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ASSOCIATION BETWEEN NEUDESIN AND IRISIN IN NEWLY DIAGNOSED TYPE-2 DIABETES MELLITUS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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LARA ABDULKHDUR KADHIM KADHIM

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By Lara Abdulkhdur Kadhim KADHIM

January 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

ASSOCIATION BETWEEN NEUDESIN AND IRISIN IN NEWLY DIAGNOSED TYPE-2 DIABETES MELLITUS

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Master of Science in Chemistry

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Type 2 diabetes is the most common form of the disease, accounting for 85 to 90 percent of all cases. It is known as "insulin resistance" when the body's capacity to react to insulin action is impaired. Experimental studies suggest that neudesin is a novel energy balance and food intake regulator with implications for obesity and its associated disorders. Obesity and type 2 diabetes are two of the most common metabolic diseases, and researchers believe that irisin's ability to transform white adipose tissue cells into brown adipose tissue cells may represent new therapy options (T2DM). In 2021-2022, 100 Iraqis were placed into two groups (50 with newly T2DM, 26 male 24 female, 50 healthy subjects as control group 25 male 25 female). Neudesin and Irisin measured by ELISA technque. the standard deviation of the mean of Neudesin and Irisin for T2DM (7.09± 1.56) and (90.75± 24.82) was significantly higher than for control (1.61± 0.75), $p<0.001$, (30.67± 10.25) $p< 0.001$ .In conclusion. Measuring serum concentration of Neudesin and Irisin could be used as suggested diagnostic biomarkers for patients with newly T2DM. Also Neudesin and Irisin very useful to predict and prevent complication of patiant with diabetes mellitus type II.

2023, 49 pages

Keywords: Neudesin, Irisin, T2DM

ÖZET

YENİ TANILAN TİP-2 DİYABET MELLİTUS'TA NEUDESIN İRİSIN ARASINDAKİ İLİŞKİ

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Ocak 2023

Tip 2 diyabet, tüm vakaların yüzde 85 ila 90'ını oluşturan hastalığın en yaygın şeklidir. Vücudun insülin etkisine tepki verme kapasitesi bozulduğunda "insülin direnci" olarak bilinir. Deneysel çalışmalar, neudesinin yeni bir enerji dengesi ve obezite ve buna bağlı bozukluklar için etkileri olan gıda alımı düzenleyicisi olduğunu göstermektedir. Obezite ve tip 2 diyabet en yaygın metabolik hastalıklardan ikisidir ve araştırmacılar, irisinin beyaz yağ dokusu hücrelerini kahverengi yağ dokusu hücrelerine dönüştürme yeteneğinin yeni tedavi seçeneklerini temsil edebileceğine inanmaktadır (T2DM). 2021-2022'de 100 Iraklı iki gruba ayrıldı (50 yeni T2DM'li, 26 erkek 24 kadın, 50 sağlıklı denek kontrol grubu olarak 25 erkek 25 kadın). ELISA tekniği ile ölçülen Neudesin ve Irisin. T2DM (7.09± 1.56) ve (90.75± 24.82) için Neudesin ve Irisin ortalamasının standart sapması kontrolden (1.61± 0.75) anlamlı derecede yüksekti, $p<0.001$, (30.67± 10.25) $p< 0.001$.Sonuç olarak . Neudesin ve Irisin'in serum konsantrasyonunun ölçülmesi, yeni T2DM'li hastalar için önerilen tanısal biyobelirteçler olarak kullanılabilir. Ayrıca Neudesin ve Irisin, tip II diyabetli hastaların komplikasyonlarını tahmin etmek ve önlemek için çok faydalıdır.

2023, 49 sayfa

Anahtar Kelimeler: Neudesin, İrisin, T2DM

PREFACE AND ACKNOWLEDGEMENTS

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Lara Abdulkhdur Kadhim KADHIM
Çankırı-2023

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LIST OF SYMBOLS

%	Percent
µL	Micro liter
am	After morning
C°	Celsius
kda	Kilo dalton
mg/dL	Milligrams per desi liter
mg/mL	Milligrams per mili letter
min	Minute
mL	Mili letter
mmol/L	Milimol per letter
ng/mL	Nano grams per mili letter
nm	Nano meter
od	Optical density
pg/mL	Pico grams per mili letter
rpm	Round per minute

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUC	Area under curve
BMI	Body mass index
CI	Confidence Intervals
CVD	Cardio vascular disease
DM	Diabetes mellitus
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme Linked immuno sorbent assay
FBS	Fasting blood sugar
GDM	Gestational diabetes mellitus
HbA1C	Glycated hemoglobin
HDL-C	High density lipoprotein cholestrol
HRP	Horseradish peroxidase conjugat
IDF	International diabetes federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
LDL-C	Low density lipoprotein cholestrol
NSAID	None steroidal anti inflammatory drugs
OGTT	Oral glucose tolerance test
P	probability
RARRES2	Retinoic acid receptor responder 2
ROC	Receiver Operator Characteristic
RPG	Random plasma glucose
SD	Standard deviation
SE	Standard Error
T2DM	Type 2 diabetes mellitus
T-CHOL	Total cholestrol
TG	Triglycerides
TIG2	Tazarotene induced gene 2
WHO	World health organization

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1. INTRODUCTION

Hyperglycemia (increased blood sugar) is a symptom of diabetes mellitus Type 2 and is caused by a deficiency in insulin synthesis by the pancreas or an increase in insulin resistance that reduces insulin's action on the body. prediabetes Fasting glucose levels range from 6.1 to 7.8 mmol/L, and impaired glucose tolerance levels range from 7.8 to 11 mmol/L, both of which are precursors to diabetes mellitus. It's now possible to analyze the variations in neuropeptide concentrations across groups with varied durations of diabetes mellitus. Neudesin and irisin are neuropeptides that control glucose metabolism and insulin resistance in humans and other animals (DM). Factor 9 Neurotrophic Neudesin, a neurotransmitter found in the brain's cortex, has been linked to changes in appetite, energy balance, mood, and sympathetic nervous system activity. Signaling pathways including mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase are also stimulated by it (PI3K). 9 The adipose tissue, heart, lungs, and kidneys all generate the hypothalamic neuropeptide neudesin, which is responsible for controlling food intake and energy metabolism. 16 Researchers have shown that the Irisin peptide may speed up the conversion of white fat to brown fat, improve insulin resistance, and reduce visceral fat in people with diabetes (Bisschop *et al*. 2001). According to an analysis of eight research, those with Type 2 Diabetes (T2DM) had lower irisin levels. A healthy control group's serum levels of the biomarkers neudisin and irisin were compared to those of individuals with type 2 DM. Researchers also examined whether these markers could serve as early-stage biomarkers for diabetes mellitus diagnosis.

2. LITERATURE REVIEW

2.1 Diabetes Mellitus

When blood glucose levels are elevated due to either a pancreatic insulin deficiency or an increase in insulin resistance, the result is hyperglycemia, which is an elevated blood glucose level. Polyuria, weight loss, polyphagia, polydipsia, and blurry eyesight are all symptoms of hyperglycemia (ADA 2016).

2.2 Epidemiology

Diabetes mellitus affected 120 million people in 2000 and is anticipated to reach 492 million people by 2025 as a result of the disease's global prevalence, affecting individuals of all ages and socioeconomic backgrounds (Hode *et al*. 2015). More than 3.5 million Iraqis have diabetes, according to the country's Ministry of Health. India and China are more likely to have had a rise in the number of instances of type-2 DM in the past. The diabetes mellitus is scourge that has involved westernized societies is think to be related with obesity and decrease with increase physical activity. (Kalra *et al*. 2013).

2.3 Classification of the Diabetes

Type 1 diabetes (DM), type 2 diabetes (DM), gestational diabetes (GDM), and other types of diabetes that originate from a genetic defect are all forms of diabetes (MODY).

1. Type 1 diabetes (T1DM) also known as "insulin dependent diabetes mellitus" (IDDM), or juvenile diabetes mellitus (Van Belle *et al*. 2011). This type of diabetes primarily result from pancreatic cell destruction that lead to insulin deficiency therefore called insulin dependent. Due to either autoimmune illness or viral exposure, beta cells will be destroyed, resulting in insulin deficiency in this kind of diabetes (SONI *et al.* 2013). Diabetics who have type 1 diabetes mellitus are plagued by the symptoms of the disease such as frequent urination and excessive thirst, along with other life-threatening

complications such as weight loss, tiredness, blurred vision, and ketoacidosis (Livingstone *et al*. 2015).

2. Type 2 diabetes, which accounts for 85-90 percent of all cases of diabetes, is the most prevalent. It caused by the body can not be response to insulin action the body impaired to use insulin that mean increase insulin body resistance.

Type 2 diabetes many factors that lead to increase insulin resistance in the body and cause type 2 DM this factor include, Adipokins, metabolic factors, life style, clinical factors free fatty acid (Taylor *et al.* 2013)

Type 2diabetes characteristic by increase body resistance for insulin also relative with insulin deficiency and associated with overweight and obese in young individual .Also related with lifestyle change and physical activity (Husain *et al*. 2010).

3. During the latter 24 to 28 weeks of pregnancy, a metabolic illness known as gestational diabetes mellitus (GDM) may develop, increasing the chance of a newborn's health problems, a cesarean birth, and even the mother's death. After giving birth, a woman may acquire type 2 diabetes (Sugiyama *et al*. 2014).

4. There are additional varieties of diabetes related to unusual conditions, such as those that are inherited in an autosomal dominant pattern and arise at an early age (before the age of 25), as well as those that are linked to other diseases, such as pancreatitis or traumatic injury to the pancreas (Kahn *et al*. 2014). Viral infection also can cause beta cell destruction like cytomegalovirus, adenovirus, coxsackie virus And some genetic syndrome is associated with diabetes like some of chromosomal abnormalities e.g. turner syndrome, down syndrome, klinefelter syndrome (Kumar *et al*. 2014). 2.4 Diabetes Mellitus Risk Factors.

2.4 Diagnosis of Diabetes Mellitus

Diagnosis criteria for T2DM are laid forth in (Table 2.1) and (Figure 2.1) by the American Diabetes Association (ADA 2020):

1. FBG ⩾126 mg/dL (6.99 mmol/L) is considered abnormal. Fasting is defined as going without food for at least eight hours.

2. During the two-hour OGTT, blood glucose levels were less than 200 mg/dL (11.1 mmol/L). Immediately after a glucose load of 75 grams of glucose dissolved in water, this test must be carried out.

3. HbA1c ⩾ 6.5% (48 mmol/mol).

4. An unexpectedly low level of blood glucose (11.1 mmol/L) of less than 200 mg/dL.

Table 2.1 Measures for diagnosis of diabetes (SONI et al. 2013, ADA *et al*. 2015)

Condition	HbA1c	Fasting blood sugar (mg/dL)	GTT mg/dL
Normal	<5.6	(<100)	(<140)
Glucose metabolism is impaired	5.7-6.4	(100-125)	(140-199)
Glycemic tolerance is impaired	5.7-6.4	(125>100)	(140-199)
Diabetes mellitus	≥6.5	≥(126)	(≥200)

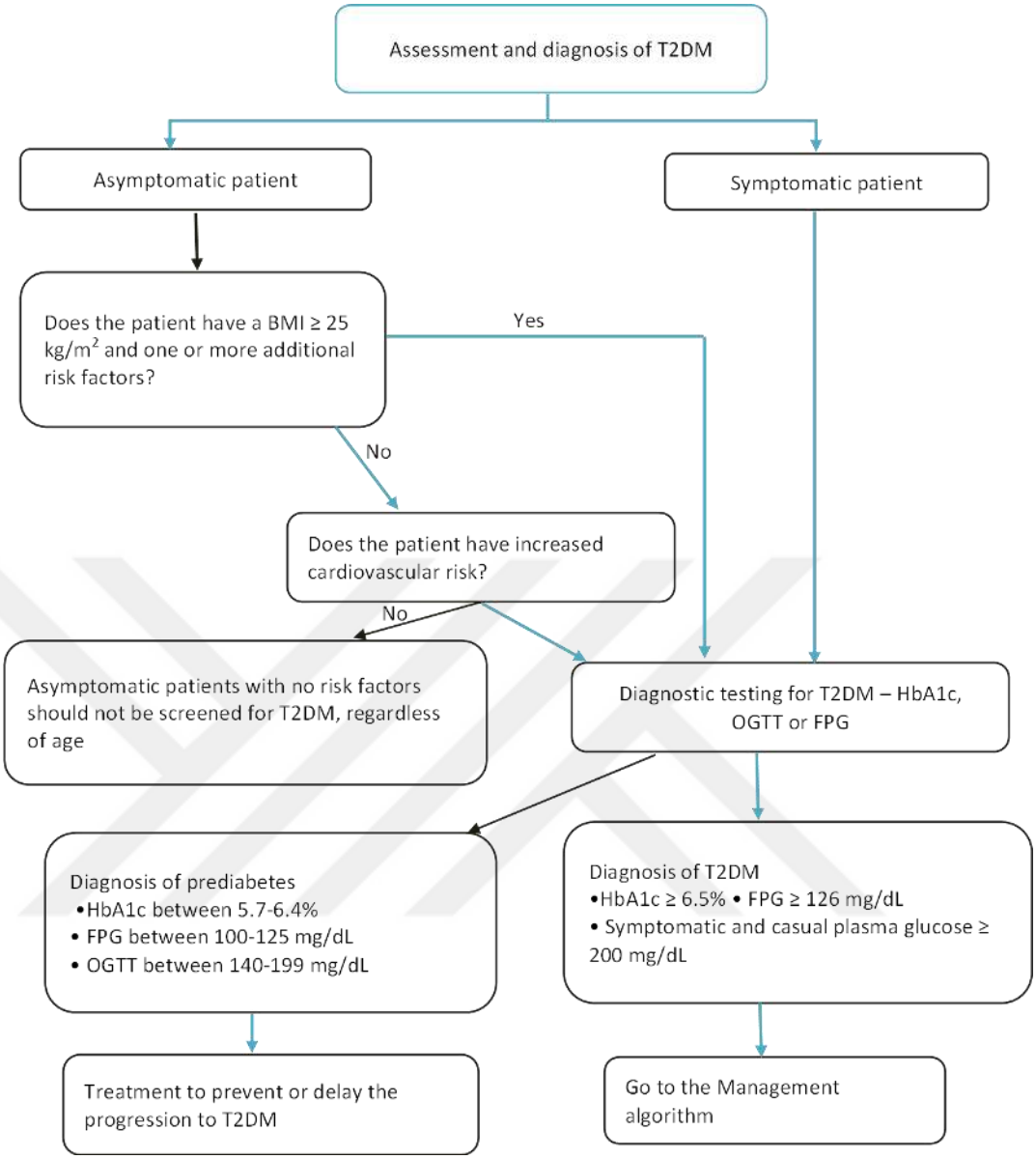


Figure 2.1 Diagnosis algorithm of type 2 diabetes mellitus in adult (Redmon *et al.* 2014)

2.5 Type 2 Diabetes Mellitus Pathophysiology

Type 2 Diabetes mellitus may be caused by pancreatic dysfunction or an increase in insulin resistance in the peripheral tissues (T2DM) (Boden *et al*. 1996). Because of a malfunction in insulin's signaling pathways in the tissue it affects (Wolfs *et al*. 2009)

After meal increase glucose level in the blood lead to stimulate beta cells of pancreas to releasing insulin that lead to decrease blood glucose level via facility glucose uptake by the body tissue that need glucose as source of energy like skeletal muscle and fat cell, increase blood glucose level lead to inhibit glucagon releasing that well lead to inhibit producing glucose from other source like glycogen break down. (Saltiel *et al*. 2001)

Liver is the major consumer of glucose and can conserve a normal level of glucose. Its obtain glucose via portal vein from circulation that contain glucose from digestive process. Liver rapidly removes large amounts of glucose after meal from circulation to the peripheral tissue and body organs that need glucose to produce energy (Foster *et al.* 2013). Improved insulin and fat synthesis are thus possible outcomes of this research. hormone-sensitive lipase (HSL) blockade also prevents fat decomposition (enzyme that breaks down fat stores). As a result, the quantity of fat in the blood is reduced. insulin has a positive influence on protein metabolism by stimulating the synthesis of protein from amino acids that are already present in the cells (Samaha *et al*. 2013).

When the level of blood glucose are decrease through fasting this lead to stimulate increase glucagon hormone level and decrease insulin level in the blood. The function of glucagon are antagonist to the function of insulin hormone, it is increase glucose level in the blood via stimulating the liver to stores glycogen,

When insulin production and insulin resistance are impaired in type 2 diabetes, glucose absorption and release by target tissues are altered, which ultimately leads to hyperglycemia (Boden *et al*. 1996).

Normal glucose homeostasis is maintained by the pancreatic -cells, which produce more insulin in response to insulin resistance. Because of the compensatory mechanism provided by -cells to maintain a high insulin demand, -cells suffer more damage and eventually die in later stages of T-2 diabetes mellitus. As a result, insulin levels dropped and blood glucose levels rose (Wolfs *et al*. 2009). Glucose levels or the development of type 2 diabetes over time (Figure 2.2) (Chen *et al*. 2017).

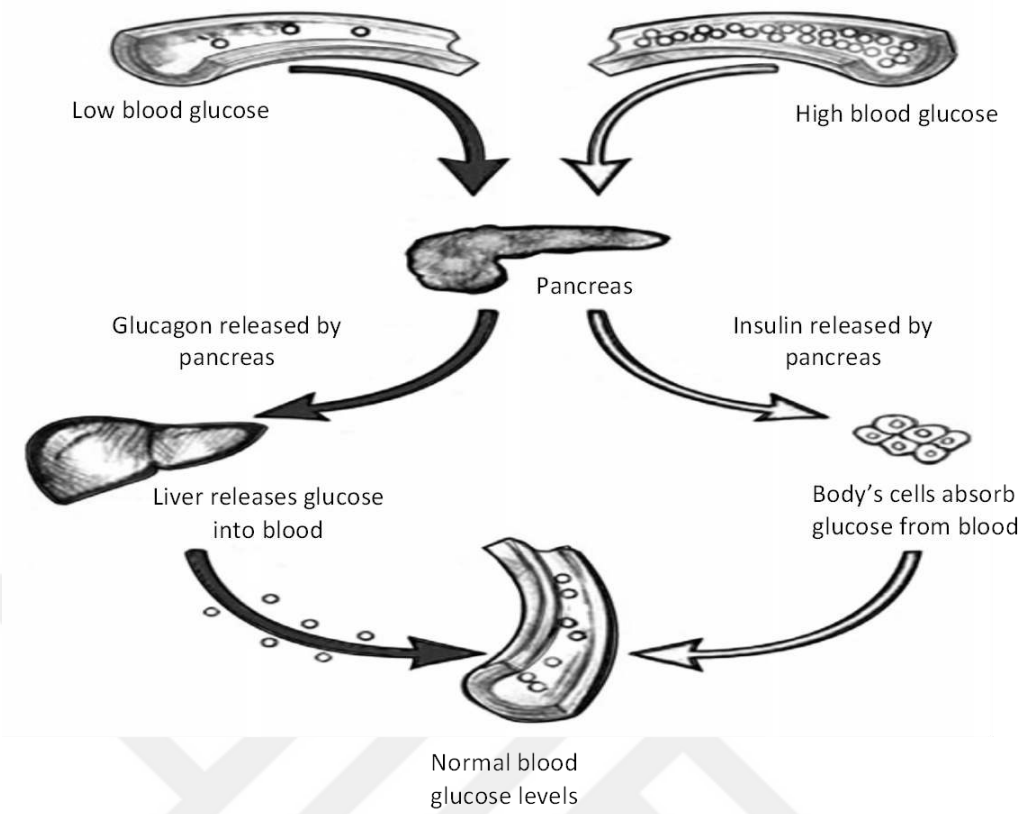


Figure 2.2 Glucagon and Insulin help to regulate the glucose levels in blood (Publication, 2014)

2.6 Index of Body Mass (BMI)

Malnutrition, an eating disorder, or other health issues might suggest a BMI of less than 18.5, whereas a BMI of 25 or more is deemed obese and a BMI of higher than 30 is considered underweight by the WHO. These BMI ranges are solely useful for statistical purposes (Table 2.2) (Cesare *et al*. 2016).

Table 2.2 The standard weight for adults that associated with body mass index is: (Cesare *et al*. 2016).

BMI	The amount of weight that a person has
Below 18.5	Underweight
18.5 – 24.9	Normal
25.0 – 29.9	Overweight
30.0 and above	Obese

2.7 Neudesin

Neudesin has just recently been recognized to have a part in the complex control of energy homeostasis. 8 Among the four proteins that share a heme/steroid binding domain with cytochrome 5-neudesin are membrane-associated progesterone receptors. It is a family of secreted proteins that includes Neudesin, Neuerricin, and Progesterone Receptor-Membrane Components 1 and 2. For instance (Kimura and *et al*. 2012) (English) It has the same 172-amino acid sequence as other vertebrates, including humans. Neuronal differentiation, cell proliferation, and cancer formation may all be linked to Neudesin's function in the body, according to study (Kimura *et al*.2005). Neudesin stimulates the signaling pathways for mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) (Ohta *et al*. 2015, Han *et al*. 2012).

2.7.1 Patophysiology of neudesin and its related to DM

Support for neural cell growth by Neudesin in the central nervous system and spinal cord is essential (Kimura *et al*. 2013). Neurotrophic effects on the hypothalamus (Byerly *et al.* 2013) and dentate gyrus (Hippocampus) of the hippocampus have also been shown (Byerly *et al*. 2013).

Neudesın-expressıng organısms ınclude adoptıve tıssue and heart and lıver kıdneys.

The principal functions of neudesin, according to 13 research, seem to be the control of neuronal function and energy metabolism (Ohta *et al*. 2015, Kimura *et al*. 2013).

Neudesin may be a novel regulator of energy balance and food intake, which has implications for obesity and its related comorbidities, according to recent study. Obesity and diabetes have not previously been shown to impact blood levels of the neuropeptide neudesin. In order to better understand its probable involvement in obesity-related metabolic abnormalities, we examined serum neudesin levels.

2.8 Irisin

Strained muscle produces Irisin, which is a protein hormone of 112 amino acids. In order to interact with other tissues, the skeletal muscle releases hormones known as myokines, which may be influenced by the kind of exercise and the level of physical exertion (Polyzos *et al*. 2013). metabolic illness may be improved by exercise's beneficial effects on myokines and their interactions with other systems in the human body (Polyzos *et al*. 2013).

Irisin, a hormone generated from muscle, increases the production of brown adipocyte cells. Striated muscle and other regions of the body communicate via Irisin. The striated muscle is able of interacting with other organs and regarded as secretory organ. a decrease in muscular activity causes a change in the muscle's structure. Contractions play a critical role in the synthesis of several proteins. Perersen and Febbraio (2012). FNDC5 concentrations are higher in muscles than in other organs in the human body as a whole (Huh *et al*. 2012). For example, Irisin is both an autocrine and paracrine myokine (Roca-Rivada *et al*. 2013).

2.8.1 Structure of irisin

Type I membrane protein FNDC5 is broken down into fibronectin type III domain number 5 by proteolysis (FNDC5) (Mahajan and Patra 2013). There are three parts to this protein: the hydrophobic carboxy-terminal domain, the two fibronectin domains, and the hydrophobic carboxy-terminal domain, as illustrated in (Figure 2.3). After being cleaved by the protease Irisin from the FNDC5 extracellular ecto domain and released into the bloodstream as a polypeptide (112 a.as),

Dimers of Irisin are depicted in Figures (1–10) B,C and feature an N-terminal region similar to that of fibronectin type–III, which has an attached four–stranded beta–sheet (Figures (1–10) B,C) (Schumacher *et al*. 2013).

A continuous eight-stranded beta sheet dimer is formed by irisin, each subunit containing four strands of beta-sheets. When it comes to tying the two halves of the dimer together, there are only two salt bridges between Arginine-75 (the first subunit) and Glu-790 (the second subunit, which represents prime) (Tryptophan-90 and Tryptophan-900). The Irisin dimer is very stable. (Shah and colleagues 2017). renal and biliary excretion of irisin from human body is carried out by these organs (Figure 2.3) (Lev *et al*. 2015).

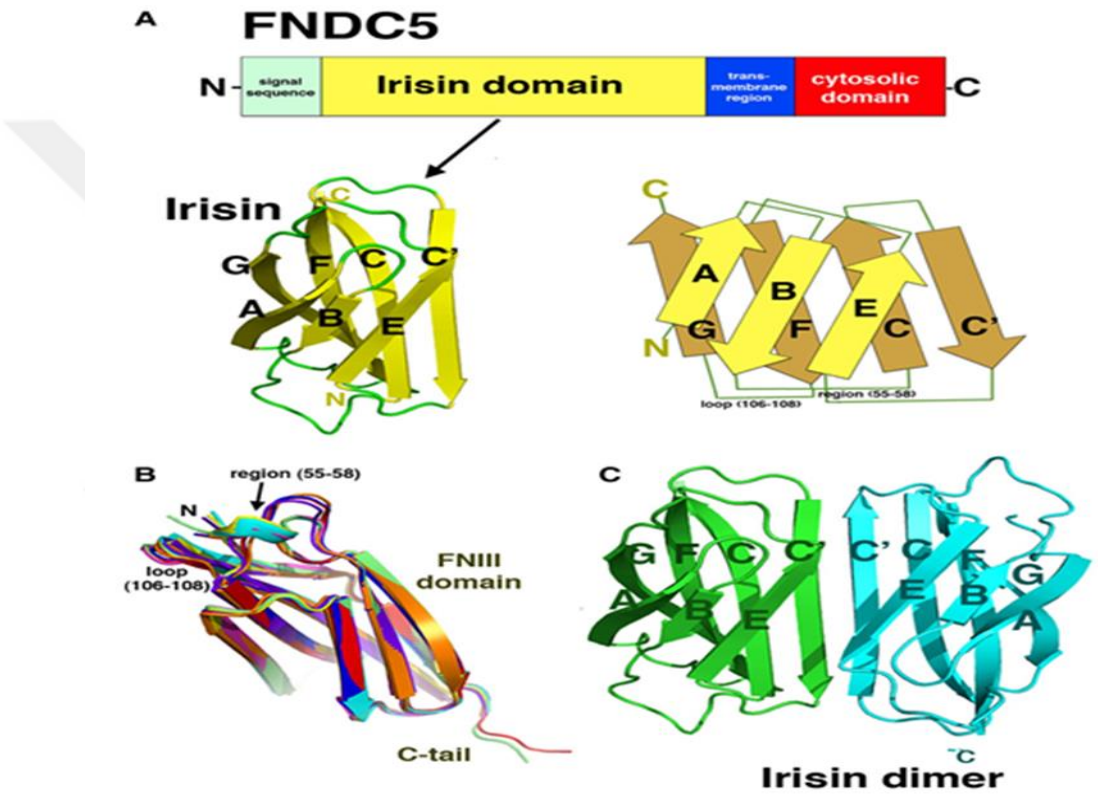


Figure 2.3 Structure of irisin (Schumacher *et al*. 2013)

A- Five fibronectin type III domains are shown in this image (FNDC5) Ectodomain claims to release Irisin, a protein with four beta-sheets sandwiched between three beta-sheets.

B- This figure depicts iridin subunits, with the locations that may be involved in protein-protein interactions highlighted.

C- The irisin dimer's structure is seen in this image.

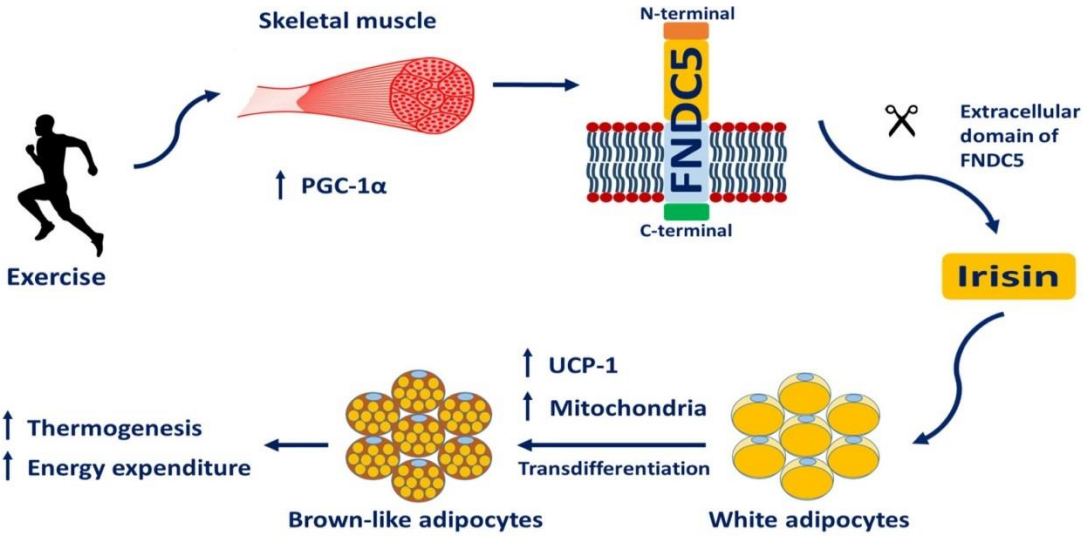
2.8.2 Irisin mechanism and function

PGC-1α (a co-activator capable of coordinating many genes in response to physiological cues and nutritional signals) is considerably elevated in the striated skeletal muscle following exercise (Ruschkke *et al*. 2010). Weight loss is caused by over-expression. Additionally, increasing the effectiveness of the insulin signaling pathways will improve insulin sensitivity (Arias-Loste *et al*. 2014).

Increased expression of Muscle PGC-1, a precursor to FNDC5, has been linked to fatty liver disease and sedentary lifestyle. The irisin is released into the circulation when FNDC5 splits into irisin and irisin interacts to a receptor on particular WAT cells (Arias-Loste *et al*. 2014). (Figure 2.4).

In adipocytes, irisin activates UCP1, which in turn activates ERK and p38 MAPK, causing browning of WAT (Zhang *et al*. 2014). Figure 2.4 shows the results.

Lipolysis is triggered by irisin through the cAMP–PKA–perilipin–HSL pathway (Xiong *et al.* 2015) and upregulation of the PNPLA2 gene, HSL, and FABP4 (Figure 2.4). Transforming white adipocytes into brown ones enhances thermogenesis and energy expenditure while also improving glucose tolerance and insulin sensitivity. This results in a decrease in body mass index (BMI) (Chen *et al.* 2015)



PGC-1α: peroxisome proliferator-activated receptor gamma coactivator-1α; FNDC5: fibronectin type III domain containing 5; UCP-1: uncoupling protein-1.

Figure 2.4 Irisin's manufacturing and action mechanisms (Buscemi *et al*. 2017)

2.8.3 Thr role of irisin in DM

FNDC5 gene produces irisin, an exercise and cold-induced hormone release in the skeletal muscle (Cunha *et al*. 2012, Lee P *et al*. 2014, Panati K *et al*. 2016). Through yet unidentified receptors, white adipose tissue (WAT) cells may be transformed into brown adipose tissue (BAT) cells in metabolic diseases such as obesity and type 2 diabetes (T2DM) (Hofmann *et al*. 2014, Sanchis-Gomar *et al*. 2012, Pyrzak *et al*. 2015, Leung *et al*. 2017). Some WAT genes are downregulated, while the expression of uncoupling protein-1 (UCP1) is increased, and other well-established BAT genes are altered as a result of irisin (Mahajan *et al*. 2013, Huh *et al*. 2014, Zhang *et al*. 2016). In order to increase calorie consumption, Irisin generates thermogenesis (Castillo-Quan *et al*. 2012). Irisin is both an adipokine and a myokine since it is secreted from adipose tissue (Moreno-Navarrete *et al*. 2013, Roca-Rivada *et al*. 2013). Exercise increases muscle irisin release, but fasting inhibits WAT release (Moreno-Navarrete *et al*. 2013). Circulating irisin and BMI (Body Mass Index) have been linked in a number of studies, with some finding a positive correlation (Stengel *et al*. 2013) and others finding no correlation (Sanchis-Gomar *et al*. 2014) or a negative correlation (Stengel *et al*. 2014).

(Sanchis-Gomar *et al*. 2014). A group of scientists are doing research (Yuksel and colleagues, 2014). Type 2 diabetes (T2DM) has not yet been linked to irisin.

3. MATERIALS AND METHODS

3.1 Material

3.1.1 Tool and instruments

An inventory of the study's tools may be found in Table 3.1.

Table 3.1 Tools and equipment were used in the experiments

No	Equipment and instruments	Type	Country
1	Pipette Tips (Yellow and Blue)	Tip	china
2	Gel Tubes	Afco	Jordan
3	EDTA Tubes	Firatmed	Turkey
4	Ependroff tubes(2mL)	Eppendroff	china
5	Body balance	balance	Germany
6	Incubator	Memmert	Germany
7	Freezer (-20°C)	Angel Antoni	Italy
8	Centrifuge	Kokusan	Germany
9	Biolyzer 300	Analytication	Germany
10	Nycocard reader II for HbA1c testing	Abbott	Norway
11	Robospin Timer	Lab Tach	Korea
12	Elisa system	Dialab	Austria

.

3.1.2 Kits

The kits (biolyzer kit , elisa kit and HbA1c kit) used in this study are shown in Table 3.2.

Table 3.2 Research tools and resources (companies and nations of origin)

No	Kit	Serial Number	Company	Country
1	Human Elisa kit of neudesin	MBS7606082	MyBioSource	China
2	Human Elisa kit of Irisin	MBS2600406	MyBioSource	China
3	Cholesterol Kit biolyzer	010520	DIALAB	Austria
4	Triglycerides Kit biolyzer	020321	DIALAB	Austria
5	Cholesterol HDL Kit biolyzer	8011/30488	DIALAB	Austria
6	HbA1c Kit	10213483	Abbott	Norway
7	Glucose kit biolyzer	050121	DIALAB	Austria

3.2 Method

3.2.1 Take a look at some of the designs

This study was carried out in the year 2022 on 100 iraqi participants divided into two groups (50 with newly type 2 Diabetes mellitus, 26 male 24 female, 50 healthy subjects as control group 25 male 25 female).

3.2.2 Collection of blood samples

Seven milliliters (7ml) of blood were taking from each subject from venous blood by using 10 mL disposable syringes between 8.00-11.00 am after fasting for 12 hours. Blood samples divided into three parts as the fallowing:

1.EDTA (ethylene diamine tetraacetic acid) (1.5 mg/mL) was added to the first portion of blood. calculating the HbA1c level.

2.The second part of blood sample put in the jel tube without any anticoagulant ant let the blood for 15 minute until clotting then separate the sample by use centrifuge for at least 10 minute at 3000 rpm to collected the serum and assay fasting blood sugar and lipid profile.

3.Third parts serum were taken after separate the sample and stored in deep freezer temperature range between (-20 c) until use. For assays of neudisin and Irisin. This test was measured in the laboratories of Medical City.

3.3 Exclusion Criteria

Exclusion criteria for this study will be pregnant women, chronic renal failure, liver diseases, thyroid diseases, type 1 DM, stablished cardiovascular diseases, diabetic kidney diseases, rheumatoid arthritis, and patients take statin and NSAID.

3.4 Determination Serum Level of Neudesin

Assay procedure: Do not dilute until you have completely mixed together all of your samples. Add the TMB Substrate to the wells of the assay once it has been equilibrated for 30 minutes at 37°C. There should be a standard curve drawn for each test.

- Standard, test, and control samples should be placed in separate wells on the pre-coated plate, which should have been diluted by half using Sample Dilution Buffer (which should be left blank). Each standard and sample should be measured twice, as recommended. Repeatedly clean the plate before adding wells for the reference material, sample, and control.

- Standards should be prepared Sample Dilution Buffer (blank) and Zero Tube (blank) are aliquoted into the standard wells together with 100ul of each of the following: 1st, 2nd, 3rd, 4th, 5th, and 6th tubes.

- Test sample wells received 100ul of diluted sample.

- Incubate the plate at 37°C for 90 minutes, then remove the lid.

- Rinse two times with Wash Buffer after removing the lid and discarding the contents of the plate. Don't ever allow the wells to dry entirely.

- 100 ul of biotin-labeled antibody working solution was added to the wells referred to before in this section (standard, test sample and blank wells). Take a well-coated plate, add the solution, and incubate at 37°C for 60 minutes.

- To clean a plate three times, remove the cover and let it rest in the wells for about 1-2 minutes each time. After that, thoroughly wash the dish to get rid of any leftover food particles.

- Each well was incubated for 30 minutes at 37°C with 100 ul of SABC Working Solution.

- Wash Buffer should be left in the wells for 1-2 minutes after each removal of the lid and washing plate.

- Each well received 90ul of TMB Substrate, which was applied, covered, and incubated for 10-20 minutes at 37°C in the dark. If the color shift is less than 30 minutes, the response time may be lowered or prolonged. In ordinary wells, the reaction may be terminated when an apparent gradient is seen

- Each well should be filled with 50 ul of Stop Solution. The color will soon begin to fade to yellow. The TMB Substrate Solution and Stop Solution should be added in the same sequence.

- OD After adding the stop solution, use a Microplate Reader to measure the O.D. absorbance at 450nm

Any other well in the system's OD450 is subtracted from the well's OD450 to arrive at its OD450 (the O.D.450 of blank well). The standard curve may be shown graphically using an O.D.450 plotted against the concentration of each standard solution (Y) (X). The standard curve may be used to interpolate the desired concentration of the samples. Using professional software, like Curve Expert 1.3 or 1.4, is the best way to do this calculation Figure 3.1.

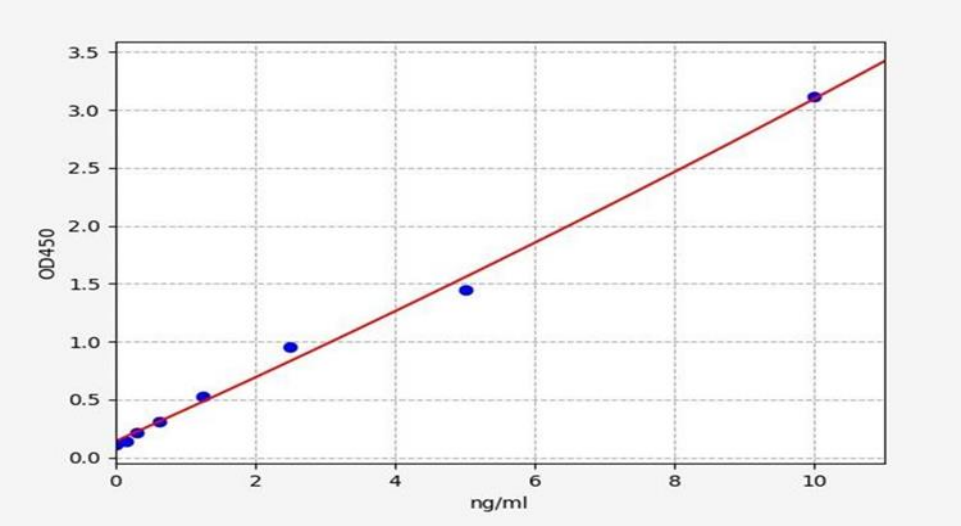


Figure 3.1 Standard curve for neudesin

3.5 Determination Serum Level of Irisin

Assay procedure: Do not dilute until you have completely mixed together all of your samples. Add the TMB Substrate to the wells of the assay once it has been equilibrated for 30 minutes at 37°C. There should be a standard curve drawn for each test.

- Standard, test, and control samples should be placed in separate wells on the pre-coated plate, which should have been diluted by half using Sample Dilution Buffer (which should be left blank). Each standard and sample should be measured twice, as recommended. Repeatedly clean the plate before adding wells for the reference material, sample, and control.

- Standards should be prepared Sample Dilution Buffer (blank) and Zero Tube (blank) are aliquoted into the standard wells together with 100ul of each of the following: 1st, 2nd, 3rd, 4th, 5th, and 6th tubes.

- Test sample wells received 100ul of diluted sample.

- The plate should be covered and incubated at 37 degrees Celsius for 90 minutes.

- Discard the plate's contents and wash the plate two more times with Wash Buffer after removing the lid. Don't ever allow the wells to dry entirely.

- A working solution containing biotin-labeled antibodies was added to the wells above (standard, test sample and blank wells). Add the solution to a well-coated plate and incubate for 60 minutes at 37°C.

- To clean a plate three times, remove the cover and let it rest in the wells for about 1-2 minutes each time. After that, thoroughly wash the dish to get rid of any leftover food particles.

- For 30 minutes, each well was incubated at 37°C with 100ul of SABC Working Solution.

- To wash the plate, remove the lid and let it in the wells for 1 to 2 minutes each time you use Wash Buffer.

- TMB Substrate was added, covered, and incubated for 10 to 20 minutes at 37°C in the dark in each well before being discarded. It is possible for the reaction time to be reduced or extended if the color change is less than 30 minutes long. As soon as an apparent gradient is seen, the reaction may be stopped in a conventional well.

- Each well was injected with 50 mL of Stop Solution. Eventually, the color will begin to become yellow. Substrate Solution should be added first, followed by Stop Solution.

- OD Use a Microplate Reader to check the O.D. absorbance at 450nm after adding the stop solution.

Any other well in the system's OD450 is subtracted from the well's OD450 to arrive at its OD450 (the O.D.450 of blank well). The standard curve may be shown graphically

using an O.D.450 plotted against the concentration of each standard solution (Y) (X). The standard curve may be used to interpolate the desired concentration of the samples. Using professional software, like Curve Expert 1.3 or 1.4, is the best way to do this calculation Figure 3.2 and Figure 3.3.

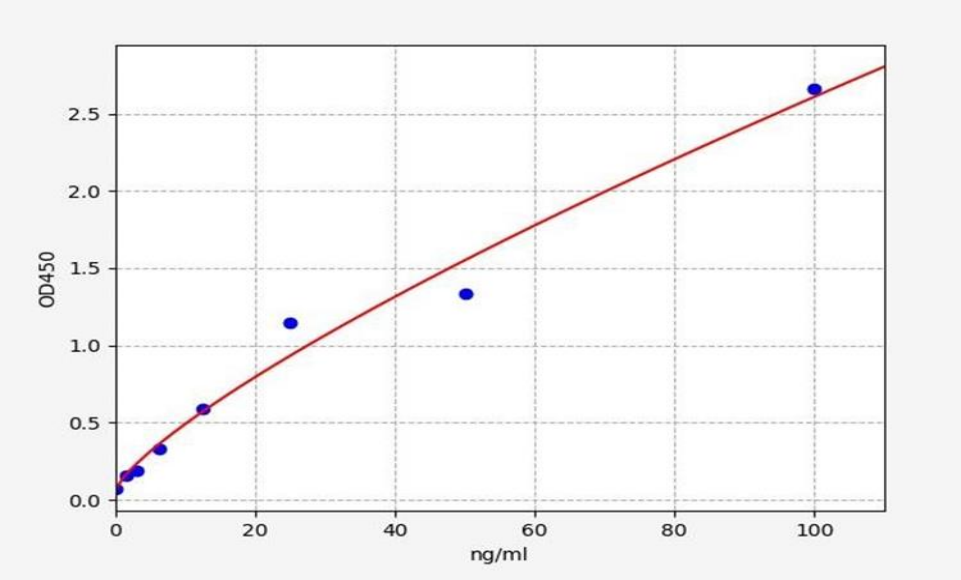


Figure 3.2 Standard curve for irisin

Figure 3.3 Biolyzer 300

3.6 Statistical Analysis

Continuous variables were represented in terms of mean and standard deviation, whilst qualitative variables were supplied in terms of the number of instances (n) and their mean and standard deviation values (percentages). The Two-Tailed Independent Samples Student's t-Test and bivariate descriptive statistics were also employed. In order to compare categorical variables, researchers utilized the chi-square test or Fisher's exact test. To determine whether the data had a normal distribution, the Kolmogorov–Smirnov test was used. Quantitative variables were correlated using Pearson's coefficient of correlation. The strength of the connections was measured according to Cohen's criterion. This means that modest effects are represented by coefficients of between 0.10 and 0.29, moderate effects are represented by those of 0.30 to 0.49, and big effects, those of 0.50 and above, are defined as (Cohen, 1988). For all of the experiments, an alpha value of 0.05 was utilized as the statistical significance threshold. Irisin and Leptin levels were correlated using a linear regression model. I used a statistical package for the social sciences to conduct all of my research (SPSS, Version 26 for Windows; SPSS, Inc., Chicago, IL, USA).

4. RESULTS AND DISCUSSION

Biomarkers (neudesine, irisin, high-sensitivity C-reactive protein, etc.) and lipid profiles, as well as fasting blood sugar levels, will be evaluated as part of this investigation. Levels of these indicators in patients with type 2 diabetes were higher than in healthy persons.

Diabetes mellitus is a chronic inflammation disease (ADA *et al*. 2016) where the fasting blood sugar > 126 mg/dL) and the HbA1c more than (6.5%). Sample collection according to the diagnosis by the consultant and this diagnosis was confirmed by done the laboratory test FBS and HbA1c.

This study involves two groups:

Group 1: 50 healthy volunteers who will provide blood samples for the study as a control.

Group 2: (type 2 diabetes mellitus) blood sample will be collected from 50 patients with type 2 DM according the WHO (HbA1C > 6.5%). the age range of the patients is (30 - 65) years.

Exclusion criteria for this study will be pregnant women, chronic renal failure. Liver diseases, Thyroid diseases, Type 1 DM, cardiovascular disease, diabetic kidney disease, Rheumatoid arthritis, and patients take none steroidal anti inflammatory drugs statins and NSAID.

4.1 Gender

There was no significant difference of gender between the groups p = 0.4016. The most frequently observed gender category within the T2DM group was Female (n = 24, 53.33%). While, the most frequently observed category of gender within the Control

group was Male (n = 25, 55.56%). Frequencies and percentages are presented in Table 4.1. The interpretation of this result due to geneder matched between patients and control groups and also within groups.

Table 4.1 Frequency table of gender by group

	Gender		
group	Female	Male	
Control	25	25	50
T2DM	24	26	50
total	49	51	100
Chi-squared	0.704		
DF	1		
Significance level	P = 0.4016		

4.2 Age

Subjects having age more than 45 years were the most frequently observed age within the T2DM group (n = 23, 51.11%). While, the most frequently observed age within the control group was ≤ 45 years (n = 38, 84.44%). Frequencies and percentages are presented in Table 4.2.

Table 4.2 Frequency table of age categories by group

		Groups	
		T2DM	Control
Age in years	>45	40	38
	≤45	10	12
	Total	50	50

The average age of the T2DM observations was 45.56 (SD = 5.21). The observations of age had an average of 42.07 (SD = 4.02) for Control. Statistical analysis revealed No significant difference between the T2DM and control groups, with $t(88) = 3.56$, $p > 0.05$. This indicates that the mean age of the T2DM and Control groups was no significant difference. Table 4.3 displays the outcomes. Figure 4.1 displays a bar plot of

the means. The interpretation of this results due to the age of all study groups were selected as age matched.

Table 4.3 Comparison of the clinical data and blood glucose parameters between T2DM and control

	T2DM	Control			
Variable	Mean± SD	Mean± SD	*t*	*p*	*d*
Age (y)	45.56± 5.21	42.07± 4.02	3.56	>0.05	0.75
BMI(kg/h²)	29.11± 4.59	26.00± 3.59	3.59	< .001	0.76
FBS (mg/dL)	168.58± 63.52	79.89± 5.77	9.33	< .001	1.97
HbA1C	8.57± 1.84	5.04± 0.45	12.48	< .001	2.63

Note. The number of participants is 90. T-statistic degrees of freedom = 88. d stands for Cohen's d.

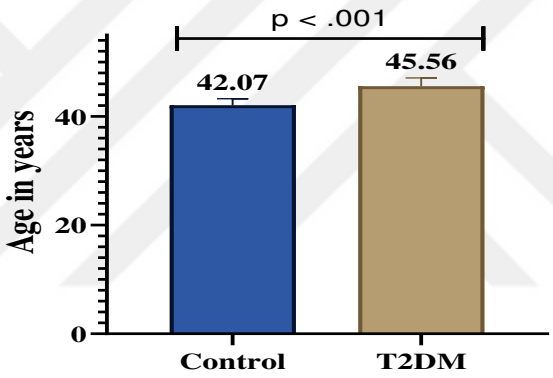


Figure 4.1 The average age of a group with 95% confidence interval error bars

4.3 BMI

In order to evaluate whether there was a statistically significant difference between the mean BMI of the T2DM and Control groups, a statistical analysis was conducted. The BMI observations for T2DM had an average of 29.11 (SD = 4.59). The BMI observations for Control had an average of 26.00 (SD = 3.59). The comparison revealed a significant difference between the T2DM and control groups, with t(88) = 3.59, p<001. This conclusion implies that the mean BMI of the T2DM and Control groups was significantly different. Table 4.3 summarizes the findings. Figure 4.2 depicts a bar plot of the means.

There was a substantial difference between the two groups' BMIs in this research, with the type 2 diabetes group weighing more than the normal control group (Bennasar-Veny *et al.* 2020). Insulin resistance is closely linked to fat storage in the body (Engin and Engin 2017). Low-grade inflammation (metaflammation) has been shown to affect insulin action and contribute to the development of insulin resistance when macrophages are present or infiltrate metabolic organs under obesity (Lauterbach and Wunderlich 2017).

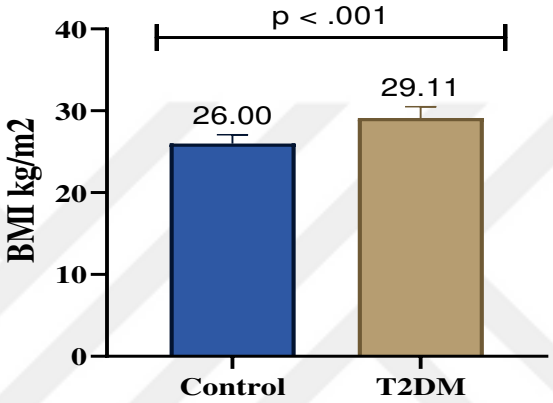


Figure 4.2 The mean BMI for each group with a 95 percent confidence interval (CI)

4.4 FBS mg/dL

With statistically significant $t(44.73) = 9.33$ and $p<.001$ difference between the T2DM and control groups, 168.58 ± 63.52 was the average of FBS measurements taken in T2DM patients. In the Control group, the average FBS was found to be 79.89 ± 5.77. The findings are summarized in Table 4.3 and Figure 4.3 is a bar graph depicting the mean.this study consistent with (Saber *et al.* 2014) in which prediabetic group have higher level of glucose than control group.

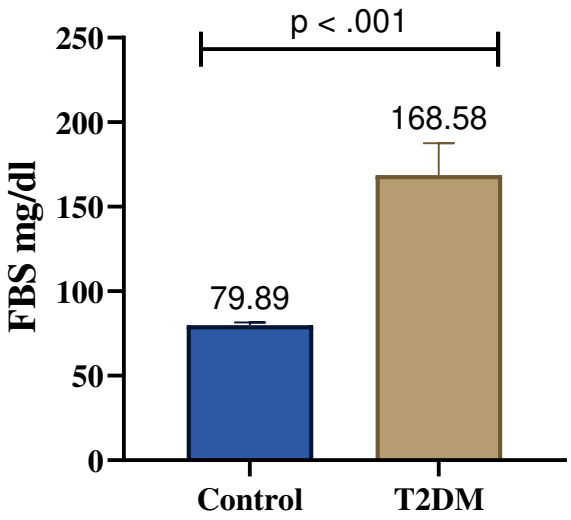


Figure 4.3 The group mean of FPS mg/dL with 95% confidence interval error bars

4.5 HbA1C

HbA1C observations for T2DM showed an average of 8.57 (SD = 1.84). The HbA1C readings for Control showed an average of 5.04 (SD = 0.45). Statistical analysis revealed a significant difference in the T2DM and control groups, t(49.20) = 12.48, p<.001. This data implies that the mean HbA1C levels varied significantly between the T2DM and Control groups. Table 4.3 summarizes the findings. Figure 4.4 depicts a bar plot of the means. and this study agreement with study which done by (Nam *et al.* 2018) who said a highly significant difference between diabetic group have higher level of HbA1c than control group.

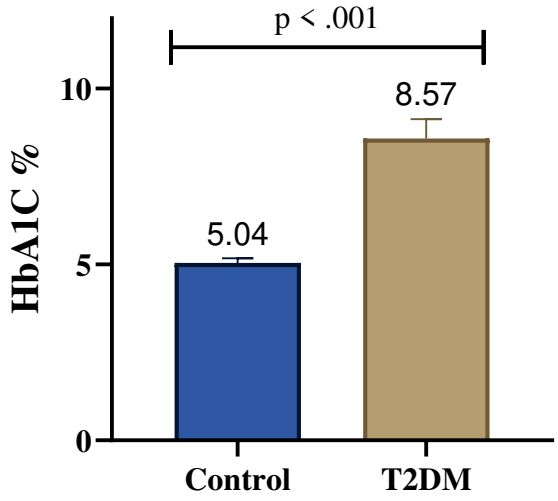


Figure 4.4 Mean HbA1C values for each group with 95% confidence intervals

4.6 Lipid Profile in all Study Groups

4.6.1 Total cholesterol

The observations of TC mg/dL for T2DM had an average of 231.02 (SD = 48.22). The observations of TC mg/dL for Control had an average of 151.16 (SD = 31.68).

The mean of TC mg/dL in the T2DM and Control groups was compared to see if there was a significant difference. Statistical analysis showed a significant difference between T2DM and control groups, $t(88) = 9.29$, $p < .001$. This finding implies that the mean of TC mg/dL differed significantly between the T2DM and Control groups. Table 4.4 summarizes the findings. Figure 4.5 depicts a bar plot of the means.

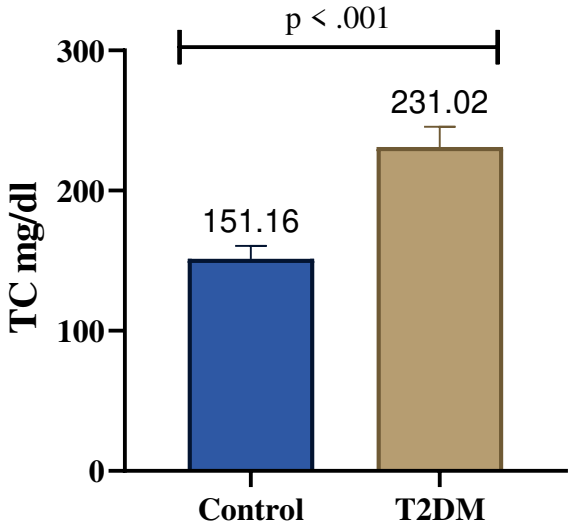


Figure 4.5 The mean TC mg/dL with 95% CI error bars for each group

4.6.2 Triglyceride (TG)

The observations of TG mg/dL for T2DM had an average of 204.62 (SD = 62.53). The observations of TG mg/dL for Control had an average of 100.33 (SD = 20.21). Statistical analysis revealed a significant difference in the T2DM and control groups, $t(53.10) = 10.65$, $p<.001$. This finding implies that the mean TG mg/dL in the T2DM and Control groups was significantly different. Table 4.4 summarizes the findings. Figure 4.6 depicts a bar plot of the means.

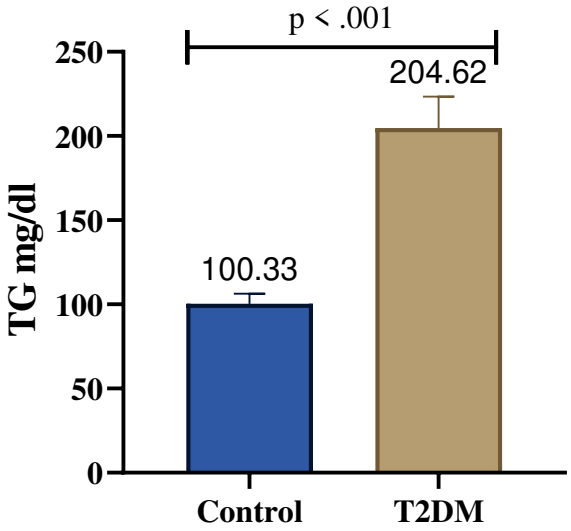


Figure 4.6 The mean TG mg/dL with 95% confidence intervals for each group

4.6.3 HDL -C

The observations of HDL mg/dL for T2DM had an average value of 42.04, with a standard deviation of 6.34. The observations of HDL mg/dL for Control had a mean value of 50.82 and a standard deviation of 6.75. The type 2 diabetes (T2DM) group and the control group were found to have statistically significant differences (t(88) = -6.36, p .001). Based on these findings, it appears that the T2DM group had significantly lower HDL levels than Control group. Table 4.4 summarizes the findings for your perusal. Figure 4.7 is a bar plot that illustrates the means of the data.

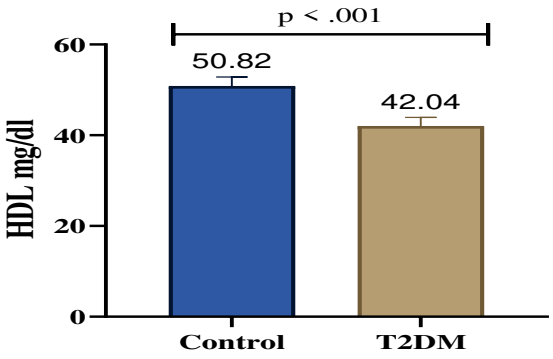


Figure 4.7 The mean HDL mg/dL with 95% confidence intervals for each group

4.6.4 LDL-C

The observations of LDL mg/dL for T2DM had a mean value of 148.05, with a standard deviation of 45.30. The observations of LDL for Control had a mean value of 85.24 and a standard deviation of 31.12. The test of statistical significance revealed a substantial gap between the T2DM and control groups, with t(88) = 7.67 and a significance level of p <.001. Based on these findings, it appears that the T2DM group had significantly higher means for their LDL than control group. Table 4.4 presents the findings in their entirety. Figure 4.8 is a bar plot that illustrates the means of the data.

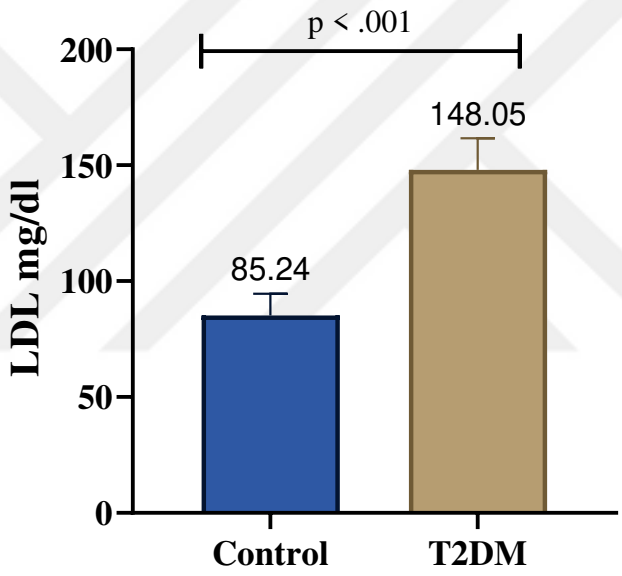


Figure 4.8 The mean LDL mg/dL with 95% CI error bars for each level of group

4.6.5 VLD-C

For T2DM, the observations of VLDL mg/dL had an average of 40.92 (SD = 12.51). For Control, the observations of VLDL mg/dL had an average of 20.07 (SD = 4.04). Statistical test showed significant difference between the T2DM and control groups, t(53.10) = 10.65, p < .001. T2DM patients had a considerably lower mean VLDL mg/dL than healthy controls. Table 4.4 summarizes the findings in this study. Figure 4.9 shows a bar graph of the mean values.

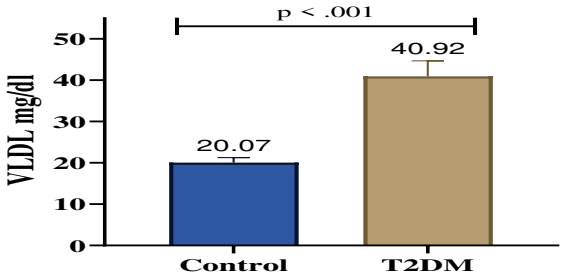


Figure 4.9 The mean of VLDL mg/dL by levels of group with 95% CI error bar

Table 4.4 Comparison of the lipid profile and the study parameters between T2DM and control

	T2DM	Control	Two-Tailed Independent Samples t-Test		
Variables	*Mean± SD*	*Mean± SD*	*t*	*P*	*d*
TC mg/dL	231.02± 48.22	151.16± 31.68	9.29	< .001	1.96
TG mg/dL	204.62± 62.53	100.33± 20.21	10.65	< .001	2.24
HDL mg/dL	42.04± 6.34	50.82± 6.75	-6.36	< .001	1.34
LDL mg/dL	148.05± 45.30	85.24± 31.12	7.67	< .001	1.62
VLDL mg/dL	40.92± 12.51	20.07± 4.04	10.65	< .001	2.24
CRP g/l	5.07± 1.32	2.50± 0.76	11.34	< .001	2.39
Irisin ng/ml	90.75± 24.82	30.67± 10.25	15.01	< .001	3.16
Neudesin ng/ml	7.09± 1.56	1.61± 0.75	21.21	< .001	4.47

Note. N = 90. Degrees of Freedom for the t-statistic = 88. d represents Cohen's d.

Previous studies (Al-Naama *et al*. 2010) and (Karim *et al*. 2014) have shown that patients with type 2 diabetes had greater concentrations of lipids and lipoproteins (excluding HDL) than the control group, and our research is in accordance with those findings, except for HDL. Lipolysis and cholesterol homeostasis are both controlled by insulin. In children and adolescents with Type 2 Diabetes Mellitus (T2DM), obesity, metabolic syndrome, and hyperglycemia aggravate the dysregulated lipid metabolism (Sunil and Ashraf 2020).

4.7 CRP g/ l

CRP measurements for T2DM showed an average of 5.07 (SD = 1.32). CRP observations for Control had an average of 2.50 (SD = 0.76). There was a significant difference between the T2DM and control groups, t(70.10) = 11.34, p.001 in statistical analysis. Based on these findings, it may be assumed that the T2DM group's mean CRP

was considerably greater than the control group's. The results are shown in Tables 4.4. The means are shown graphically in Figure 4.10 as a bar graph. CRP levels were greater in the type 2 diabetics compared to those in the control group in this study, which is in line with earlier findings (Kanmani *et al.* 2019). Patients with prediabetes mellitus had greater CRP levels than those in the control group, according to a study (Wang Z. *et al*.2016). Inflammation, hyperglycemia, oxidative stress, and type 2 diabetes are all connected to high CRP levels. Anti-inflammatory mediators and reactive oxygen species are generated in response to oxidative stress (Oguntibeju, 2019).

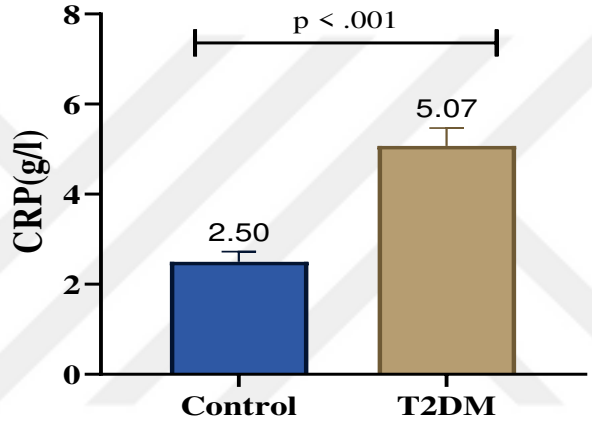


Figure 4.10 Arithmetic mean of CRP for each group, with 95% confidence intervals

4.8 Irisin

For T2DM, the observations of Irisin had an average of 90.75 (SD = 24.82). For Control, the observations of Irisin had an average of 30.67 (SD = 10.25). Statistical test showed significant difference between the T2DM and control groups, $t(58.57) = 15.01$, $p < .001$. This finding suggests the mean of Irisin had significantly high levels in T2DM as compared to the control group. The results are presented in Table 4.4. A bar plot of the means is presented in Figure 4.11.

T2DM patients had higher blood irisin concentrations than healthy participants, which is contrary to prior research that found lower irisin concentrations among patients with diabetes. Choi et al. found that newly diagnosed T2DM patients were younger and had a

lower BMI than the current study's participants (Choi *et al.* 2013). As a result, it's possible that the variations in irisin levels seen across studies are influenced by these behavioral traits. Although the research by Liu *et al*. had similar cohort characteristics, the greater mean BMI in this study may have accounted for the variations in irisin readings (Liu *et al.* 2013). The lack of measurements of muscle mass and body fat in prior research makes it difficult to draw any conclusions.

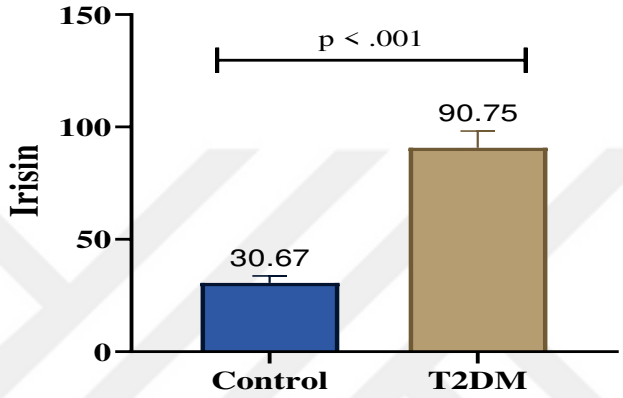


Figure 4.11 Irisin levels per group with 95% CI error bars for the mean

4.9 Neudesin

Neudesin's observations for T2DM had an average of 7.09 (SD = 1.56). While, for Control, it had an average of 1.61 (SD = 0.75). The Welch's t-test was done to see if there was a significant difference in Neudesin levels between T2DM and controls; the results were significant, $t(63.47) = 21.21$, $p < .001$. This finding implies that the mean of Neudesin was significantly higher in the T2DM than control group. Table 4.4 summarizes the findings. Figure 4.12 depicts a bar plot of the means. Ovgu Karatas *et al*.2021 found a rise in serum neudesin in Type-2 DM patients with a fresh diagnosis, which is consistent with this study's findings. Obesity and insulin resistance may be to blame for this rise.

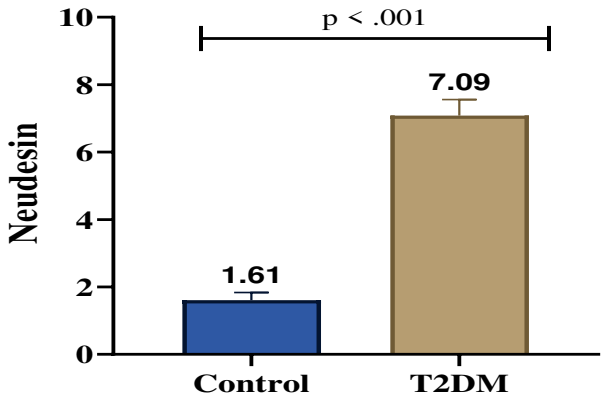


Figure 4.12 The group mean of Neudesin, with 95% confidence intervals for the error bars

4.10 Pearson Correlation Analysis T2DM Group

Irisin, Neudesin, CRP correlated with age, BMI, FPS, HbA1C, TC, TG, HDL, LDL, and VLDL using Pearson correlation analysis. A significant positive correlation of r = 0.78 was found between Irisin and Neudesin, indicating a strong effect size (p< 0.001, 95%CI = [.62,.87]). This suggests that if Irisin levels rise, so will Neudesin. A significant positive correlation of.48 was found between CRP and BMI, indicating a moderate effect size (p =.044, 95%CI = [.22,.68]). A significant positive correlation of r = 0.61 was found between CRP and FPS mg/dL, indicating a strong impact size (p< 0.001, 95%CI = [.39,.77]). This shows that while FPS rises, CRP rises as well. A significant positive correlation of r = 0.53 was found between CRP and TG mg/dL, indicating a considerable impact size (p =.011, 95%CI = [.28,.71]). This shows that while CRP rises, TG mg/dL rises as well. A significant positive correlation of r= 0.53 was found between CRP and VLDL mg/dL, indicating a large impact size (p =.011, 95%CI = [.28,.71]). This shows that as CRP rises, VLDL mg/dL rises as well. There were no additional significant relationships discovered. The correlation data are shown in Table 4.5.

Table 4.5 Pearson correlation results among irisin, neudesin, CRP, AGE, BMI, FPS, HbA1C, TC , TG, HDL, LDL, and VLDL in T2DM patients

Combination	r	95.00% CI	N	p
Irisin-Neudesin	.78	[.62, .87]	45	< .001
Irisin-CRP	.07	[-.23, .36]	45	1.000
Irisin-AGE	.35	[.07, .59]	45	.653
Irisin-BMI	-.08	[-.37, .22]	45	1.000
Irisin-FPS	.13	[-.17, .41]	45	1.000
Irisin-HbA1C	.12	[-.18, .40]	45	1.000
Irisin-TC	.22	[-.08, .48]	45	1.000
Irisin-TG	.26	[-.04, .52]	45	1.000
Irisin-HDL	-.25	[-.51, .04]	45	1.000
Irisin-LDL	.20	[-.10, .47]	45	1.000
Irisin-VLDL	.26	[-.04, .52]	45	1.000
Neudesin-CRP	.19	[-.11, .46]	45	1.000
Neudesin-AGE	.34	[.05, .58]	45	.787
Neudesin-BMI	.19	[-.11, .45]	45	1.000
Neudesin-FPS	.26	[-.04, .51]	45	1.000
Neudesin-HbA1C	.27	[-.02, .52]	45	1.000
Neudesin-TC	.33	[.04, .57]	45	.890
Neudesin-TG	.33	[.05, .57]	45	.841
Neudesin-HDL	-.36	[-.59, -.07]	45	.601
Neudesin-LDL	.31	[.01, .55]	45	1.000
Neudesin-VLDL	.33	[.05, .57]	45	.841
CRP-AGE	.18	[-.12, .45]	45	1.000
CRP-BMI	.48	[.22, .68]	45	.044
CRP-FPS	.61	[.39, .77]	45	< .001
CRP-HbA1C	.46	[.19, .66]	45	.070
CRP-TC mg/dL	.31	[.02, .55]	45	1.000
CRP-TG mg/dL	.53	[.28, .71]	45	.011
CRP-HDL mg/dL	-.45	[-.66, -.19]	45	.083
CRP-LDL mg/dL	.25	[-.05, .50]	45	1.000
CRP-VLDL mg/dL	.53	[.28, .71]	45	.011

4.11 Pearson Correlation Analysis Control

Irisin, Neudesin, CRP, AGE, BMI, FPS mg/dL, HbA1C, TC mg/dL, TG mg/dL, HDL mg/dL, LDL mg/dL, and VLDL mg/dL were all correlated using Pearson correlation analysis. The correlations' outcomes were investigated. A significant positive correlation of r = 0.82 was found between Irisin and Neudesin, indicating a large effect size (p.001, 95 % Confidence interval = [.69,.90]). This suggests that if Irisin levels rise, so will Neudesin. A strong positive correlation of .77 was found between TC mg/dL and LDL mg/dL, indicating a large effect size (p.001, 95 % Confidence interval = [.62,.87]). This shows that as TC mg/dL increases, so does LDL mg/dL. A significant positive

correlation of 1.00 was found between TG mg/dL and VLDL mg/dL, indicating a strong effect size (p.001, 95 percent Confidence interval = [1.00, 1.00]). This shows that as TG mg/dL increases, so does VLDL mg/dL. There were no additional significant relationships observed. The correlation data are shown in Table 4.6.

Table 4.6 Pearson correlation results among irisin, neudesin, CRP, AGE, BMI, FPS mg/dL, HbA1C, TC mg/dL, TG mg/dL, HDL mg/dL, LDL mg/dL, and VLDL

Combination	r	95.00% CI	n	p
Irisin-Neudesin	.82	[.69, .90]	45	< 0.001
Irisin- CRP	.20	[-.10, .46]	45	1.000
Irisin-AGE	.15	[-.15, .42]	45	1.000
Irisin-BMI	-.14	[-.42, .16]	45	1.000
Irisin-FPS mg/dL	.25	[-.05, .51]	45	1.000
Irisin-HbA1C	-.28	[-.53, .02]	45	1.000
Irisin-TC mg/dL	.31	[.02, .55]	45	1.000
Irisin-TG mg/dL	-.05	[-.34, .24]	45	1.000
Irisin-HDL mg/dL	.21	[-.09, .48]	45	1.000
Irisin-LDL mg/dL	.23	[-.07, .49]	45	1.000
Irisin-VLDL mg/dL	-.05	[-.34, .24]	45	1.000
Neudesin- CRP	.05	[-.25, .34]	45	1.000
Neudesin-AGE	-.00	[-.30, .29]	45	1.000
Neudesin-BMI	-.13	[-.40, .17]	45	1.000
Neudesin-FPS mg/dL	.27	[-.02, .52]	45	1.000
Neudesin-HbA1C	-.27	[-.53, .02]	45	1.000
Neudesin-TC mg/dL	.20	[-.10, .47]	45	1.000
Neudesin-TG mg/dL	.05	[-.25, .34]	45	1.000
Neudesin-HDL mg/dL	.15	[-.15, .43]	45	1.000
Neudesin-LDL mg/dL	.07	[-.23, .36]	45	1.000
Neudesin-VLDL mg/dL	.05	[-.25, .34]	45	1.000
CRP-AGE	.09	[-.21, .38]	45	1.000
CRP-BMI	.06	[-.24, .35]	45	1.000
CRP-FPS mg/dL	.04	[-.26, .33]	45	1.000
CRP-HbA1C	-.35	[-.58, -.06]	45	1.000
CRP-TC mg/dL	.28	[-.01, .53]	45	1.000
CRP-TG mg/dL	-.02	[-.31, .27]	45	1.000
CRP-HDL mg/dL	.31	[.02, .56]	45	1.000
CRP-LDL mg/dL	.11	[-.19, .39]	45	1.000
CRP-VLDL mg/dL	-.02	[-.31, .27]	45	1.000

4.12 Linear Regression Analysis T2DM Group

In order to determine whether or not Neudesin was able to significantly predict Irisin, a linear regression analysis was carried out. The results of the linear regression model

were found to be significant, with F(1,43) = 64.98, p< .001, and R2 =.60. These results indicate that roughly 60.18 % of the variance in Irisin can be explained by Neudesin. Irisin may be predicted with a high degree of accuracy by Neudesin (B = 12.34, t(43) = 8.06, p <.001). This suggests that the value of Irisin will increase by 12.34 units on average whenever there is a one-unit increase in the amount of Neudesin. The findings of the regression model are outlined in Table 4.7, and Figure 4.13.

Table 4.7 Prediction of irisin based on linear regression with neudesin

Variable	B	SE	95.00% CI	β	t	p
(Intercept)	3.28	11.10	[-19.11, 25.68]	0.00	0.30	.769
Neudesin ng/ml	12.34	1.53	[9.25, 15.43]	0.78	8.06	< .001

Note. Results: F(1,43) = 64.98, p < .001, R2 = .60

Unstandardized Regression Equation: Irisin = 3.28 + 12.34*Neudesin

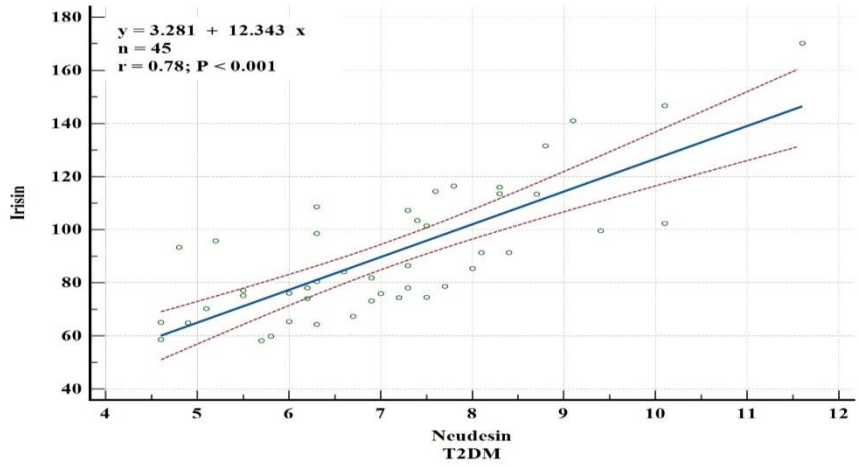


Figure 4.13 Scatter plot showing simple linear regression between Irisin and Neudesin in type 2 DM patients. A regression line with 95% CI boundaries were applied to ease the interpretation

4.13 ROC Analysis

Roc analysis was used to evaluate the sensitivity and specificity of the studied parameters in detection diabetic patients from control and calculating the Area under curve for each parameter. The results showed excellent AUC= 1.00 for all parameters

studied, details of the ROC characteristics are summarized in Table 4.8 and Figure 4.14 below.

Table 4.8 AUCs for Irisin, Neudisin, HbA1c and FBS to detect T2DM

Variable	AUC	SE	95% CI	Cutoffs	Sensitivity	Specificity
Irisin ng/ml	1.000	0.000698	0.959 to 1.000	>53.5	100.00	97.78
Neudesin ng/ml	1.000	0.000	0.960 to 1.000	>3.8	100.00	100.00
HbA1C%	1.000	0.000	0.960 to 1.000	>6.1	100.00	100.00
FBS	1.000	0.000	0.960 to 1.000	>98	100.00	100.00

"AUC" stands for "Area Under the Receiver Operating Characteristic Curve." "SE" stands for "Standard Error."

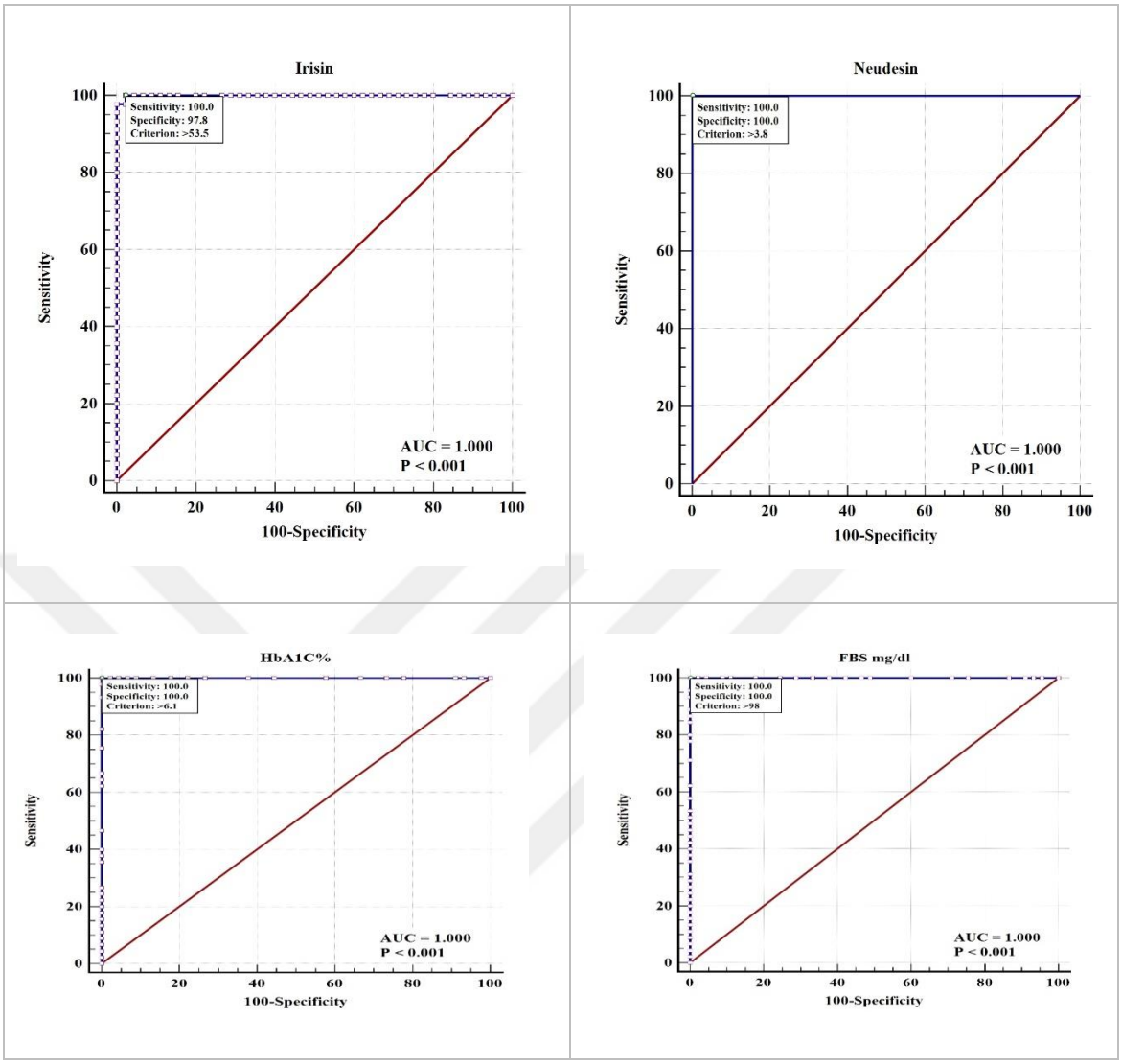


Figure 4.14 Irisin, Neudisin, HbA1c, and FBS were analyzed for their capacity to identify T2DM patients using ROC curves. Receiver Operating Characteristic Curve AUC

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

1.Measuring sera concentration of Neudesin and Irisin could be used as suggested diagnostic biomarkers for patients with newly T2DM.

2.Measurment of Neudesin and Irisin very useful to predict and prevent complication of patiant with diabetes mellitus type II.

3.Direct and highly significant correlation of Neudesin and Irisin with obesity, that may be help for using Neudesin and Irisin as early diagnostic marker of DM in obese patients.

4. The lipid profile and obesity were shown to have a strong association with these biomarkers, suggesting a link between them and diabetes complications such cardiovascular disease, atherosclerosis and stroke.

5.As Neudesin level differed significantly between the type 2 diabetic group compared to healthy controls, it can be concluded that high neudesin level exacerbated inflammation, insulin resistance, oxidative stress and other factors that contribute to the pathogenesis of type 2 diabetes mellitus.

5.2 Recommendations

1. Study Neudesin and Irisin in urine sample of patients with type 2 DM in a large sample study.

2. Patients with complications of type 2 diabetes should have their Neudesin and Irisin levels checked.

3. Detecting the mutation in the genes coding for Neudesin and Irisin in type 2 DM patients with a larger sample size from different center.

4. Compare the markers variable Neudesin and Irisin in patients' sera with T2DM before and after treatment.

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