

**A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY**

**BIOCHEMICAL STUDY OF LEPTIN, APELIN, IRISIN AND
INSULIN HORMONES IN DIAGNOSED PATIENTS WITH
HYPOTHYROIDISM**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY**

**BY
HARITH KHALEEL HAMAD AL-FAHDAWI**

ÇANKIRI

2023

BIOCHEMICAL STUDY OF LEPTIN, APELIN, IRISIN AND INSULIN
HORMONES IN DIAGNOSED PATIENTS WITH HYPOTHYROIDISM

By Harith Khaleel Hamad AL-FAHDAWI

February 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

Advisor : Prof. Dr. Volkan EYÜPOGLU

Examining Committee Members:

Chairman : Assoc. Prof. Dr. Şevki ADEM
Chemistry
Çankırı Karatekin University

Member : Asst. Prof. Dr. Ümit YIRTICI
Medical Laboratory
Kırıkkale University

Member : Prof. Dr. Volkan EYÜPOGLU
Chemistry
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

I hereby declare that all data and information in this thesis was gathered and presented in accordance with academic rules and ethical conduct. I further affirm that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Harith Khaleel Hamad AL-FAHDAWI

ABSTRACT

BIOCHEMICAL STUDY OF LEPTIN, APELIN, IRISIN AND INSULIN HORMONES IN DIAGNOSED PATIENTS WITH HYPOTHYROIDISM

Harith Khaleel Hamad AL-FAHDAWI

Master of Science in Chemistry

Advisor: Prof. Dr. Volkan EYÜPOGLU

February 2023

The relationship between Leptin, Apelin, Irisin, Insulin, and thyroid hormones (TH) in the blood and whether TH affects their levels were not yet known. From this perspective, it was important to examine whether the levels of these hormones fluctuate in thyroid disease (TD). Because it was not known how common TD was in Iraq. Diagnosis of subclinical and overt hypothyroidism was based on the results of blood tests that measure thyroid stimulating hormone (TSH), thyroxine hormone (T4) and triiodothyroxine hormone (T3). Patients were classified according to their TH levels at the time of examination. There were 90 participants in this study, divided into 3 groups: 30 euthyroid subjects (group I), 60 subclinical hypothyroid patients (group 2) and 55 patients with overt hypothyroidism (group 3). Mean levels of Leptin and Insulin were statistically significantly higher, whereas Apelin levels were statistically significantly lower in the overt hypothyroid group. Mean Irisin levels did not differ between groups. In terms of the correlation coefficient, Leptin, Apelin and Insulin revealed a significant positive correlation with TSH in the three groups and a significant negative correlation with T4 in individuals with overt hypothyroidism. Irisin levels were substantially positively correlated with T4 and significantly negatively correlated with TSH in the overt and subclinical hypothyroid groups. Thus, these findings support the role of these hormones in human hypothyroidism.

2023, 64 pages

Keywords: Hypothyroidism, Leptin, Apelin, Irisin, Insulin, TSH, T3, T4

ÖZET

HİPOTİROİDİZM TANI ALAN HASTALARDA LEPTİN, APELİN, İRİSİN VE İNSÜLİN HORMONLARININ BİYOKİMYASAL İNCELENMESİ

Harith Khaleel Hamad AL-FAHDAWI

Kimya, Yüksek Lisans

Tez Danışmanı: Prof. Dr. Volkan EYÜPOGLU

Şubat 2023

Leptin, apelin, irisin, insülin ve kandaki tiroid hormonları (TH) arasındaki ilişki ve bunların düzeylerini etkileyip etkilemediği henüz bilinmiyordu. Bu açıdan tiroid hastalığında (TD) bu hormonların düzeylerini etkileyip etkilemediğinin incelenmesi önemliydi. Subklinik ve aşikar hipotiroidizm teşhisi, tiroid uyarıcı hormon (TSH), tiroksin hormonu (T4) ve triiyodotiroksin hormonu (T3) düzeylerini ölçen kan testlerinin sonuçlarına dayanıyordu. Hastalar muayene anındaki TH seviyelerine göre sınıflandırıldı. Bu çalışmada 3 gruba ayrılan 90 katılımcı vardı: 30 tiroid hastası (grup 1), 60 subklinik hipotiroid hastası (grup 2) ve aşikâr hipotiroidizmi olan 55 hasta (grup 3). Belirgin hipotiroidi grubunda ortalama Leptin ve İnsülin seviyeleri istatistiksel olarak anlamlı derecede yüksek, Apelin seviyeleri ise istatistiksel olarak anlamlı derecede düşüktü. Ortalama irisin seviyeleri gruplar arasında farklılık göstermedi. Korelasyon katsayısı açısından Leptin, Apelin ve İnsülin, üç grupta TSH ile anlamlı pozitif korelasyon ve aşikar hipotiroidisi olan bireylerde T4 ile anlamlı negatif korelasyon gösterdi. Aşikar ve subklinik hipotiroid gruplarında irisin düzeyleri T4 ile büyük ölçüde pozitif, TSH ile anlamlı derecede negatif korelasyon gösterdi. Dolayısıyla, bu bulgular bu hormonların insan hipotiroidizmindeki rolünü desteklemektedir.

2023, 64 sayfa

Anahtar Kelimeler: Hipotiroidizm, Leptin, Apelin, İrisin, İnsülin, TSH, T3, T4

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Prof. Dr. Volkan EYÜPOĞLU, for his patience, guidance and understanding.

Harith Khaleel Hamad AL-FAHDAWI

Çankırı-2023



CONTENTS

ABSTRACT	i
ÖZET.....	ii
PREFACE AND ACKNOWLEDGEMENTS	iii
CONTENTS.....	iv
LIST OF SYMBOLS	vii
LIST OF ABBREVIATIONS	viii
LIST OF FIGURES	ix
LIST OF TABLES	x
1. INTRODUCTION	1
1.1 Aim of Study	2
2. LITERATURE REVIEW	3
2.1 Thyroid Gland	3
2.1.1 Physiology of thyroid gland	4
2.2 Thyroid hormones	5
2.2.1 Thyroid hormones production.....	5
2.2.2 Thyroid hormones synthesis	6
2.2.3 Thyroid hormones mechanism of action	7
2.2.4 Thyroid hormones regulation.....	8
2.2.5 Thyroid hormones function	9
2.2.6 Thyroid disorders	10
2.2.7 Thyroid hormones and TSH.....	14
2.3 Hypothyroidism	15
2.3.1 Screening and diagnosis of hypothyroidism.....	15
2.3.2 Symptoms of hypothyroidism.....	16
2.4 Leptin hormone relation to hypothyroidism.....	17
2.5 Apelin hormone relation to hypothyroidism.....	18
2.6 Irisin hormone relation to hypothyroidism.....	20
2.7 Fasting Insulin and HOMA-IR levels relation to hypothyroidism	20
3. MATERIALS AND METHODS	23
3.1 Study Design	23
3.2 Sample Collection	24

3.3 General Information	24
3.4 Materials.....	25
3.4.1 Equipments.....	25
3.5 Reagents for ECLIA.....	26
3.5.1 TSH elecsys kit	26
3.5.2 Total T3 elecsys kit	27
3.5.3 Total T4 elecsys kit	28
3.5.4 Insulin elecsys kit	29
3.6 Reagents for glucose and lipid profile	30
3.6.1 Serum glucose elecsys kit	30
3.6.2 Serum cholesterol Elecsys kit	31
3.6.3 Serum triglyceride elecsys kit.....	31
3.6.4 Serum HDL elecsys kit	32
3.6.5 Serum LDL-cholesterol elecsys kit.....	33
3.7 Reagents for ELISA	35
3.7.1 Leptin ELISA kit	35
3.7.2 Apelin ELISA kit	35
3.7.3 Irisin ELISA kit	36
3.8 Statistics Analysis	37
4. RESULTS.....	38
4.1 Patients categorizing and thyroid hormone concentrations	38
4.1.1 The level of TSH.....	38
4.1.2 The level of T4.....	39
4.1.3 The level of T3.....	40
4.2 The level of age and gender distributions.....	41
4.3 The level of BMI	42
4.4 The level of serum Leptin concentrations	43
4.5 The level of serum Apelin concentrations	44
4.6 The level of serum Irisin concentrations	45
4.7 The level of serum Insulin, glucose and HOMA concentrations.....	46
4.8 The level of serum lipid profile concentrations.....	49
4.8.1 Serum cholesterol levels	49
4.8.2 Serum TG levels.....	50

4.8.3 Serum HDL-cholesterol levels	51
4.8.4 Serum LDL-cholesterol levels.....	52
4.9 The result of correlation	53
5. DISCASSION.....	55
6. CONCLUSIONS AND RECOMMENDATION.....	59
REFERENCES.....	60
CURRICULUM VITAE	64



LIST OF SYMBOLS

%	Percent
±	Plus – minus
°C	Degrees Celsius
μg	Microgram
μIU	Microliter International Unit
μL	Microliter
dL	Deciliter
g	Gram
kDa	Kilodalton
L	Liter
mg	Milligram
mL	Milliliter
nm	Nanometer
nmol	Nanomole
pg	Picogram
rpm	Revolution per minute

LIST OF ABBREVIATIONS

APJ	G-protein coupled angiotensin-like 1
BMI	Body mass index
D	Deiodinase
DIT	Diiodotyrosine
ECLIA	Electrochemiluminescence immunoassays
ELISA	Enzyme-linked immunosorbent assay
FDNC5	Fibronectin type III domain-containing 5
FT3	Free triiodotyronine
FT4	Free tetraiodotyronine
HDL	High density lipoprotein
HOMA	Homeostatic model assessment
HPLC	High performance liquid chromatography
IR	Insulin resistance
LDL	Low density lipoprotein
MIT	Monoiodotyrosine
T2	Diiodo-L-thyronine
T3	Triiodothyroxin hormone
T4	Thyroxin hormone
Tg	Thyroglobulin
TG	Triglyceride
TH	Thyroid hormones
TPO	Thyroid peroxidase
TRH	Hypothalamic thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
VLDL	Very low density lipoprotein

LIST OF FIGURES

Figure 2.1 Macroscopic view of the posterior thyroid gland (Benvenga et al. 2018).....	4
Figure 2.2 Thyroid hormone regulation (Guariglia 2021)	9
Figure 2.3 Hypothyroidism causes (Guariglia 2021).....	12
Figure 2.4 The amino acid sequence of apelin isoforms (O'Carroll et al. 2013).....	19
Figure 4.1 Chart of TSH values in patients' groups and control	39
Figure 4.2 Chart of T4 values in patients' groups and control.....	40
Figure 4.3 Chart of T3 values in patients' groups and control.....	41
Figure 4.4 Chart of age in patients' groups and control	42
Figure 4.5 Chart of BMI in patients' groups and control	43
Figure 4.6 Chart of Leptin values in patients' groups and control	44
Figure 4.7 Chart of Apelin values in patients' groups and control.....	45
Figure 4.8 Chart of Irisin values in patients' groups and control	46
Figure 4.9 Chart of Insulin values in patients' groups and control	47
Figure 4.10 Chart of glucose values in patients' groups and control	48
Figure 4.11 Chart of HOMA-IR values in patients' groups and control	49
Figure 4.12 Chart of cholesterol values in patients' groups and control	50
Figure 4.13 Chart of TG values in patients' groups and control	51
Figure 4.14 Chart of HDL-cholesterol values in patients' groups and control	52
Figure 4.15 Chart of LDL-cholesterol values in patients' groups and control.....	53

LIST OF TABLES

Table 3.1 Expected values of HDL	33
Table 3.2 Expected values of LDL	34
Table 4.1 Classification of clinical status of thyroid	38
Table 4.2 TSH values in patients' groups and control	39
Table 4.3 T4 values in patients' groups and control.....	40
Table 4.4 T3 values in patients' groups and control.....	41
Table 4.5 Age and gender distribution in patients' groups and control.....	42
Table 4.6 BMI in patients' groups and control.....	43
Table 4.7 Leptin values in patients' groups and control.....	44
Table 4.8 Apelin values in patients' groups and control	45
Table 4.9 Irisin values in patients' groups and control.....	46
Table 4.10 Insulin values in patients' groups and control	47
Table 4.11 Glucose values in patients' groups and control	47
Table 4.12 HOMA-IR values in patients' groups and control.....	48
Table 4.13 Cholesterol values in patients' groups and control.....	49
Table 4.14 TG values in patients' groups and control.....	50
Table 4.15 HDL-cholesterol values in patients' groups and control	51
Table 4.16 LDL-cholesterol values in patients' groups and control	52
Table 4.17 The result of correlation for hormone in Euthyroid group.....	53
Table 4.18 The result of correlation for hormone in Subclinical Hypothyroidism group	54
Table 4.19 The result of correlation for hormone in Overt Hypothyroidism group	54

1. INTRODUCTION

It was believed that 200 million people worldwide have thyroid problems, and up to 60% of those suffering from thyroid dysfunction are unaware of their illness (Alzahrani *et al.* 2020). In fact, data from the Third National Health and Nutrition Examination Survey (NHANES III) showed that the prevalence of hypothyroidism in the general population was 4.6%, compared to 9.5% had elevated TSH levels (Canaris *et al.* 2000). Iodine deficiency is the most common cause of TD, with 2 billion people worldwide believed to have inadequate iodine intake. According to statistics provided by (Alzahrani *et al.* 2020), the prevalence of overt hypothyroidism in the general population in Western countries ranges from 0.2% to 5.3%, and the prevalence of subclinical hypothyroidism were it varies between 1% and 10% of the population. The prevalence of clinical hypothyroidism was higher in the elderly and women. Signs and symptoms suggestive of thyroid dysfunction were non-diagnostic and non-specific, especially in the early stages of the disease. Diagnosis is therefore based on blood levels of TSH and free thyroxine (FT4), and deficiencies in these hormones can have profound clinical effects in various body organs. Symptoms range from mild to life-threatening (myxedema coma) (Wilson *et al.* 2021). Due to the non-specific nature of hypothyroidism symptoms, up to 60% of people with TD are unaware of their illness, can exacerbate chronic diseases of hypothyroidism. Hypothyroidism was underdiagnosed and treated differently, but was known to be prevalent throughout the Arabian Gulf countries (Alzahrani *et al.* 2020). The prevalence of hypothyroidism in Iraqis has not been determined by national surveys. The prevalence of hypothyroidism in Iraqis has not been well defined by national surveys. Leptin was once thought to be an obesity-preventing hormone, but recent studies point to an important interaction between TH and Leptin to help regulate the transition from fed to starved states. It has also been shown to be a signal (Zimmermann-Belsing *et al.* 2003). By affecting specific receptors in the paraventricular hypothalamic nucleus, Leptin stimulates the thyroid gland and increases Hypothalamic thyrotropin-releasing hormone (TRH) release in people. Mutations in the Leptin receptor are associated with central hypothyroidism in humans (Menendez *et al.* 2003). When hypothyroid people were compared with

controls or/and euthyroid subjects, blood Leptin was found to be elevated in 5 studies, decreased in 3 studies, and unchanged in 8 studies (Zimmermann-Belsing *et al.* 2003). Patients with hypothyroidism, especially those who are obese, have elevated levels of Leptin and Insulin resistance (IR). Leptin levels do not affect IR, but are positively associated with BMI in obese children and hypothyroid adolescents (El Amrousy *et al.* 2022). Therefore, in this study, we focused on the correlation of Leptin and IR with hypothyroidism. Irisin, was a recently discovered adipomyokine. It was produced by extracellular cleavage of fibronectin type-3 domain-containing-5 (FDNC5). Overexpression of FDNC5/Irisin has been demonstrated to cause browning of white adipose tissue and increase thermogenesis, helping to reduce IR and improve glucose homeostasis. Many tissues, including thyroid tissue, were found to contain FDNC5, the precursor of Irisin (Yasar *et al.* 2019). Increased energy expenditure and browning of adipose tissue are both effects of TH and Irisin (Panagiotou *et al.* 2016). Apelin was the endogenous ligand for the G protein-coupled angiotensin-like-1 (APJ) receptor, the primary source of which is adipose tissue. APJ was expressed in brain, adrenal gland, endothelium, plasma cells, adipose tissue, kidney, liver, gastrointestinal system, and heart. Apelin's effects on diabetes, IR, and hypertension were investigated (Yasar *et al.* 2019). Weight changes, thermogenesis, and lipolysis of adipose tissue are common in people with TD. Although both Apelin and TH serve important functions in metabolism (Gürel *et al.* 2015), it was still unknown whether blood levels of the two hormones were related or whether TH influences Apelin levels. From this perspective, it was important to examine whether Apelin levels are altered in hypothyroidism.

1.1 Aim of Study

1. To measure blood levels of the hormones Leptin, Apelin, Irisin, and Insulin in patients with subclinical and overt hypothyroidism and compare them with normothyroid values (euthyroidism) in Iraqi adults with hypothyroidism.
2. To investigate the associations between thyroid hormones and Leptin, Apelin, Irisin, and Insulin parameters in Iraqi participants with both subclinical and overt hypothyroidism.

2. LITERATURE REVIEW

2.1 Thyroid Gland

The thyroid gland, an essential butterfly shaped endocrine gland, was located in the lower neck. It lies below the larynx and was found on the front and sides of the trachea. In addition to being important for calcium metabolism, it stimulates physical and cognitive development and regulates the essential metabolic rate. It is a gland with left and right lobes joined by an isthmus, an intermediary tissue. The pyramidal lobe (third lobe), may project from the isthmus. The levator thyroid muscle, a band of fibrous/fibrous muscles, runs from the body of the hyoid bone to the isthmus. Lobe weighs approximately 25 g and measures (5 x 2.5 x 2.5 cm). It extends from the first thoracic vertebra to the fifth cervical vertebra. The 5th ring of the trachea is reached by the lung lobes, which start at the thyroid cartilage's center. The isthmus connecting the second and third tracheal rings is 1.2×1.2 cm in size. In women, it increases during menstruation and pregnancy.

Men have a heavier thyroid than women. A newborn's thyroid weighs about 1 g and increases by about 1 g each year until the age of 15. It is an H-shaped, flexible, reddish parenchyma organ with two left and right lobes and an isthmus connecting them (Fig. 2.1). The lower half (called the inferior horn) reaches the level of the fifth or sixth tracheal ring, and the upper limb (called the superior horn) lies lateral to the inferior constrictor and posterior to the sternothyroid muscle. The carotid sheath and its components are covered by glands in the posterolateral part. Pyramidal lobes (Morgagni or Laruetto pyramids), emerging from either the lobe or the upper region of the isthmus and pointing upwards, usually to the left, are found in about 50% of people (Figure 2.1) (Benvenga *et al.* 2018).

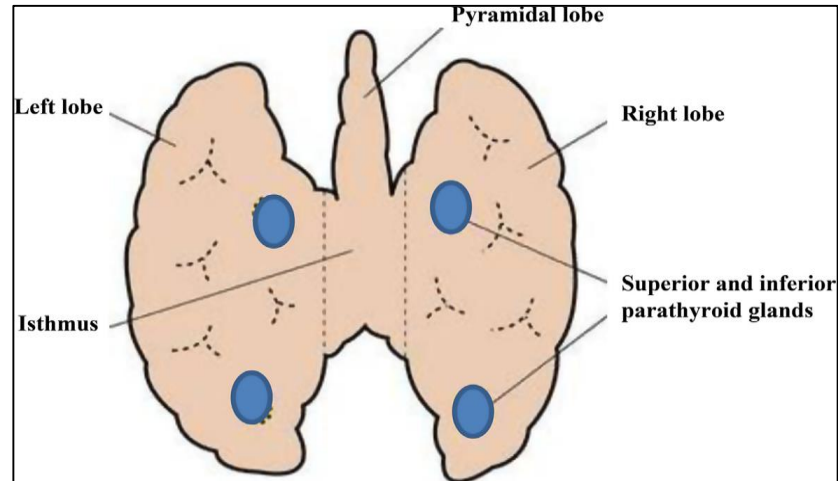


Figure 2.1 Macroscopic view of the posterior thyroid gland (Benvenga et al. 2018)

2.1.1 Physiology of thyroid gland

In primary hypothyroidism, the thyroid produces less TH and TSH rises as a protective mechanism. Pituitary abnormalities resulting in reduced TSH secretion and lower T3 and/or T4 concentration are the cause of secondary hypothyroidism. Tertiary hypothyroidism is caused by hypothalamic abnormalities that reduce TRH, TSH, and levels of T3/T4 (Brent 2012).

Hyperthyroidism: Thyroid disorders can lead to excessive synthesis of T3 and T4 and a corresponding decrease in TSH. Thyroid-irritating adenomas can also cause excessive T3 and T4 production and uncontrolled TSH production. Rarely, TH is produced ectopically, resulting in elevated T3 and T4 and decreased TSH (Brent 2012).

Graves' disease can lead to hyperthyroidism if thyroid tumors (such as toxic adenoma) or excess TSH are secreted, or if exogenous T3 or T4 is used (Stathatos 2012).

The Wolf-Tchaikoff effect occurs when increasing iodine doses initially stimulate TH production in people with autoimmune thyroiditis. Negative feedback causes TH production to stop if the iodine content is further elevated. The thyroid gland can prevent this loss of iodine by releasing and leaking iodine in people with functioning

thyroid tissue. When increased, the thyroid gland is unable to adapt and compensate for decreased TH production, and patients develop hypothyroidism (Stathatos 2012).

2.2 Thyroid hormones

The biological activity of TH molecules was modified by deiodinases, which can convert T4 to 3,5,3'-T3, inactivate T3 to 3,3'-diiodo-L-thyronine (T2), or convert T4 to reverse triiodothyronine (rT3). The current paradigm assumes that the deiodinases not only contribute to the balance of circulating T3, but also offer TH signaling dynamic control. Cells that express type 2 deiodinase (D2), the activating deiodinase, have enhanced TH signaling because of intracellular accumulation of T3, whereas type 3 deiodinase (D3), the inactivating deiodinase, is expressed by the opposite type of cell. D2 and D3 have been studied using cell, animal, and human models because they are expressed in areas that are important for metabolism, including brown adipose tissue, liver, and the skeletal muscle. Measuring the amount of free thyroid hormone has important diagnostic implications because only a small fraction of the circulating hormone is free (unbound) and physiologically active. An important criterion is the amount of free T3 and T4, since THs are inactive while bound (Russo *et al.* 2021).

2.2.1 Thyroid hormones production

Thyroid hormones (mainly T4 and T3) are very important for healthy development and adult physiology. However, finding compounds that adversely affect the thyroid system through interaction with the thyroid system is difficult because of many problems. Only about 10% of the representing individual's differences in serum concentrations of hormones. Therefore, population studies examining associations between chemical exposures and serum thyroid hormones must be large enough to account for this internal variability (Zoeller 2021).

The hypothalamic-pituitary-thyroid (HPT) axis mediates feedback processes that control the production of THs. Decreased TH levels increase hypothalamic TRH synthesis and

increase anterior pituitary TSH output. TSH stimulates thyroid cells to produce THs. TH synthesis requires thyroglobulin (Tg) synthesis, thyroid peroxidase (TPO), and active iodide uptake by the sodium/iodide symporter (NIS). Tg proteolysis releases T4 and T3. The thyroid gland releases T4 in fairly large amounts (ratio of about 14:1). D1 and D2 act in target tissues to convert the majority of T4 to T3. More than 99.7% of TH produced in plasma is associated with plasma proteins. The remaining 0.3% is in unbound (free) forms (FT4 and FT3). THs are physiologically active in their unbound state. Fluctuations in TSH and TH levels may indicate changes in the course of normal thyroid function (Babi Leko *et al.* 2021).

2.2.2 Thyroid hormones synthesis

Below were the five phases of thyroid hormones synthesis:

1. **Thyroglobulin Synthesis:** Thyroid cells within the thyroid follicle make a protein known as Tg. Tg is an iodine-free precursor protein that is retained in the follicular cavity. The rough endoplasmic reticulum is where it is made. It is packed into vesicles by the Golgi apparatus and subsequently exocytosed into the follicle lumen.
2. **Iodide uptake:** Phosphorylation of protein kinase A increases the ability of the basolateral $\text{Na}^+\text{-I}^-$ symporter to transport iodide from the bloodstream to thyroid cells under the control of $\text{Na}^+\text{-K}^+\text{-ATPase}$. Iodide then moves through the cell's apex and basolateral regions before diffusing to the colloid through the pendrin carrier.
3. **Thyroglobulin iodination:** TPO is phosphorylated and activated by protein kinase A. Oxidation, organization and coupling reactions are the three functions of TPO.
 - a. **Oxidation:** Iodide (I^-) is oxidized by TPO using hydrogen peroxide to produce iodine (I_2). The tip enzyme NADPH oxidase produces hydrogen peroxide for TPO.

- b. Organization: The tyrosine residue of the Tg is connected to I₂ by TPO. Produces DI and monoiodotyrosine (MIT) and diiodotyrosine (DIT). Iodine-containing tyrosine residues are present at one tyrosine residue in MIT and two tyrosine residues in DIT.
 - c. Coupling reaction: TPO binds to an iodinated tyrosine residue to generate T3 and T4. T4 is produced when two DIT molecules combine and T3 produce from MIT and DIT.
4. Storage: TH is retained in the follicular lumen by binding to Tg.
5. Release: Thyroid cells release TH into a network of fenestrated capillaries in the manner described below.
- a. Thyroglobulin iodide is taken up by thyroid cells by endocytosis.
 - b. Endosomes carrying iodinated thyroglobulin and lysosomes fuse.
 - c. Tg is degraded to MIT, DIT, T3, and T4 by endolysosomal proteases.
 - d. MCT8 transporter discharges 20% T3 and 80% of T4 into permeable capillaries.
 - e. Molecular iodine is removed from DIT and MIT by deiodinase. Iodine is recovered and distributed in an intracellular iodide pool (Brent 2012, Benvenga *et al.* 2018).

2.2.3 Thyroid hormones mechanism of action

The processes by which TH affect many target peripheral tissues are diverse. Briefly, D1 or D2 catalyzes the enzymatic activity that converts the thyroid's major output, T4, to the active hormone, T3. D3 has the ability to disable T4 and T3. T4 and T3 enter cells via specific membrane transporters, and T3 from blood or intracellular conversion of T4 to T3 binds to TH receptors of subtype 1, $\beta 1$ or $\beta 2$ found in the nucleus to regulate the

transcription of target genes. Various non-classical pathways have been described recently, but this is the conventional one. Cytosolic or mitochondrial TH receptors (TRs) may be involved in mediating TH effects and binding TH to nonspecific membrane proteins leading to cascades of signaling pathways.

These unconventional effectors reportedly play important roles in the effects of THs on cardiac metabolism. To discriminate between T3 motivate by canonical and non-canonical pathways, the authors of that fruitful study utilized genetically modified mice. They showed that deoxyribonucleic acid binding was not required for the immediate hypoglycemic effects of T3, but was dependent on TR. The phosphatidylinositol 3-kinase signaling cascade is activated as a result of its effects. The same non-canonical signaling mechanism contributes to the reduction of T3 in liver and serum triglycerides. In addition, non-canonical signaling pathways play an important role in the effects of T3 on the external regulation of metabolic rate, energy expenditure, and heart rate (Teixeira *et al.* 2020).

2.2.4 Thyroid hormones regulation

The major product released by the thyroid follicle, T4, and the by-product, the active hormone T3, are collectively called TH. Deiodination of T4 in peripheral tissues produces the majority of T3. Lifelong tissue function and development of many tissues, especially the central nervous system, are dependent on TH. Although the hypothalamic-pituitary thyroid axis controls TH production, the biological actions of TH are primarily controlled at the level of target tissues (Teumer *et al.* 2018). TSH controls the synthesis of T4 and T3. In the negative feedback loop formed by the thyroid and TSH (Figure 2.2), high T4 levels reduce TSH production and vice versa. Thyrotrophic-releasing hormone (TRH), produced by the hypothalamus and released at a high rate under cold-like conditions, itself regulates the production of TSH (when metabolism accelerates, more heat is released). Elevated somatostatin, glucocorticoid and sex hormone (estrogen and testosterone) levels, and excessively high blood iodide levels all inhibit TSH production (Benvenga *et al.* 2018).

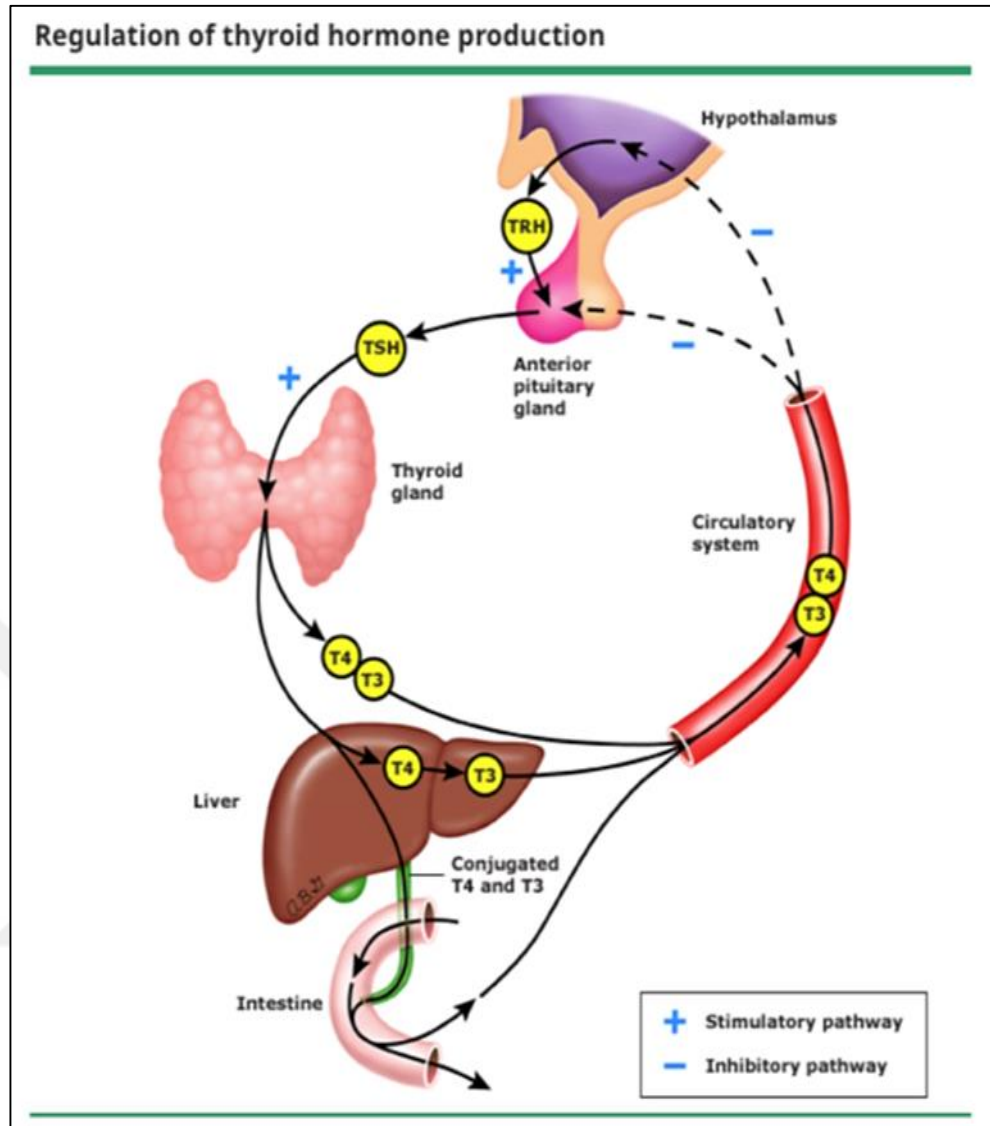


Figure 2.2 Thyroid hormone regulation (Guariglia 2021)

2.2.5 Thyroid hormones function

The following list contains some of the important roles of thyroid hormones.

1. Supports the general grow, develop and differentiate of the body's tissues.
2. Regulates the body's metabolic rate.
3. Essential for calcium metabolism.
4. Support children's CNS function and general CNS growth.

5. Promotes mental and physical development.
6. Promote contractions and heart rate.
7. Helps build calcium and phosphate in bones, strengthening bones.
8. Lower blood calcium levels.
9. Regulates protein, fat and carbohydrate metabolism.
10. It also helps metabolize vitamins.
11. Control body heat and cold.
12. Helps break down triglycerides and cholesterol.
13. Maintain electrolyte balance.
14. Helps produce red blood cells.
15. They increase mitochondrial metabolism.
16. Allows cells and tissues to use more oxygen.
17. Influence people's attitudes and behavior.
18. Promotes intestinal motility.
19. It also makes beta-adrenergic receptors more sensitive to catecholamines.

As a result, almost every cell in the body is affected by TH. They also play important roles in the formation, growth, and operation of the majority of the body's tissues and organs.

2.2.6 Thyroid disorders

Hypothyroidism and hyperthyroidism are the two major forms of TD. Both problems can be caused by other diseases that affect thyroid function. TD can occur in the following situations:

Hyperthyroidism: Thyrotoxicosis is the clinical condition of excess TH circulating regardless of source, whereas hyperthyroidism is associated with increased TH production and release from the thyroid gland. Defined by low serum levels of TSH and high levels of FT4, FT3, or both characterize thyrotoxicosis. As a result, there are two subgroups of thyrotoxicosis. Those with hyperthyroidism, such as Graves' disease and

Plummer's disease, and those without hyperthyroidism, including subacute and indolent thyroiditis (Yanai 2019).

Hypothyroidism: This disorder results from an anatomical or functional abnormality that causes decreased production of THs. Clinical manifestations of the disease include myxedema in adults and cretinism in infants. Hypothyroidism can affect a person's quality of life by causing weight fluctuations, weakness, fatigue and depressive symptoms. Insufficient dietary iodine intake is the leading cause of hypothyroidism in underdeveloped areas of the world (Figure 2.3). As a result, some countries are trying to increase iodine consumption by iodizing salt. Hashimoto's disease is the most typical cause of hypothyroidism in regions of the world where individuals consume adequate amounts of iodine. Levothyroxine often needs to be taken for a very long period of time, sometimes lifelong, to treat hypothyroidism. Levothyroxine replenishes a person's thyroid hormone levels and improves mood. However, the dosage should be changed frequently for optimal results. Furthermore, many hypothyroid patients are unaware of their condition (Chiovato and Carlé 2019).

Hashimoto's thyroiditis: Hashimoto's thyroiditis is the most frequent cause of hypothyroidism where adequate iodine is available. It was generated by the breakdown of the thyroid gland by the immune system. Thyroid follicular cell death is caused by CD8⁺ T lymphocytes. TH1 cells attract and activate macrophages by releasing IFN- γ . Patients may have a nontender, symmetrical, painless goiter in the early stages of the disease. Thyroid follicles are damaged and can rupture if inflammation continues. Patients with thyroid follicular rupture may be asymptomatic or develop ashtoxicosis (ruptured follicles secrete TH, causing symptoms of hyperthyroidism). Depending on the degree of fibrosis, the thyroid gland may grow or shrink as the condition progresses. As a result, patients may begin to experience symptoms of hypothyroidism. Anti-thyroid autoantibodies are also produced in addition to cell-mediated cytotoxicity, leading to antibody-dependent cell-mediated cytotoxicity (Brent 2012).

Although 10–20% of the general population have thyroid antibodies, thyroid antibody positivity is virtually ubiquitous (>95%) in patients with overt hypothyroidism and

subclinical hypothyroidism. present in 50% of patients with the disease (Chiovato and Carlé 2019). Thyroid function tests, antibody detection, and ultrasound are used to identify Hashimoto's thyroiditis. Radioactive iodine absorption testing and fine needle aspiration can be used to rule out cancer (Brent 2012).



Figure 2.3 Hypothyroidism causes (Guariglia 2021)

Graves disease: is an autoimmune condition brought on by TSH receptor antibody generation. It is the most typical cause of hyperthyroidism. The creation of TH release and thyroid growth-promoting TSH receptor antibodies is responsible for this autoimmune condition. Patients have abnormally elevated levels of T4 and T3 and decreased TSH. Diagnosis is confirmed by a positive TSH receptor immunoglobulin G (IgG) test. Thyroid function and growth are improved by IgG against the TSH receptor. Patients frequently exhibit symptoms of generalized goiter and hyperthyroidism. Antibodies to TSH receptors can promote fibroblast proliferation and adipocyte development in ocular fibroblasts. As a result, hyaluronic acid and glycosaminoglycans (GAG) are produced more frequently, increasing the amount of intraorbital fat and muscle tissue. Eye movement problems cause proptosis, retraction of eyelids, and double vision. An additional symptom of Graves' disease is tibialis anterior muscle edema. GAG is deposited in connective tissue as a result of stimulation of dermal

fibroblasts. Anti-TPO antibodies are high in seventy percent of Graves' disease patients (Brent 2012).

Goiter: This disease causes an abnormal swelling of the thyroid gland. Broadly speaking, there are three forms of goiter: diffuse, multinodular, and uninodular. Depending on the parameters used to characterize this form of goiter, the incidence of retrosternal goiter ranges from 0.2- 45% of all thyroidectomies. Most patients report some form of pulmonary symptoms associated with goiter, and symptoms are often related to the compressibility of the surrounding structural mass. Testing of thyroid function, chest x-ray radiography, and computed tomography are often included in the diagnostic evaluation. Surgery is the only effective treatment for retrosternal goiter. This is because drug therapy usually fails in these situations (Knobel 2021).

Colloidal nodular goiter: It is the most common non neoplastic lesion of the thyroid. The thyroid follicles in this type of goiter are adjacent to squamous follicular cells and their lumen is filled with many colloids. The main mitotic factor is TSH. Substances that increase TSH promote the development of nodules. As a result of iodine deficiency, thyroid nodules become more common in areas where goiter is endemic. For the same reasons that the incidence of thyroid cancer is increasing, so is the prevalence of nodules. Publications around the world discuss the increasing prevalence of thyroid cancer, especially related to papillary cancer. This large outbreak has several causes. In particular, metabolic syndrome and IR play important roles in the current increased incidence of nodular goiter and papillary thyroid cancer (Simsir *et al.* 2020).

Thyroid cancer: Both parafollicular C cells or follicular epithelium can cause thyroid cancer. Compression that moves painless nodules and nearby structures: Papillary, follicular, undifferentiated, and medullary carcinoma are four different types of thyroid cancer that may appear (Brent 2012).

2.2.7 Thyroid hormones and TSH

One of the important thyroid tests is the TSH. The most accurate way to identify thyroid problems is the TSH test. The controversy regarding the laboratory reference range for TSH has largely been resolved, and a range of approximately 0.4- 4.0 mU/L is now widely recognized. Except for individuals with pituitary dysfunction with inaccurate TSH, additional testing such as FT3, FT4, or thyroid antibodies is of little value if TSH levels are within the reference range (Walsh 2016). TRH, which is released by the hypothalamus, increases the pituitary gland's production of TSH. A negative feedback loop created by elevated FT4 and FT3 prevents TRH and TSH from being released. Iodine absorption and T3 and T4 secretion are thus decreased. TSH synthesis is also inhibited by other hormones such as glucocorticoids, somatostatin, and dopamine. Stress, exercise and cold all boost TRH release. TSH and the FT4 test are the first procedures of preference to check for any these establish whether the aberration originates peripherally from the pituitary (secondary), mostly from the primary hypothyroidism, or from the hypothalamus (tertiary). If (primary) hypothyroidism is suspected, the thyroid gland does not release sufficient TH. The result is a decrease in FT4 levels and an appropriate increase in TSH levels. In primary hyperthyroidism, FT4 levels are abnormally high and TSH levels are moderately low. TSH receptor antibody and TPOAb are two other laboratory tests that can be used to diagnose Graves' disease and Hashimoto's thyroiditis, respectively (Brent 2012).

Overt and subclinical hypothyroidism with TSH levels above 10 mU/L can be treated without additional testing. The health consequences of asymptomatic hypothyroidism with slightly elevated TSH levels (4 to 10 mU/L) are still unknown, especially in the elderly. Treatment or monitoring is suitable alternatives (Walsh 2016). With normal levels of FT3 and FT4, subclinical hypothyroidism is identified by elevated blood TSH levels. The prevalence of subclinical hypothyroidism ranges from 4% to 10% in the general population and 7% to 26% in the elderly. About 2–5% of patients with subclinical hypothyroidism develop overt hypothyroidism each year (Yasar *et al.* 2019).

In most cases, overt hypothyroidism (high TSH, low FT4) is symptomatic, easily diagnosed, and treatable without further testing. Elevated TSH levels with normal FT4 is a more frequent symptom in general practice. This may be a sign of subclinical hypothyroidism caused by autoimmune TD, but it is also a sign of systemic non-TD, especially while the patient is recovering. Possibly, in more than 50% of patients with moderately high TSH (about up to 10 mU/L), medication is not always necessary, as TSH often returns to normal without treatment. Instead, TSH, FT4, and TPOAb tests should be repeated in 6 to 8 weeks and treated if abnormalities persist. Screening for hypothyroidism does not require thyroid imaging, including ultrasound. Uncertainty surrounds the value of diagnosing and managing subclinical hypothyroidism. Thyroxine has erratic effects on symptoms and quality of life in randomized controlled trials, especially in the elderly and in those with slightly elevated TSH levels up to 10 mU/L. Untreated subclinical hypothyroidism with TSH levels above 10 mU/L increases the risk of cardiovascular disease. Young and middle-aged patients may benefit more from subclinical hypothyroidism treatment than older patients (Walsh 2016).

2.3 Hypothyroidism

2.3.1 Screening and diagnosis of hypothyroidism

Regarding regular testing for adult hypothyroidism in the population, there was no agreement. Symptoms are common, but presence of many characteristic symptoms can raise suspicion of hypothyroidism. All patients with hypercholesterolemia, infants, pregnant women, and members of high-risk groups (such those with goiter, as those with a family history of the condition or those who tested positive for anti-TPOAb), should be screened for hypothyroidism. Repeat TSH testing after 1-3 months should confirm the diagnosis of subclinical hypothyroidism (Alzahrani *et al.* 2020).

Ninety percent of all subclinical hypothyroidism patients had TSH levels 4-10 mIU/L. TSH levels can raise with age, and modest increases in TSH levels may be typical of the elderly. Within 5 years, 62% of TSH levels between 4 to 10 mIU/L normalize naturally. TSH levels are subject to biological variation. Stress and short-term illness can increase

levels. Due to biological variability in TSH measurements, a second blood test should be performed to confirm the diagnosis after a single detection of an abnormal TSH level (Bekkering *et al.* 2019).

Depending on symptoms and FT4 levels, individuals with elevated TSH levels are divided into two groups: overt and subclinical hypothyroidism. Patients with overt hypothyroidism should receive thyroid replacement therapy with levothyroxine. In individuals with subclinical hypothyroidism, TSH levels should be checked after 1-3 months. Levothyroxine can be started if both TSH values are above 10 mIU/L. Otherwise; these patients should be checked for TPOAb levels. Levothyroxine can be started in TPOAb-positive patients with TSH levels above normal but below 10 mIU/L. Patients without TPOAb with two elevated TSH measurements (above the upper limits of the normal range but >10 mIU/L). Levothyroxine should be taken into consideration if the patient has goiter, is pregnant, is infertile, or has any of these conditions (Alzahrani *et al.* 2020). Due to the lack of specificity in common clinical signs, diagnosis of hypothyroidism relies primarily on laboratory investigations. A subclinical hypothyroidism is biochemically defined as normal FT4 levels associated with elevated TSH levels; whereas primary hypothyroidism is associated with elevated TSH levels and FT4. Secondary hypothyroidism characterized by low FT4 concentration and inappropriate elevation of TSH levels (Gaitonde *et al.* 2012).

A suitable screening test for ambulatory hypothyroidism is the measurement of TSH test (as opposed to free or total T4). However, TSH may not be a useful tool for diagnosing hypothyroidism if pituitary or hypothalamic disease is known or suspected. In hospitalized patients, as there are many other factors in patients with acute or chronic disease hyperthyroidism that affect TSH secretion; and in patients taking medications or having underlying medical conditions that affect TSH test (Gaitonde *et al.* 2012).

2.3.2 Symptoms of hypothyroidism

A general decline in basal metabolic rate can manifest as cognitive slowing, baldness, dry skin, increased elevated triglycerides and low-density lipoprotein. When dealing

with psychotic individuals who display apathy and cognitive decline, hypothyroidism should be checked out. Hypothyroidism reduces sympathetic activity and affects bradycardia, constipation, and sweating. Patients may develop myopathy and reduced cardiac output as a result of downregulated transcription of sarcolemmal genes.

Hypothyroidism can cause hyperprolactinemia. Hypothalamic TRH triggers the release of prolactin and TSH. GnRH, LH, FSH, and testosterone release are all inhibited by prolactin. Breast tissue may also enlarge as a result of prolactin. Patients with hypothyroidism may develop myxedema due to reduced ability of the reticular layer of the dermis to remove complex glycosaminoglycans and hyaluronic acid. First tibialis anterior, non-depressed edema. Patients with hypothyroidism may develop edema as their condition worsens. Bradycardia, cold aversion, fatigue, weight gain, and other signs of slowed metabolic rate: Anorexia, dry skin, hair loss, constipation, slow relaxation of deep tendon reflexes, confinement, cramps, stiffness, myopathy

Symptoms of generalized myxedema: pretibial and periorbital edema, trouble speaking words and hoarseness and myxedema heart disease. Symptoms of: depression, poor memory and concentration, goiter, persistent neonatal jaundice, hypertension, umbilical hernia, hypotonia, and poor eating development are all symptoms of congenital hypothyroidism (Brent 2012).

2.4 Leptin hormone relation to hypothyroidism

The obesity gene produces the protein Leptin, which is produced primarily by adipocytes. Leptin suppresses food intake, causes weight loss, and has a profound effect on energy balance. Leptin, secreted into adipose tissue, regulates IR, appetite, and obesity (El Amrousy *et al.* 2022). Leptin levels are inversely related to total adipose tissue mass, so losing weight lowers serum leptin levels. The majority of studies examining weight loss in obese individuals show decreased blood TSH and T3 levels, which may be related to decreased Leptin levels (Al Mohareb *et al.* 2021). By affecting specific receptors in the paraventricular hypothalamic nucleus, Leptin stimulates the thyroid gland and increases TRH release in people. Human Leptin receptor mutations

are associated with central hypothyroidism (Teixeira *et al.* 2020, Menendez *et al.* 2003). Decreased lipolysis and oxygen consumption are all effects of hypothyroidism and may also affect adipose tissue function (Teixeira *et al.* 2020, Duntas 2002). Children and adolescents with hypothyroidism, especially those who are obese, have elevated levels of Leptin and IR. Leptin levels do not affect IR, but are positively associated with BMI in obese children and hypothyroid adolescents (El Amrousy *et al.* 2022).

Even after considering several variables such as autoimmunity, Leptin pulsatility, Leptin-binding protein, fertility, estrogen, cortisol, beta 3 adrenergic agonists, Insulin and thermogenesis, explanations for conflicting results are could not identify. However, Leptin and the thyroid are thought to be related, possibly due to Leptin's effects on the negative feedback regulation of TH and effects on thermogenesis.

Leptin has been proposed to control TRH production in the hypothalamus when Insulin is present. Peripherally, T3 suppresses the accumulation of Leptin mRNA in Insulin-starved adipocytes, whereas the opposite is probably true in the Insulin-presence fed state. Furthermore, Leptin itself can promote T3 synthesis by activating the conversion of T4 to T3 in rats. Increased T3 production, as seen in thyrotoxicosis, stimulates the production of fever (Zimmermann-Belsing *et al.* 2003).

Leptin has also been demonstrated to control the ability of the midhypothalamus to produce neurohormones, including those produced by TRH in the periventricular nucleus. An further study, Leptin stimulated TRH by directly and indirectly activating the signal transducer and Janus kinase and activator of transcription pathway. It has been demonstrated that increased release of TRH leads to a rise in TSH production from the pituitary gland and promtes thyroid growth and function (Teixeira *et al.* 2020).

2.5 Apelin hormone relation to hypothyroidism

Adipose tissue is the major source of Apelin, the APJ is expressed in human adrenal glands, endothelium, heart, liver, lung, kidney, adipose tissue, gastrointestinal system,

brain and plasma cells (Yasar *et al.* 2019). The Apelin gene produces human Apelin. This peptide contains a 77-amino acid precursor called pre-proapelin, which may have several chemical forms with different biological functions. Apelin-36 (pre-pro-apelin-42-77), Apelin-17 (pre-pro-apelin-61-77), and Apelin-13 (pre-pro-apelin-65-77) and pyroglutamate-apelin-13 (Figure 2.4) is the active form. A form of natural pre-proapelin produced as a result of enzymatic hydrolysis (Read *et al.* 2020). Apelin receptors are determined in the hypothalamus and thyroid, but their role in the hypothalamus-pituitary-thyroid axis and their mechanism of action on energy metabolism are still unclear. It is also unclear whether there is a relationship between blood levels of Apelin and hypothyroidism, and whether TH affects Apelin levels. (Erden *et al.* 2018).

The study by (Erden *et al.* 2018) was to investigate the potential effects of apelin-13 administration on the the hypothalamus-pituitary-thyroid axis and energy metabolism. According to this study, blood FT3 and FT4 levels were significantly reduced in the apelin-13 treated group ($p < 0.05$). A study by (Gürel *et al.* 2015) found that the hypothyroid group had the highest mean Apelin levels and the control group the lowest, but there was no statistically significant difference in mean Apelin levels between groups.

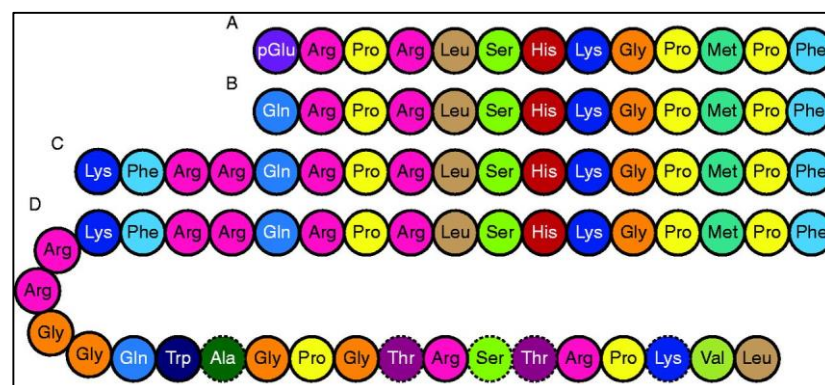


Figure 2.4 The amino acid sequence of apelin isoforms (O'Carroll *et al.* 2013)

2.6 Irisin hormone relation to hypothyroidism

Irisin is the proteolytically degraded version of FNDC5. Since its discovery, Irisin has received much attention due to its extensive physiopathological functions in several metabolic diseases. Irisin contains antioxidant, anti-inflammatory, and anti-apoptotic properties. Therefore, Irisin can be used as a diagnostic and therapeutic target for diabetes, obesity, non-alcoholic fatty liver disease, osteoporosis, and even cancer to treat or detect these conditions. The peroxisome proliferator-activated receptor gamma and uncoupling protein-1 are expressed as a result of Irisin. In human adipocytes, T3 and Irisin have the ability to promote uncoupling protein-1 expression, reduce lipid synthesis, and prevent DNA damage (Eshraghian and Jahromi 2014).

It is possible to speculate that Irisin levels may be altered in conditions of TD, based on the comparable physiological roles and potential relevance of THs and Irisin, and there is conflicting information about the role of Irisin in patients with hypothyroidism and hyperthyroidism. A thorough evaluation of the currently available information on Irisin levels in individuals with TD is needed. This is because they are potentially new diagnostic markers and regulatory hormones of metabolism. The metabolism of the human body is greatly influenced by Irisin and TH. Based on similarities, numerous studies have been conducted to investigate variations in Irisin levels in people with hypothyroidism and hyperthyroidism (Eshraghian and Jahromi 2014). Correlation analysis results showed no association between Irisin and TH (Panagiotou *et al.* 2016, Eshraghian and Jahromi 2014).

2.7 Fasting Insulin and HOMA-IR levels relation to hypothyroidism

Insulin resistance is characterized by decreased sensitivity of muscle, adipose tissue, liver, and other body tissues to Insulin despite normal or elevated blood levels of the hormone. IR occurs when the biological effects of Insulin on liver, skeletal muscle, adipose tissue, and other peripheral tissues are lower than they should be. IR in hypothyroidism has been proposed to result from peripheral-IR occurring in skeletal muscle and adipose tissue as a result of altered expression of glucose transporters. The

development of IR can be influenced by alterations in glucose transporters in the cell plasma membrane, which could be caused by a deficiency of TH. TH deficiency is also associated with alterations in peripheral blood flow (El Amrousy *et al.* 2022).

Insulin resistance can be asymptomatic or manifest in many disorders, including impaired type 2 DM, glucose tolerance, hypertriglyceridemia, hypercholesterolemia, obesity, and arterial hypertension. Simultaneous measurement of Insulin and glucose levels in serum is the basis of several methods to assess IR. Glucose and insulin measurements are taken at baseline or after intravenous injection of a fixed amount of glucose or Insulin (Gierach *et al.* 2014).

Abnormalities of glucose metabolism have been shown to be present in TD, including either overt hyperthyroidism or overt hypothyroidism. The severity of these problems is inversely correlated with disease severity. Whether subclinical hyperthyroidism or hypothyroidism contributes to carbohydrate problems is still a matter of debate. Glucose intolerance in hyperthyroidism may be caused primarily by hepatic-IR, whereas evidence for hypothyroidism indicates that peripheral-IR predominates (Gierach *et al.* 2014).

Clinical hypothyroidism is considered a risk factor for IR. In hypothyroidism, the rate of glucose absorption in the intestine is reduced, adrenergic activity is reduced, less glycogen is broken down by the liver and muscle, and gluconeogenesis and basal insulin production are also reduced. Increased amounts of free fatty acids, decreased glucose absorption, and enhanced glucose oxidation are all associated with increased postprandial insulin secretion despite the presence of whole-body peripheral-IR. (Gierach *et al.* 2014).

In a study conducted by (El Amrousy *et al.* 2022), who established HOMA-IR and fasting blood Insulin levels were significantly higher with hypothyroidism, compared with controls. It is more related to hypothyroidism than BMI. Another study by (Vyakaranam *et al.* 2014), however, reported that HOMA-IR is strongly inversely correlated with FT3 and FT4, but rather positively correlated with TSH. These findings

may serve as a reminder that variations in TSH, FT3 and FT4 can lead to the development of IR in hypothyroidism. IR and metabolic complications such as type 2 DM and hypertriglyceridemia are associated with hypothyroidism and subclinical hypothyroidism. However, a study by (Al Mohareb *et al.* 2021) failed to find an association between HOMA-IR-measured and TSH levels, FT4, FT3 and Leptin in obese subjects.



3. MATERIALS AND METHODS

3.1 Study design

Blood was drawn from 90 participants, including 30 healthy volunteers and 60 hypothyroid patients. Their ages varied (from 26 to 66). All patients presented in this study underwent a hospital visit by a physician. The criteria used to diagnose thyroid disease were based on symptoms, signs, and biochemical examination of thyroid dysfunction. This study suggests that TSH levels directly correspond to thyroid activity, its synthesis and release of T4 and T3, which could lead to improved diagnosis in individuals with hypothyroidism and euthyroidism. As a result, TSH was chosen as an indicator of thyroid health. All patient samples were measured for T3, T4, Leptin, Apelin, Irisin, Insulin, glucose, cholesterol, TG, LDL-cholesterol and HDL-cholesterol. All participants in this study were divided into three groups:

❖ Group one: Euthyroid state (control group)

Subjects were classified as normal if their TSH, T3, and T4 measurements were within the normal range (i.e., TSH= 0.27-4.2 IU/L; T3= 0.846 - 2.02 ng/mL; T4= male 5.13-14.06 µg/dL, women 4.8–11.6 µg/dL). Thirty patients had normal thyroid function (euthyroidism) and were of age from 26 to 61 years (all of these people were not diabetic, hypertensive, smoking, diabetic, or atherosclerotic) and were not treated with lipid-lowering drugs or antioxidants. No vitamin supplements were used. People who had undergone surgery, significant trauma, or clinical disease were excluded from the study.

❖ Group two: Subclinical hypothyroidism

The combination of elevated TSH and normal blood T3 and T4 levels allowed the diagnosis of subclinical hypothyroidism. There were 24 patients with subclinical hypothyroidism, with ages ranging from 29 to 65 years.

❖ Group three: Overt hypothyroidism

Clinical features and their hormonal analysis determined the diagnosis. High serum TSH levels and low serum T3 and T4 levels were the key determinants of the biochemical assays. The ages of the 36 patients with overt hypothyroidism ranged from (34 to 71) years with a mean standard deviation of (45.3 ± 7.0) years.

3.2 Sample collection

After 8 to 12 hours of fasting, (5 to 10) mL of peripheral blood was aspirated into sterile tubes by venipuncture for 30 minutes and allowed to clot in vitro. Serum lipid and glucose profiles were measured immediately after serum was separated from blood cells by centrifugation at 4000 rpm for 12 min at room temperature. Serum should be frozen and kept at a temperature of -60°C for estimation of hormone assays. Between April 2022 and August 2022, these samples were collected at the AL Fallujah Teaching Hospital in Anbar Governorate, Iraq.

3.3 General Information

Each patient's specific information such as name, age, and other details were entered into a questionnaire form (Appendix 1) for documentation. Patient height (cm) and weight (kg) were recorded using a balance (Beurer). Other diseases and whether patients were taking medications for thyroid or other diseases were also recorded. All study groups participated in person-interviews to complete the questionnaire.

Roche Diagnostics has created and made available a semi-automated electrochemiluminescence immunoassay (ECLIA) for measuring TSH, total T3, total T4, Insulin, and lipid profiles in whole blood (GmbH, Mannheim, Germany). The Cobas e 411 Analyzer was a fully automated analyzer that uses proprietary ECLA technology to analyze immunoassays. It was designed for quantitative and qualitative in vitro test results for various applications.

Serum Leptin, Apelin, and Irisin levels were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Calculate insulin sensitivity using the homeostasis model assessment (HOMA) method using the following formula;

$$\text{HOMA-IR} = \text{Fasting insulin} \times \text{fasting glucose} / 405$$

where glucose was measured in mg/dL and applied to where the fasting serum Insulin concentration ($\mu\text{U/L}$). A HOMA score greater than 2.7, according to the definition of IR provided by Matthews *et al.* (1985).

We calculated the BMI for each individual using the formula $\text{BMI} = \text{weight (Kg)} / \text{height (m}^2\text{)}$. Patients were considered obese if their BMI was greater than 30 kg/m^2 . Patients were classified as non-obese if their BMI was less than 30 kg/m^2 .

~Inclusion Criteria: Iraqi patients must be at least 20 years of age.

~Exclusion Criteria: Exclusion criteria include; Pituitary or hypothalamic disease, including secondary hypothyroidism. major surgery within 2 weeks after enrollment; serious mental health problems; GIT malabsorption disease; pregnancy; alcohol abuse; Thyroidectomized patients receiving L-T4 treatment; and DM (serum fasting blood glucose $> 126 \text{ mg/dL}$ twice), hypertension (current or previous use of antihypertensive medications), cardiovascular disease, acute and chronic renal disease, neurological disease, or other chronic disease or cancer.

3.4 Materials

3.4.1 Equipments

Instrument/ Company:

A- Absorbance Microplate Reader (ELISA reader)/ GENEX Laboratories, Florida- USA.

B- Microplate washer (ELISA washer)/ GENEX Laboratories, Florida- USA

C- Clinical Chemistry Analyzer (cobas e 411) Roche Diagnostics (GmbH, Mannheim, Germany).

D- Incubator Fisher Scientific/ U.S.A

E- Refrigerator with Freezer LG / Korea

F- Centrifuge/Germany

G- Micropipettes (different volumes)

H. Multichannelpipette(50-300 μ L)

I. Balance (Beurer/ Germany)

J. Meter (measuring 3m/10ft/China)

3.5 Reagents for ECLIA

The TSH, total T3, total T4, insulin and lipid profile were measured according to Elecsys kits and cobas e411 immunoassay analyzers.

3.5.1 TSH elecsys kit

A Roche Cobas e 411 analyzer was used to test TSH levels using Elecsys technology, kit reference number 08429324-1820/Germany.

Test principle: - sandwich theory.

~It took 18 minutes to complete the test.

~In the first incubation, a sandwich complex is formed when 50 μ L of sample, a biotinylated monoclonal TSH-specific antibody, and a ruthenium complex-tagged monoclonal TSH-specific antibody react.

~In the second incubation, the complexes are bound to the solid phase by the interaction between biotin and streptavidin after addition of streptavidin coated micro particles.

~The reaction mixture is drawn into the measuring cell and magnetically attracted micro particles adhere to the electrode surface. Then use Pro-Cell/Pro-Cell M to remove any remaining unbound compounds. A photomultiplier tube measures the chemiluminescence produced when a voltage is applied to the electrodes.

~Results are obtained by comparing a master curve provided by a reagent barcode or electronic barcode with a calibration curve generated instrument-specifically by two-point calibration.

Expected values: 0.270-4.20 μ IU/mL

3.5.2 Total T3 elecsys kit

A Roche Cobas e 411 analyzer was used to test T3 levels using Elecsys technology, kit reference number 09007725-190/Germany.

Test principle: - Competition theory.

~It took 18 minutes to complete the test.

~In the first incubation, bound T3 is released from bound proteins in the sample by ANS using 30 μ L of sample and a T3-specific antibody tagged with a ruthenium complex.

~In the second incubation, as a result of the addition of streptavidin-coated micro particles and biotinylated T3, the still free binding sites of the labeled antibody are occupied during the second incubation. This forms an antibody-hapten complex. The interaction of biotin and streptavidin binds the complete complex to the solid phase.

~The reaction mixture is drawn into the measuring cell and magnetically attracted micro particles adhere to the electrode surface. Then use Pro-Cell/Pro-Cell M to remove any remaining unbound compounds. A photomultiplier tube measures the chemiluminescence produced when a voltage is applied to the electrodes.

~Results are obtained by comparing a master curve provided by a reagent barcode or electronic barcode with a calibration curve generated instrument-specifically by two-point calibration.

Expected values: 0.846-2.02ng/mL.

3.5.3 Total T4 elecsys kit

A Roche Cobas e 411 analyzer was used to test T4 levels using Elecsys technology, kit reference number 09007741-021/Germany.

Test principle:- Competition theory.

~It took 18 minutes to complete the test.

~In the first incubation, bound T3 is released from bound proteins in the sample by ANS using 15 μ L of sample and a T4-specific antibody tagged with a ruthenium complex.

~In the second incubation, as a result of the addition of streptavidin-coated micro particles and biotinylated T4, the still free binding sites of the labeled antibody are occupied during the second incubation. This forms an antibody hapten complex. The interaction of biotin and streptavidin binds the complete complex to the solid phase.

~The reaction mixture is drawn into the measuring cell and magnetically attracted micro particles adhere to the electrode surface. Then use Pro-Cell/Pro-Cell M to remove any

remaining unbound compounds. A photomultiplier tube measures the chemiluminescence produced when a voltage is applied to the electrodes.

~Results are obtained by comparing a master curve provided by a reagent barcode or electronic barcode with a calibration curve generated instrument-specifically by two-point calibration.

Expected values: male 5.13-14.06 µg/dL, female 4.8- 11.6 µg/dL.

3.5.4 Insulin elecsys kit

A Roche Cobas e 411 analyzer was used to test Insulin levels using Elecsys technology, kit reference number; 12017547-120 (Germany).

Test principle Sandwich theory.

~It took 18 minutes to complete the test.

~In the first incubation, a sandwich complex is formed when 20 µL of sample, a biotinylated monoclonal insulin -specific antibody, and a ruthenium complex-tagged monoclonal insulin -specific antibody react.

~In the second incubation, the complexes are bound to the solid phase by the interaction between biotin and streptavidin after addition of streptavidin coated micro particles.

~The reaction mixture is drawn into the measuring cell and magnetically attracted micro particles adhere to the electrode surface. Then use Pro-Cell/Pro-Cell M to remove any remaining unbound compounds. A photomultiplier tube measures the chemiluminescence produced when a voltage is applied to the electrodes.

~Results are obtained by comparing a master curve provided by a reagent barcode or electronic barcode with a calibration curve generated instrument-specifically by two-point calibration. Expected values: 2.6-24.9 $\mu\text{U/mL}$.

3.6 Reagents for glucose and lipid profile

3.6.1 Serum glucose elecsys kit

A Roche Cobas e 411 analyzer was used to test glucose levels using Elecsys technology, kit reference number (04657527/Germany).

Test principle UV test: Hexokinase-based enzyme referencing method. Using ATP, hexokinase catalyzes the conversion of glucose to glucose-6-phosphate (Equation 3.1).



In the presence of NADP, glucose-6-phosphate dehydrogenase converts glucose-6-phosphate to gluconate-6-phosphate (Equation 3.2). Others of carbohydrates are not oxidized. The rate of NADPH production during the reaction is photometrically monitored and is directly proportional to glucose concentration.



Expected values: Serum Fasting Glucose 4.11-6.05 mmol/L (74-109 mg/dL)

Adults =4.11-5.89 mmol/L (74-106 mg/dL)

3.6.2 Serum cholesterol Elecsys kit

A Roche Cobas e 411 analyzer was used to test cholesterol levels using Elecsys technology, kit reference number 04718917.

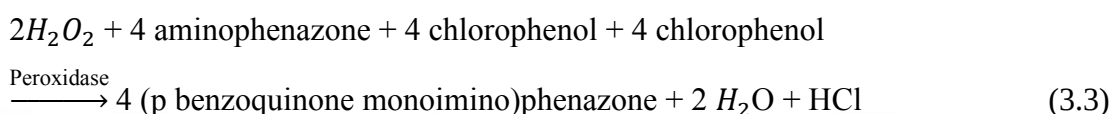
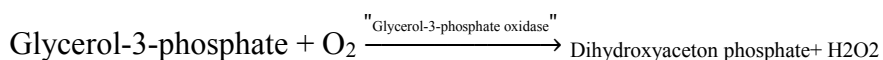
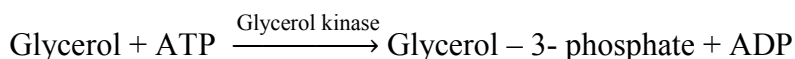
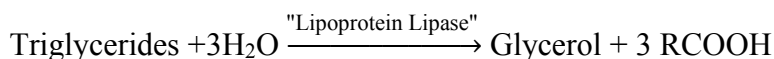
Test principle: Many researchers have investigated the use of enzymes to measure cholesterol. Cholesterol esterase converts cholesterol and free fatty acids from cholesterol esters by enzymatic hydrolysis. Cholesterol oxidase then converts free cholesterol, including what was originally present, to cholest-4-en-3-one and hydrogen peroxide. A chromophore (quinoneimine dye) is formed when hydrogen peroxide, hydroxybenzoic acid, and 4-aminoantipyrine are mixed. This dye can be measured at 500 nm.

3.6.3 Serum triglyceride elecsys kit

A Roche Cobas e 411 analyzer was used to test triglyceride levels using Elecsys technology, kit reference number 04657594.

Test principle: Enzymatic colorimetric test: Triglycerides are three long-chain fatty acid esters of the trihydric alcohol glycerol. Some are produced in the liver and some are obtained from the diet. Triglyceride measurements are used to diagnose and treat individuals with DM, liver obstruction, lipid metabolism problems, nephrosis, and various other endocrine disorders.

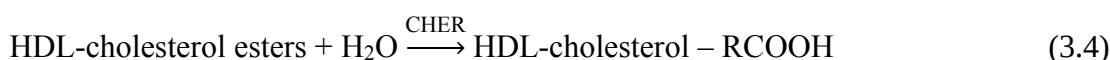
This process is based on the rapid and complete hydrolysis of triglycerides to glycerol by lipoprotein lipase from bacteria, followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. Under the catalytic activity of peroxidase, the hydrogen peroxide produced in turn combines with 4-aminophenazone and 4-chlorophenol (Equation 3.3) to produce a red color (Trinder end-point reaction). It is possible to photometrically detect the color intensity of the red dye produced, which is directly proportional to the triglyceride content.



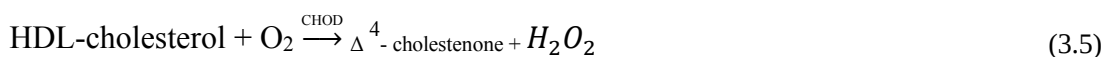
Expected values: < 1.70 mmol/L (< 150 mg/dL)

3.6.4 Serum HDL elecsys kit

A Roche Cobas e 411 analyzer was used to test HDL levels using Elecsys technology, kit reference number 07528604. Test principle: Enzymatic colorimetric test that is homogeneous. LDL-cholesterol, VLDL, and chylomicrons, which are non-HDL lipoproteins, were combined with polyanions and surfactants to create water-soluble complexes. This combination inhibits the enzymatic conversion of CHER and CHOD to non-HDL lipoproteins. Finally, CHER and CHOD can only interact with HDL particles. CHER and CHOD work together to enzymatically determine HDL cholesterol content (Equation 3.4). CHER quantitatively converts cholesterol esters into free cholesterol and fatty acids.



Cholesterol oxidase converts cholesterol to 4-cholestenone and hydrogen peroxide in the presence of oxygen (Equation 3.5).



The generated hydrogen peroxide reacts with EMSEa and 4-aminoantipyrine in the presence of peroxidase to produce a dye (Equation 3.6). The color intensity of this dye was measured photometrically and directly correlates with the level of cholesterol in the blood (Table 3.1).



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

Table 3.1 Expected values of HDL

Expected values	No risk	Moderate risk	High risk
Men	> 1.68 mmol/L	1.15 - 1.68 mmol/L	< 1.15 mmol/L
	(> 65 mg/dL)	(45 - 65 mg/dL)	(< 45 mg/dL)
Women	> 1.45 mmol/L	0.90 - 1.45 mmol/L	< 0.90 mmol/L
	(> 55 mg/dL)	(35 - 55 mg/dL)	(< 35 mg/dL)

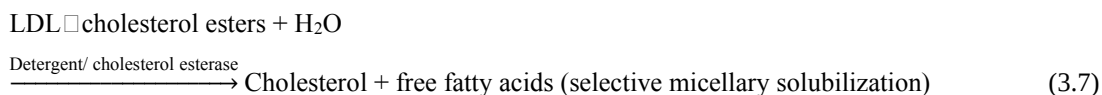
3.6.5 Serum LDL-cholesterol elecsys kit

A Roche Cobas e 411 analyzer was used to test LDL-cholesterol levels using Elecsys technology, kit reference number 07005806.

Test principle: Enzymatic colorimetric test that is homogeneous.

Based on a cholesterol enzymatic technique, the amount of free cholesterol and cholesterol esters in LDL-cholesterol was determined. Using cholesterol esterase and cholesterol oxidase in the presence of detergents that specifically solubilize only LDL-

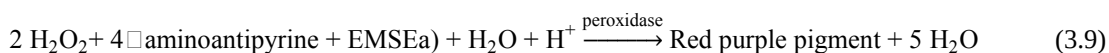
cholesterol. Detergents and sugar molecules block enzymatic responses to lipoproteins other than LDL-cholesterol (Equation 3.7). The amount of cholesterol in HDL-cholesterol, VLDL, and chylomicrons was unknown. Cholesterol esterase by quantitatively converting cholesterol esters into free cholesterol and fatty acids.



Cholesterol oxidase converts cholesterol to Δ^4 -cholestenone and hydrogen peroxide in the presence of oxygen (Equation 3.8).



In the presence of peroxidase, the hydrogen peroxide produced combines with EMSE and 4'-aminoantipyrine to produce a reddish-purple color (Equation 3.9). The color intensity of this dye was measured photometrically and was directly related to the level of cholesterol in the blood.



Expected values: Risk indices for coronary heart disease levels (Table 3.2).

Table 3.2 Expected values of LDL

Adult levels	
Optimal	< 2.59 mmol/L (< 100 mg/dL)
Near optimal/above optimal	2.59-3.34 mmol/L (100-129 mg/dL)
Borderline high	3.37-4.12 mmol/L (130-159 mg/dL)
High	4.14-4.89 mmol/L (160-189 mg/dL)
Very high	≥ 4.92 mmol/L (≥ 190 mg/dL)

3.7 Reagents for ELISA

Leptin, Apelin and Irisin parameters were tested using commercial ELISA microwell kits according to the manufacturer's instructions.

3.7.1 Leptin ELISA kit

Human LEP (leptin) ELISA Kit E-EL-H 6017 of Elab Science Biotechnology Co., Ltd. was used for Leptin kit measurement.

Test principle: The ELISA kit employs the Sandwich-ELISA test method. The micro ELISA plate included in this kit was precoated with an antibody against human LEP. Wells of micro ELISA plates are filled with standards or samples and specific antibodies. An avidin-horseradish peroxidase (HRP) combination and a biotinylated detection antibody specific for human LEP were then sequentially added to each microplate well. Unused parts are removed during washing. A substrate solution is applied to each well. Avidin-HRP conjugate, biotinylated detection antibody, and human LEP will all be present in blue-colored wells. The enzyme-substrate process was halted by the addition of the stop solution, which also causes the color to become yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm plus 2 nm. LEP was linear. By comparing the sample OD to the standard curve, the concentration of human LEP in the sample can be determined.

Detection Range: 15.63~1000 pg/mL

3.7.2 Apelin ELISA kit

The intended use of the ELISA kit was the in vitro quantitative determination of human Apelin concentrations in serum, plasma, and other body fluids.

Inter Assay CV% < 4.93%

Intra Assay CV% < 5.07%

Test principle: The ELISA kit adopts Competitive-ELISA method. The micro ELISA plates in the kit were pre-coated with human apelin. A fixed amount of human apelin on a solid support competes with human apelin in a sample or standard for sites on a biotinylated detection antibody specific for human apelin during the reaction. Excess conjugate and unbound samples or standards are removed from the plate and each microplate well was loaded with avidin conjugated to HRP and incubated. A TMB substrate solution is then added to each well. Injection of a stop solution terminates the enzyme-substrate reaction and the color change was detected spectrophotometrically at a wavelength of 450 nm + 2 nm. The OD of the samples was then compared to the standard curve to determine the concentration of human apelin in the samples.

Sensitivity 37.50 pg/mL

Detection Range 62.50-4000 pg/mL

3.7.3 Irisin ELISA kit

Human Irisin ELISA Kit E-EL-H 6120 of Elab-Science Biotechnology Co., Ltd. was used for Irisin measurement.

Test Principle: The ELISA kit employs the Sandwich-ELISA test method. The micro ELISA plate included in this kit was precoated with an antibody against human Irisin. Wells of micro ELISA plates were filled with standards or samples and specific antibodies. Avidin-HRP combination and a biotinylated detection antibody specific for human Irisin were then sequentially added to each microplate well. Unused parts were removed during washing. A substrate solution was applied to each well. Avidin-HRP conjugate, biotinylated detection antibody, and human Irisin will all be present in blue-colored wells. The enzyme-substrate process is halted by the addition of the stop solution, which also causes the color to become yellow. The OD was measured

spectrophotometrically at a wavelength of 450 nm plus 2 nm. Irisin was linear. By comparing the sample OD to the standard curve, the concentration of human Irisin in the sample can be determined.

Sensitivity 9.38 pg/mL

Detection Range 15.63-1000 pg/mL

3.8 Statistics Analysis

Statistical Analysis System-2019 has been adopted. Use a t-test to compare the underlying assumptions of two variables. In each study, statistical significance was determined at $P < 0.05$.

Means $\pm$ SD was used to represent the result. Student-t test was used to compare patient and control groups. Chi-square test was used for nominal variables. A paired-samples t-test was used to compare Bazal values in study groups with post-treatment values. Pearson's correlation analysis was used to determine correlations between MPV and other parameters. Independent factors affecting carotid IMT were discovered using multiple regression analysis. Statistics were considered significant at $P < 0.05$. Analyzes were performed using the statistical program SPSS version 20.

4. RESULTS

4.1 Patients categorizing and thyroid hormone concentrations

Table 4.1 shows the clinical characteristics of the patient and control groups. Patients were divided according to their TH levels. There are three classes (euthyroidism; normal TSH, normal total T3, normal total T4, subclinical hypothyroidism; high TSH, normal total T3, normal total T4, and overt hypothyroidism; high TSH, low total T3 and low total T4).

Table 4.1 Classification of clinical status of thyroid

Thyroid Status (n: Number of patients)	T3 ng/mL	T4 µg/dL	TSH µIU/L
Euthyroidism (n=30)	0.846-2.02	4.8-11.6	0.27-4.2
Subclinical Hypothyroidism (n=24)	N	N	> 4.2
Overt Hypothyroidism (n=36)	<0.846	<4.8	> 4.2

N: Normal levels of thyroid hormones

4.1.1 The level of TSH

As shown in Table 4.2 and Figure 4.1, mean serum TSH levels in the overt hypothyroid group (13.50 ± 4.05 µIU/mL) were significantly higher than those in the euthyroid group ($2.831.041$ IU/mL) and those in the subclinical hypothyroid group (7.66 ± 2.71 µIU/mL).

The findings also revealed that serum TSH levels in the subclinical hypothyroid group were significantly higher than those in the euthyroid group.

Table 4.2 TSH values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
TSH (μ IU/mL) mean $\pm$ SD	2.83 $\pm$ 1.04	7.66 $\pm$ 2.71	13.50 $\pm$ 4.05
Euthyroidism vs. Subclinical Hypothyroidism (t= 8.954, P< 0.001) Euthyroidism vs. Overt Hypothyroidism (t= 14.013, P< 0.0001) subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 6.180, P< 0.0001)			

n: Number of patients.

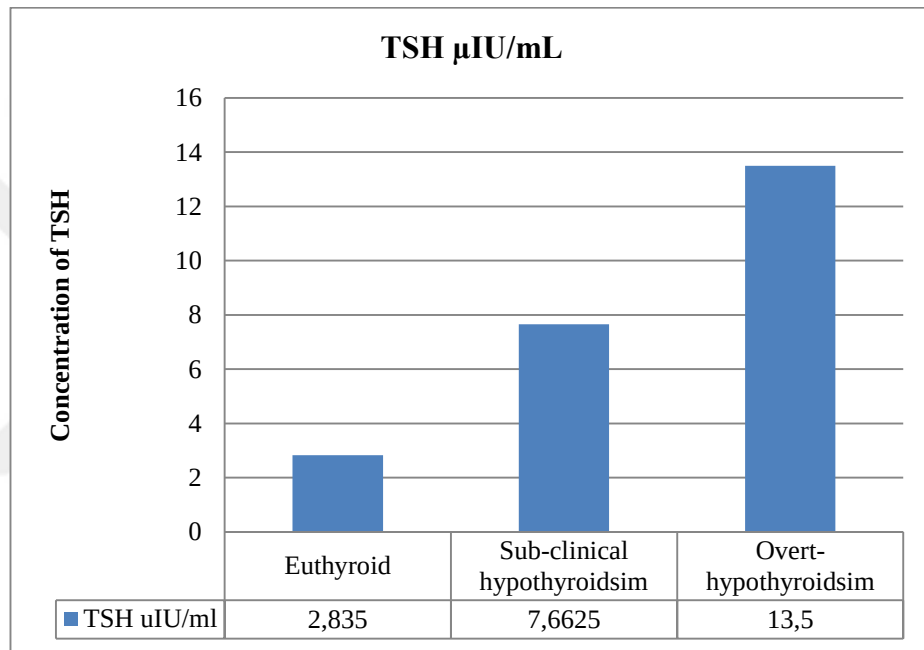


Figure 4.1 Chart of TSH values in patients' groups and control

4.1.2 The level of T4

As shown in Table 4.3 and Figure 4.2, mean serum T4 levels in the overt hypothyroid group (1.91 ± 1.29 μ g/dL) were significantly lower than those in the euthyroid group (8.17 ± 2.25 μ g/dL), and those in the subclinical hypothyroid group (7.02 ± 1.77 μ g/dL).

The findings also revealed that serum T4 levels in the subclinical hypothyroid group were significantly lower than those in the euthyroid group.

Table 4.3 T4 values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
T4 (µg/dL) mean±SD	8.1700±2.2562	7.0208±1.7754	1.9139±1.2920
Euthyroidism vs. Subclinical Hypothyroidism(t= 2.039, P= 0.0465) Euthyroidism vs. Overt Hypothyroidism (t=14.104, P= 0.0001) subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 12.899, P< 0.0001)			

n: Number of patients.

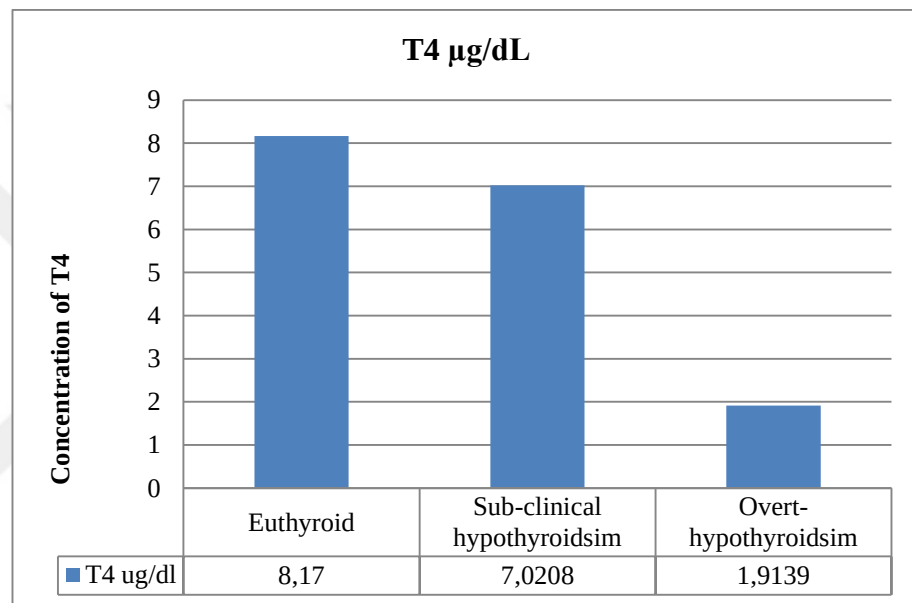


Figure 4.2 Chart of T4 values in patients' groups and control

4.1.3 The level of T3

As shown in Table 4.4 and Figure 4.3, mean serum T3 levels in the overt hypothyroid group (0.62 ± 0.39 ng/mL) were significantly lower than those in the euthyroid group (1.23 ± 0.30 ng/mL), and those in the subclinical hypothyroid group (1.22 ± 0.31 ng/mL).

The findings also revealed that serum T3 levels in the subclinical hypothyroid group were non-significantly lower than those in the euthyroid group.

Table 4.4 T3 values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
T3 (ng/mL) mean±SD	1.23±0.30	1.22±0.31	0.626±0.399
Euthyroidism vs. Subclinical Hypothyroidism(t= 1.175, P= 0.867) Euthyroidism vs. Overt Hypothyroidism (t= 6.825, P< 0.0001) Subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 6.056, P< 0.0001)			

n: Number of patients.

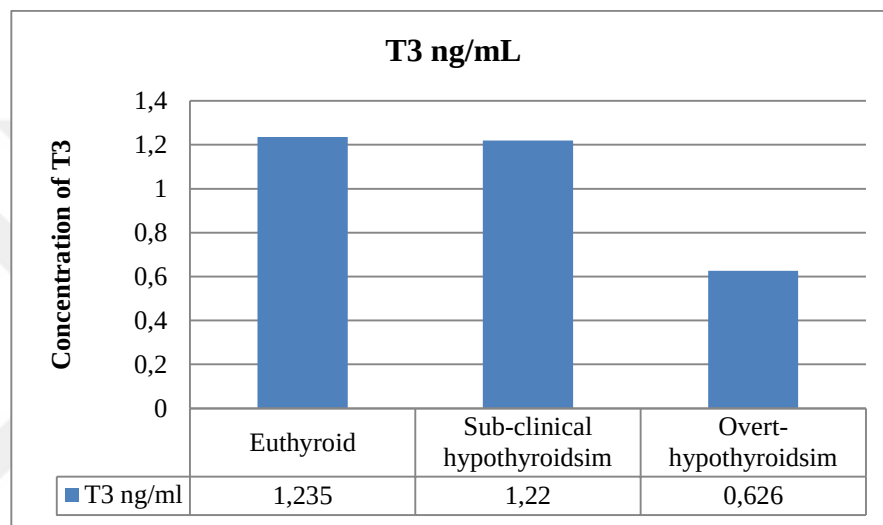


Figure 4.3 Chart of T3 values in patients' groups and control

4.2 The level of age and gender distributions

As shown in Table 4.5 and Figure 4.4, the mean age in the overt hypothyroid group (50.25 ± 9.88 years) were significantly ($P = 0.0107$) higher than those in the euthyroid group (43.46 ± 11.07 years) and non-significantly ($P = 0.139$) higher than those in the subclinical hypothyroidism group (46.50 ± 8.90 years).

The findings also revealed that age in the subclinical hypothyroid group were non-significantly ($P = 0.281$) higher than those in the euthyroid group.

Across all patient categories, Table 4.5 also revealed that female patients had a higher frequency of thyroid dysfunction than male patients.

Table 4.5 Age and gender distribution in patients' groups and control

Diagnosis	Number of patient			Age (years)
Groups	Male	Female	Total	mean±SD
Euthyroidism	12 (40%)	18 (60%)	30 (100%)	43.46 ±n 11.07
Subclinical Hypothyroidism	9 (38%)	15 (62%)	24 (100%)	46.50 ±8.90
Overt Hyperthyroidism	14 (39%)	22 (61%)	36 (100%)	50.25 ± 9.88
Euthyroidism vs. Subclinical Hypothyroidism (t= 1.089, P= 0.281)				
Euthyroidism vs. Overt Hypothyroidism (t= 2.629, P = 0.0107)				
Subclinical Hypothyroidism vs. Overthyroidism (t= 1.497, P= 0.139)				

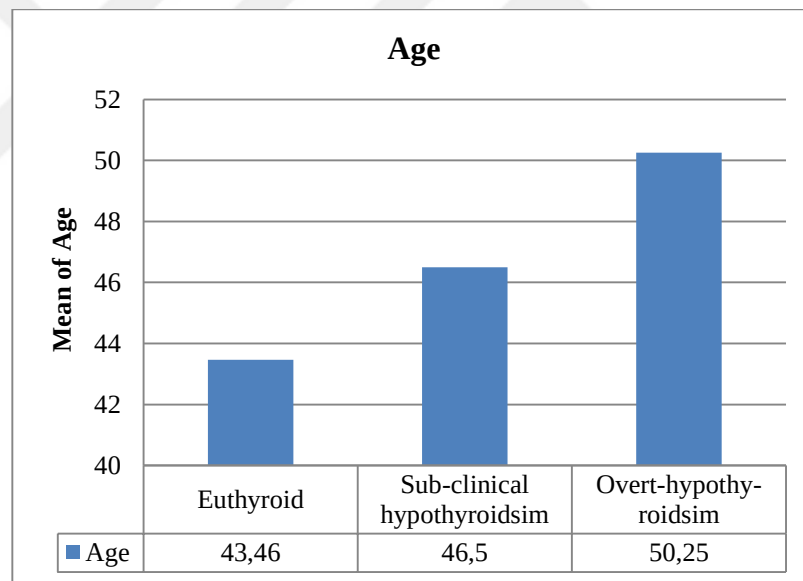


Figure 4.4 Chart of age in patients' groups and control

4.3 The level of BMI

As shown in Table 4.6 and Figure 4.5, mean BMI in the overt hypothyroid group ($29.25 \pm 3.28 \text{ kg/m}^2$) were significantly higher than those in the euthyroid group ($27.72 \pm 2.68 \text{ kg/m}^2$) and those in the subclinical hypothyroid group ($27.66 \pm 2.71 \text{ kg/m}^2$).

The findings also revealed that BMI in the subclinical hypothyroid group (28.59 ± 2.85 kg/m²) were non-significantly when compared with euthyroid group and overt hypothyroidism group.

Table 4.6 BMI in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
BMI (kg/m ²) mean $\pm$ SD	27.72 $\pm$ 2.68	28.59 $\pm$ 2.85	29.25 $\pm$ 3.28
Euthyroidism vs. Subclinical Hypothyroidism (t= 1.140, P= 0.259) Euthyroidism vs. Overt Hypothyroidism (t= 2.02, P= 0.0476) subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 0.802, P= 0.425)			

n: Number of patients.

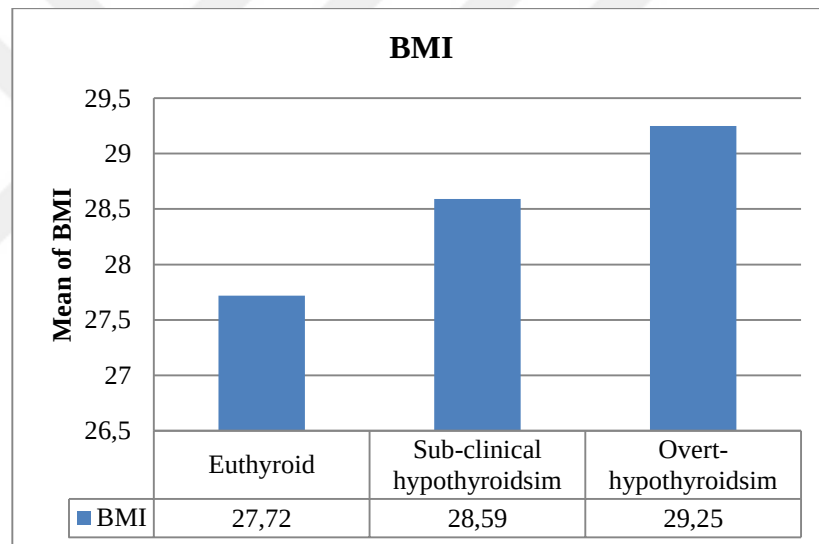


Figure 4.5 Chart of BMI in patients' groups and control

4.4 The level of serum Leptin concentrations

As shown in Table 4.7 and Figure 4.6, mean serum leptin levels in the overt hypothyroid group (184.05 ± 80.65 pg/mL) was significantly higher than those in the euthyroid group (145.50 ± 69.06 pg/mL).

The findings also revealed that serum leptin levels in the subclinical hypothyroid group (157.29 ± 85.94 pg/mL) were non-significantly when compared with euthyroid group and overt hypothyroidism group.

Table 4.7 Leptin values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
Leptin (pg/mL) mean $\pm$ SD	145.50 $\pm$ 69.06	157.29 $\pm$ 85.94	184.05 $\pm$ 80.65
Euthyroidism vs. Subclinical Hypothyroidism (t= 0.559, P= 0.5784)			
Euthyroidism vs. Overt Hypothyroidism (t= 2.062, P= 0.0432)			
Subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 1.227, P= 0.224)			

n: Number of patients.

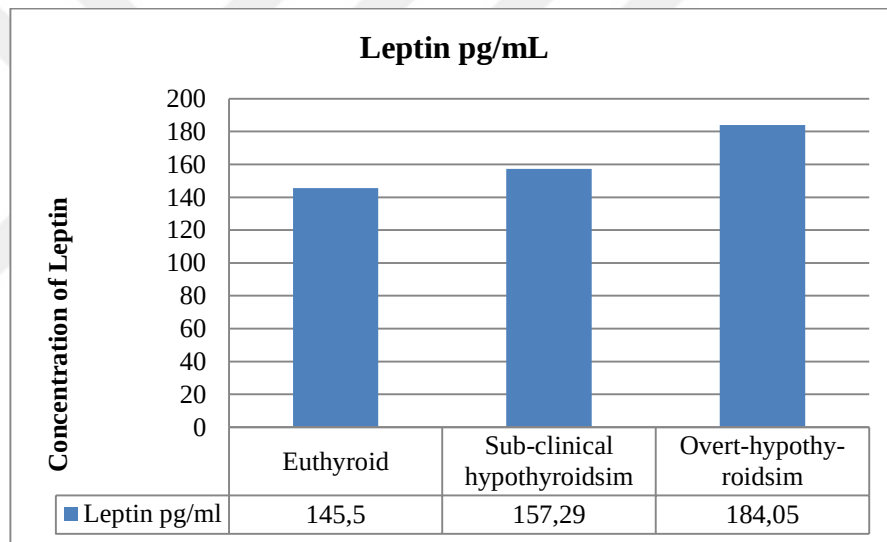


Figure 4.6 Chart of Leptin values in patients' groups and control

4.5 The level of serum Apelin concentrations

As shown in Table 4.8 and Figure 4.7, mean serum apelin levels in the overt hypothyroid group (293.33 ± 142.28 pg/mL) was significantly lower than those in the euthyroid group (370.83 ± 161.20 pg/mL).

The findings also revealed that serum apelin levels in the subclinical hypothyroid group (321.25 ± 155.89 pg/mL) were non-significantly when compared with euthyroid group and overt hypothyroidism group.

Table 4.8 Apelin values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
Apelin (pg/mL) mean $\pm$ SD	370.83 $\pm$ 161.20	321.25 $\pm$ 155.89	293.33 $\pm$ 142.28
Euthyroidism vs. Subclinical Hypothyroidism(t= 1.140, P= 0.2597) Euthyroidism vs. Overt Hypothyroidism (t= 2.07, P= 0.0421) subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 0.717, P= 0.4765)			

n: Number of patients.

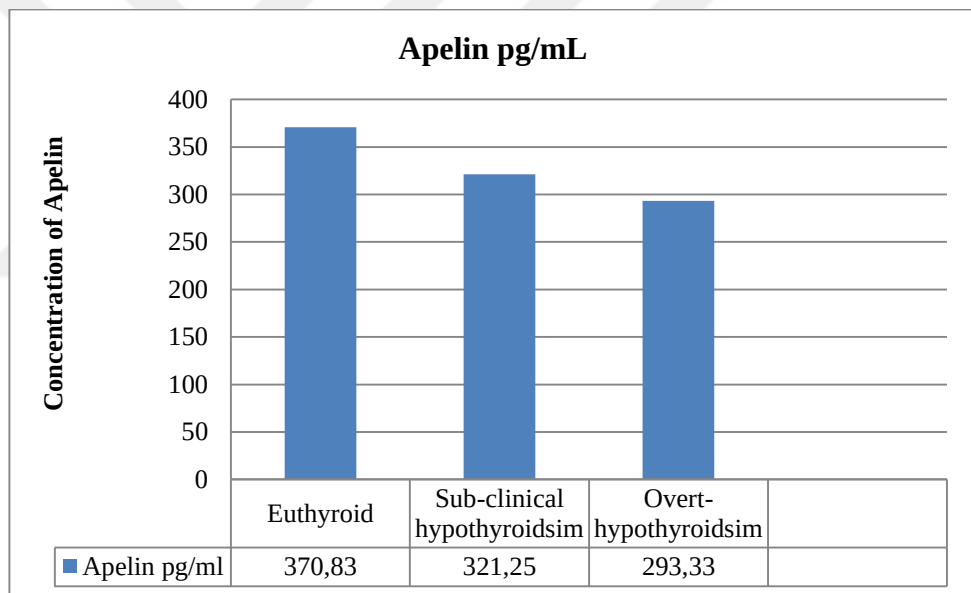


Figure 4.7 Chart of Apelin values in patients' groups and control

4.6 The level of serum Irisin concentrations

As shown in Table 4.9 and Figure 4.8, serum irisin concentration results were it was similar among the overt hypothyroidism group (2.44 ± 1.02 μ g /mL), subclinical hypothyroidism group (2.69 ± 0.90 μ g /mL) and euthyroidism group (2.95 ± 1.15 μ g /mL).

Table 4.9 Irisin values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
irisin ($\mu\text{g}/\text{mL}$) mean $\pm$ SD	2.95 $\pm$ 1.15	2.69 $\pm$ 0.90	2.44 $\pm$ 1.022
Euthyroidism vs. Subclinical Hypothyroidism (t= 0.909, P= 0.3677) Euthyroidism vs. Overt Hypothyroidism (t= 1.899, P= 0.0620) Subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 0.960, P= 0.3411)			

n: Number of patients.

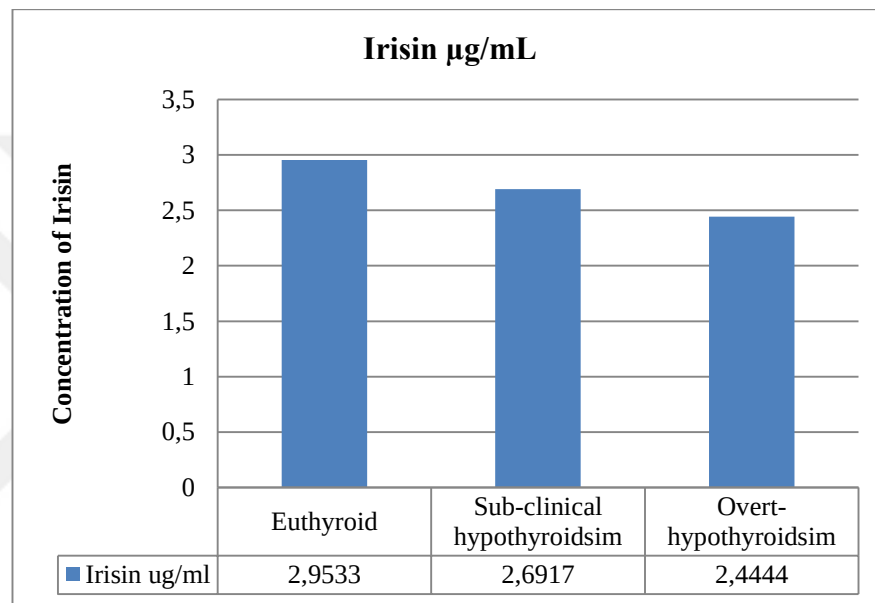


Figure 4.8 Chart of Irisin values in patients' groups and control

4.7 The level of serum Insulin, glucose and HOMA concentrations

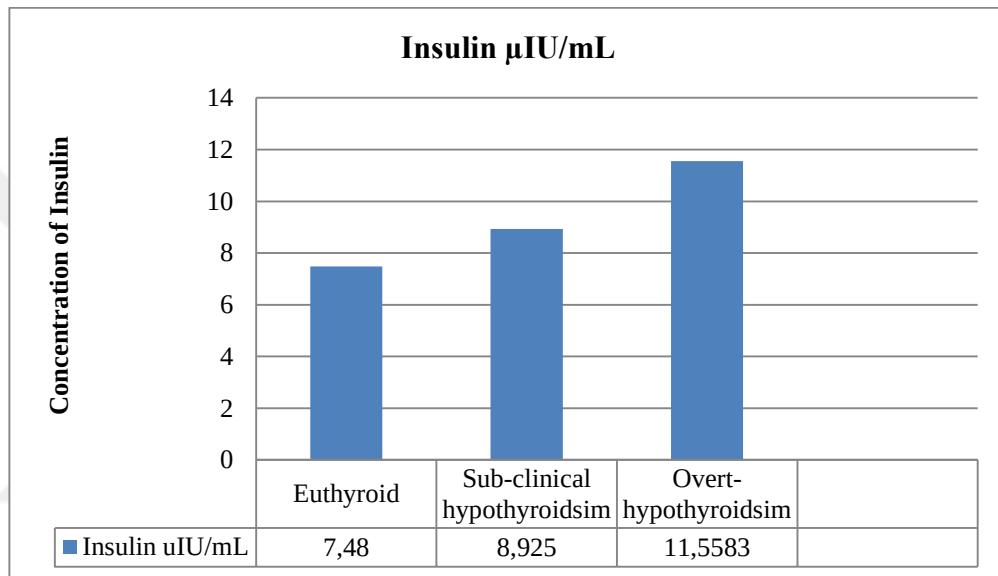
As shown in Table 4.10 and Figure 4.9, mean serum insulin levels in the overt hypothyroid group (11.55 $\pm$ 4.05 $\mu\text{IU}/\text{mL}$) were significantly higher than those in the euthyroid group (7.48 $\pm$ 3.18 $\mu\text{IU}/\text{mL}$), and those in the subclinical hypothyroid group (8.92 $\pm$ 4.97 $\mu\text{IU}/\text{mL}$).

The findings also revealed that serum insulin levels in the subclinical hypothyroid group were non-significantly lower than those in the euthyroid group.

Table 4.10 Insulin values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
Insulin ($\mu\text{IU/mL}$) mean $\pm$ SD	7.48 $\pm$ 3.18	8.92 $\pm$ 4.97	11.55 $\pm$ 4.05
Euthyroidism vs. Subclinical Hypothyroidism(t= 1.294,P= 0.203) Euthyroidism vs. Overt Hypothyroidism (t= 4.477,P< 0.001) Subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 1.250, P= 0.0283)			

n: Number of patients.

**Figure 4.9** Chart of Insulin values in patients' groups and control

As shown in Table 4.11 and Figure 4.10, serum glucose concentration results were it was similar among the overt hypothyroidism group (86 $\pm$ 6.8 mg/dL), subclinical hypothyroidism group (85 $\pm$ 7.7 mg/dL) and euthyroidism group (83 $\pm$ 8.1 mg/dL).

Table 4.11 Glucose values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
glucose (mg/dL) mean $\pm$ SD	83 $\pm$ 8.1	85 $\pm$ 7.7	86 $\pm$ 6.8
Euthyroidism vs. Subclinical Hypothyroidism(t= 0.614, P= 0.542) Euthyroidism vs. Overt Hypothyroidism (t= 1.590, P= 0.116) Subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 0.840, P= 0.4043)			

n: Number of patients.

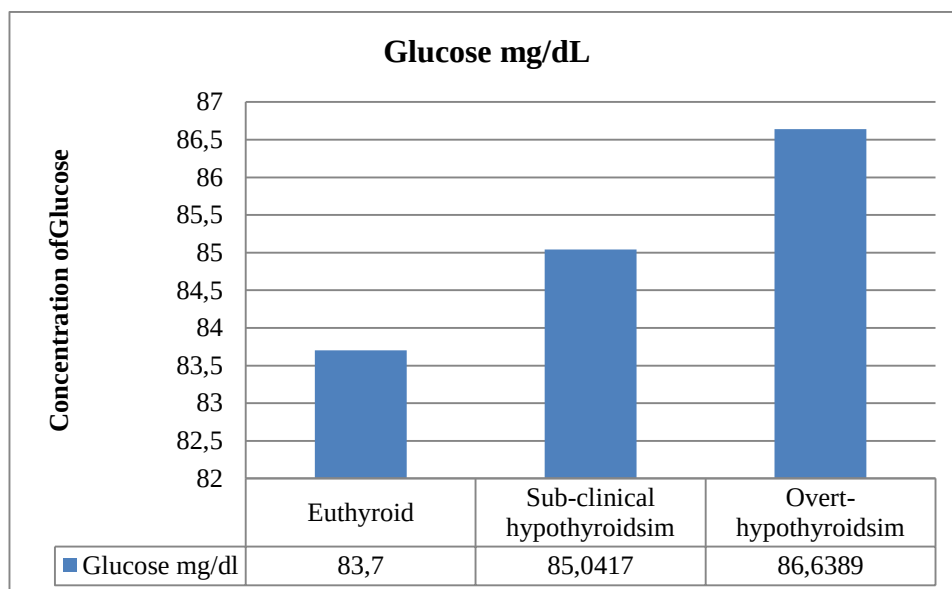


Figure 4.10 Chart of glucose values in patients' groups and control

As shown in Table 4.12 and Figure 4.11, mean HOMA-IR levels in the overt hypothyroid group (2.56 ± 1.09) were significantly higher than those in the euthyroid group (1.56 ± 0.74), and those in the subclinical hypothyroid group (1.92 ± 1.22).

The findings also revealed that HOMA-IR levels in the subclinical hypothyroid group were non-significantly lower than those in the euthyroid group.

Table 4.12 HOMA-IR values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
HOMA-IR mean $\pm$ SD	1.56 $\pm$ 0.74	1.92 $\pm$ 1.22	2.56 $\pm$ 1.09
Euthyroidism vs. Subclinical Hypothyroidism(t= 1.367,P= 0.1775)			
Euthyroidism vs. Overt Hypothyroidism (t= 4.301, P= 0.0001)			
Subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 2.120, P= 0.0383)			

n: Number of patients.

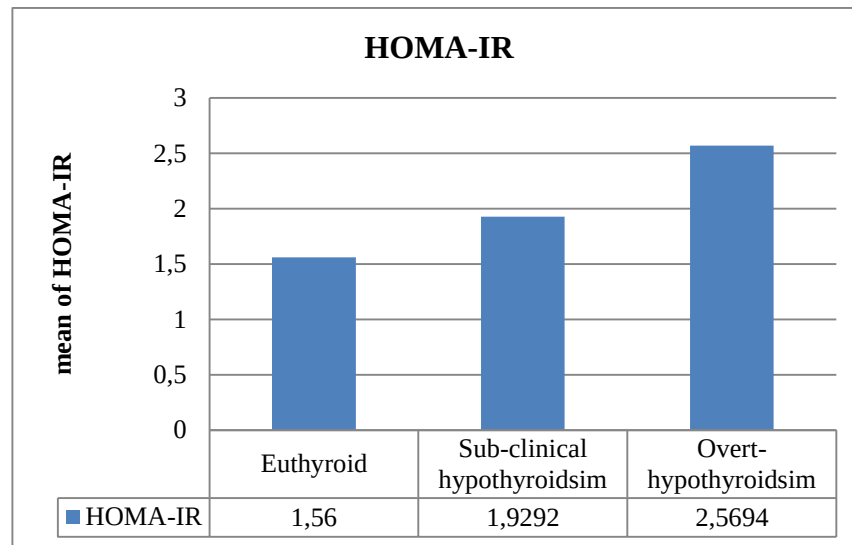


Figure 4.11 Chart of HOMA-IR values in patients' groups and control

4.8 The level of serum lipid profile concentrations

4.8.1 Serum cholesterol levels

As shown in Table 4.13 and Figure 4.12, mean serum cholesterol levels in the overt hypothyroid group (214 ± 53 mg/dL) was significantly higher than those in the euthyroid group (189 ± 25 mg/dL).

The findings also revealed that serum cholesterol levels in the subclinical hypothyroid group (202 ± 42 mg/dL) were non-significantly when compared with euthyroid group and overt hypothyroidism group.

Table 4.13 Cholesterol values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
Cholesterol (mg/dL) mean $\pm$ SD	189 $\pm$ 25	202 $\pm$ 42	214 $\pm$ 53
Euthyroidism vs. Subclinical Hypothyroidism(t= 1.34, P= 0.183)			
Euthyroidism vs. Overt Hypothyroidism (t= 2.34, P= 0.0221)			
Subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 0.953, P= 0.344)			

n: Number of patients.

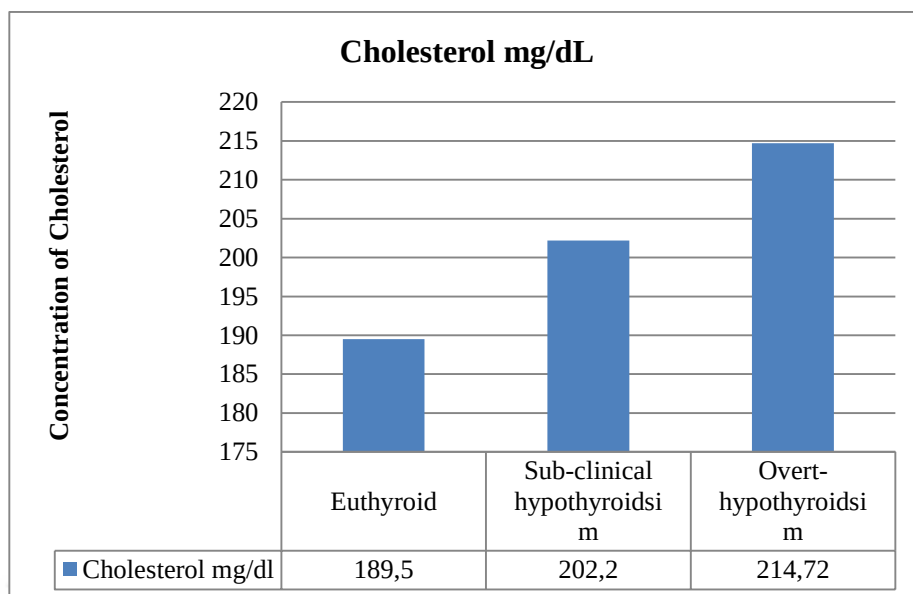


Figure 4.12 Chart of cholesterol values in patients' groups and control

4.8.2 Serum TG levels

As shown in Table 4.14 and Figure 4.13, serum TG concentration results were it was higher, but non-significant among the overt hypothyroidism group (199 ± 81.8 mg/dL), subclinical hypothyroidism group (178 ± 66.9 mg/dL) and euthyroidism group (163 ± 59.7 mg/dL).

Table 4.14 TG values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
TG (mg/dL) mean $\pm$ SD	163 $\pm$ 59.7	178 $\pm$ 66.9	199 $\pm$ 81.8
Euthyroidism vs. Subclinical Hypothyroidism (t = 0.842, P= 0.403) Euthyroidism vs. Overt Hypothyroidism (t=1.965, P= 0.533) Subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 1.033, P= 0.306)			

n: Number of patients.

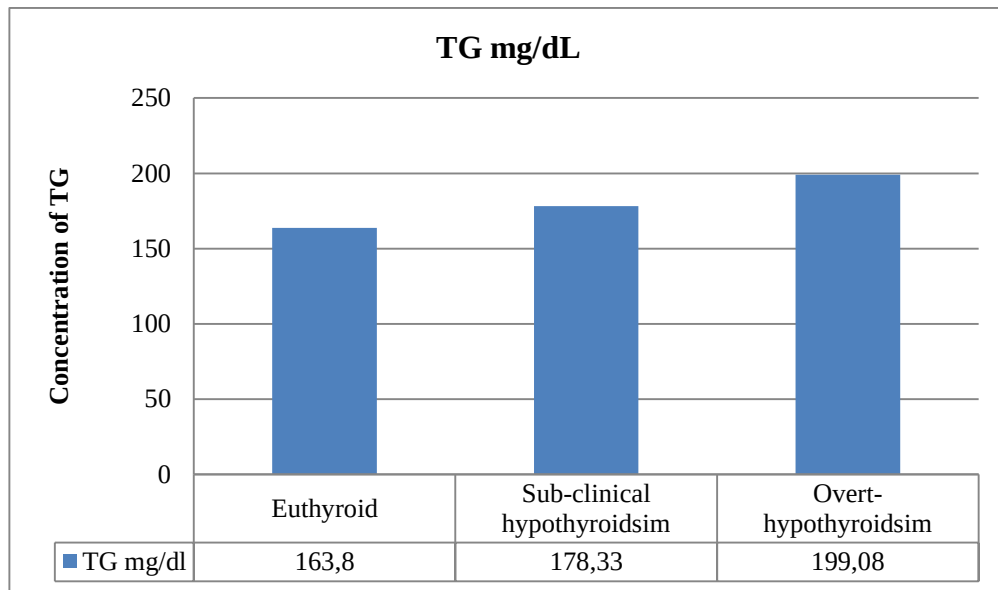


Figure 4.13 Chart of TG values in patients' groups and control

4.8.3 Serum HDL-cholesterol levels

As shown in Table 4.15 and Figure 4.14, mean serum HDL-cholesterol levels in the overt hypothyroid group (36.8 ± 8.9 mg/dL) were significantly lower than those in the euthyroid group (44.7 ± 5.93 mg/dL), and those in the subclinical hypothyroid group (42.1 ± 7.8 mg/dL).

The findings also revealed that serum HDL-cholesterol levels in the subclinical hypothyroid group were non-significantly lower than those in the euthyroid group.

Table 4.15 HDL-cholesterol values in patients' groups and control

Groups Parameter	Euthyroidism (n=30)	Subclinical Hypothyroidism (n=24)	Overt Hypothyroidism (n=36)
HDL-cholesterol (mg/dL) mean $\pm$ SD	44.7 $\pm$ 5.93	42.1 $\pm$ 7.8	36.8 $\pm$ 8.9
Euthyroidism vs. Subclinical Hypothyroidism (t = 1.38, P= 0.177)			
Euthyroidism vs. Overt Hypothyroidism (t= 4.155, P= 0.0001)			
Subclinical Hypothyroidism vs. Overt Hypothyroidism (t= 2.377, P= 0.0208)			

n: Number of patients.

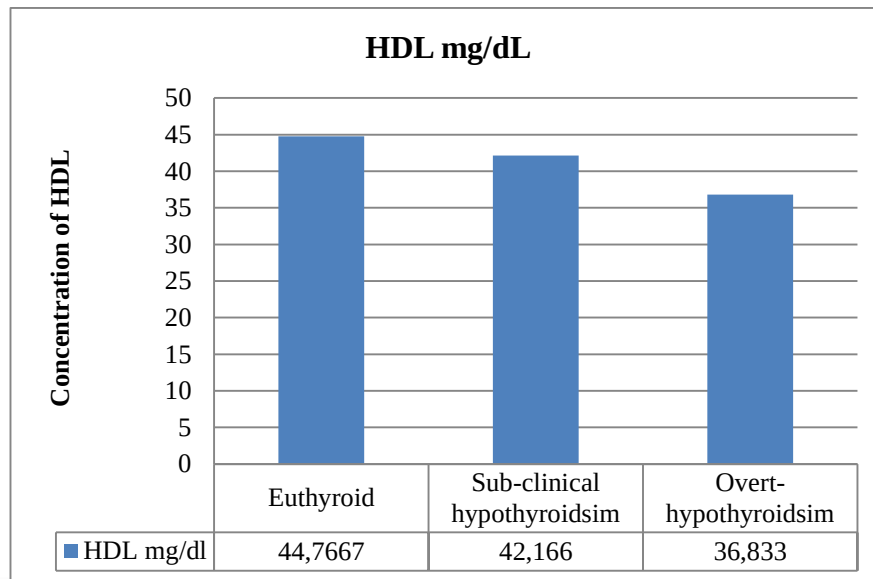


Figure 4.14 Chart of HDL-cholesterol values in patients' groups and control

4.8.4 Serum LDL-cholesterol levels

As shown in Table 4.16 and Figure 4.15, mean serum LDL-cholesterol levels in the overt hypothyroid group (136.6 ± 50.12 mg/dL) was significantly higher than those in the euthyroid group (111.4 ± 20.29 mg/dL).

The findings also revealed that serum LDL-cholesterol levels in the subclinical hypothyroid group (121.5 ± 37.72 mg/dL) were non-significantly when compared with euthyroid group and overt hypothyroidism group.

Table 4.16 LDL-cholesterol values in patients' groups and control

Groups Parameter	Euthyroidis m (n=30)	Subclinical Hypothyroidisim (n=24)	Overt Hypothyroidisim (n=36)
LDL-cholesterol (mg/dL) mean $\pm$ SD	111.4 $\pm$ 20.29	121.5 $\pm$ 37.72	136.6 $\pm$ 50.12
Euthyroidism vs. Subclinical Hypothyroidisim(t= 1.255, P= 0.215)			
Euthyroidism vs. Overt Hypothyroidisim (t= 2.583, P=0.0121)			
Subclinical Hypothyroidisim vs. Overt Hypothyroidisim (t= 1.261, P= 0.212)			

n: Number of patients.

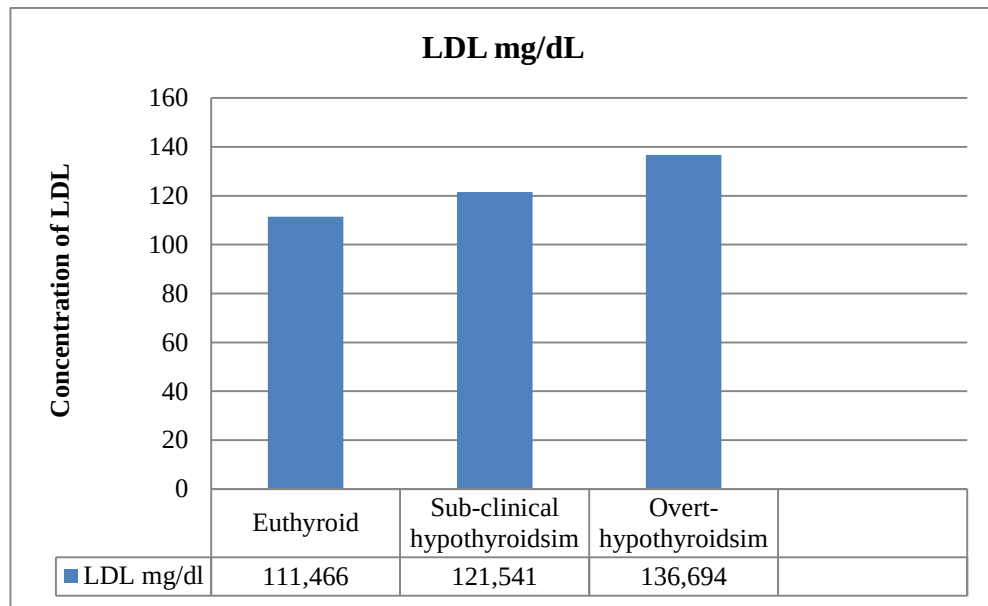


Figure 4.15 Chart of LDL-cholesterol values in patients' groups and control

4.9 The result of correlation

In the three groups of study, leptin, apelin, and insulin were significantly positively correlated with TSH, whereas only overt hypothyroidism was significantly negatively correlated with T4. The fact that irisin levels are substantially positively correlated with T4 in overt hypothyroidism and significantly negatively correlated with TSH in the subclinical hypothyroid group, as shown below the Table 4.17, 4.18, and 4.19.

Table 4.17 The result of correlation for hormone in Euthyroid group

Parameters	Euthyroid group					
	T3		T4		TSH	
	r	p	r	p	r	p
Leptin (pg/mL)	-0.07	0.699	-0.347	0.056	0.55	0.004
Apelin (pg/mL)	0.005	0.97	-0.347	0.060	0.383	0.036
Irisin (µg/mL)	-.019	0.922	0.177	0.348	-0.137	0.470
Insulin (µIU/mL)	-0.289	0.121	-0.299	0.224	0.582	0.0008

R: Correlation coefficient. P: Significance Level

Table 4.18 The result of correlation for hormone in Subclinical Hypothyroidism group

Parameters	Subclinical Hypothyroidism group					
	T3		T4		TSH	
	r	p	r	p	r	p
Leptin (pg/mL)	0.082	0.709	-0.121	0.582	0.578	0.0039
Apelin (pg/mL)	-0.022	0.919	-0.174	0.310	0.626	0.001
Irisin (μ g/mL)	-0.324	0.122	-0.013	0.952	-0.479	0.017
Insulin (μ IU/mL)	0.206	0.333	-0.037	0.830	0.766	0.0001

R: Correlation coefficient. P: Significance Level

Table 4.19 The result of correlation for hormone in Overt Hypothyroidism group

Parameters	Overt Hypothyroidism group					
	T3		T4		TSH	
	r	p	r	p	r	p
Leptin (pg/mL)	-0.312	0.062	-0.506	0.0116	0.357	0.032
Apelin (pg/mL)	0.070	0.683	-0.345	0.039	0.514	0.001
Irisin (μ g/mL)	0.141	0.411	0.336	0.045	-0.350	0.040
Insulin (μ IU/mL)	0.053	0.757	-0.484	0.0166	0.367	0.027

R: Correlation coefficient. P: Significance Level

5. DISCUSSION

Depending on the amount of thyroid hormones, thyroid dysfunction can be classified as overt or subclinical hypothyroidism. Overt hypothyroidism was determined to be present in individuals with elevated TSH and low T4 and T3 levels. TSH levels were elevated and subclinical hypothyroidism was identified by normal T3 and T4 values (Demers and Spencer 2003). Overt hypothyroidism was much less common than subclinical hypothyroidism. Subclinical hypothyroidism was thought to affect 4.3% to 9% of the general population. Females and older age groups have a higher frequency of subclinical hypothyroidism. In addition, subclinical hypothyroidism can develop into overt hypothyroidism. Higher levels of TSH were associated with faster rates of development (Rizos *et al.* 2011). The frequency of hypothyroidism increases with age and was more common in women (Canaris *et al.* 2000). These results are consistent with our results.

When comparing the overt hypothyroid group with the euthyroid control group, the mean results in Tables 4.5 and 4.6 revealed a significant rise in age and BMI. This finding may be explained by the fact that most patients with overt hypothyroidism experience weight gain (Gürel *et al.* 2015), whereas subclinical hypothyroidism and euthyroidism did not. In a related study, (Gürel *et al.* 2015) found no statistically significant differences between hypothyroidism, subclinical hypothyroidism and healthy controls groups with respect to age or BMI.

The protein leptin was produced primarily by adipocytes. Leptin suppresses food intake, causes weight loss, and has a profound effect on energy balance. Leptin affects IR, hunger, and obesity (El Amrousy *et al.* 2022). Adipose tissue also was the major source of apelin (Read *et al.* 2020). TSH and TH-receptors are found in adipose tissue, while Apelin receptors are found in the thyroid. Furthermore, Adipose tissue produces the hormone leptin in direct proportion to the quantity of adipose tissue in the body. Leptin predominantly influences hypothalamus neurons to cause satiety (Teixeira *et al.* 2020). Therefore, there may be a relationship between thyroid status, TD, and Leptin and Apelin, as changes in TH and TSH can affect Leptin and Apelin release (Gürel *et al.*

2015). Statistical analysis of the results showed that there were only significant differences in serum Leptin and Apelin levels in the overtly hypothyroid group compared to normal thyroid (euthyroidism), which was consistent with the results of previous studies (El Amrousy *et al.* 2022). Leptin in people with hypothyroidism has been tested by several investigators, and serum Leptin was higher in five studies, lower in three, and invariant at eight times compared with controls or euthyroid subjects (Zimmermann-Belsing *et al.* 2003). In the data presented in Tables 4.7 and 4.8, there were no discernible differences in serum leptin and apelin levels between the subclinical hypothyroidism when compared with overt hypothyroidism and with euthyroidism groups. These findings may be explained by the fact that subclinical hypothyroidism, which acts as a precursor to overt hypothyroidism. According to (Zorlu *et al.* 2014), a study of subclinical hypothyroidism and TD found that patients' Apelin levels did not differ from those of healthy controls.

Leptin and Apelin were shown to have a statistically significant relationship with TSH in thyroid disease based on data at Tables 4.17 to 4.19. A study by (El Amrousy *et al.* 2022), showed a substantial positive association between leptin and TSH levels in hypothyroid individuals. TSH can directly promote leptin synthesis in adipose tissue by stimulating TSH receptors on the surface of adipocytes. On the other hand, leptin regulates TRH synthesis in the hypothalamus and TSH release in the anterior pituitary (Teixeira *et al.* 2022). Apelin also plays an important role in controlling food and water intake by the hypothalamus and endocrine system. TSH production was inhibited by Apelin and its receptors in the hypothalamic-pituitary axis. Pituitary TSH production is reduced by Apelin (Matta and Saedii 2022). Furthermore, Apelin treatment results in modest and significant reductions in TSH levels in hypothyroid and euthyroid rats, presumably through APJ receptors in the hypothalamic-pituitary axis (Badavi *et al.* 2022).

According to our findings, there was no statistically significant difference between the group mean irisin levels ($P > 0.05$). This finding supports the findings of (Eshraghian and Jahromi 2014, Panagiotou *et al.* 2016). Correlation studies show that there were a positive correlation between Irisin levels and T4, and a possible negative correlation

between Irisin levels and TSH. Our findings are consistent with those of (Eshraghian and Jahromi 2014) established a negative association between Irisin levels and TSH, and a positive correlation between Irisin levels and T4.

The development of IR and glucose metabolism were greatly influenced by TH. IR was associated with both overt and subclinical hypothyroidisms. Available evidence indicates that peripheral tissue IR predominates in hypothyroidism (Gierach *et al.* 2014). In hypothyroidism, the rate of glucose absorption in the intestine was reduced, adrenergic activity was reduced, less glycogen was broken down by the liver and muscle, and gluconeogenesis and basal Insulin production were also reduced. Increased amounts of free fatty acids, decreased glucose absorption, and enhanced glucose oxidation were all associated with increased postprandial insulin secretion despite the presence of whole-body peripheral IR (Gierach *et al.* 2014). HOMA-IR and fasting serum insulin were significantly higher in subjects with hypothyroidism, regardless of obesity, compared with controls in the study by (El Amrousy *et al.* 2022, established the link between IR and hypothyroidism. Another study by (Vyakaranam *et al.* 2014) showed that HOMA-IR was fairly positively related to TSH, but negatively correlated with FT3 and FT4. These findings may be consistent with our results in this thesis.

Compared with euthyroid individuals, patients with overt hypothyroidism had significantly higher values of cholesterol (Table 4.14) and LDL- cholesterol (Table 4.17) and lower levels of HDL- cholesterol (Table 4.16). This was consistent with the results of (Pearce 2012), who noted that more than 90% of patients with overt hypothyroidism have hyperlipidemia, and serum total cholesterol and LDL- cholesterol levels were elevated by about 30% in those with overt hypothyroidism. Another study by (Canaris et al. 2000) established that patients with hypothyroidism have elevated levels of TC and LDL-C. Lipid profile, cholesterol and LDL- cholesterol levels were elevated in overt hypothyroidism patients. This was caused by decreased activity of LDL- cholesterol receptors and also affects LDL- cholesterol and IDL catabolism. Furthermore, overt hypothyroidism was associated with decreased LPL activity, which leads to decreased clearance of TG-rich lipoproteins. As a result, individuals with overt hypothyroidism may also have elevated TG levels, elevated VLDL levels (Rizos *et al.*

2011). Although the effects of overt hypothyroidism on HDL- cholesterol vary between studies, the majority of the data show that HDL- cholesterol levels were normal or slightly elevated from normal in study by (Pierce 2012). According to our results in the Table 4.14, there was no significant difference in mean serum TG levels between groups. These finding supports the study of (Pearce 2012) who found that overt hypothyroidism can normalize or elevate serum TG and VLDL levels. Adverse effects on lipids are known side effects of hypothyroidism. In fact, a linear increase in total cholesterol, LDL- cholesterol, and TG levels and a linear decrease in HDL- cholesterol levels have been shown with increasing TSH even within the normal range of TSH values. An early step in cholesterol production was the induction of 3-hydroxy-3-methylglutaryl-coenzyme A reductase by thyroid hormone. T3 also influences the activation of LDL- cholesterol receptor genes, which upregulates the LDL- cholesterol receptor. T3 directly binds to specific TH-sensitive sites and thus activates genes. In addition, T3 was involved in protecting LDL- cholesterol from oxidation. In fact, secondary dyslipidemia was often caused by hypothyroidism (Rizos *et al.* 2011).

Patients with clinically or potentially abnormal thyroid function were more likely to develop heart problems, due to altered lipid metabolism, and musculoskeletal problems (Caturegli *et al.* 2014). TSH has been shown to directly affect metabolism in studies similar to those described by (Badavi *et al.* 2022). Increased plasma total cholesterol and LDL- cholesterol levels and LDL- cholesterol /HDL- cholesterol ratios were found in individuals with overt hypothyroidism and were commonly associated with atherosclerosis and cardiac dysfunction. There were no discernible differences in subclinical hypothyroidism compared with euthyroid subjects with respect to total cholesterol, TG, LDL- cholesterol, or HDL- cholesterol (Pearce 2012) reported that subclinical hypothyroidism, defined as 1-11% of all patients with dyslipidemia. Although some studies have shown that people with subclinical hypothyroidism have higher levels of total cholesterol and LDL- cholesterol, other studies have shown no relationship between subclinical hypothyroidism and these lipid markers. Overall, there was a weak correlation between blood levels of Leptin, Apelin, Irisin, and Insulin and serum TSH. Our findings suggest that assessment of these parameters may be important and should be included in future screening of adult patients with hypothyroidism.

6. CONCLUSIONS AND RECOMMENDATION

1. The overt hypothyroid group had the highest mean Leptin, Insulin, and HOMA values, and the euthyroid group had the lowest. In contrast, the group with overt hypothyroidism had the lowest Apelin levels, while the group with euthyroidism had the highest.
2. Compared to the euthyroid group, both patient groups (overt and subclinical hypothyroidism) had the lowest mean HDL- cholesterol levels and the highest cholesterol and LDL-cholesterol levels.
3. Mean Irisin, glucose, and TG levels were not statistically significantly different across groups.
4. In the three study groups, Leptin, Apelin, and Insulin showed strong positive correlations with TSH, and negative correlations with T4 in the overt hypothyroid group only.
5. Irisin levels were statistically significant inversely associated with TSH in both patient groups (overt and asymptomatic hypothyroidism) and statistically significant inversely with T4 in overt hypothyroidism only.

In the future, potential associations between thyroid hormones (particularly serum TSH concentrations) and serum levels of Leptin, Apelin, and Insulin will drive these tools into the screening of adult patients with hypothyroidism. It was recommended that a large patient's population were needed to generate better data to validate our findings.

REFERENCES

- Ahmed, A., Abeer, A. A., Al-Raof, A. B. D. and Yasser, M. 2018. Role of changes in some adipokines with obesity in relation to thyroid function in early diagnosed patients with subclinical hypothyroidism. *The Medical Journal of Cairo University*, (86): 4657-4665.
- Al Mohareb, O., Al Saqaaby, M., Ekhzaimy, A., Hamza, M., AlMalki, M. H., Bamehriz, F. and Brema, I. 2021. The Relationship Between Thyroid Function and Body Composition, Leptin, Adiponectin, and Insulin Sensitivity in Morbidly Obese Euthyroid Subjects Compared to Non-obese Subjects. *Clinical Medicine Insights: Endocrinology and Diabetes*, (14): 11-23.
- Alzahrani, A. S., Al Mourad, M., Hafez, K., Almaghamsy, A. M., Alamri, F. A., Al Juhani, N. R. and Abdelhamid, E. 2020. Diagnosis and Management of Hypothyroidism in Gulf Cooperation Council (GCC) Countries. *Advances in Therapy*, 37(7): 3097-3111.
- Babić Leko, M., Gunjača, I., Pleić, N. and Zemunik, T. 2021. Environmental factors affecting thyroid-stimulating hormone and thyroid hormone levels. *International Journal of Molecular Sciences*, 22(12): 6521.
- Badavi, M., Grootveld, M., Jafari, F., Dianat, M. and Faraji Shahrivar, F. 2022. Supplement therapy with apelin for improving the TSH level and lipid disorders in PTU- induced hypothyroid rats. *Biotechnology and Applied Biochemistry*, 69(2): 668-675.
- Bekkering, G. E., Agoritsas, T., Lytvyn, L., Heen, A. F., Feller, M., Moutzouri, E. and Vermandere, M. 2019. Thyroid hormones treatment for subclinical hypothyroidism: A Clinical Practice Guideline, 365.
- Benvenga, S., Tuccari, G., Ieni, A. and Vita, R. 2018. Thyroid gland: anatomy and physiology. *Encyclopedia of Endocrine Diseases*, 4:382-390.
- Brent, G. A. 2012. Mechanisms of thyroid hormone action. *The Journal of Clinical Investigation*, 122(9): 3035-3043.
- Canaris, G. J., Manowitz, N. R., Mayor, G. and Ridgway, E. C. 2000. The Colorado thyroid disease prevalence study. *Archives of Internal Medicine*, 160(4): 526-534.

- Caturegli, P., De Remigis, A. and Rose, N. R. 2014. Hashimoto thyroiditis: clinical and diagnostic criteria. *Autoimmunity Reviews*, 13(4-5): 391-397.
- Chiovato, L., Magri, F. and Carlé, A. 2019. Hypothyroidism in context: where we've been and where we're going. *Advances in Therapy*, 36(2): 47-58.
- Demers, L. M. and Spencer, C. A. 2003. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. *Clinical Endocrinology*, 58(2): 138-140.
- Duntas, L. H. 2002. Thyroid disease and lipids. *Thyroid*, 12(4): 287-293.
- Duntas, L. H. and Yen, P. M. 2019. Diagnosis and treatment of hypothyroidism in the elderly. *Endocrine*, 66(1): 63-69.
- El Amrousy, D., El-Afify, D. and Salah, S. 2022. Insulin resistance, leptin and adiponectin in lean and hypothyroid children and adolescents with obesity. *BMC Pediatrics*, 22(1): 1-6.
- Engels, K., Rakov, H., Hönes, G. S., Brix, K., Köhrle, J., Zwanziger, D. and Führer-Sakel, D. 2019. Aging alters phenotypic traits of thyroid dysfunction in male mice with divergent effects on complex systems but preserved thyroid hormone action in target organs. *The Journals of Gerontology*, 74(8): 1162-1169.
- Erden, Y., Tekin, S., Tekin, C., Ozyalin, F., Yilmaz, U., Onalan, E. E. and Sandal, S. 2018. Effect of intracerebroventricular administration of apelin-13 on the hypothalamus–pituitary–thyroid axis and peripheral uncoupling proteins. *International Journal of Peptide Research and Therapeutics*, 24(4): 511-517.
- Eshraghian, A. and Jahromi, A. H. 2014. Non-alcoholic fatty liver disease and thyroid dysfunction: a systematic review. *World Journal of Gastroenterology*, 20(25): 8102.
- Gaitonde, D. Y., Rowley, K. D. and Sweeney, L. B. 2012. Hypothyroidism: an update. *South African Family Practice*, 54(5): 384-390.
- Gierach, M., Gierach, J. and Junik, R. 2014. Insulin resistance and thyroid disorders. *Endokrynologia Polska*, 65(1): 70-76.
- Guariglia, M. D. 2021. Management and Diagnosis of Hypothyroidism. Department
- Gürel, A., Doğantekin, A., Özkan, Y. and Aydın, S. 2015. Serum apelin levels in patients with thyroid dysfunction. *International Journal of Clinical and Experimental Medicine*, 8(9): 16394.

- Kar, K. and Sinha, S. 2017. Variations of adipokines and insulin resistance in primary hypothyroidism. *Journal of Clinical and Diagnostic Research*, 11(8): BC07.
- Knobel, M. 2021. An overview of retrosternal goiter. *Journal of Endocrinological Investigation*, 44(4): 679-691.
- Matta, R. A. and Saedii, A. A. 2022. Cross-Talk between Apelin, Insulin Resistance, Thyroid Hormones, and Cardio-metabolic Risk Factors in Polycystic Ovary Syndrome. *The Egyptian Journal of Hospital Medicine*, 87(1): 1635-1643.
- Menendez, C., Baldelli, R., Camina, J. P., Escudero, B., Peino, R., Dieguez, C. and Casanueva, F. F. 2003. TSH stimulates leptin secretion by a direct effect on adipocytes. *Journal of Endocrinology*, 176(1): 7-12.
- O'Carroll, A. M., Lolait, S. J., Harris, L. E. and Pope, G. R. 2013. The apelin receptor APJ: journey from an orphan to a multifaceted regulator of homeostasis. *J Endocrin.*, 219(1): 13-35.
- of Family & Community Medicine Presentations and Grand Rounds. 504:1-39.
- Panagiotou, G., Pazaitou-Panayiotou, K., Paschou, S. A., Komninou, D., Kalogeris, N., Vryonidou, A. and Mantzoros, C. S. 2016. Changes in thyroid hormone levels within the normal and/or subclinical hyper-or hypothyroid range do not affect circulating irisin levels in humans. *Thyroid*, 26(8): 1039-1045.
- Pearce, E. N. 2012. Update in lipid alterations in subclinical hypothyroidism. *The Journal of Clinical Endocrinology and Metabolism*, 97(2): 326-333.
- Rakov, H., De Angelis, M., Renko, K., Hönes, G. S., Zwanziger, D., Moeller, L. C. and Führer, D. 2019. Aging is associated with low thyroid state and organ-specific sensitivity to thyroxine. *Thyroid*, 29(12): 1723-1733.
- Rizos, C. V., Elisaf, M. S. and Liberopoulos, E. N. 2011. Effects of thyroid dysfunction on lipid profile. *The Open Cardiovascular Medicine Journal*, 5: 70-76.
- Roos, A., Bakker, S. J., Links, T. P., Gans, R. O. and Wolffenbuttel, B. H. 2007. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. *The Journal of Clinical Endocrinology and Metabolism*, 92(2): 491-496.
- Russo, S. C., Salas-Lucia, F. and Bianco, A. C. 2021. Deiodinases and the metabolic code for thyroid hormone action. *Endocrinology*, 162(8): bqab059.

- Simsir, I. Y., Cetinkalp, S. and Kabalak, T. 2020. Review of factors contributing to nodular goiter and thyroid carcinoma. *Medical Principles and Practice*, 29(1): 1-5.
- Stathatos, N. 2012. Thyroid physiology. *Medical Clinics*, 96(2): 165-173.
- Teixeira, P. D. F. D. S., Dos Santos, P. B. and Pazos-Moura, C. C. 2020. The role of thyroid hormone in metabolism and metabolic syndrome. *Therapeutic Advances in Endocrinology and Metabolism*, 11, 2042018820917869.
- Teumer, A., Chaker, L., Groeneweg, S., Li, Y., Di Munno, C., Barbieri, C. and Medici, M. 2018. Genome-wide analyses identify a role for SLC17A4 and AADAT in thyroid hormone regulation. *Nature Communications*, 9(1): 4455.
- Vyakaranam, S., Vanaparthi, S., Nori, S., Palarapu, S. and Bhongir, A. V. 2014. Study of insulin resistance in subclinical hypothyroidism. *International Journal of Health Sciences and Research*, 4(9): 147-155.
- Walsh, J. P. 2016. Managing thyroid disease in general practice. *Medical Journal of Australia*, 205(4): 179-184.
- Wilson, S. A., Stem, L. A. and Bruehlman, R. D. 2021. Hypothyroidism: diagnosis and treatment. *American Family Physician*, 103(10): 605-613.
- Yanai, H. 2019. Differential diagnosis of thyrotoxicosis. *Journal of Endocrinology and Metabolism*, 9(5): 127-132.
- Yasar, H. Y., Demirpence, M., Colak, A., Yurdakul, L., Zeytinli, M., Turkon, H. and Yasar, E. 2019. Serum irisin and apelin levels and markers of atherosclerosis in patients with subclinical hypothyroidism. *Archives of Endocrinology and Metabolism*, 63: 16-21.
- Zimmermann-Belsing, T., Brabant, G., Holst, J. J. and Feldt-Rasmussen, U. 2003. Circulating leptin and thyroid dysfunction. *European Journal of Endocrinology*, 149(4): 257-272.
- Zoeller, R. T. 2021. Endocrine disrupting chemicals and thyroid hormone action. In *Advances in Pharmacology*, (92): 401-417.
- Zorlu, M., Kiskac, M., Karatoprak, C., Kesgin, S., Cakirca, M., Yildiz, K. and Erkoc, R. 2014. Assessment of serum apelin and lipocalin-2 levels in patients with subclinical hypothyroidism. In *Annales Dendocrinologie*, 75(1): 10-14.

CURRICULUM VITAE

Personal Information

Name and Surname : Harith Khaleel Hamad AL-FAHDAWI

Education

MSc Çankırı Karatekin University
Graduate School of Natural and Applied Sciences 2021-2023
Department of Chemistry

Undergraduate AL-Anbar University
Faculty of Engineering 1999-2003
Department of Chemistry