin-like growth factor II (IGF-II) is also produced from ProIGF-II. IGF-II is a member of the insulin family that regulates cell growth, differentiation and metabolism. Since preptin is derived from proIGF-II, which forms IGF-II, studies have been conducted to determine the biological activity of preptin. Synthetic preptin increases glucose-stimulated insulin secretion from βTC6-F7 cells of the pancreas in a concentration-dependent and saturable manner.Therefore, the effects of preptin on insulin secretion from βTC6-F7 cells were investigated (Buchanan et al.,2001).

1.7.3. Preptin and Metabolic Diseases

1.7.3.1. Preptin and insulin

Preptin is a peptide increases insulin secretion. The precursor of preptin is ProIGF-II. Insulin-like growth factor II (IGF-II) is also produced from ProIGF-II. Insulin-like growth factor II is a member of the insulin family. Cell growth, differentiation and metabolism are regulated by the effect of insulin-like growth factor II. In immunohistochemical studies with normal and diabetic rats, it has been shown that ProIGF-II, the precursor of preptin, is in the same localization as insulin in the secretory granules of rats. Synthetic preptin increases insulin secretion from glucose-stimulated ßTC6-F7 cells in a concentration-dependent and saturable manner (Buchanan et al.,2001, Höög et al.,1997). preptin is a physiological enhancer of glucose-induced insulin secretion. Preptin has been reported to increase rather than initiate insulin secretion. Preptin is secreted together with insulin from pancreatic beta cells in response

to glucose (Buchanan et al.,2001, Höög et al.,1997). In the pancreas, the maximum amount of preptin that completely binds with anti-preptin-immunoglobulin under experimental conditions was determined as 20 ng/min. plasma preptin levels are higher in patients with Type 2 DM than control group and impaired glucose tolerance. In addition, plasma preptin levels were found to be lower in men than in women. (Buchanan et al.,2001) The activity of preptin released together with insulin and amylin on bone was evaluated. While it has been shown to be anabolic both in vivo and in vitro, it has been shown not to affect osteoclast activity (Zhao et al.,2008).

1.7.4. The Effect of Exercise on Preptin

It is thought that preptin, which has a much smaller number of studies compared to irisin, may be related to the adaptations in energy metabolism due to exercise, since it increases insulin secretion. However, there are only two studies examining the effect of exercise on preptin (Mohammad Rahimi et al.,2020, Safarimosavi et al.,2018). In the first of these studies, Safarimosavi et al. (Safarimosavi et al.,2018) reported that an exercise program applied 3 days a week for 12 weeks in prediabetic patients decreased the resting preptin level (10% high-intensity exercise, 14% exercise at anaerobic threshold) (Safarimosavi et al.,2018). In addition, it was reported that the participants' insulin resistance indices improved by 35% in both exercise groups (Safarimosavi et al.,2018). These findings show that preptin level is affected by exercise intensity and the decrease observed in preptin due to exercise is related to the improvement in insulin sensitivity. In another study, Gholam et al. (Mohammad Rahimi et al.,2020) examined the effects of exercise programs defined simultaneously, including 12 weeks of aerobic, strength, and aerobic-strength training together, on preptin in obese adults with metabolic syndrome (Mohammad Rahimi et al.,2020). In addition, participants' glucose, insulin, insulin resistance index and glycosylated haemoglobin (HbA1c) were evaluated. Research results show that all exercise programs significantly reduce preptin levels, but the decrease that occurs after simultaneous and aerobic exercise program is higher (Mohammad Rahimi et al.,2020). In addition, like preptin results, it has been shown that only simultaneous and aerobic exercise programs significantly reduce fasting insulin, fasting glucose, and glycosylated haemoglobin (HbA1c) values, and these decreases are associated with a decrease in preptin (Mohammad Rahimi et al.,2020).

CHAPTER TWO

2. Literature review

Irisin, adropin and preptin are novel peptides that stay involved in the guideline of strength metabolism, but the exact conditions and conditions under which they are changed are still under investigation.

Irisin is a thermogenic protein, consisting of 112 amino acids, weighing 12587 Da, and causing energy expenditure by converting white adipose tissue to brown adipose tissue. In 2012, Böstrom et al. This protein, discovered by FNDC5 (fibronectin type III domain-containing 5), a cell membrane protein, is formed by enzyme cleavage (Boström et al.,2012). Although it is of muscle origin, it is also synthesized in adipose tissue and some other tissues (stomach, liver, testis, ovary, kidneys, myelin sheath, heart muscle). However, its expression in muscle is 200 times higher than in adipose tissue (Moreno-Navarrete et al.,2013). White adipose tissue is adipose tissue that stores energy to ensure long-term survival of humans. It helps to provide energy by releasing fatty acids and triglycerides in case of hunger. White adipose tissue consists of cells with large, single, and spherical lipid vacuoles, a peripherally located nucleus and few mitochondria. White adipose tissue has endocrine activity and is known to secrete various factors and hormones such as leptin and adiponectin. Brown adipose tissue; It consists of a centrally located nucleus and numerous mitochondria, which are small, multilocular lipid droplets. Brown adipose tissue is well vascularized and innervated by the sympathetic nervous system. Mitochondria of brown adipose tissue are characterized by the presence of UCP-1 (uncoupling protein-1). When this protein is activated, it initiates mitochondrial respiration for ATP synthesis and causes heat production. Thus, nutrient stores are consumed and energy is consumed (Tews and Wabitsch, 2011).

Adropin is a small peptide with cardiovascular functions and regulation of metabolic homeostasis. In 2008, Kumar et al., which was detected Adropin, is composed of 76 amino acids with a molecular weight of 4999 Da and is involved in the regulation of lipid metabolism. It is synthesized from liver, brain, and endothelial cells (Kumar et al.,2008, Lovren et al.,2010). The primary sequence of adropin cDNA from different species turned out to be highly conserved. The amino acid sequence of adropin in humans, rats, and mice is 100% identical. ENCHO gene expression in the liver has

been shown to be increased in rats fed a high-fat diet and genetically obese. In addition, systemic adropin administration reduced hepatosteatosis and it was argued that the expression of hepatic lipogenic genes was regulated by adropin. Again, in the same study, it was discussed that adropin has a protective effect against obesity-dependent hyperinsulinemia (Kumar et al.,2008). In another study conducted in mice genetically deprived of adropin, it was shown that although the food intake and energy expenditure of the mice were normal, their adipose tissues increased by 50% (Ganesh Kumar et al.,2012).

Preptin, in 2001 Bucham et al. It was found to be synthesized from β cells of the pancreas by It is a peptide originating from Pro IGF II (pro-insulin-like-growth factor II) and consisting of 34 amino acids (Buchanan et al.,2001). It has been reported that preptin is secreted from β cells together with insulin, increasing insulin secretion in cases where blood glucose level rises, and thus plays a role in the regulation of glucose metabolism. It is thought to be the fourth important active pancreatic hormone in glucose homeostasis (Buchanan et al.,2001). In a small number of studies, it was observed that preptin level increased both in patients with impaired glucose tolerance type II diabetes and in patients with gestational diabetes (Dogan et al.,2014, Celik et al.,2011). The positive relationship between body weight and bone density has been demonstrated in numerous clinical and laboratory studies. Along with insulin resistance, elevated preptin level and increased osteocalcin concentration have been found to be associated with obesity and overweight (El-Eshmawy et al.,2015).

CHAPTER THREE

3. MATERIALS AND METHODS

This study was carried out at university hastanesi in tokat and Gaziosmanpaşa University Faculty of Medicine, department of medical biochemistry. The study was initiated after the decision of Gaziosmanpaşa University Clinical Research Ethics Committee dated 29.04.2021 and numbered 2021/08.

3.1. Determination of experimental groups

Body mass index (BMI) was used to determine overweight or obese groups,T2DM and non-obese groups. BMI can be easily calculated by dividing body weight in kilograms by the square of height in meters [Body weight/height 2].

3.2. The criteria of volunteers to be included in the study

1.Overweight or obese people to be taken in the study should be BMI >30kg/m2

2. Non obese or normal weight for the control group should be BMI <25 kg / m2.

3. T2DM people taken in the study should be BMI >30kg/m2

4.Volunteers to be between the ages of 18-50.

3.3. The criteria for exclusion of volunteers from the study

1. Use of drugs that affect body weight, such as glucocorticoids

2. Being a hypertension patient

3. Having coronary heart disease

4. Having heart failure and Cerebrovascular diseases

5. Persons under the age of 18 and over the age of 50 will not be included in the study.

The results to be obtained with these measurements will be determined by the SPSS package program and $p<0.05$ will be considered statistically significant.

3.4. Collection1of blood samples

blood was drawn from the brachial vein into 5 ml gel tubes from all groups included in the study. In order to work in serum samples of all groups, the blood taken into gel flat tubes was centrifuged for 10 minutes at 3500 rpm at 4°C. The serum portion was separated into Eppendorf tubes containing aprotinin, a protease inhibitor, to be studied, and stored in a deep freezer at -20°C until the study day. After the blood collection was completed, Irisin, adropin and preptin levels will be analysed in serum samples prepared using commercially available ELISA kits.

3.5. Biochemical1Analysis:

3.5.1. Measurement of1Serum Irisin:

Cusabio brand kit (Catalogue No: CSB- EQ027943HU) was used to determine the amount of serum irisin and the studies were performed in Tokat Gaziosmanpaşa University Faculty of Medicine, Department of medical biochemistry.

Test Principle:

This ELISA kit uses the competition (Sandwich-ELISA) principle. The microplate provided in this kit is pre-coated with an antibody specific to FNDC5. Samples or standards are added to microplate wells coated with adropin-specific biotin-detection antibody. The HRP conjugate is added to each microplate and incubated. Unbound components are removed by washing. TMB substrate solution is added. Wells with only FNDC5-containing biotinylated detection antibody and added avidin-HRP conjugate appear in blue. The enzyme-substrate reaction is terminated by adding a stop solution and the colour changes to yellow. The colour change is measured using a spectrophotometer at a wavelength of 450 nm ± 10 nm. The concentration of irisin in the samples is determined by comparing the O.D of the samples with the standard curve.

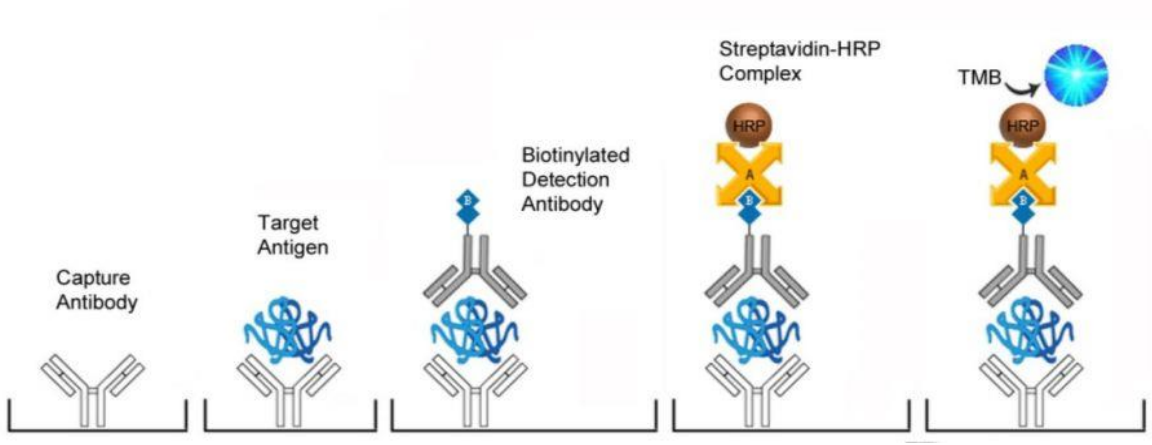


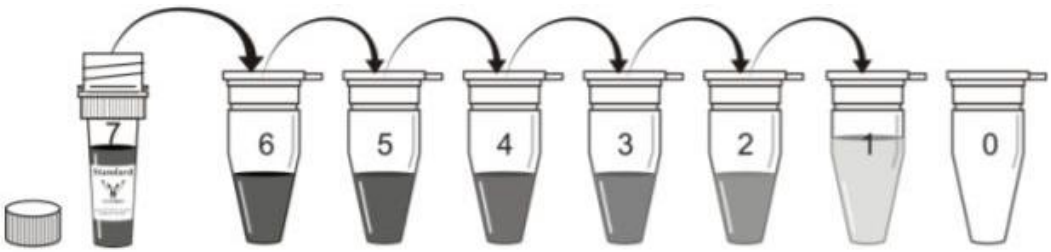
Figure 3.5. of (Sandwich-ELISA) principle

Test Procedure:

All materials and samples to be used in the analysis were brought to room temperature (18-25 °C) before use. Test reactants were prepared as follows;

-200 ng/ml Human Adropin Standard Solution; Reconstitute the Standard with 1.0mL of Standard Diluent, The tube was kept for 10 minutes at room temperature and mixed.

-To prepare 3.12 ng/ml human irisin standard solution from 200 ng/ml, prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. 7 points of diluted standard such as 200ng/mL, 100ng/mL, 50ng/mL,25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.12ng/mL, and the last EP tubes with Standard Diluent is the blank as 0 ng/mL.



Tube	S7	S6	S5	S4	S3	S2	S1	S0
ng/ml	200	100	50	25	12.5	6.25	3.12	0

Figure 3.5.1. Standard Diluent of human irisin Elisa kit

-Preparation of Detection Reagents A and B; Stock Detection A and B are slightly mixed. Afterwards, the Analysis was diluted 1:100 with diluent buffers A and B.

-Preparation of Washing Solution; 20 ml of washing solution (30X) was diluted with 580 ml of distilled water.

- 200ng/mL, 100ng/mL, 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.12ng/mL each of the wells reserved for the standard of the precoated 96-well plate 0.1 ml of the human irisn standard solution was added.

- One well is reserved as a blank in the plate.

- 0.1 ml sample was loaded into the wells of the plate reserved for the sample. The plate is closed with a plate sealer.

- The plate was incubated for 1 hour at 37 degrees.

- At the end of the incubation period, the wells were aspirated and 100µL of Detection reactant A was added, the plate was sealed with a plate sealer and incubated for 1 hour at 37°C.

- At the end of the incubation period, the wells were aspirated again and washed 3 times with 350 µL of washing solution.

- Then 100 µL of Detection reactant B was added to each well, covered with Plate plate sealer and incubated at 37°C for 30 minutes.

- At the end of the incubation period, the wells were aspirated and then washed 5 times with 350 µL of washing solution.

- Then 90 µL of substrate solution was added, the plate was covered with a sealer and incubated for 10-20 minutes at 37°C in the dark. The colour of the plate has started to turn blue.

-Finally, 50 µL of stop solution was added, the colour immediately turned yellow, and then the plate was immediately read in a plate-to-plate reader at 450 nm.

3.5.2. Measurement of Serum Adropin:

Human Adropin (ENHO) ELISA kit (Catalogue No: SEN-5307Hu) was used to determine the serum adropin amount and the studies were carried out in Tokat Gaziosmanpaşa University Faculty of Medicine, Department of medical biochemistry.

Test Principle:

Test principle This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human AD. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human AD and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human AD, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in colour. The enzyme-substrate reaction is terminated by the addition of stop solution and the colour turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human AD. You can calculate the concentration of Human AD in the samples by comparing the OD of the samples to the standard curve.

Test Procedure:

All materials and samples to be used in the analysis were brought to room temperature (18-25 °C) before use. Test reactants were prepared as follows;

-2000 pg/ml Human Adropin Standard Solution; Reconstitute the Standard with 1.0mL of Standard Diluent, The tube was kept for 10 minutes at room temperature and mixed.

-To prepare 31.2 pg/ml human adropin standard solution from 2000 pg/ml, prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. 7 points of diluted standard such as 2,000pg/mL, 1,000pg/mL, 500pg/mL,250pg/mL, 125pg/mL, 62.5pg/mL, 31.2pg/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL.

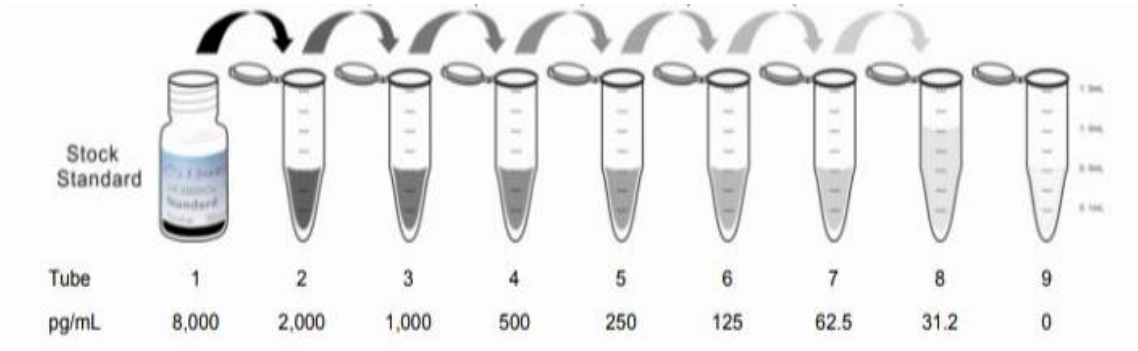


Figure 3.5.2. Standard Diluent of human adropin Elisa kit

-Preparation of Detection Reagents A and B; Stock Detection A and B bulb lightly mixed. Afterwards, the Analysis was diluted 1:100 with diluent buffers A and B.

-Preparation of Washing Solution; 20 ml of washing solution (30X) was diluted with 580 ml of distilled water.

- 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml and 0.312 ng/ml each of the wells reserved for the standard of the precoated 96-well plate 0.1 ml of human adropin standard solution was added.

- One wells is reserved as a blank in the plate.

- 0.1 ml sample was loaded into the wells of the plate reserved for the sample. The plate is closed with a plate sealer.

- The plate was incubated for 1 hour at 37 degrees.

- At the end of the incubation period, the wells were aspirated and 100µL of Detection reactant A was added, the plate was sealed with a plate sealer and incubated for 1 hour at 37°C.

- At the end of the incubation period, the wells were aspirated again and washed 3 times with 350 µL of washing solution.

- Then 100 µL of Detection reactant B was added to each well, covered with Plate plate sealer and incubated at 37°C for 30 minutes.

- At the end of the incubation period, the wells were aspirated and then washed 5 times with 350 µL of washing solution.

- Then 90 µL of substrate solution was added, the plate was covered with a sealer and incubated in the dark at 37 degrees for 10-20 minutes. The color of the plate has started to turn blue.

- Finally, 50 µL of stop solution was added, the color immediately turned yellow, and then the plate was immediately read at 450 nm in a plate-to-plate reader.

3.5.3. Measurement of Serum Preptin

The Sunred brand Human Preptin ELISA kit (201-12-1449) was used to determine the serum preptin amount, and the studies were performed in Tokat Gaziosmanpaşa University Faculty of Medicine, Department of medical biochemistry.

Test Principle:

Preptin was studied in accordance with the manufacturer's instructions by ELISA method from previously obtained serum samples. The technique can be summarized as follows. The double antibody sandwich ELISA technique was used to measure the amount of preptin in the samples. Antibodies specific for preptin were plated onto the microplate. Samples and standards were pipetted into the wells and preptins bound with immobilized antibodies were found. After removing unbound substances by washing, biotin-labelled and streptavidin-HRP-conjugated antibodies specific for preptin were added to the wells to form immune complexes. After incubation at 37°C for 60 minutes, washing was done to remove unbound enzymes. Subsequently, the colour of the liquid turned blue with the addition of chromogen A and B solutions. The colour eventually turned yellow under the influence of acid. The brightness of the colour formed and the preptin concentration in the samples were positively related. Then, reading was done at 450 nm on the microplate reader. Results were obtained in ng/ml and data were recorded.

Test Procedure:

All materials and samples to be used in the analysis were brought to room temperature (18-25 °C) before use.

Test reactants were prepared as follows;

-160 ng/ml Human preptin Standard Solution; Reconstitute the Standard with 1.0mL of Standard Diluent, The tube was kept for 10 minutes at room temperature and mixed.

-To prepare 0.312 ng/ml human preptin standard solution from 160 ng/ml, prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double

dilution series. Mix each tube thoroughly before the next transfer. 7 points of diluted standard such as 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml and 0.312 ng/ml, and the last EP tubes with Standard Diluent is the blank as 0 ng/mL.

-Preparation of Detection Reagents A and B; Stock Detection A and B bulb lightly mixed. Afterwards, the Analysis was diluted 1:100 with diluent buffers A and B.

-Preparation of Washing Solution; 20 ml. wash solution (30X) was diluted with 580 ml of distilled water.

- 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml and 0.312 ng/ml each of the wells reserved for the standard of the precoated 96-well plate 0.1 ml of human preptin standard solution. has been added.

- One well is reserved as a blank in the plate.

- 0.1 ml into the wells of the plate reserved for the sample. sample uploaded. The plate is closed with a plate sealer.

- The plate was incubated for 1 hour at 37 degrees.

- At the end of the incubation period, the wells were aspirated and 100µL of Detection reactant A was added, the plate was sealed with a plate sealer and incubated for 1 hour at 37°C.

- At the end of the incubation period, the wells were aspirated again and washed 3 times with 350µL of washing solution.

- Then 100µL of Detection reactant B was added to each well, the plate was sealed with a plate sealer and incubated at 37°C for 30 minutes.

- At the end of the incubation period, the wells were aspirated and then washed 5 times with 350µL of washing solution.

- Then 90µL of substrate solution was added, the plate was covered with a sealer and incubated for 10-20 minutes at 37°C in the dark. The colour of the plate has started to turn blue.

- Finally, 50µL of stop solution was added, the colour immediately turned yellow, and then the plate was immediately read in a plate-to-plate reader at 450 nm.

Statistical analysis

Statistical analyses were performed with IBM SPSS software ver. 24.0 (IBM Co., Armonk, NY, USA).

program was used for statistical analysis. Obtained data were recorded as mean±SD. Analysis of variation Kolmogorov-Smirnov and Shapiro-Wilk test was used to check the conformity of numerical variables with normal distribution. İRİSİN (NG/ML), ADROPİN (PG/ML) and PREPTİN (NG/L) in three groups of(control), (obesity and T2DM), (obesity and T2DM) were compared as follows: 1-1 control and obesity, 1-2 control and T2DM, 1-3 obesity and T2DM and 1-4 control, obesity and T2DM. ANOVA test and Post Hoc Duncan test were used when a significant difference was detected in the comparison between groups and in the evaluation of demographic data, since the variables were normally distributed. t-test was used for in-group comparison of repeated measures. Values with $P < 0.05$ were considered statistically significant.

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CHAPTER FOURE
4. RESULTS

4.1. İRİSİN (NG/ML)

In this section IRİSİN in three groups of control, obesity and T2DM, obesity and T2DM were compared as follows: 4-1 control and obesity, 4-2 control and T2DM, 4-3 obesity and T2DM ,4-4 control, obesity and T2DM. The t-test and ANOVA tests were used for statistical analysis. To use data for t-test and ANOVA the distribution of the data must be normal. Therefore, the normality of the data was examined using two methods of Kolmogorov-Smirnov and Shapiro-Wilk (Table4.1).

Table 4.1: Tests of Normality for İRİSİN (NG/ML) of control, obesity and T2DM groups.

Parameter	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	P	Statistic	df	p
control	0.016	59	0.256	0.997	59	1.000
obesity	0.020	59	0.200	0.997	59	1.000
T2DM	0.016	59	0.232	0.997	59	1.000

The results of the Tests of Normality are shown in table (4.1.) The normality of data showed that the values are normal ($p > 0.05$) which means the results of the t-test and ANOVA are reliable in this study.

4.1.1. Control and Obesity

Table4.1.1: T-test results for compare means of İRİSİN (NG /ML) between control and obesity groups.

Parameter	Mean	Std. Deviation	Mean Difference	95% Confidence Interval of the Difference		t	df	p
				Lower	Upper			
İRİSİN control	22.283	18.202	11.975	7.312	16.637	5.139	59	0.000
İRİSİN obesity	10.308	7.301						

The results of t-test showed that the mean of İRİSİN (NG / ML) in the control and obesity groups were significantly different ($p < 0.05$, $t = 5.139$, $MD = 11.975$) (Table 4.1.1). Also, according to the mean values, İRİSİN (NG/ML) in the control group ($M=22.283$) was higher than the obesity group ($M=10.308$). Therefore, the level of İRİSİN (NG/ML) in the obesity group decreased compared to the control group (Figure 4.1.1).

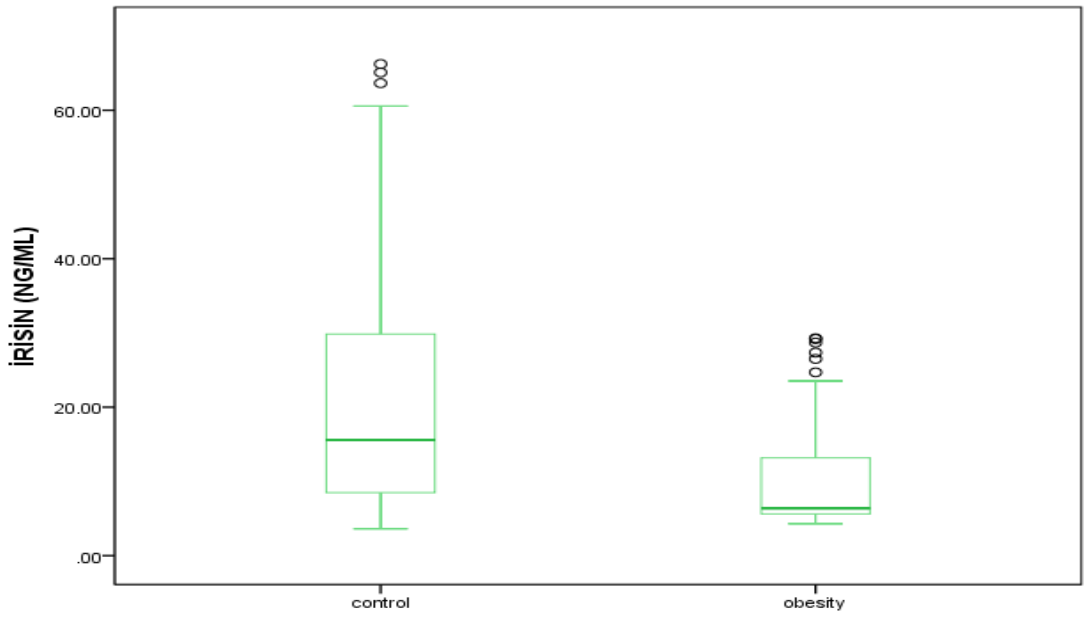

Figure4.1.1: compare means of İRİSİN (NG / ML) between control and obesity groups.

4.1.2 Control and T2DM

Table: T-test results for compare means of İRİSİN (NG / ML) between control and T2DM groups.

Parameter	Mean	Std. Deviation	Mean Differencs	95% Confidence Interval of the Difference		t	df	p
				Lower	Upper			
İRİSİN control	22.283	18.202	11.999	6.727	17.270	4.555	59	0.000
İRİSİN T2DM	10.285	7.406						

The results of t-test showed that the mean of İRİSİN (NG/ML) in the control group and T2DM were significantly different ($p < 0.05$, $t = 4.555$, $MD = 11.99$) (Table 4.1.2). Also, according to the mean values, the level of İRİSİN (NG/ML) in the control group ($M = 22.283$) was higher than the T2DM group ($M = 10.285$). Therefore, the level of İRİSİN (NG/ML) in the T2DM group decreased compared to the control group (Figure4.1. 2).

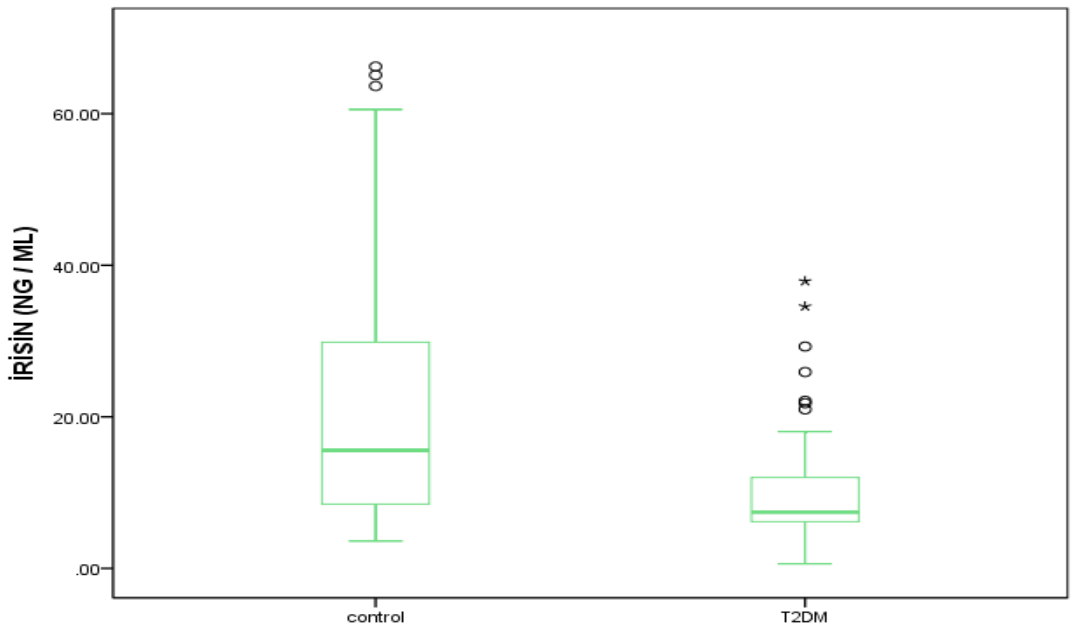

Figure4.1.2: compare means of İRİSİN (NG / ML) between control and T2DM groups.

4.1.3. Obesity and T2DM

Table 4.1.3. T-test results for compare means of İRİSİN (NG /ML) between obesity and T2DM groups.

Parameter	Mean	Std. Deviation	Mean Differences	95% Confidence Interval of the Difference		t	df	p
				Lower	Upper			
İRİSİN obesity	10.308	7.301	0.023	-2.662	2.710	0.018	59	0.986
İRİSİN T2DM	10.285	7.406						

The results of t-test showed that the mean of İRİSİN (NG / ML) in the obesity and T2DM groups were not significantly different (p >0.05, t =0.018, MD =0.023) (Table 4.1.3) (Figure 4.1.3).

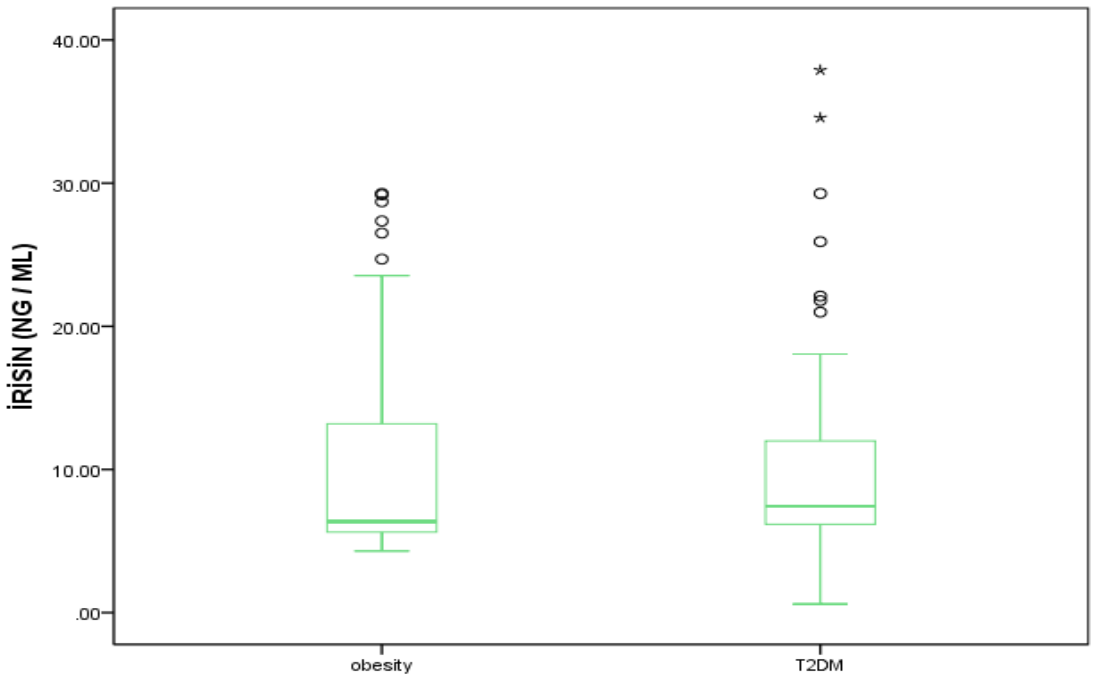

Figure4.1.3: compare means of İRİSİN (NG / ML) between obesity and T2DM groups.

4.1.4 Control ،Obesity and T2DM

Table 4.1.4. ANOVA results for compare means of İRİSİN (NG / ML) between control ،obesity and T2DM groups.

Parameter	Sum of Squares	df	Mean Square	F	p
Between Groups	5747.439	2	2873.720	19.617	0.000
Within Groups	25928.440	177	146.488		
Total	31675.879	179			

ANOVA results showed that the mean İRİSİN (NG/ML) in the three groups of control, obesity and T2DM were significantly different ($p < 0.05$, $F = 19.617$) (Table 4.1.4).

Table 4.1.5. Post Hoc Tests (Duncan Test) results for compare means of İRİSİN (NG / ML) between control ،obesity and T2DM groups.

Parameter	control	obesity	T2DM
İRİSİN (NG / ML)	22.2834^{a*}	10.308^{b}	10.285^{b}

^{*a} Values with the same superscript are not significantly differently ($p > 0.05$).

According to Duncan test results, the highest mean İRİSİN (NG / ML) belonged to the control group (M =22.283) and the lowest mean obtained to the T2DM group (M=10.285). On the other hand, there was a significant difference between the mean İRİSİN (NG/ML), obesity and T2DM (Figure 4.1.4. and Table 4.1.5.).

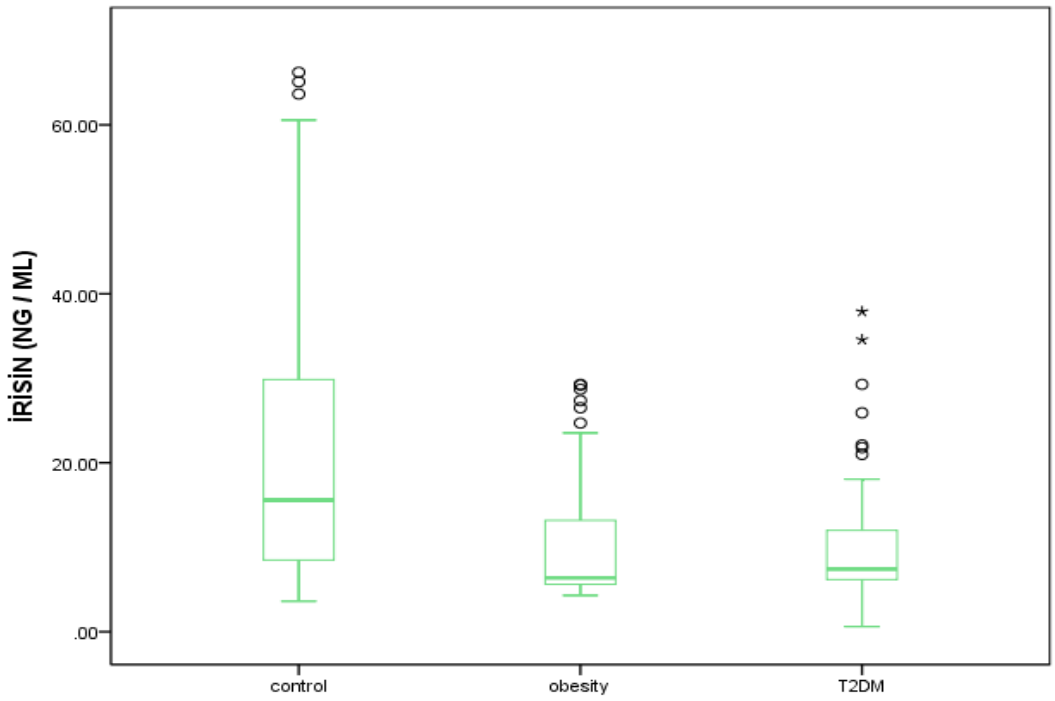

Figure 4.1.5. compare means of İRİSİN (NG / ML) between control ،obesity and T2DM groups.

4.2. ADROPİN (PG/ML)

In this section ADROPİN (PG/ML) in three groups of control, obesity and T2DM, obesity and T2DM were compared. the normality of the data was examined using two methods of Kolmogorov-Smirnov and Shapiro-Wilk (Table 4.2.).

Table 4.2. Tests of Normality for ADROPİN (PG/ML) of control, obesity and T2DM groups.

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Parameter	Statistic	df	p	Statistic	df	p
control	0.024	59	0.210	0.995	59	0.993
obesity	0.022	59	0.223	0.994	59	0.999
T2DM	0.016	59	0.232	0.995	59	0.998

The results of the Tests of Normality are shown in table (4.1.) The normality of data showed that the values are normal ($p > 0.05$) which means the results of the t-test and ANOVA are reliable in this study.

4.2.1 Control and obesity

Table4.2.1: T-test results for compare means of ADROPİN (PG/ML) between control and obesity groups.

Parameter	Mean	Std. Deviation	Mean Differences	95% Confidence Interval of the Difference		t	df	p
				Lower	Upper			
ADROPİN control	340.486	229.901	74.623	0.9001	148.347	2.025	59	0.047
ADROPİN obesity	265.862	181.736						

The results of t-test showed that the mean of ADROPİN (PG/ML) in the control and obesity groups were significantly different ($p < 0.05$, $t = 2.025$, $MD = 74.623$) (Table 4.2.1). Also, according to the mean values, ADROPİN (PG/ML) in the control group ($M=340.486$) was higher than the obesity group ($M=265.862$). Therefore, the level of ADROPİN (PG/ML) in the obesity group decreased compared to the control group (Figure 4.2.1).

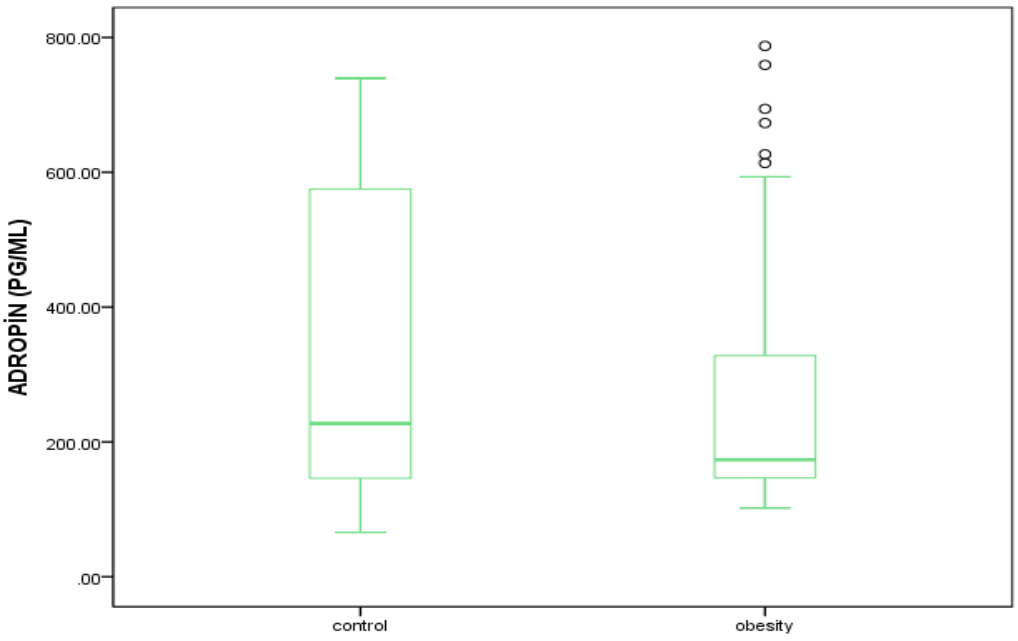

Figure4.2.1. compare means of ADROPİN (PG/ML) between control and obesity groups.

4.2.2. Control and T2DM

Table 4.2.2 T-test results for compare means of ADROPİN (PG/ML) between control and T2DM groups.

Parameter	Mean	Std. Deviation	Mean Differences	95% Confidence Interval of the Difference		t	df	p
				Lower	Upper			
ADROPİN control	340.462	229.901	89.108	10.071	168.144	2.256	59	0.028
ADROPİN T2DM	251.378	165.645						

The results of t-test showed that the mean of ADROPİN (PG/ML) in the control group and T2DM were significantly different ($p < 0.05$, $t=2.256$, $MD=89.108$) (Table 4.2.2). Also, according to the mean values, the level of ADROPİN (PG/ML) in the control group ($M=340.462$) was higher than the T2DM group ($M=251.378$). Therefore, the level of ADROPİN (PG/ML) in the T2DM group decreased compared to the control group (Figure 4.2.2).

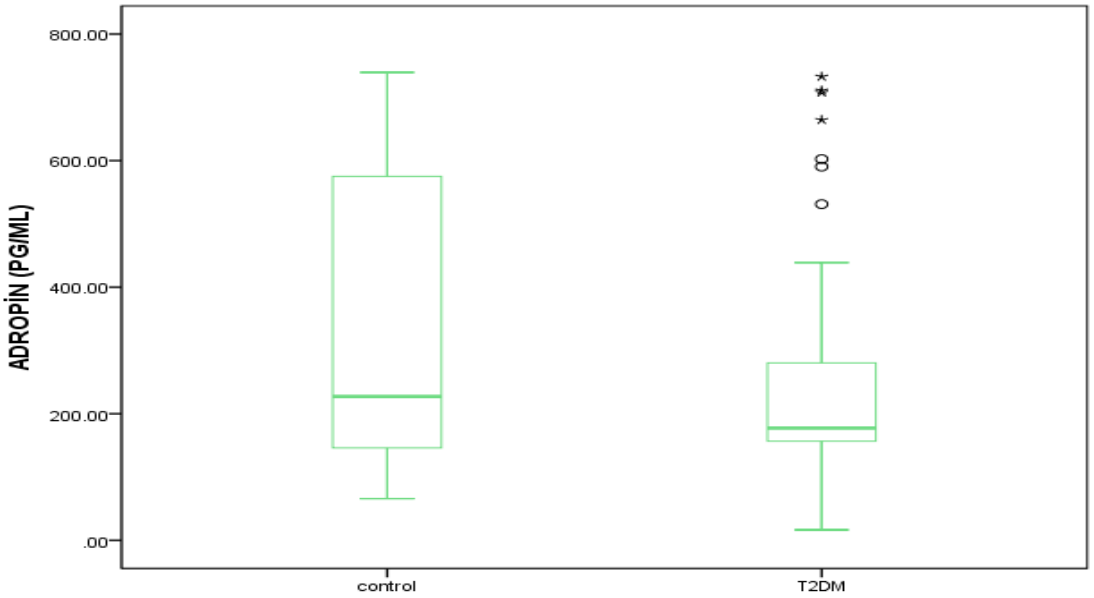

Figure4.2.2. compare means of ADROPİN (PG/ML) between control and T2DM groups.

4.2.3 Obesity and T2DM

Table 4.2.3: T-test results for compare means of İRİSİN (NG /ML) between obesity and T2DM groups.

Parameter	Mean	Std. Deviation	Mean Differences	95% Confidence Interval of the Difference		t	df	p
				Lower	Upper			
ADROPİN obesity	265.862	181.736	14.484	-47.285	79.254	0.469	59	0.641
ADROPİN T2DM	251.378	165.645						

The results of t-test showed that the mean of ADROPİN (PG/ML) in the obesity and T2DM groups were not significantly different (p >0.05, t =0.469, MD =14.484) (Table 4.2.3) (Figure 4.2.3).

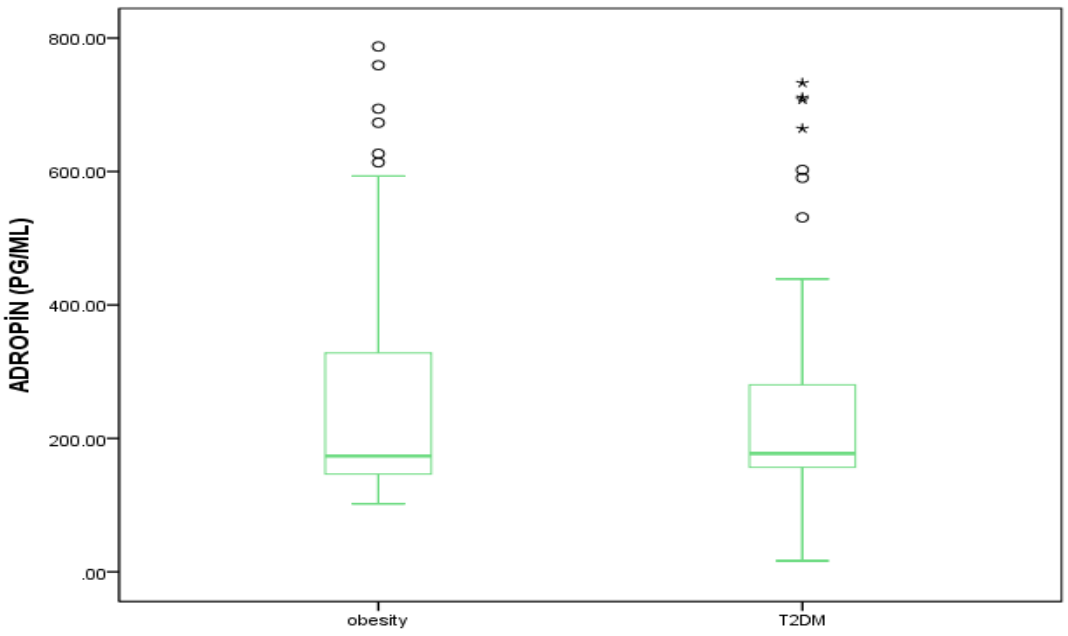

Figure4.2.3. compare means of ADROPİN (PG/ML) between obesity and T2DM groups.

4.2.4. Control ،obesity and T2DM

Table 4.2.4. ANOVA results for compare means of ADROPİN (PG/ML) between control ،obesity and T2DM groups.

Parameter	Sum of Squares	Df	Mean Square	F	p
Between Groups	274375.501	2	137187.751	3.632	.028
Within Groups	6685942.729	177	37773.688		
Total	6960318.231	179			

ANOVA results showed that the mean ADROPİN (PG/ML) in the three groups of control, obesity and T2DM were significantly different ($p < 0.05$, $F = 3.632$) (Table 4.2.4).

Table 4.2.5. Post Hoc Tests (Duncan Test) results for compare means of ADROPİN (PG/ML) between control ،obesity and T2DM.

Parameter	control	obesity	T2DM
ADROPİN (PG/ML)	340.486^{a*}	265.862^{b}	251.378^{b}

*a Values with the same superscript are not significantly differently ($p > 0.05$).

According to Duncan test results, the highest mean ADROPİN (PG/ML) belonged to the control group (M =340.486) and the lowest mean obtained to the T2DM group (M=251.378). On the other hand, there was a significant difference between the mean ADROPİN (PG/ML), obesity and T2DM (Figure 4.2.4 and Table 4.2.5).

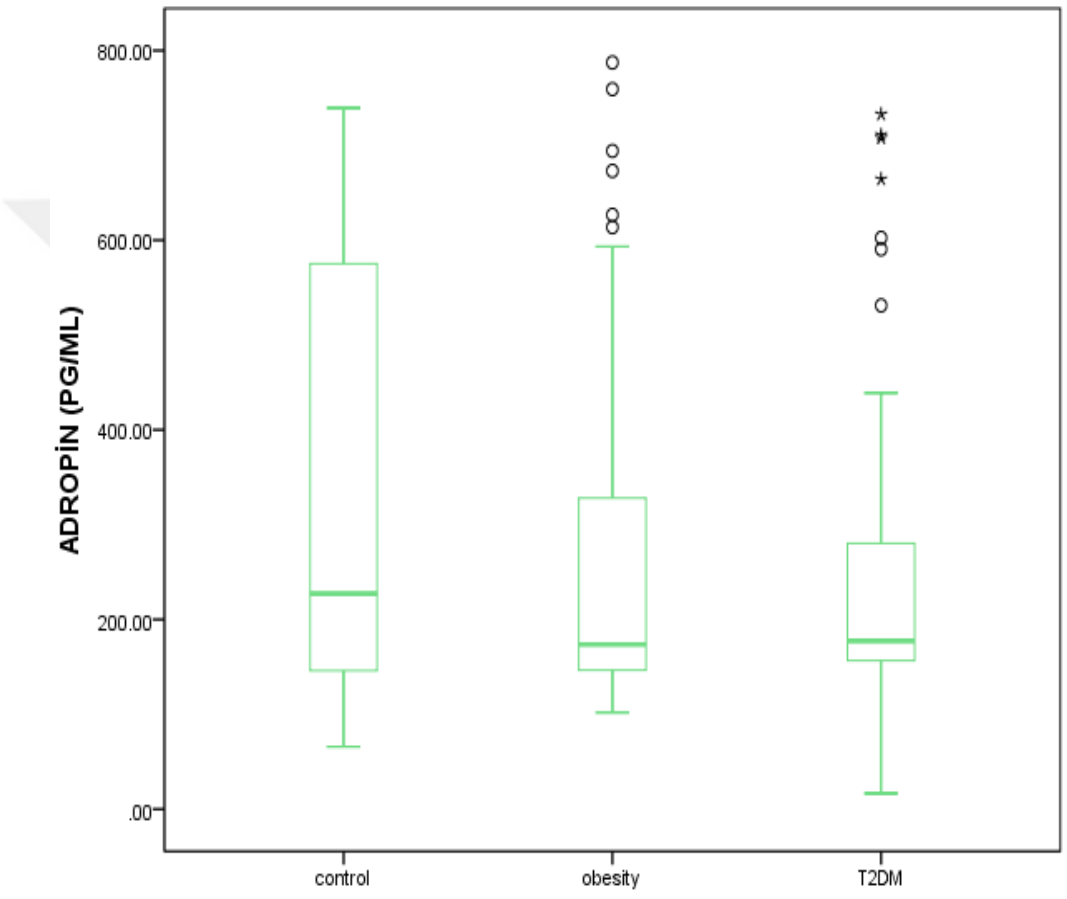

Figure4.2.5. compare means of ADROPİN (PG/ML) between control ،obesity and T2DM groups.

4.3. PREPTİN (NG/L)

In this section PREPTİN (NG/L) in three groups of control, obesity and T2DM, obesity and T2DM were compared. The normality of the data was examined using two methods of Kolmogorov-Smirnov and Shapiro-Wilk (Table 4.3).

Table 4.3. Tests of Normality for PREPTİN (NG/L) of control, obesity and T2DM groups.

Parameter	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	p	Statistic	df	p
control	0.023	59	0.201	0.994	59	0.992
obesity	0.021	59	0.200	0.996	59	0.999
T2DM	0.022	59	0.203	0.996	59	0.999

The results of the Tests of Normality are shown in table (4.1). The normality of data showed that the values are normal ($p > 0.05$) which means the results of the t-test and ANOVA are reliable in this study.

4.3.1. Control and obesity

Table 4.3.1. T-test results for compare means of PREPTİN (NG/L) between control and obesity groups.

Parameter	Mean	Std. Deviation	Mean Differences	95% Confidence Interval of the Difference		t	df	p
				Lower	Upper			
PREPTİN (control	312.723	283.274	-136.197	-233.669	-38.724	-2.795	59	0.007
PREPTİN obesity	448.920	296.394						

The results of t-test showed that the mean of PREPTİN (NG/L) in the control and obesity groups were significantly different ($p < 0.05$, $t = -2.795$, $MD = -136.197$) (Table 4.3.1). Also, according to the mean values, PREPTİN (NG/L) in the obesity group (M=448.920) was higher than the control group (M=312.723). Therefore, level of PREPTİN (NG/L) in the obesity group increased compared to the control group (Figure4.3.1).

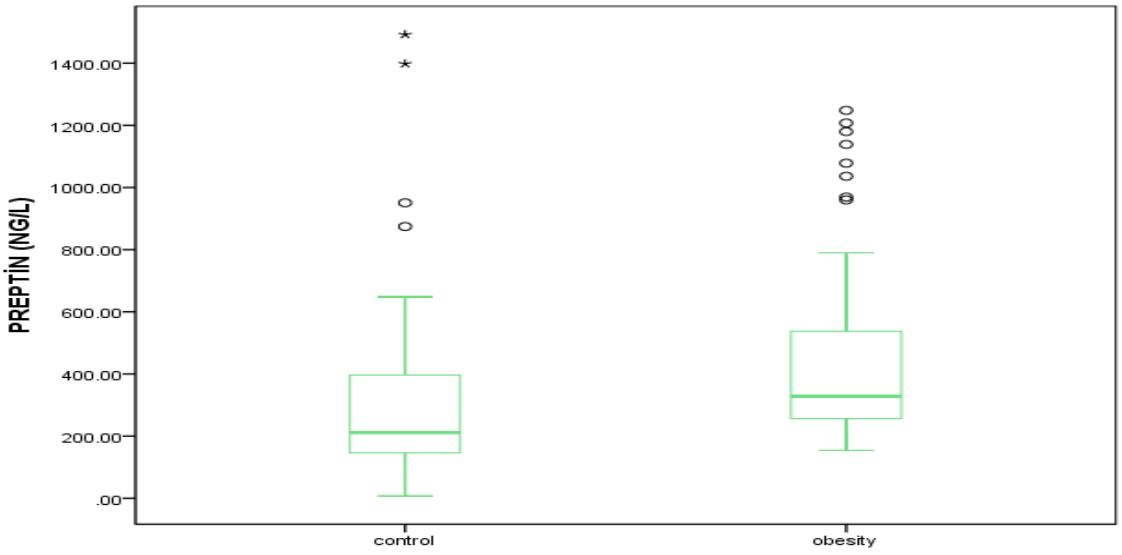

Figure 4.3.1. compare means of PREPTİN (NG/L) between control and obesity groups.

4.3.2 Control and T2DM

Table 4.3.2. T-test results for compare means of PREPTİN (NG/L) between control and T2DM groups.

Parameter	Mean	Std. Deviation	Mean Differences	95% Confidence Interval of the Difference		t	df	p
				Lower	Upper			
PREPTİN control	312.723	283.274	-196.211	-313.325	-79.096	-3.352	59	0.001
PREPTİN T2DM	508.934	312.506						

The results of t-test showed that the mean of PREPTİN (NG/L) in the control group and T2DM were significantly different ($p < 0.05$, $t = -3.352$, $MD = -196.211$) (Table 4.3.2). Also, according to the mean values, the level of PREPTİN (NG/L) in the T2DM group ($M = 508.934$) was higher than the control group ($M = 312.723$). Therefore, the level of PREPTİN (NG/L) in the T2DM group increased compared to the control group (Figure 4.3.2).

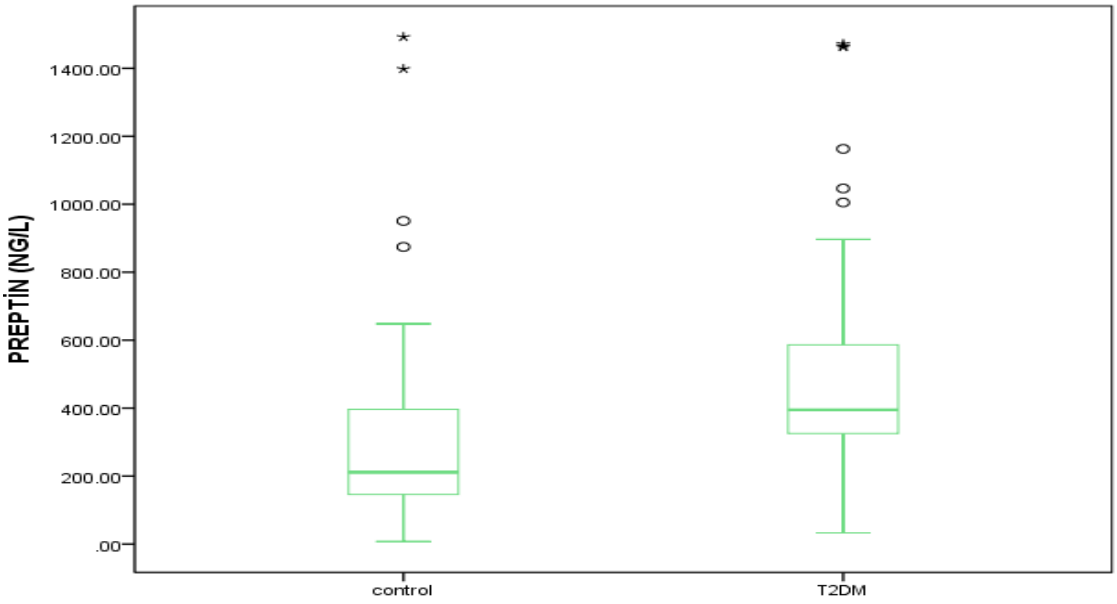

Figure4.3.2. compare means of PREPTİN (NG/L) between control and T2DM groups.

4.3.3 Obesity and T2DM

Table 4.3.3. T-test results for compare means of İRİSİN (NG /ML) between obesity and T2DM groups.

Parameter	Mean	Std. Deviation	Mean Differences	95% Confidence Interval of the Difference		t	df	p
				Lower	Upper			
PREPTİN Obesity	448.920	296.394	-60.014	-166.956	46.928	-1.122	59	0.266
PREPTİN T2DM	508.934	312.506						

The results of t-test showed that the mean of PREPTİN (NG/L) in the obesity and T2DM groups were not significantly different (p >0.05, t = -1.122, MD = -60.014) (Table 4.3.3) (Figure4.3.3).

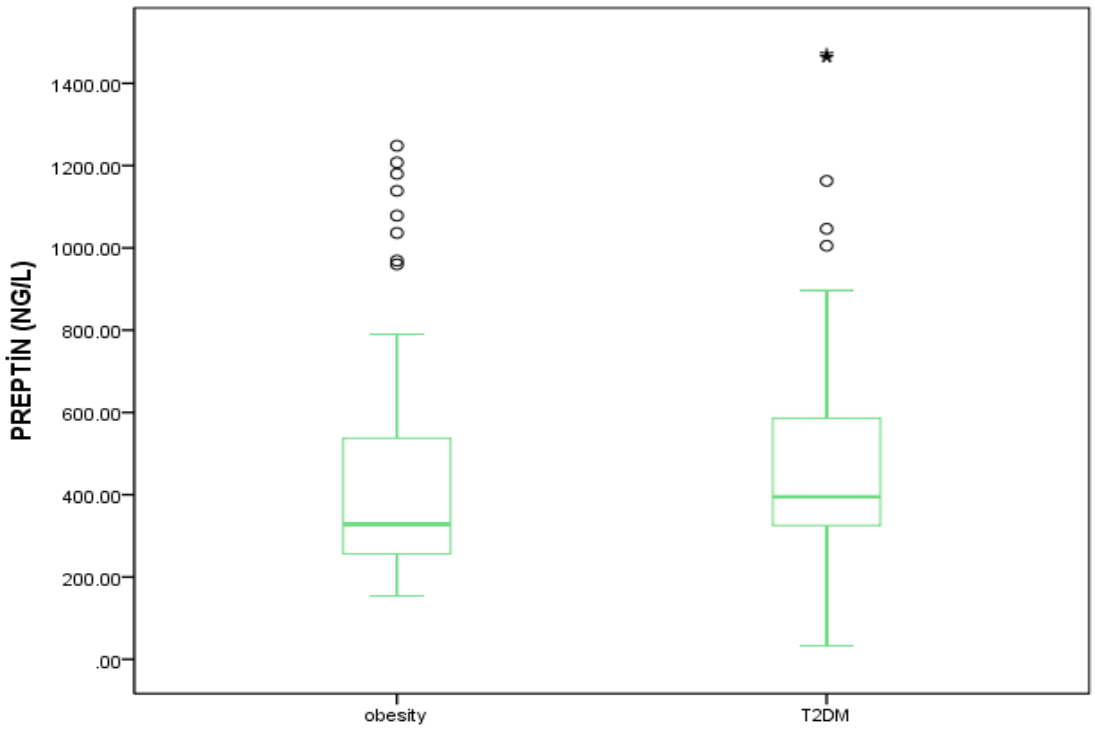

Figure 4.3.3. compare means of PREPTİN (NG/L) between obesity and T2DM groups.

4.3.4 Control ،obesity and T2DM

Table4.3.4. ANOVA results for compare means of PREPTİN (NG/L) between control ،obesity and T2DM groups.

Parameter	Sum of Squares	df	Mean Square	F	p
Between Groups	1213004.005	2	606502.002	6.846	0.001
Within Groups	15649494.85	177	88584.716		
Total	16892498.85	179			

ANOVA results showed that the mean PREPTİN (NG/L) in the three groups of control, obesity and T2DM were significantly different ($p < 0.05$, $F = 6.846$) (Table 4.3.4).

Table 4.3.5 Post Hoc Tests (Duncan Test) results for compare means of PREPTİN (NG/L) between control ،obesity and T2DM.

Parameter	control	obesity	T2DM
PREPTİN (NG/L)	312.723^{a*}	448.920^{b}	508.934^{b}

***a** Values with the same superscript are not significantly differently ($p > 0.05$).

According to Duncan test results, the highest mean PREPTİN (NG/L) belonged to the T2DM group (M =508.934) and the lowest mean obtained to the control group (M=312.723). On the other hand, there was a significant difference between the mean PREPTİN (NG/L), obesity and T2DM (Figure 4.3.5 and Table 4.3.5).

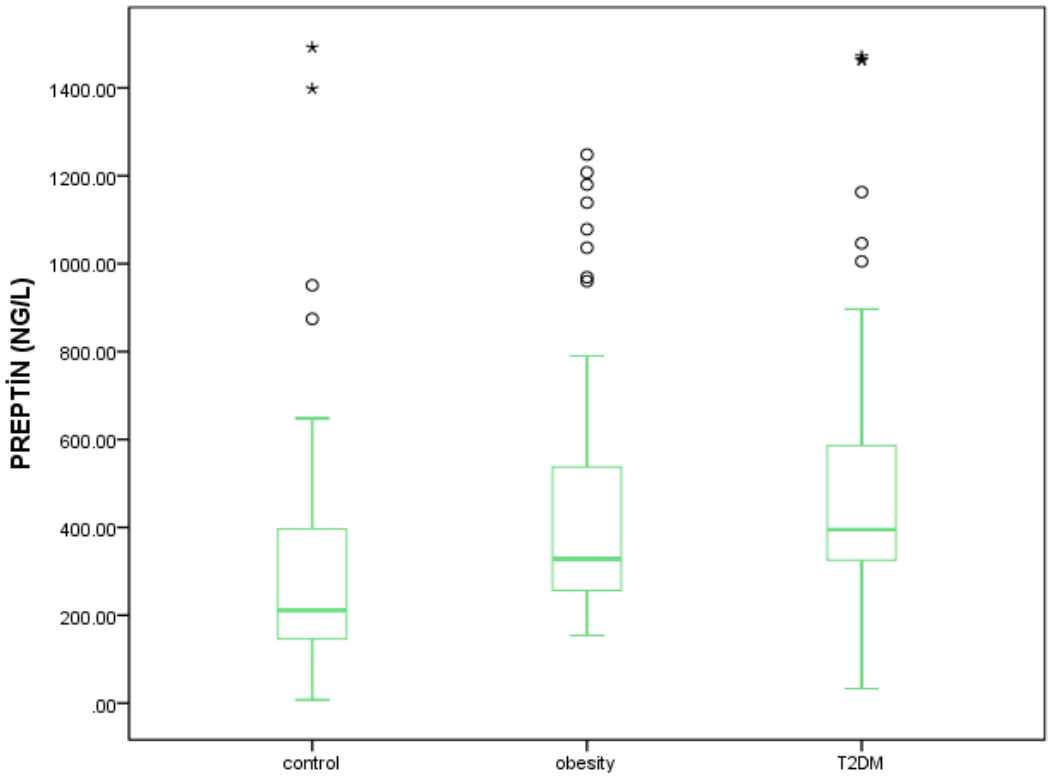

Figure4.3.5. compare means of PREPTİN (NG/L) between control ،obesity and T2DM groups.

4.4. Obesity (İRİSİN, ADROPİN, PREPTİN)

Table 4.4.: ANOVA results for compare means of obesity between İRİSİN, ADROPİN, PREPTİN groups.

Parameter	Sum of Squares	df	Mean Square	F	p
Between Groups	5823975.849	2	5823975.849	72.239	0.000
Within Groups	7134917.984	177	7134917.984		
Total	12958893.833	179			

ANOVA results showed that the mean obesity in the three groups of İRİSİN, ADROPİN, PREPTİN were significantly different ($p < 0.05$, $F = 72.239$) (Table 4.4.).

Table 4.4.1: Post Hoc Tests (Duncan Test) results for compare means of obesity between İRİSİN, ADROPİN, PREPTİN.

Parameter	İRİSİN	ADROPİN	PREPTİN
Obesity	10.308^{C*}	265.862^{b}	448.920^{a}

***c** Values with the same superscript are not significantly differently ($p > 0.05$).

According to Duncan test results, the highest mean obesity belonged to the PREPTİN (M =448.920) and the lowest mean obtained to the İRİSİN (M=10.308). (Figure 4.4 and Table 4.4.1).

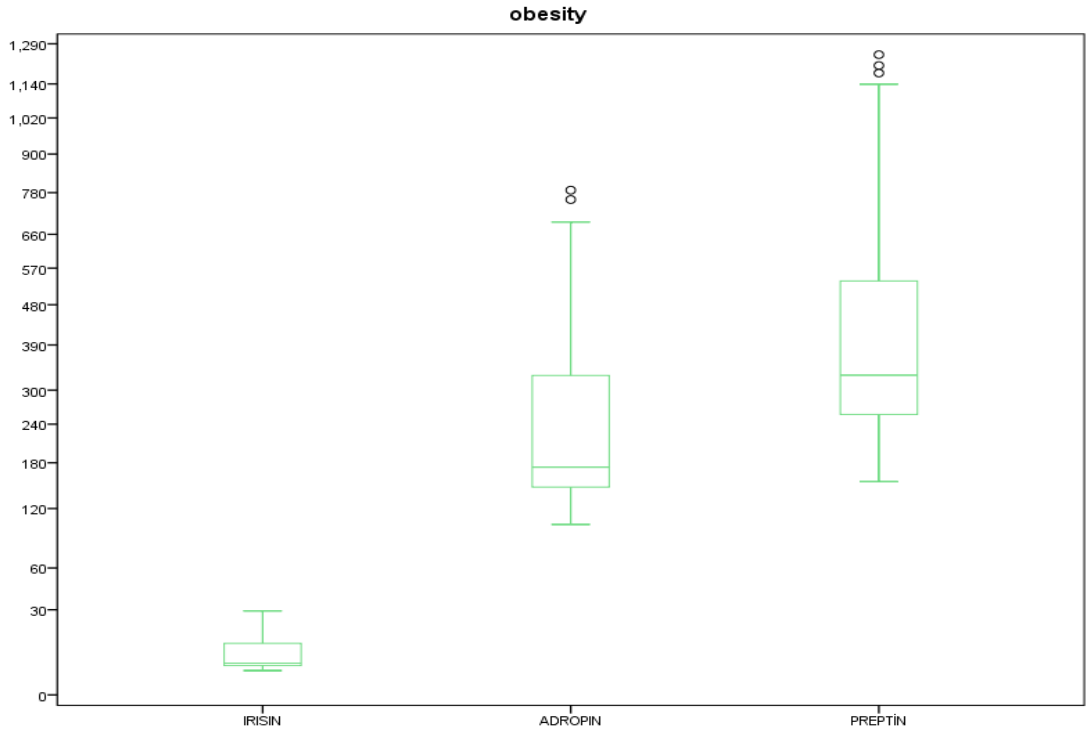

Figure 4.4: compare means of obesity between İRİSİN, ADROPİN, PREPTİN.

4.5. T2DM (İRİSİN, ADROPİN, PREPTİN)

Table 4.5: ANOVA results for compare means of T2DM between İRİSİN, ADROPİN, PREPTİN groups.

Parameter	Sum of Squares	df	Mean Square	F	p
Between Groups	7462269.471	2	3731134.736	89.437	0.000
Within Groups	7384056.476	177	41717.833		
Total	14846325.947	179			

ANOVA results showed that the mean T2DM in the three groups of İRİSİN, ADROPİN, PREPTİN were significantly different ($p < 0.05$, $F = 89.437$) (Table4.5.).

Table 4.5.1: Post Hoc Tests (Duncan Test) results for compare means of T2DM between İRİSİN, ADROPİN, PREPTİN.

Parameter	İRİSİN	ADROPİN	PREPTİN
T2DM	10.284^{C*}	251.378^{b}	508.934^{a}

^{*c} Values with the same superscript are not significantly differently ($p > 0.05$).

According to Duncan test results, the highest mean T2DM belonged to the PREPTİN (M =508.934) and the lowest mean obtained to the İRİSİN (M=10.284). (Figure 4.5 and Table 4.5.1).

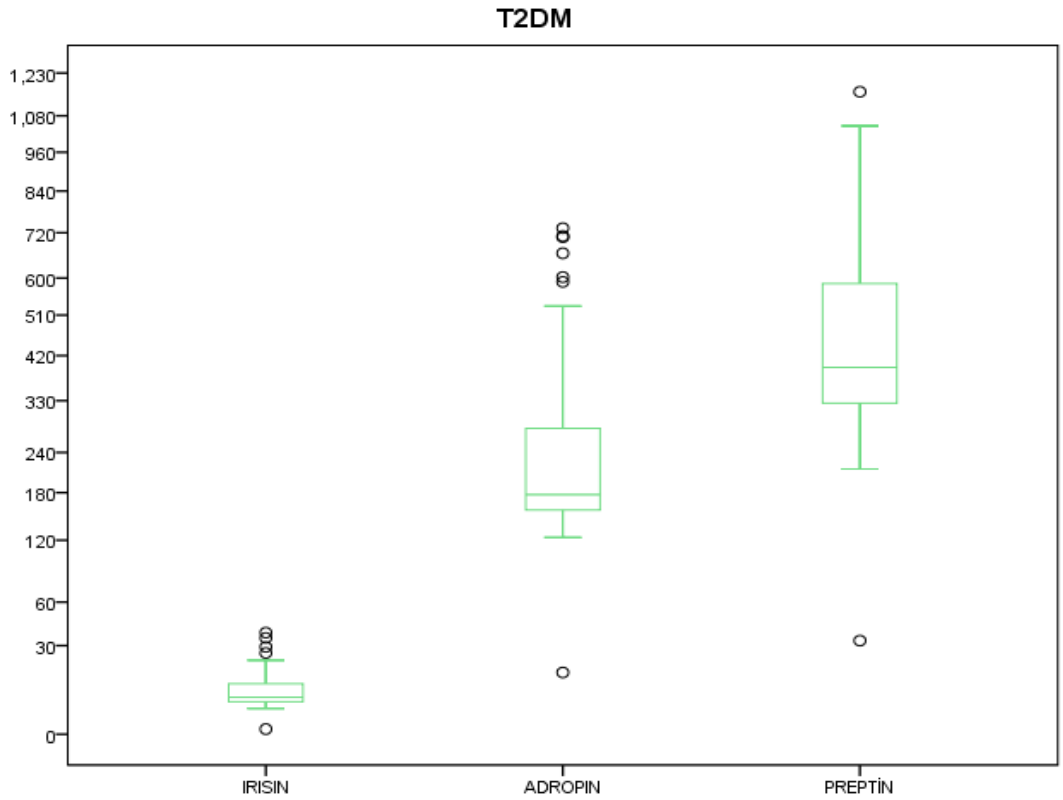

Figure 4.5: compare means of T2DM between İRİSİN, ADROPİN, PREPTİN.

4.6.Post Hoc Tests (Duncan Test) results for compare means of İRİSİN (NG / ML), ADROPİN (PG/ML), PREPTİN (NG/L)

Parameter	control	obesity	T2DM
İRİSİN (NG / ML)	22.2834[a*]	10.308[b]	10. 285[b]
ADROPİN (PG/ML)	340.486[a*]	265.862[b]	251.378[b]
PREPTİN (NG/L)	312.723[a*]	448.920[b]	508.934[b]

4.7. Table of compare Mean±SD of İRİSİN, ADROPİ, PREPTİN.

Parameter	Control	Obesity	T2DM
	Mean±SD	Mean±SD	Mean±SD
İRİSİN (NG / ML)	22.283±18.202	10.308±7.301	10.285±7.406
ADROPİN (PG/ML)	340.486±229.901	265.862±181.736	251.378±165.645
PREPTİN (NG/L)	312.723±283.274	448.920±296.394	508.934±312.506

Table 4.8. Distribution of Variables by Groups (n=180)

	Groups						p	Differences of Post-Hoc
	Normal		Obesity		T2DM			
	Mean±SD	Median(Min-Max)	Mean±SD	Median(Min-Max)	Mean±SD	Median(Min-Max)		
Age	31,57±10,04	28,50(19,00-58,00)	36,75±7,63	37,50(21,00-49,00)	35,12±9,49	34,50(19,00-49,00)	F:7,422;0,001*	1-2 1-3
Weight	59,07±8,59	59,00(42,00-85,00)	95,80±13,46	95,00(70,00-127,00)	90,83±9,47	90,00(70,00-120,00)	F:185,682;<0,001*	1-2 1-3
Hight	166,10±9,29	163,50(150,00-190,00)	159,80±7,37	160,00(141,00-175,00)	160,68±8,21	160,00(141,00-185,00)	F:12,213; <0,001*	1-2 1-3
BMI	21,35±1,59	21,40(18,60-24,80)	38,08±5,10	37,95(30,00-49,90)	35,27±4,05	34,95(26,90-45,30)	F:307,866; <0,001*	1-2 1-3
Irisin	22,28±18,20	15,59(3,60-66,22)	10,31±7,30	6,36(4,30-29,29)	10,28±7,41	7,42(0,60-37,90)	KW:25,561; <0,001*	1-2 1-3
Adropin	340,39±229,90	227,27(65,66-739,42)	265,86±181,74	173,47(101,94-787,42)	251,38±165,65	177,25(16,51-732,77)	KW:1,406; 0,495	
Preptin	312,72±283,27	211,37(7,63-1492,15)	448,92±296,39	328,45(153,93-1248,33)	508,93±312,51	395,03(32,95-1471,82)	KW:25,852; <0,001*	1-2 1-3

F: One-Way Analysis of Variance (Anova)

KW: Kruskal Wallis Analysis of Variance

* p value is significant at the 0.05 level

Pairwise comparisons were made with Tamhane's T2 or Mann Whitney U Test with Bonferroni Correction.

Table 4.9. Distribution of Values of T2DM Patients (n=180)

	Average	Standard deviation	Median	Minimum	Maximum
HBA1C	7,62	1,70	7,20	5,10	11,50
Glucose	163,58	64,70	149,75	83,80	369,00
İnsulin resistance	7,33	7,36	5,52	1,00	44,70
TG mg/dl	175,62	99,03	132,30	35,30	486,00
LDL mg/dl	116,95	36,91	115,20	50,70	232,70
BUN mg/dl	21,05	40,31	15,85	0,92	324,70
Cr mg/dl	5,44	25,20	0,78	0,30	141,00
Na mmol/l	134,25	24,50	139,00	4,20	149,00
K mmol/l	7,87	17,45	4,65	2,77	103,80
Cl mmol/l	97,64	19,91	102,05	9,30	111,40
ALT U/l	22,02	13,29	19,05	10,20	108,10
AST U/L	20,98	11,96	18,65	9,32	98,00

Table 4.10. Distribution of Groups by Gender (n=180)

		Gender		p
		Female	Male	
Groups	Normal	46(33,3)	14(33,3)	χ2:4,741;0,093
	Obesity	51(37,0)	9(21,0)	
	T2DM	41(29,7)	19(45,0)	

p: Chi-Square Test

Table 4.11. Relationships of the Variables According to the Control Group (n=180)

		Adropin	Preptin
Irisin	r	0,849	0,167
	p	<0,001*	0,201
Adropin	r		0,200
	p		<0,126

r: Pearson Correlation Coefficient

* p value is significant at the 0.05 level.

Table 4.12. Relationships of Variables by Obesity Group (n=180)

		Adropin	Preptin
Irisin	r	0,986	0,978
	p	<0,001*	<0,001*
Adropin	r		0,982
	p		<0,001*

r: Pearson Correlation Coefficient

* p value is significant at the 0.05 level.

Table 4.13. Relationships of Variables by T2DM Group (n=180)

		Adropin	Preptin
Irisin	r	0,960	0,956
	p	<0,001*	<0,001*
Adropin	r		0,927
	p		<0,001*

r: Pearson Correlation Coefficient

* p value is significant at the 0.05 level.

Interpretation of the correlation coefficient (r);

If r<0.2, very weak correlation or no correlation.

If r is between 0.2-0.4, weak correlation.

If r is between 0.4-0.6, moderate correlation.

If r is between 0.6-0.8, high correlation.

If r is 0.8>, it is interpreted that there is a very high correlation.

Discussion :

In this study, we report the association of irisin, Adropin and preptin concentrations regarding healthy(control), obesity and obesity patients with type 2 diabetes mellitus. The first is a cross Sectional study stratified by effect of irisin in healthy, obesity and obesity patients with type 2 diabetes mellitus.

Obesity is "Abnormal or excessive fat accumulation in adipose tissues to the extent that it impairs health." Made in the form. In other words, obesity is a complex, multifactorial disease characterized by an increase in body fat and consequent behavioural, endocrine, and metabolic changes. Obesity occurs when the amount of energy taken from food exceeds the amount of energy consumed by metabolism and physical activity, so that the fat mass in the body increases compared to the lean body mass. Excess fat in obese individuals paves the way for many important diseases that affect systems such as the cardiovascular system, respiratory system, hormonal system, and digestive system. Obesity has been shown to increase the risk of developing HT, type 2 DM, dyslipidemia, cardiovascular diseases, and certain types of cancer (colon, breast, gallbladder) (WHO.,2013).

Diabetes Mellitus cause disorders in carbohydrates, oil, and protein metabolism caused by flaws in insulin secretion or defects in both insulin and insulin effect. Hyperglycemia plays a role in disease-related complications (Inzucchi ET AL.,2012). Various pathogenic processes play a role in the development of diabetes. These pathogenic processes constitute a range that ranges from the lack of insulin to resisting the insulin effect of the beta cells of the pancreas. The symptoms of prominent hyperglycemia consist of polyuria, polyidypsy, nocturia, unexplained weight loss, sometimes polyvasm, and visual impairment. Sensitivity and growth disorders to certain infections may also accompany chronic hyperglycemia (Harvey ET AL.,2011).

Irisin may be a cleavage item of layer protein FNDC5, discharged by muscle tissue and has Been proposed to imitate the favourable metabolic impacts of work out by interceding the cross conversation Between contracting muscle and other tissues organs especially the fat tissue (Aydin ET AL.,2014). Irisin has been Proposed to induce beige/brown fat tissue change by expanding the

mitochondrial substance and so the uncoupling protein-1 (UCP1) concentrations, which, in turn increments thermogenic Work of the changing white fat tissue. These beige/brown cells have defensive part Against hypothermia, diabetes and corpulence and move forward glucose homeostasis (Aydin ET AL.,2014). Expanded expression Of irisin is seen in skeletal muscle and blood concentrations of mice after intense work out (Butler ET AL.,2015). Therefore, irisin's potential role in Metabolic health has gained significant attention since its discovery.

It is thought that irisin will have a role in promoting lipid mobilization and glucose uptake by increasing UCP1, enabling the differentiation of white adipocyte into beige adipocytes, and improving adipose tissue capacity (Moreno-Navarrete et al.,2013). Therefore, it is suggested that irisin levels may be associated with overweight or obesity in different groups of people. However, this relationship remains controversial due to conflicting results reported. In a study conducted on 81 individuals, 40 of whom were healthy controls, it was reported that circulating irisin was significantly lower in obese individuals compared to healthy controls. irisin may play a role in the regulation of endothelial function in obesity (Hou et al.,2015).

.Our findings show that serum irisin are significantly lower in obesity compare to healthy controls ,also irisin are significantly decreased in the patients with type2 diabetes mellitus compare to healthy controls. The result depends the role of irisin in promoting lipid mobilization and glucose uptake by increasing UCP1.

In another study involving 75 metabolically healthy non-obese adults and 51 metabolically healthy obese adults, serum irisin levels were found to be significantly lower in the metabolically healthy obese group, and irisin was negatively correlated with BMI (Liu et al.,2017).

It has been shown that irisin exhibits therapeutic potential in insulin resistance and T2DM by stimulating browning of white adipose tissue, promoting glucose uptake in skeletal muscle and heart , improving hepatic glucose, and improving pancreatic cell function (Zhang et al.,2014, Liu et al.,2017, Park et al.,2015). irisin can increase glucose tolerance and reduce insulin resistance (Qiao et al.,2016, Zhu et al.,2015 , Ates et al.,2017).

In cross-sectional studies, irisin levels in the diabetic state seem to diverge between the two types of diabetes. While irisin levels have been reported to be higher in

type 1 diabetes mellitus (T1DM) than in controls (Ates et al.,2017, Espes et al.,2015), in many studies including 2 meta-analyses (Du X-L et al..,2016, Zhang et al..,2016) patients with T2DM were found to be lower than controls (Shelbaya et al..,2018, Shelbaya et al..,2016). Liu et al. In his study on 156 individuals, 96 of whom had T2DM, he found that circulating irisin in T2DM patients was significantly lower than in non-diabetic controls. It is thought that this observed decrease may be caused by the decrease in muscle function and PGC-1α expression in T2DM (Liu et al..,2017).

Adropin is encoded by the energy homeostasis-related gene symbolized as "Enho", which is mostly expressed in the liver and brain. Enho is located on the 9p13,3 chromosome and plays a role in the regulation of glucose homeostasis and lipid metabolism (Kumar et al..,2008).

Adropin consists of 76 amino acids and its molecular weight is 4.5 kDa (Kumar et al..,2008). Adropin 1-33 is a secretory signal peptide, while adropin 34-76 is a peptide hormone. Adropin34-76 has been shown to be biologically active when administered to mice and cultured cells (Kumar et al..,2008, Gao et al.., 2015). The N-terminus of amino acids 1-91 is localized in the cytoplasm. The 9-30 region of amino acids is the transmembrane domain (Li L et al..,2016). The C terminal of amino acids 30-76 is localized outside the surface of the plasma membrane (Wong C-M et al..,2014).

The aa sequence of adropin is one-to-one in humans, mice, and rats (Kumar et al..,2008). The half-life of adropin has not yet been determined. However, its half-life is assumed to be only a few minutes (Aydin et al..,2014).

effect of Adropin in healthy, obesity and obesity patients with type 2 diabetes mellitus.The result show that level of adropin in the obesity groups and T2DM groups are significantly decrease than the control group.The result related to that adropin have a role in the regulation of glucose homeostasis and lipid metabolism .

In many studies, it was reported that adropin decreased in preobese and obese individuals and that adropin was negatively correlated with BMI (Zang et al.,2018, Kałużna..,2016), In a study conducted with 64 obese 36 nonobese children aged 11 to 16 years, serum adropin levels of obese children were found to be significantly lower compared to nonobese children. Butler et al. In a study conducted

by Adropin, serum adropin levels were found to be lower in obese patients (Butler et al..,2012). In a study of 64 obese children, serum adropin levels were found to be lower in obese children (Sayin et al..,2014). it was suggested that adropin may be an independent marker in obesity (Chen R-M et al..,2019).

In most human studies, serum adropin levels were decreased in individuals with T2DM (Zhang et al..,2018), Zhang et al. They evaluated the serum adropin level in 116 T2DM patients and 60 healthy individuals with normal glucose tolerance. It has been reported that adropin is Decrease in the type 2 diabetes mellitus group (Zhang et al..,2018). LinLingzhen Wu et al. A total of 392 patients were assigned into the type 2 diabetic and healthy patients, Serum adropin level was significantly decreased in type 2 diabetic patients than healthy patients (Wu et al..,2014). adropin was inversely related to model assessment-estimated insulin resistance (HOMA-IR) values (Chen et al..,2019, zang et al..,2018).

Serum adropin levels were found to be low not only in T2DM, but also in children with T1DM and women with GDM (Yildirim et al.,2014, Beigi et al.,2015). Low adropin levels in GDM have been explained by carbohydrate intolerance (Yildirim et al.,2014). Ebru Celik et al. The study includes 20 pregnant women with GDM and 20 gestational age healthy pregnant women. serum adropin were lower in the GDM group. (Celik et al.,2013)

Preptin is a peptide increases insulin secretion. The precursor of preptin is ProIGF-II. Insulin-like growth1factor II1(IGF-II) is also produced from ProIGF-II. Insulin-like growth factor II is a member of the insulin family. Cell growth, differentiation and metabolism are regulated by the effect of insulin-like growth factor II. In immunohistochemical studies with normal and diabetic rats, it has been shown that ProIGF-II, the precursor1of preptin, is in the same localization as insulin in the secretory granules of rats. Synthetic preptin increases insulin secretion from glucose-stimulated ßTC6-F7 cells in a concentration-dependent and saturable manner (Buchanan et al.,2001, Höög et al.,1997). preptin is a physiological enhancer of glucose-induced insulin secretion. Preptin has been reported to increase rather than initiate insulin secretion. Preptin is secreted together with insulin from pancreatic beta cells in response to glucose (Buchanan et al.,2001, Höög et al.,1997). In the pancreas, the maximum amount of preptin that

completely binds with1anti-preptin-immunoglobulin under experimental conditions was determined as 20 ng/min. (Buchanan et al.,2001).

In our study demonstrated that serum preptin higher in obesity and patients with Type2 diabetes mellitus compered to the control group . Along with insulin resistance, elevated preptin level and increased osteocalcin concentration have been associated with obesity and overweight. Therefore, it suggest that it can be a clinical benefit for obesity and T2DM.

In the study conducted by 100 obesity and 50 healthy controls found serum preptin level is significantly higher than healthy controls.(El-Eshmawy et al..,2018).In the study of Özkan, Yusuf et al. Reported serum preptin level is significantly higher in the obesity patients than control(Özkan, Yusuf 2013). In the study Salih et al ,conducted 64 patients with T2DM and 38 healthy controls, reported that serum preptin level is significantly1higher in the patient with T2DM compare with the healthy controls(Salih et al..,2020).In the finally preptin May important role in weight1management and prevention1of diabetes1mellitus. In the finally preptin May important role in weight management and prevention of diabetes mellitus.

CHAPTER FIVE

Conclusion:

With this work; It was aimed to investigate the relationship of these three endocrine factors with energy homeostasis by comparing the concentrations of irisin, adropin and preptin in obese and non-obese groups. The fact that all three of the parameters planned to be looked at are related to energy metabolism and that obesity is an increasingly important health problem has been effective in the planning of this study. However, when planning the study, it was also considered that there were no publications showing how irisin, preptin and adropin levels changed with exercise. This study is also important with the aim of identifying new physiological actors involved in the regulation of energy homeostasis. In addition, no study was found in which irisin, adropin and preptin were evaluated together. Physiological mechanisms related to energy metabolism that may arise as a result of the realization of this study may also shed light on the search for new therapeutic agents related to obesity in the future.

Obese and non-obese people will be included in the study. Obesity is a disease that occurs as a result of the body entering much more energy than it is expended. Today, due to the sedentary lifestyle of people, their energy expenditure is very limited. It is known that exercise provides many benefits to our body. Exercise increases energy expenditure in the body and ensures the maintenance of energy balance. It has been reported that some newly discovered peptides are involved in the regulation of energy balance in the body. In this study, the concentrations of these peptides in obese and non-obese subjects was compared. To the best of our knowledge, this is the first trial investigation of IRISIN, ADROPIN, AND PREPTIN IN OBESE AND NON-OBESE INDIVIDUALS. Our findings show

that serum irisin and Adropin are significantly lower in obesity and patients with type 2 diabetes mellitus compare to healthy controls.

The present study demonstrated that serum irisin and adropin was correlated with decreased risk of developing T2DM and Wight management. Hence, serum irisin and adropin should be utilized as a biomarker for assessing the risk of developing T2DM and control obesity.

In our study demonstrated that serum preptin higher in obesity and patients with Type2 diabetes mellitus. Preptin may play an important role in weight control and diabetes prevention.

Reference

Affourtit C, Quinlan CL, Brand MD,2012. Measurement of proton leak and electron leak in isolated mitochondria. Mitochondrial bioenergetics: Springer; p. 165-82.

American Diabetes Association,2017. Classification and diagnosis of diabetes. Diabetes Care.;40(1):11-24.

American Diabetes Association, 2013. Diagnosis and classification of diabetes mellitus. Diabetes Care; 36: 67-74.

American Diabetes Association, 2014. Diagnosis and classification of diabetes mellitus. Diabetes care;37(1):S81-S90.

Anastasilakis AD, Polyzos SA, Saridakis ZG, Kynigopoulos G, Skouvaklidou EC, Molyvas D, et al, 2014. Circulating irisin in healthy, young individuals: day-night rhythm, effects of food intake and exercise, and associations with gender, physical activity, diet, and body composition. The Journal of clinical endocrinology and metabolism.;99(9):3247-55.

Assyov Y, Gateva A, Tsakova A, Kamenov Z, 2016. Irisin in the glucose continuum. Experimental and Clinical Endocrinology & Diabetes.;124(01):22-7.

Ates I, Arikan M, Erdogan K, Kaplan M, Yuksel M, Topcuoglu C, et al, 2017. Factors associated with increased irisin levels in the type 1 diabetes mellitus. Endocrine regulations.;51(1):1-7.

Aydin S, Aydin S, Kuloglu T, Yilmaz M, Kalayci M, Sahin İ, et al, 2013. Alterations of irisin concentrations in saliva and serum of obese and normal-weight subjects, before and after 45 min of a Turkish bath or running. Peptides.;50:13-8.

Aydin S, Kuloglu T, Aydin S, Eren MN, Yilmaz M, Kalayci M, et al, 2013. Expression of adropin in rat brain, cerebellum, kidneys, heart, liver, and pancreas in streptozotocin-induced diabetes. Molecular and cellular biochemistry.;380(1-2):73-81.

Aydin S, Kuloglu T, Aydin S. A, 2014. comprehensive immunohistochemical examination of the distribution of the fat-burning protein irisin in biological tissues. Peptides;61: 130-136.

Aydin S, Kuloglu T, Aydin S, 2013. Copeptin, adropin and irisin concentrations in breast milk and plasma of healthy women and those with gestational diabetes mellitus. Peptides.;47:66-70.

Aydin S, 2013. Presence of adropin, nesfatin-1, apelin-12, ghrelins and salusins peptides in the milk, cheese whey and plasma of dairy cows. Peptides.;43:83-7.

Aydin S, 2014.Three new players in energy regulation: preptin, adropin and irisin. Peptides.;56:94-110.

Beigi A, Shirzad N, Nikpour F, Nasli Esfahani E, Emamgholipour S, Bandarian F, 2015 . Association between serum adropin levels and gestational diabetes mellitus; a case– control study. Gynecological Endocrinology.;31(12):939-41.

Björntorp P, 1993. Visceral obesity: a “civilization” syndrome. Obes Res; 1: 206-222.

Bluher S, Panagiotou G, Petroff D, Markert J, Wagner A, Klemm T, et al, 2014 . Effects of a 1-year exercise and lifestyle intervention on irisin, adipokines, and inflammatory markers in obese children. Obesity (Silver Spring).;22(7):1701-8.

Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al, 2012. A PGC1dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature;481:463–8.

Bray GA, Ryan DH, 2006. Medical Approaches to Treatment of the Obese Patients. In: Mantzoros CS, Editors. Obesity and Diabetes. 1 st Ed. New Jersey: Humana Press,; 457- 469.

BresaterLE, Welin L, Romanus B, 1996. Foot pathology and risk factors for diabetic foot Disease in elderly men.Diabetes Res Clin Pract; 32:103-9.

Buchanan CM, Phillips AR, Cooper GJ, 2001. Preptin derived from proinsulin-like growth factor II (proIGF-II) is secreted from pancreatic islet beta-cells and enhances insulin secretion. Biochem J;360:431–9.

Buchanan CM, Phillips AR, Cooper GJ, 2001. Preptin derived from proinsulin-like growth factor II (proIGF-II) is secreted from pancreatic islet beta-cells and enhances insulin secretion. Biochem J; 360: 431-439.

Burant CF, 2004. Tip 2 Diyabetin Tıbbi Tedavisi. Amerikan Diyabet Cemiyeti. Uzel B (Çevirmen) s.3-15, İstanbul, Sigma Publishing Danışmanlık ve Organizasyon: 3- 15.

Butler AA, St-Onge MP, Siebert EA, Medici V, Stanhope KL, Have, 2015l PJ. Differential Responses of Plasma Adropin Concentrations To Dietary Glucose or Fructose Consumption In Humans. Sci Rep.;5:14691.

Butler AA, Tam CS, Stanhope KL, Wolfe BM, Ali MR, O'Keeffe M, et al, 2012. Low circulating adropin concentrations with obesity and aging correlate with risk factors for metabolic disease and increase after gastric bypass surgery in humans. The Journal of Clinical Endocrinology & Metabolism.;97(10):3783-91.

Celik E, Yilmaz E, Celik O, Ulas M, Turkcuoglu I, Karaer A, et al, 2013. Maternal and fetal adropin levels in gestational diabetes mellitus. Journal of perinatal medicine.;41(4):375-80.

Celik O, Celik N, Hascalik S, Sahin I, Aydin S, Ozerol E, 2011. An appraisal of serum preptin levels in PCOS. Fertil Steril;95:314–6.

Chen J, Fang L, Song K, Wang X, Huang Y, Chai S, et al, 2016. Serum irisin level is higher and related with insulin in acanthosis nigricans-related obesity. Experimental and Clinical Endocrinology & Diabetes.;124(03):203-7.

Chen N, Li Q, Liu J, Jia S, 2016. Irisin, an exercise-induced myokine as a metabolic regulator: an updated narrative review. Diabetes/metabolism research and reviews.;32(1):51-9.

Chen R-M, Yuan X, Ouyang Q, Lin X-Q, Ai Z-Z, Zhang Y, et al, 2019. Adropin and glucagon-like peptide-2 are associated with glucose metabolism in obese children. World Journal of Pediatrics.;15(6):565-71.

Choi ES, Kim MK, Song MK, Kim JM, Kim ES, Chung WJ, et al, 2014. Association between serum irisin levels and non-alcoholic fatty liver disease in health screen examinees. PLoS One.;9(10):e110680.

Cinti S, 2017. UCP1 protein: The molecular hub of adipose organ plasticity. Biochimie.;134:71-6.

Cohn JN, 2001. Arterial compliance to stratify cardiovascular risk: more precision in therapeutic decision making. Oxford University Press;.

Costa JV, Duarte JS, 2006. Adipose tissue and adipokines. Acta medica portuguesa.;19(3):251-6.

Crujeiras AB, Pardo M, Arturo RR, Santiago NC, Zulet MA, Martínez JA, et al, . 2014. Longitudinal variation of circulating irisin after an energy restriction-induced weight loss and following weight regain in obese men and women. American Journal of Human Biology;26(2):198-207.

Cui L, Qiao T, Xu F, Li Z, Chen T, Su H, et al, 2020. Circulating irisin levels of prenatal and postnatal patients with gestational diabetes mellitus: A systematic review and meta-analysis. Cytokine.;126:154924.

Daskalopoulou SS, Cooke AB, Gomez YH, Mutter AF, Filippaios A, Mesfum ET, et al, 2014. Plasma irisin levels progressively increase in response to increasing exercise workloads in young, healthy, active subjects. Eur J Endocrinol.;171(3):343-52.

de Macedo SM, Lelis DF, Mendes KL, Fraga CAC, Brandi IV, Feltenberger JD, et al, 2017. Effects of Dietary Macronutrient Composition on FNDC5 and Irisin in Mice Skeletal Muscle. Metabolic syndrome and related disorders.;15(4):161-9.82.

Diabetes Association, 2013. Diagnosis and classification of diabetes mellitus. Diabetes Care; 36: 67-74.

Dogan FB, Cicek D, Aydin S, Dertlioglu SB, Halisdemir N, Ucak H, Demir B, Erden İ, 2014. Serum Preptin and Amylin Values in Psoriasis Vulgaris and Behcet's Patients. Journal of Clinical Laboratory Analysis 00: 1–4.

Du X-L, Jiang W-X, 2016. Lower circulating irisin level in patients with diabetes mellitus: a systematic review and meta-analysis. Hormone and Metabolic Research.;48(10):644-52.

Ebert T, Stepan H, Schrey S, Kralisch S, Hindricks J, Hopf L, et al, 2014. Serum levels of irisin in gestational diabetes mellitus during pregnancy and after delivery. Cytokine.;65(2):153-8.

Egan B, Zierath JR, 2013. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab.;17(2):162-84.

El-Eshmawy M, Abdel Aal I, 2015 .Relationships between preptin and osteocalcin in obese, overweight, and normal weight adults. Appl Physiol Nutr Metab. Mar;40(3):218-22.

Erol O, Erkal N, Ellidağ HY, İsenlik BS, Aydın Ö, Derbent AU, et al, 2016. Irisin as an early marker for predicting gestational diabetes mellitus: a prospective study. The Journal of Maternal-Fetal & Neonatal Medicine.;29(22):3590-5.

Espes D, Lau J, Carlsson P-O, 2015. Increased levels of irisin in people with long-standing Type 1 diabetes. Diabetic medicine.;32(9):1172-6.

Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003. Report of the Expert Commitee on the Diagnosis and Classification of Diabetes. Diabetes Care; 26: 5-20.

Ferrer-Martínez A, Ruiz-Lozano P, Chien KR. Mouse PeP, 2002. a novel peroxisomal protein linked to myoblast differentiation and development. Developmental dynamics: an official publication of the American Association of Anatomists.;224(2):154-67.

Fonseca-Alaniz MH, Takada J, Alonso-Vale MIC, Lima FB. The adipose tissue as a regulatory center of the metabolism. Arquivos Brasileiros de Endocrinologia & Metabologia. 2006;50(2):216-29..

Fox J, Rioux BV, Goulet EDB, Johanssen NM, Swift DL, Bouchard DR, et al, 2018. Effect of an acute exercise bout on immediate post-exercise irisin concentration in adults: A meta-analysis. Scandinavian journal of medicine & science in sports.;28(1):16-28.

Frayn KN, 2001. Adipose tissue and the insulin resistance syndrome. Proceedings of the Nutrition Society.;60(3):375-80.

Fujie S, Hasegawa N, Kurihara T, Sanada K, Hamaoka T, Iemitsu M, 2017. Association between aerobic exercise training effects of serum adropin level, arterial stiffness, and adiposity in obese elderly adults. Appl Physiol Nutr Metab.;42(1):8-14.

Fujie S, Hasegawa N, Sato K, Fujita S, Sanada K, Hamaoka T, et al, 2015. Aerobic exercise training-induced changes in serum adropin level are associated with reduced arterial stiffness in middle-aged and older adults. Am J Physiol Heart Circ Physiol.;309(10):H1642-7.

Ganesh Kumar K, Zhang J, Gao S, Rossi J, McGuinness OP, Halem HH, et al, 2012..Adropin deficiency is associated with increased adiposity and insulin resistance. Obesity (Silver Spring);20:1394–402.

Ganesh Kumar K, Zhang J, Gao S, Rossi J, McGuinness OP, Halem HH, et al, 2012.Adropin deficiency is associated with increased adiposity and insulin resistance.Obesity (Silver Spring); 20: 1394–1402.

Gao S, McMillan RP, Jacas J, Zhu Q, Li X, Kumar GK, et al, 2014. Regulation of substrate oxidation preferences in muscle by the peptide hormone adropin. Diabetes.;63(10):3242-52.

Gao S, McMillan RP, Zhu Q, Lopaschuk GD, Hulver MW, Butler AA. Therapeutic effects of adropin on glucose tolerance and substrate utilization in diet- induced obese mice with insulin resistance. Molecular metabolism.;4(4):310-24.

Gao S, McMillan RP, Zhu Q, Lopaschuk GD, Hulver MW, Butler AA, 2015. Therapeutic effects of adropin on glucose tolerance and substrate utilization in diet- induced obese mice with insulin resistance. Mol Metab. ;4(4):310-24.

Garcés MF, Peralta JJ, Ruiz-Linares CE, Lozano AR, Poveda NE, Torres- Sierra AL, et al, 2014. Irisin levels during pregnancy and changes associated with the development of preeclampsia. The Journal of Clinical Endocrinology & Metabolism.;99(6):2113-9.

García-Fontana B, Reyes-García R, Morales-Santana S, Ávila-Rubio V, Muñoz-Garach A, Rozas-Moreno P, et al, 2016. Relationship between myostatin and irisin in type 2 diabetes mellitus: a compensatory mechanism to an unfavourable metabolic state? Endocrine.;52(1):54-62.

Gibbons GH, 1997. Endothelial function as a determinant of vascular function and structure: a new therapeutic target. The American journal of cardiology.;79(5):3- 8.

Gillen JB, Percival ME, Ludzki A, Tarnopolsky MA, Gibala MJ, 2013. Interval training in the fed or fasted state improves body composition and muscle oxidative capacity in overweight women. Obesity (Silver Spring).;21(11):2249-55.

Giordano A, Smorlesi A, Frontini A, Barbatelli G, Cinti S, 2014. Mechanisms in endocrinology: white, brown and pink adipocytes: the extraordinary plasticity of the adipose organ. European Journal of Endocrinology.;170(5):R159-R71.

Goossens GH, 2008. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. Physiology & behavior.;94(2):206-18.

Gouda W, Mageed L, Shaker Y, Hamimy W, Afify M, 2018. Assessment of Serum Vitamin D and Irisin Levels in Obese Patients. Clinical laboratory.;64(11).

Gozal D, Kheirandish-Gozal L, Bhattacharjee R, Molero-Ramirez H, Tan H- L, Bandla HP, 2013. Circulating adropin concentrations in pediatric obstructive sleep apnea: potential relevance to endothelial function. The Journal of pediatrics.;163(4):1122-6.

Grygiel-Górniak B, Puszczewicz M. A, 2017. review on irisin, a new protagonist that mediates muscle-adipose-bone-neuron connectivity. Eur Rev Med Pharmacol Sci.;21(20):4687-93.

Harvey RA, Ferrier DR (editors), 2011. Diabetes Mellitus. Lippincott's Illustrated Reviews: Biochemistry. Fifth edition, Baltimore: Lippincott Williams and Wilkins,: 337-348.

Hee Park K, Zaichenko L, Brinkoetter M, Thakkar B, Sahin-Efe A, Joung KE, et al, 2013. Circulating irisin in relation to insulin resistance and the metabolic syndrome. The Journal of Clinical Endocrinology & Metabolism.;98(12):4899-907.

Höög A, Hu W, Abdel-Halim SM, Falkmer S, Qing L, Grimelius L, 1997. Ultrastructural localization of insulin-like growth factor-2 (IGF-2) to the secretory granules of insulin cells: a study in normal and diabetic (GK) rats. Ultrastruct Pathol; 21: 457-466.

Horvath TL, 2005. The hardship of obesity: a soft-wired hypothalamus. Nat Neurosci; 8: 561-565.

Hosseini A, Shanaki M, Emamgholipour S, Nakhjavani M, Razi F, Golmohammadi T. Elevated serum levels of adropin in patients with type 2 diabetes mellitus and its association with insulin resistance. Journal of Biology and Today's World.;5(3):44-9.

Hou N, Han F, Sun X, 2015. The relationship between circulating irisin levels and endothelial function in lean and obese subjects. Clinical endocrinology .;83(3):339-43.

Hu W, Chen L, 2016. Association of serum adropin concentrations with diabetic nephropathy. Mediators of inflammation.

Huh JH, Ahn SV, Choi JH, Koh SB, Chung CH, 2016. High serum irisin level as an independent predictor of diabetes mellitus: a longitudinal population-based study. Medicine.;95(23).

Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE, et al, 2012. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism.;61(12):1725-38.

Huh JY, Siopi A, Mougios V, Park KH, Mantzoros CS, 2015. Irisin in response to exercise in humans with and without metabolic syndrome. The Journal of Clinical Endocrinology & Metabolism.;100(3):E453-E7.

International Diabetes Federation, 2017. Diabetes Atlas – 8th Edition. International Diabetes Federation;

Inzucchi SE, Sherwin RS. Diabetes Mellitus. Goldman L, Schafer AI, 2012 (editors). Goldman's Cecil Medicine. Twenty-fourth edition, Philadelphia: Elsevier Saunders.

Ishibashi J, Seale P, 2015. Functions of Prdm16 in thermogenic fat cells. Temperature.;2(1):65-72.

Jang HB, Kim H-J, Kang JH, Park SI, Park KH, Lee H-J, 2017. Association of circulating irisin levels with metabolic and metabolite profiles of Korean adolescents. Metabolism.;73:100-8.

Jastroch M, Divakaruni AS, Mookerjee S, Treberg JR, Brand MD, 2010. Mitochondrial proton and electron leaks. Essays in biochemistry.;47:53-67.

Jensen MD, 2008. Role of body fat distribution and the metabolic complications of obesity. The Journal of Clinical Endocrinology & Metabolism .

Jia J, Yu F, Wei W-P, Yang P, Zhang R, Sheng Y, et al, 2019. Relationship between circulating irisin levels and overweight/obesity: A meta-analysis. World Journal of Clinical Cases.;7(12):1444-55.

Kahn CR, Weir GC, King GL, Moses AC, Smith RJ, Jacobson AM. Joslin's Diabetes Mellitus, 2005. Fourteenth edition, Boston: Lippincott Williams and Wilkins,: 331-338.

Kałużna M, Hoppe K, Schwermer K, Ibrahim AY, Pawlaczyk K, Ziemnicka K, 2016. Adropin and irisin levels in relation to nutrition, body composition, and insulin resistance in patients with end-stage renal disease on chronic hemodialysis and peritoneal dialysis. Pol Arch Med Wewn.;126(7-8):474-82.

Kiess W, Petzold S, Töpfer M, Garten A, Blüher S, Kapellen T, et al, 2008. Adipocytes and adipose tissue. Best practice & research Clinical endocrinology & metabolism.;22(1):135-53.

Ko B-J, Park KH, Shin S, Zaichenko L, Davis CR, Crowell JA, et al, 2016. Diet quality and diet patterns in relation to circulating cardiometabolic biomarkers. Clinical Nutrition.;35(2):484-90.

Kraemer RR, Shockett P, Webb ND, Shah U, Castracane VD, 2014. A transient elevated irisin blood concentration in response to prolonged, moderate aerobic exercise in young men and women. Horm Metab Res.;46(2):150-4.

Kraemer RR, Shockett PE, Webb ND, Shah U, Castracane VD, 2014. A Transient Elevated Irisin Blood Concentration in Response to Prolonged, Moderate Aerobic Exercise in Young Men and Women. Medicine and science in sports and exercise.;46(5):404.

Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA, Chouljenko VN,et al, 2008. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. Cell Metab;8:468–81.

Kurdiova T, Balaz M, Mayer A, Maderova D, Belan V, Wolfrum C, et al, 2014. Exercise-mimicking treatment fails to increase Fndc5 mRNA & irisin secretion in primary human myotubes. Peptides.;56:1-7.

Kuzmicki M, Telejko B, Lipinska D, Pliszka J, Szamatowicz M, Wilk J, et al, 2014. Serum irisin concentration in women with gestational diabetes. Gynecological Endocrinology.;30(9):636-9.

Kwok KH, Lam KS, Xu A, 2016. Heterogeneity of white adipose tissue: molecular basis and clinical implications. Experimental & molecular medicine.;48(3):e215-e.

Lecker SH, Zavin A, Cao P, Arena R, Allsup K, Daniels KM, et al, 2012. Expression of the irisin precursor FNDC5 in skeletal muscle correlates with aerobic exercise

performance in patients with heart failure. Circulation: Heart Failure.;5(6):812-8.

Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, et al, 2014. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell metabolism.;19(2):302-9.

Levin ME, 1996. Foot lesions in patients with diabetes mellitus. End Met Clin North Amer; 25: 447-462.

Li L, Xie W, Zheng XL, Yin WD, Tang CK, 2016. A novel peptide adropin in cardiovascular diseases. Clin Chim Acta.;453:107-13.

Lian A, Wu K, Liu T, Jiang N, Jiang Q, 2016. Adropin induction of lipoprotein lipase expression in tilapia hepatocytes. J Mol Endocrinol.;56(1):11-22.

Little JP, Gillen JB, Percival ME, Safdar A, Tarnopolsky MA, Punthakee Z, et al, 2011. Low- volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. J Appl Physiol.;111(6):1554-60.

Liu B-W, Yin F-Z, Qi X-M, Fan D-M, Zhang Y, 2017. The Levels of Serum Irisin as a Predictor of Insulin Resistance in Han Chinese Adults with Metabolically Healthy Obesity. Clinical laboratory.;63(5):881-6.

Liu J-J, Wong MD, Toy WC, Tan CS, Liu S, Ng XW, et al, 2013. Lower circulating irisin is associated with type 2 diabetes mellitus. Journal of Diabetes and its Complications.;27(4):365-9.

Liu S, Du F, Li X, Wang M, Duan R, Zhang J, et al, 2017. Effects and underlying mechanisms of irisin on the proliferation and apoptosis of pancreatic β cells. PloS one.;12(4):e0175498.

Loffler D, Muller U, Scheuermann K, Friebe D, Gesing J, Bielitz J, et al, 2015. Serum Irisin Levels Are Regulated by Acute Strenuous Exercise. J Clin Endocrinol Metab.;100(4):1289-99.

Lovren F, Pan Y, Quan A, Singh KK, Shukla PC, Gupta M, et al, 2010. Adropin is a novel regulator of endothelial function. Circulation;122:S185–92.

Lovren F, Pan Y, Quan A, Singh KK, Shukla PC, Gupta M, et al, 2010. Adropin is a novel regulator of endothelial function. Circulation.;122(11_suppl_1):S185-S92.

Lu J, Xiang G, Liu M, Mei W, Xiang L, Dong J. Irisin protects against endothelial injury and ameliorates atherosclerosis in apolipoprotein E-Null diabetic mice. Atherosclerosis. 2015;243(2):438-48.

Lyznicki JM, Young DC, Riggs JA, Davis RM. Obesity: assessment and management in primary care. Am Fam Physician 2001; 63: 2185-2196.

Mahajan RD, Patra SK. Irisin, a novel myokine responsible for exercise induced browning of white adipose tissue. Indian J Clin Biochem. 2013;28(1):102-3.

Mahgoub MO, D'Souza C, Al Darmaki RS, Baniyas MM, Adeghate E. An update on the role of irisin in the regulation of endocrine and metabolic functions. Peptides. 2018;104:15-23.

Maillard F, Rousset S, Pereira B, Traore A, de Pradel Del Amaze P, Boirie Y, et al. High-intensity interval training reduces abdominal fat mass in postmenopausal women with type 2 diabetes. Diabetes Metab. 2016.

Matsuzawa Y. The metabolic syndrome and adipocytokines. FEBS letters. 2006;580(12):2917-21.

Mirghani Dirar A, Doupis J. Gestational diabetes from A to Z. World journal of Diabetes. 2017;8(12):489-511.

Mohammad Rahimi GR, Bijeh N, Rashidlamir A. Effects of exercise training on serum preptin, undercarboxylated osteocalcin and high molecular weight adiponectin in adults with metabolic syndrome. Experimental physiology. 2020;105(3):449-59.

Montanari T, Pošćić N, Colitti M. Factors involved in white-to-brown adipose tissue conversion and in thermogenesis: a review. Obesity reviews. 2017;18(5):495-513.

Moreno M, Moreno-Navarrete JM, Serrano M, Ortega F, Delgado E, Sanchez-Ragnarsson C, et al. Circulating irisin levels are positively associated with metabolic risk factors in sedentary subjects. PLoS One. 2015;10(4):e0124100.

Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, RicartW, Fernandez-Real JM (2013) Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab 98:E769–E778.

Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, et al, 2013. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. The Journal of Clinical Endocrinology & Metabolism.;98(4):E769-E78.

Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW, 2006. Central nervous system control of food intake and body weight. Nature; 443: 289-295.

Natalicchio A, Marrano N, Biondi G, Spagnuolo R, Labarbuta R, Porreca I, et al, 2017. The myokine irisin is released in response to saturated fatty acids and promotes pancreatic β-cell survival and insulin secretion. Diabetes.;66(11):2849-56.

Nedergaard J, Cannon B, 2014. The browning of white adipose tissue: some burning issues. Cell metabolism.;20(3):396-407.

Norheim F, Langleite TM, Hjorth M, Holen T, Kielland A, Stadheim HK, et al, 2014. The effects of acute and chronic exercise on PGC-1alpha, irisin and browning of subcutaneous adipose tissue in humans. FEBS J.;281(3):739-49.

Oelmann S, Nauck M, Völzke H, Bahls M, Friedrich N, 2016. Circulating irisin concentrations are associated with a favourable lipid profile in the general population. PloS one.;11(4):e0154319.

Organization WHO, 2000. Obesity: preventing and managing the global epidemic: World Health Organization;

Ozbay S, Ulupınar S, Şebin E, Altınkaynak K, 2020. Acute and chronic effects of aerobic exercise on serum irisin, adropin, and cholesterol levels in the winter season: Indoor training versus outdoor training. Chin J Physiol. Jan-Feb;63(1):21-26.

Ozkan Y, Timurkan ES, Aydin S, Sahin I, Timurkan M, Citil C, Kalayci M, Yilmaz M, Aksoy A, Catak Z, 2013. Acylated and desacylated ghrelin, preptin, leptin, and nesfatin-1 Peptide changes related to the body mass index. Int J Endocrinol.;2013:236085.. Epub 2013 Nov 25.

Palizban A-A, Yazdani A-H, Jahanbani-Ardakani H, 2019. Role of rs7903146 polymorphism and adropin serum level in patients with diabetes mellitus; a case– control study from Isfahan, Iran. Archives of Physiology and Biochemistry.

Panati K, Narala VR, Narasimha VR, Derangula M, Tatireddigari VRA, Yeguvapalli S, 2018. Expression, purification and biological characterisation of recombinant human irisin (12.5 kDa). Journal of Genetic Engineering and Biotechnology.;16(2):459-66.

Park KH, Zaichenko L, Peter P, Davis CR, Crowell JA, Mantzoros CS, 2014. Diet quality is associated with circulating C-reactive protein but not irisin levels in humans. Metabolism.;63(2):233-41.

Park M-J, Kim D-I, Choi J-H, Heo Y-R, Park S-H, 2015. New role of irisin in hepatocytes: The protective effect of hepatic steatosis in vitro. Cellular signalling.;27(9):1831-9.

Partridge CG, Fawcett GL, Wang B, Semenkovich CF, Cheverud JM, 2014. The effect of dietary fat intake on hepatic gene expression in LG/J AND SM/J mice. BMC genomics.;15(1):99.

Pedersen B, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al, 2003. Searching for the exercise factor: is IL-6 a candidate? Journal of Muscle Research & Cell Motility.;24(2-3):113.

Pekkala S, Wiklund PK, Hulmi JJ, Ahtiainen JP, Horttanainen M, Pollanen E, et al, 2013. Are skeletal muscle FNDC5 gene expression and irisin release regulated by exercise and related to health? J Physiol.;591(Pt 21):5393-400.

Perakakis N, Triantafyllou GA, Fernández-Real JM, Huh JY, Park KH, Seufert J, et al, 2017. Physiology and role of irisin in glucose homeostasis. Nature reviews endocrinology.;13(6):324.

Pérez-Sotelo D, Roca-Rivada A, Baamonde I, Baltar J, Castro A, Domínguez E, et al, 2017. Lack of adipocyte-Fndc5/irisin expression and secretion reduces thermogenesis and enhances adipogenesis. Scientific reports.;7(1):16289.109.

Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J, 2010. Chronic peroxisome proliferator-activated receptor γ (PPARγ) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. Journal of Biological Chemistry.;285(10):7153-64.

Piya MK, Harte AL, Sivakumar K, Tripathi G, Voyias PD, James S, et al, 2014. The identification of irisin in human cerebrospinal fluid: influence of adiposity, metabolic markers, and gestational diabetes. American Journal of Physiology-Endocrinology and Metabolism.;306(5):E512-E8.

Polyzos SA, Mathew H, Mantzoros CS, 2015. Irisin: A true, circulating hormone. Metabolism.;64(12):1611-8.

Qiao X, Nie Y, Ma Y, Chen Y, Cheng R, Yin W, et al, 2016. Irisin promotes osteoblast proliferation and differentiation via activating the MAP kinase signaling pathways. Scientific reports.;6:18732.

Qiu S, Cai X, Sun Z, Schumann U, Zügel M, Steinacker JM, 2015. Chronic exercise training and circulating irisin in adults: a meta-analysis. Sports medicine.;45(11):1577-88.

Qiu S, Cai X, Yin H, Zügel M, Sun Z, Steinacker JM, et al, 2016. Association between circulating irisin and insulin resistance in non-diabetic adults: a meta- analysis. Metabolism.;65(6):825-34.

Racette SB, Deusinger SS, Deusinger RH, 2003. Obesity: overview of prevalence, etiology, and treatment. Phys Ther; 83: 276-288.

Rizk FH, Elshweikh SA, Abd El-Naby AY, 2015. Irisin levels in relation to metabolic and liver functions in Egyptian patients with metabolic syndrome. Canadian journal of physiology and pharmacology.;94(4):359-62.

Roca-Rivada A, Castelao C, Senin LL, Landrove MO, Baltar J, Crujeiras AB, et al, 2013. FNDC5/irisin is not only a myokine but also an adipokine. PloS one.;8(4):e60563.

Safarimosavi S, Mohebbi H, Rohani H, 2018. High-Intensity Interval vs. Continuous Endurance Training: Preventive Effects on Hormonal Changes and Physiological Adaptations in Prediabetes Patients. Journal of strength and conditioning research.

Salih, H.M. & Hamzah, M.I. & Malik, A.S.. (2020). EXPRESSION OF PREPTIN IN IRAQI PATIENTS WITH TYPE 2 DIABETES NEPHROPATHY. Biochemical and Cellular Archives. 20. 5723-5728.

Sanchis-Gomar F, Alis R, Rampinini E, Bosio A, Ferioli D, La Torre A, et al, 2015. Adropin and apelin fluctuations throughout a season in professional soccer players: Are they related with performance? Peptides.

Sanchis-Gomar F, Lippi G, Mayero S, Perez-Quilis C, Garcia-Gimenez JL, 2012. Irisin: a new potential hormonal target for the treatment of obesity and type 2 diabetes. J Diabetes.;4(3):196.

Satman I, Yilmaz T, Sengül A, Salman S, Salman F, Uygur S, et al, 2002. Population-based study of diabetes and risk characteristics in Turkey: results of the Turkish diabetes epidemiology study (TURDEP). Diabetes Care,; 25(9):1551-6.

Sayin O, Tokgoz Y, Arslan N, 2014. Investigation of adropin and leptin levels in pediatric obesity-related nonalcoholic fatty liver disease. J Pediatr Endocrinol Metab.;27(5-6):479-84.

Shelbaya S, Abu Shady MM, Nasr MS, Bekhet MM, Mageed YA-A, Abbas M, 2018. Study of irisin hormone level in type 2 diabetic patients and patients with diabetic nephropathy. Current diabetes reviews.;14(5):481-6.

Shoukry A, Shalaby SM, Bdeer SEA, Mahmoud AA, Mousa MM, Khalifa A, 2016. Circulating serum irisin levels in obesity and type 2 diabetes mellitus. IUBMB life.;68(7):544-56.

Soori R, Amini AA, Choobineh S, Eskandari A, Behjat A, Ghram A, et al, 2019. Exercise attenuates myocardial fibrosis and increases angiogenesis-related molecules in the myocardium of aged rats. Arch Physiol Biochem.

Sriwijitkamol A, Coletta DK, Wajcberg E, Balbontin GB, Reyna SM, Barrientes J, et al, 2007. Effect of acute exercise on AMPK signaling in skeletal muscle of subjects with type 2 diabetes: a time-course and dose-response study. Diabetes.;56(3):836-48.

Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF, 2013. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity–correlation with body mass index. Peptides.;39:125-30.

St-Onge MP, Shechter A, Shlisky J, Tam CS, Gao S, Ravussin E, et al, 2014. Fasting plasma adropin concentrations correlate with fat consumption in human females. Obesity.;22(4):1056-63.

Talanian JL, Galloway SD, Heigenhauser GJ, Bonen A, Spriet LL, 2007. Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. J Appl Physiol (1985).;102(4):1439-47.

Tang S, Zhang R, Jiang F, Wang J, Chen M, Peng D, et al, 2015. Circulating irisin levels are associated with lipid and uric acid metabolism in a Chinese population. Clinical and Experimental Pharmacology and Physiology.;42(9):896-901.

Tentolouris A, Eleftheriadou I, Tsilingiris D, Anastasiou IA, Kosta OA, Mourouzis I, et al, 2018. Plasma Irisin Levels in Subjects with Type 1 Diabetes: Comparison with Healthy Controls. Hormone and Metabolic Research.;50(11):803-10.

Teufel A, Malik N, Mukhopadhyay M, Westphal H, 2002. Frcp1 and Frcp2, two novel fibronectin type III repeat containing genes. Gene.;297(1-2):79-83.

Tews,D.,and Wabitsch,M.(2011).Renaissance of Brown adipose tissue. Horm.Res.Paediatr. 75, 231–239.

Tibana R, da Cunha Nascimento D, De Souza NF, de Souza V, de Sousa Neto I, Voltarelli F, et al, 2017. Irisin levels are not associated to resistance training-induced alterations in body mass composition in older untrained women with and without obesity. The journal of nutrition, health & aging.;21(3):241-6.

Timmons JA, Baar K, Davidsen PK, Atherton PJ, 2012. Is irisin a human exercise gene? Nature.;488(7413):E9.

Tsuchiya Y, Ando D, Goto K, Kiuchi M, Yamakita M, Koyama K, 2014. High-intensity exercise causes greater irisin response compared with low-intensity exercise under similar energy consumption. The Tohoku journal of experimental medicine.;233(2):135-40.

Tsuchiya Y, Ando D, Goto K, Kiuchi M, Yamakita M, Koyama K, 2014. High-intensity exercise causes greater irisin response compared with low-intensity exercise under similar energy consumption. The Tohoku journal of experimental medicine.;233(2):135-40.

Tsuchiya Y, Ijichi T, Goto K, 2016. Effect of sprint training on resting serum irisin concentration - Sprint training once daily vs. twice every other day. Metab-Clin Exp.;65(4):492-5.

Varela-Rodriguez BM, Pena-Bello L, Juiz-Valina P, Vidal-Bretal B, Cordido F, Sangiao-Alvarellos S, 2016. FNDC5 expression and circulating irisin levels are

modified by diet and hormonal conditions in hypothalamus, adipose tissue and muscle. Scientific reports.;6:29898.

Vaughan RA, Gannon NP, Mermier CM, Conn CA, 2015. Irisin, a unique non-inflammatory myokine in stimulating skeletal muscle metabolism. J Physiol Biochem.;71(4):679-89.

Villarroya F, 2010. Irisin, turning up the heat. Cell Metab. 2012;15(3):277-8.

Whyte LJ, Gill JM, Cathcart AJ. Effect of 2 weeks of sprint interval training on health-related outcomes in sedentary overweight/obese men. Metabolism.;59(10):1421-8.

Wisse BE, Kim F, Schwartz MW, 2007. An integrative view of obesity. Science.;318(5852):928-9.

Wong C-M, Wang Y, Lee JTH, Huang Z, Wu D, Xu A, et al, 2014. Adropin is a brain membrane-bound protein regulating physical activity via the NB-3/Notch signaling pathway in mice. Journal of Biological Chemistry.;289(37):25976-86.

Word health organization website,2013. "What are the health consequences of being overweight? http://www.who.int/features/qa/49/en/index.html.

World Health Organization Europe2005 . The Challenge of Obesity in the WHO European Region. Copenhagen, Bucharest,. http://www.euro.who.int/document/mediacentre/fs1305e.pdf.

World Health Organization, 2004.. Obesity: Preventing and Managing the Global Epidemic, Report of a WHO Consultation. Singapore, WHO;

Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, et al, 2013. Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. Cell metabolism.;18(5):649-59.

Wrann CD, 2015. FNDC5/Irisin–their role in the nervous system and as a mediator for beneficial effects of exercise on the brain. Brain plasticity.;1(1):55-61.

Wu L, Fang J, Chen L, Zhao Z, Luo Y, Lin C, et al, 2014. Low serum adropin is associated with coronary atherosclerosis in type 2 diabetic and non-diabetic patients. Clinical chemistry and laboratory medicine.;52(5):751-8.

Yildirim B, Celik O, Aydin S, 2014. Adropin: a key component and potential gatekeeper of metabolic disturbances in policystic ovarian syndrome. Clin Exp Obstet Gynecol.;41(3):310-2.

Yosaee S, Soltani S, Sekhavati E, Jazayeri S, 2016. Adropin-a novel biomarker of heart disease: a systematic review article. Iranian journal of public health.;45(12):1568.

You WP, Henneberg M, 2016. Type 1 diabetes prevalence increasing globally and Regionally: the role of natural selection and life expectancy at birth. BMJ open Diabetes research & care;4(1): 000161.

Young RA, 1976 . Fat, energy and mammalian survival. American Zoologist .;16(4):699-710.

Zang H, Jiang F, Cheng X, Xu H, Hu X, 2018. Serum adropin levels are decreased in Chinese type 2 diabetic patients and negatively correlated with body mass index. Endocrine journal.;65(7):685-91.

Zhang C, Ding Z, Lv G, Li J, Zhou P, Zhang J, 2016. Lower irisin level in patients with type 2 diabetes mellitus: A case-control study and meta-analysis: 2 型糖尿病患者的低鸢尾素水平：一项病例对照研究和 meta 分析. Journal of diabetes.;8(1):56-62.

Zhang H, Jiang L, Yang YJ, Ge RK, Zhou M, Hu H, et al, 2017. Aerobic exercise improves endothelial function and serum adropin levels in obese adolescents independent of body weight loss. Sci Rep.;7(1):17717.

Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, et al, 2014. Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling. Diabetes.;63(2):514-25.

Zhao LJ, Jiang H, Papasian CJ, Maulik D, Drees B, Hamilton J, et al, 2008. Correlation of obesity and osteoporosis: effect of fat mass on the determination of osteoporosis. J Bone Miner Res; 23: 17-29.

Zhu D, Wang H, Zhang J, Zhang X, Xin C, Zhang F, et al, 2015. Irisin improves endothelial function in type 2 diabetes through reducing oxidative/nitrative stresses. Journal of molecular and cellular cardiology.;87:138-47.