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INVESTIGATION OF SERUM ISTHMIN-1 PROTEIN LEVELS AND ITS RELATIONSHIP WITH ENDOGENOUS LIPID METABOLISM IN HYPERTRIGLYCERIDEMIC INDIVIDUALS

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MASTER'S THESIS

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DECLARATION

I declare that this thesis was accomplished and written in accordance with the standards of Karadeniz Technical University Graduate School of Health Sciences Thesis Preparation and Writing Guide. The present thesis is original scientific research carried out by committing to academic and ethical rules. I confirm that I have cited the sources of any information or comments that were not obtained by the present study in the list of references, and I have not acted in violation of patents and copyrights during the study and writing of the thesis.

10/*07/2025*

Kawther Ameen Muhammed Saeed ALEDRESI

Dedication

I would like to dedicate this thesis to my beloved parents, Prof. Dr. Ameen Muhammed Saeed ALEDRESI and Dr. Shatha Fawzi Mahmood AL-KHAYAT, for their unwavering encouragement and support throughout my journey. I would also like to thank my sisters, Maryam, Alaa, and Shifaa, for their love and compassion. Additionally, I am grateful to my dear friend Entesar AMER, I would like to thank all my friends and the teaching staff at Karadeniz Technical University for their help and support.

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LIST of ABBREVIATIONS, SYMBOLS and FORMULA

Abbreviations

ACC	Acetyl-CoA Carboxylase
Acetyl-CoA	Acetyl-Coenzyme A
ADP	Adenosine Diphosphate
AHA/ACC	American Heart Association/American College of Cardiology
AIP	Atherogenic Index of Plasma
Akt	Protein Kinase B
ALT	Alanine Aminotransferase
AMOP	Adhesion-Associated Domain in MUC4 and Other Proteins
Apo B100	Apolipoprotein B 100
Apo B48	Apolipoprotein B48
AST	Aspartate Aminotransferase
ATGL	Adipose Triglyceride Lipase
ATP	Adenosine Triphosphate
AUC	Area Under Curve
BC71	Proapoptotic Cyclic Peptide
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
C	Cysteine
CAD	Coronary Artery Disease
CE	Cholesterol Ester
CHREBPβ	Carbohydrate Response Element Binding Protein
CM	Chylomicron
DAG	Diacylglycerol
DBP	Diastolic Blood Pressure
DNL	*De Novo* Lipogenesis
DSPN	Diabetic Sensorimotor Peripheral Neuropathy
EAS	European Atherosclerosis Society
EC	Endothelial Cell
ESC	European Society of Cardiology
eGFR	Estimated Glomerular Filtration Rate

ELISA	Enzyme-Linked Immunosorbent Assay
eWAT	Epidymal White Adipose Tissues
F	Female
FAS	Fatty Acid Synthase
FFA	Free Fatty Acids
FLD	Fatty Liver Disease
G3P	Glycerol-3-Phosphate
GDM	Gestational Diabetic Mellitus
GLUT2	Glucose Transporter 2
GLUT4	Glucose Transporter Protein 4
GRP78	Glucose- Regulated Protein 78
HC	Hip Circumference
HDL-C	High-Density Lipoprotein Cholesterol
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HSC	Hematopoietic Stem Cell
HSL	Hormone-Sensitive Lipase
HTG	Hypertriglyceridemia
HTHC	Hypercholesterolemic Hypertriglyceridemia
HTNC	Normorcholesterolemic Hyperteriglyceridemia
IDL	Intermediate-Density Lipoprotein
INSR	Insulin Receptor
IQR	Interquartile Range
IR	Insulin Resistance
ISM1	Isthmin-1
ISM2	Isthmin-2
iWAT	Inguinal White Adipose Tissues
LDH	Lactate Dehydrogenase
LDL-C	Low-Density Lipoprotein Cholesterol
LpL	Lipoprotein Lipase
M	Male
MADB	4-Aminophenazone N, N-Bis(4-Sulfobutyl)- 3,5-Dimethylaniline, Disodium Salt
MAG	Monoacylglycerol

MAP	Mean Arterial Pressure
METS-IR	Metabolic Score for Insulin Resistance
MGL	Monoacylglycerol Lipase
MHB	Midbrain-Hindbrain Boundary
mTOR	Mammalian Target of Rapamycin
MTTP	Microsomal Triglyceride Transfer Protein
MUC4	Mucin-4
N	Asparagine
NAD^+	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
NEFFA	Non-Esterified Free Fatty Acid
NF-Kb	Nuclear Factor Kappa
OD	Optical Density
P53	Tumor Suppressor Protein
PGC1β	Peroxisome Proliferator-Activated Receptor γ Coactivator 1β
PI3K	Phosphoinositide 3-Kinase
PKC	Protein Kinase C
Q	Glutamine
R	Arginine
RLP	Remnant Lipoprotein
ROC	Receiver-Operating Characteristic Curve
ROS	Reactive Oxygen Species
SBP	Systolic Blood Pressure
SD	Standard Deviation
SP	Signal Peptide
SPSS	Statistical Package for Social Sciences
SREBP-1c	Sterol Regulatory Element-Binding Protein 1c
T2DM	Type 2 Diabetic Mellitus
TC	Total Cholesterol
TG	Triglyceride
TRL	Triglyceride-Rich Lipoprotein
Trp/W	Tryptophan
TSR1	Thrombospondin Type 1 Repeat

TyGi	Triglyceride Index
VEGF	Vascular Endothelial Growth Factor
VLDL	Very Low-Density Lipoprotein
WC	Waist Circumference

Symbols

&	And
%	Percentage
α	Alpha
β	Beta
γ	Gamma

Formula

CO_2	Carbon dioxide
Ca^{2+}	Calcium
HCO_3^-	Bicarbonate
H_2O	Water
H_2O_2	Hydrogen peroxide
Na^+	Sodium
NH_3	Ammonia
O_2	Oxygen

ABSTRACT

Investigation of Serum Isthmin-1 Protein Levels and Its Relationship with Endogenous Lipid Metabolism in Hypertriglyceridemic Individuals

Hypertriglyceridemia (HTG) is a type of dyslipidemia defined by elevated triglyceride (TG) levels, typically exceeding 150 mg/dL. It is an important medical condition to study as it is the primary cause of various complications, including atherosclerosis, insulin resistance (IR), metabolic syndrome and pancreatitis. This study aimed to quantify the levels of isthmin-1 (ISM1) protein in individuals with HTG and to evaluate its relationship with the biochemical parameters. The study involved 80 individuals who were divided into two groups: Control (n = 40) and HTG (n = 40). The HTG group was further divided into two subgroups: normocholesterolemic hypertriglyceridemia (HTNC=20) and hypercholesterolemic hypertriglyceridemia (HTHC=20). The levels of serum ISM1, apolipoprotein B 100 (Apo B100), and microsomal triglyceride transfer protein (MTTP) were determined using commercial sandwich enzyme-linked immunosorbent assay **(**ELISA) kits. A colorimetric assay kit was utilized to assess non-esterified free fatty acid (NEFFA) levels in the samples. ISM1 levels in HTG group, HTNC, and HTHC subgroups were found to be higher than in the control group ($p < 0.05$). In addition, ISM1 showed positive correlations with most of the demographic and biochemical parameters including insulin resistance indices, ($p < 0.05$). It has been suggested that ISM1 may have a pivotal role in developing hypertriglyceridemia and insulin resistance.

Keywords: Hypertriglyceridemia, Insulin, Isthmin-1

ÖZET

Hipertrigliseridemik Bireylerde Serum İstmin-1 Protein Düzeyleri ve Endojen Lipid Metabolizmasıyla İlişkisinin İncelenmesi

Hipertrigliseridemi (HTG), tipik olarak 150 mg/dL'yi aşan yüksek trigliserid (TG) düzeyleri ile tanımlanan bir dislipidemi türüdür. Ateroskleroz, insülin direnci (IR), metabolik sendrom ve pankreatit gibi çeşitli komplikasyonların birincil nedeni olması nedeniyle araştırılması gereken önemli tıbbi durumdur. Bu çalışma, HTG'li bireylerde istmin-1 (ISM1) proteini düzeylerini belirlemeyi ve biyokimyasal parametrelerle ilişkisini değerlendirmeyi amaçlamıştır. Çalışmaya iki gruba ayrılan 80 kişi dahil edildi: Kontrol (n = 40) ve HTG (n = 40). HTG grubu ayrıca iki alt gruba da ayrıldı: normorkolesterolemik hipertrigliseridemi (HTNC=20) ve hiperkolesterolemik hipertrigliseridemi (HTHC = 20). Serum ISM1, apolipoprotein B 100 (Apo B100) ve mikrozomal trigliserit transfer protein (MTTP) düzeyleri ticari sandviç enzim-bağlı immünosorbent testi (ELISA) kitleri kullanılarak belirlendi. Numunelerdeki esterleşmemiş serbest yağ asidi (NEFFA) düzeylerini değerlendirmek için bir kolorimetrik analiz kiti kullanıldı. HTG grubunda, HTNC ve HTHC alt gruplarında ISM1 düzeyleri kontrol grubundan daha yüksek bulundu ($p < 0,05$). Ek olarak, ISM1, insülin direnci endeksleri de dahil olmak üzere demografik ve biyokimyasal parametrelerin çoğuyla anlamlı şekilde pozitif korelasyonlar gösterdi ($p < 0,05$). ISM1'in hipertrigliseridemi ve insülin direncinin gelişmesinde önemli bir rol oynayabileceği öne sürülmüştür.

Anahtar Sözcükler: Hipertrigliseridemi, İnsülin, Istmin-1

1. INTRODUCTION and AIM

Dyslipidemia is the term given to various abnormalities in lipid metabolisms, one of which is HTG. The European Atherosclerosis Society (EAS) and the European Society of Cardiology (ESC) define mild HTG as having TG levels that are equal to or exceed 150 mg/dL (1.7 mmol/L) (1), which give rise to the accumulation of triglyceride-rich lipoproteins (TRLs), remnant lipoproteins (RLPs), elevated low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) levels (2).

The primary source of TGs is obtained from diets and carried in the blood in the form of chylomicrons (CMs). However, TG are also produced endogenously in the liver and adipose tissues by the *de novo* lipogenesis pathway (DNL) and carried in the blood with the assistance of very low-density lipoproteins (VLDLs) (3). HTG is categorized into two distinct classifications: primary HTG and secondary HTG (4). Primary HTG is primarily caused by genetic defects in the proteins that facilitate TG lipolysis. On the other hand, secondary HTG originates from environmental factors such as obesity, diabetes, or drug abuse (4). Severe HTG is considered a serious disorder as abnormal aggregation of lipids in the blood vessels can increase blood viscosity, vasoconstriction in capillary beds, and thrombus formation, thus increasing the risk of atherosclerosis, coronary artery disease (CAD), pancreatic necrosis, IR, fatty liver diseases (FLDs), metabolic syndromes, and nephrotic syndromes (5).

Insulin is the main polypeptide hormone that is released from the pancreas and plays a pivotal role in the uptake of glucose through the activation of the phosphoinositide 3-kinase / protein kinase B pathway (PI3K/Akt) and translocation of glucose transporter protein 4 (GLUT4) to the plasma membrane (6,7). In conditions marked by an accumulation of visceral body fat, an elevated risk of HTG and the release of free fatty acids (FFA) occur. This, in turn, impairs the response of insulin-sensitive organs, ultimately leading to IR and hyperinsulinemia (8, 9). In the same context, IR increases the sensitivity of adipose tissues' hormone-sensitive lipase (HSL), thereby augmenting lipolysis and the release of FFA from adipose tissues, which leads to stimulation of large TG-rich VLDLs synthesis and HTG. Thus, HTG and IR show an intertwined relationship (10).

ISM1 protein was first identified in 2002 (11) at the midbrain-hindbrain boundary (MHB), commonly referred to as the isthmus of the brain. It is derived from adipose

tissues and exhibits prominent expressions in various organs, like liver, lung, skeletal muscles and kidney (12). Recent studies have asserted that ISM1 is an insulin- like adipokine that enhances adipocyte glucose uptake through activating P13K-AKT signaling pathway by binding to a receptor distinct from that of insulin, however, it diminishes hepatic lipogenesis, encouraging hepatocytes to shift from lipogenic to protein synthesis pathways (12-14). As a result, this adipokine has been considered a pivotal advancement in the treatment of many diseases related to the irregulated metabolic process in the body, like IR, metabolic disorders and HTG (15, 16).

This study aims to determine the ISM1 protein levels in hypertriglyceridemic individuals and to evaluate its relationship with lipid parameters, insulin and insulin resistance indices.

2. LITERATURE REVIEW

2.1. Hypertriglyceridemia

2.1.1. Triglyceride Metabolism

TG is a type of lipid formed from three fatty acids connected to a glycerol backbone through ester bonds. These lipids are primarily stored in adipose tissues and act as an important source of energy during the fasting periods (17).

Dietary fats increase the concentration of TGs carried in CMs and it is considered as the primary source of TGs; thus, the DNL in liver and adipose tissues makes a minor contribution to maintaining TG levels within appropriate limits (3). After consuming a fat-rich diet, TG, along with other fats, such as cholesterol, phospholipids, and fat-soluble vitamins, are first digested in the stomach by gastric lipase and lingual lipase. Once these lipids enter the small intestine are further digested by pancreatic lipase, cholesterol ester (CE) hydrolase, and phospholipase A2. The result components from the mentioned enzymes are monoacylglycerol (MAG), FFA, free cholesterol, and lysophospholipids (4). These lipids are emulsified by bile salt to form micelles that can pass the brush border and enter the intestinal mucosal cells (3). In the intestine, these lipids will reesterify into TG, CE, and phospholipids facilitated by the action of acyltransferase enzyme (4). Then they aggregate into CMs and acquire apolipoprotein B48 (Apo B48) from MTTP. Finally, the CMs leave the small intestine and travel to the peripheral organs, where they can liberate their FFA and glycerol and form CM remnants by the action of lipoprotein lipase (LpL) (3, 4, 18). CM remnants can be taken back by the liver, where they are further hydrolyzed to CE and FFA by lysosomes (4).

DNL is the process of synthesizing lipids from carbohydrate sources. This process is typically active after the intake of high-carbohydrate diets or during fasting. In DNL, fatty acids are synthesized from acetyl-Coenzyme A (acetyl-CoA) molecules originating from carbohydrate metabolism. This process mainly occurs in the liver and adipose tissues (19). DNL recruits a series of enzymes that begin with the conversion of citrate to acetyl-CoA by adenosine triphosphate (ATP)-citrate lyase, then acetyl-CoA is carboxylated to malonyl-CoA by acetyl-CoA carboxylase (ACC). The fatty acid synthase (FAS) enzyme is the rate-limiting factor in the synthesis of fatty acids from malonyl-CoA. In the liver, the combination of the newly synthesized fatty acids and glycerol-3-phosphate (G3P) forms TGs, which are then packaged with CE and phospholipids in VLDLs. This lipoprotein

carries the ApoB100 that's also obtained from MTTP. The production of both CMs and VLDLs requires MTTP to be transported to the blood vessels (3, 20, 21). When VLDLs reach specific tissues, the lipoproteins are broken down by LpL into fatty acids and glycerol (18, 20, 22, 23). The remaining VLDL after hydrolyzing by LPL are referred to as intermediate-density lipoproteins (IDLs), after which they are converted to LDLs and are taken up by the liver and other tissues (4, 22, 24).

During exercise or a fasting state, the TGs stored in the adipose tissues will be hydrolyzed by the action of adipose triglyceride lipase (ATGL/Desnutrin), HSL, and monoacylglycerol lipase (MGL). This process will release FFA and glycerol that supply the organs with the energy and heat they need (25).

2.1.2. Effects of Hypertriglyceridemia

HTG, which is a type of dyslipidemia, is a lipid deterioration characterized by high levels of TRLs, RLPs, increased levels of LDL-C, and reduced levels of high-density lipoprotein cholesterol (HDL-C). This lipid perturbance can significantly increase the risk for CAD and atherosclerosis (26).

According to the American Heart Association/American College of Cardiology (AHA/ACC) and the National Lipid Association, TG values classified as normal (< 150 mg/dL), borderline elevated (150-199 mg/dL), high (200-499 mg/dL) and very high (≥ 500 mg/dL). Furthermore, the Endocrine Society considers values of 1000 to 1999 mg/dL to be severe and greater than 2000 mg/dL to be very severe. Additionally, EAS states that values less than 100 mg/dL are optimal. The ECG declares that levels less than 150 mg/dL are normal. Both the EAS and ESC deem that TG levels above 880 mg/dL to be severe. HTG is recognized as a serious metabolic disorder that raises the risk of atherosclerosis and related heart diseases (1, 27-29).

HTG can be categorized into two types: primary HTG and secondary HTG. Primary HTG is typically related to genetic disturbances that can be classified according to Fredrickson and Lees' classification into five categories, which are familial hyperchylomicronemia, familial hypercholesterolemia, familial combined hypercholesterolemia, familial dysbetalipoproteinemia, familial hyperlipidemia, and endogenous HTG (4, 30). These inherited lipid disorders usually display deficiencies in the LDL receptors, mutations in LpL and its activation and maturation factors, or defects in Apo B. On the other hand, secondary HTG is primarily caused by daily behaviors and/or

clinical conditions, for instance, excessive dietary intake of food rich in TGs, obesity, diabetes, pregnancy, metabolic syndromes, kidney disorders, alcohol, and drug abuse (4, 31). Considering all these issues together can result in the accumulation or decreased clearance of TRLs and RLPs (32).

Additionally, HTG shows a significant risk factor for the incidence of pancreatic necrosis and mortality. The surplus and irregular hydrolysis of TG into fatty acids by pancreatic lipase can decrease the bicarbonate (HCO_3^-) and fluid secretion of pancreatic ductal cells, increase Calcium (Ca^{2+}) levels, deplete ATP, and impair mitochondrial complexes in pancreatic acinar cells, eventually leading to deterioration in pancreatic ducts and wounding of the pancreatic acinar cells. Additionally, pancreatic ischemia and possible metabolic acidosis are other intricate complications caused by acute pancreatitis, which are associated with increased blood viscosity and HTG (1,33).

Consequently, HTG poses a threat to blood vessel integrity and increases the risk of atherosclerosis, CAD, IR, FLD, metabolic syndromes, nephrotic syndromes, and pancreatitis (5, 34).

2.1.3. Insulin Resistance and Hypertriglyceridemia

Insulin is an endocrine polypeptide hormone that is released from the β- islet cells of Langerhans of the pancreas. The synthesis of insulin is preceded by the production of preproinsulin in the endoplasmic reticulum and proinsulin in the Golgi apparatus; the outcome of these two products is insulin and C- peptide. This hormone is made of 51 amino acids and is composed of two polypeptide chains, which are the α and β chains. Two disulfide bonds interlink these polypeptide chains. As an anabolic hormone, insulin plays crucial roles in the liver, muscles, and adipose tissues. It promotes important metabolic processes such as glycolysis, lipogenesis, protein synthesis, and glycogenesis, while simultaneously suppressing lipolysis and gluconeogenesis in the liver (6, 7, 35, 36).

During nutrient availability, the release of insulin is stimulated from the β- islet cells of the pancreas following the binding of glucose to the glucose transporter 2 (GLUT2) receptor situated on the plasma membrane of the pancreas. This initiates membrane depolarization and activation of the Ca^{2+} channel, promoting insulin secretion. Once released, insulin binds to the insulin receptor (INSR) on the target cell. This interaction triggers conformational changes on the receptor, resulting in receptor dimerization and intracellular phosphorylation of the tyrosine residue of the β- subunit of

INSR. This process switches on a series of reactions that activate the PI3K/Akt pathway, facilitate the translocation of GLUT4 to the plasma membrane, and initiate the absorption of glucose into the cell (6, 7, 36, 37).

Insulin resistance is the impairment of the insulin-sensitive tissues' response to the presence and stimulation of insulin. Thus, insulin cannot induce glucose uptake by the cells, ultimately resulting in suppression of lipogenesis and glycogen synthesis in the liver, adipose tissues, and skeletal muscles. During this condition, pancreatic cells enhance the release of insulin to compensate for the deterioration in the insulin response. As a result, fasting plasma insulin levels rise, which is referred to as hyperinsulinemia (8, 37, 38).

IR worsens HTG and increases liver lipogenesis by increasing the production of TRL and decreasing its clearance (39). Under IR, the sensitivity of adipose tissues to insulin declines, causing an increase in the expression of HSL, the latter stimulates lipolysis and increases the secretion of FFA. Plasma FFA, along with TRL remnants, are taken by the liver, increasing the synthesis of hepatic TGs (10, 40). In the liver, the DNL pathway is initiated, where the production of TGs is activated, accompanied by a reduction in the degradation of Apo B100, stimulating the activation of MTTP, and eventually facilitating the assembly and the secretion of VLDL (10, 41). The elevated plasma levels of large TG-rich VLDL particles (VLDL1) enhance the activity of cholesterol ester transfer protein (CETP). CETP initiates the exchange of TGs from the VLDL1 with CE in LDL-C and HDL-C, producing triglyceride-rich LDL and triglyceride-rich HDL. The prolonged retention of triglyceride-rich LDL leads to the emergence of sdLDL. Ultimately, the sdLDL undergoes several atherogenic modifications that result in the formation of oxidized LDL, electronegative LDL, desialylated LDL, and glycated LDL in plasma. Additionally, IR also stimulates the activity of MTTP in the intestine and promotes the formation of CMs, diminishes the activity of LpL, and triggers Apo CIII secretion, resulting in decreased hydrolysis of CM and VLDL. Further, this results in the aggregation of fasting and postprandial TGs (10, 41).

In the same context, excess calorie intake boosts the accumulation of visceral fats, which will stimulate the secretion of inflammatory factors, increasing lipolysis, expanding the storage of adipocytes in visceral fats, and raising plasma TGs levels, in turn increasing the risk of HTG and other metabolic disorders (41). HTG, obesity, and diabetic dyslipidemia exhibit elevated production of FFA, which give rise to lipotoxic intermediates

that can interfere with insulin signaling and trigger IR (9, 42). These metabolic intermediates, including diacylglycerol (DAG), ceramides, and acylcarnitine, cause disturbances in the metabolic process and mitochondrial functions, eventually leading to IR, decreased glycogen synthesis, increased gluconeogenesis from the liver, and cellular apoptosis. DAG activates protein kinase C (PKC), which restrains the phosphorylation of INSR and disrupts insulin signaling (41). Ceramides exert their toxic effects by interacting with mitochondria, activating the mitochondrial apoptotic pathway, and stimulating the production of reactive oxygen species (ROS), which can lead to cellular apoptosis and IR. Acylcarnitine is important in transporting long-chain fatty acids across the mitochondrial membrane. Any defects in this process can cause inflammation in mitochondrial permeabilization, cellular and plasma accumulation of long chain acylcarnitine, incomplete fatty acid oxidation, and impairment in insulin signaling (9).

Briefly, HTG and IR show a bidirectional relationship. Elevated TG levels can produce toxic NEFFA that deteriorates insulin signaling. Conversely, IR can promote hepatic TGs synthesis and accumulation, reinforcing a vicious cycle between HTG and IR.

2.2. Isthmin-1

2.2.1. General Features of Isthmin-1

ISM1 belongs to a novel protein family first observed by Pera et al. in 2002 during an experiment on Xenopus laevis (the clawed frog) embryos (11). This protein exhibits prominent expression at the MHB, (also known as the isthmus of the brain); hence, its name was derived from that structure (11). In 2021, ISM1 was determined as an adipokine via a combination of bioinformatic analysis and expression data from mature brown and white adipocytes (13, 14, 16, 43).

ISM1 is a member of the *ISM1* gene family, which also includes isthmin-2 (ISM2), known as tail 1. In humans, *ISM1* exists on chromosome 20, while *ISM2* on 14q24.3. Both *ISM* genes encode secreted proteins featuring thrombospondin type 1 repeat (TSR1) and adhesion-associated domain in mucin-4 (MUC4) and other proteins (AMOP) domains. A study asserted that ISM1's AMOP domain has a distinct structure resembling bacterial streptavidin. Moreover, the human *ISM1* gene is located on chromosome 20 and encodes a ~60 kDa protein with 499 amino acids that contain 3α helices and 2β- sheets. Both ISM1 and ISM2 possess an amino (N)-terminal signal peptide (SP), a single copy of the TSR1 domain in the central region, and a conserved (C)- terminal AMOP domain. Notably, TSR1

and AMOP domains of ISM1 exhibit a high degree of conservation across various species (44, 45).

ISM1 is derived from white adipocytes and shows an important role in modulating insulin sensitivity of adipocytes, ameliorating IR, and improving metabolic disorders (15, 16). It is widely distributed throughout the body, with notable expression in key areas including the brain and lungs. Strong expressions are detected in the bronchi and alveoli's epithelium, as well as in specific regions of the brain, such as the cerebellum, cerebral cortex, and hippocampus. Moreover, ISM1 is expressed to varying degrees in organs like the eyes, kidneys, skeletal muscles, and heart (12). *ISM1* is an extremely well-preserved gene across species and is highly expressed in immune barrier tissues, including the skin, mucosal tissues, lymphocyte populations, and hematopoietic cells (46). Notably, its expression in CD+4 T cells significantly increases when these cells are polarized to the T helper 17 cells lineage in vitro, suggesting a crucial role for ISM1 in various pathophysiological processes as an adipokine (44). On the other hand, *ISM2* is primarily expressed in the placenta. It is important in choriocarcinoma and preeclampsia (44).

ISM1 has been found to potentially bind to two receptors: the low-affinity alpha- v beta- 5 (αvβ5) integrin and the high-affinity glucose-regulated protein 78 (GRP78) receptor. Integrins (transmembrane receptors) facilitate cell adhesion to matrix molecules and have significant roles in processes like angiogenesis and inflammation. Consequently, both receptors can facilitate the internalization of ISM1, thereby expediting the apoptosis of activated human umbilical endothelial cells (ECs) and tumor cells (15).

ISM1, a secreted protein, has been identified as being subject to N-glycosylation through biochemical analysis. This modification occurs at two specific sites, N39 and N285. Intriguingly, when site-directed mutagenesis was used to mimic the substitution of Asparagine (N) with Glutamine (Q) at these positions, it resulted in a reduction in the size of the ISM1 protein. This reduction strongly suggests that N-glycosylation is a requisite for the secretion of ISM1, a notion further corroborated by experiments involving tunicamycin treatment. Furthermore, a separate study pinpointed two C-mannosylation sites within the TSR1 domain, namely Tryptophan (Trp)223 and (Trp)226. In a mutagenesis study, it was demonstrated that C-mannosylation plays a crucial role in facilitating N-glycosylation of ISM. Notably, C-mannosylation has been associated with aiding in protein folding and stabilization, particularly within the TSR1 domain (47, 45).

2.2.2. TSR1 and AMOP Domains in ISM1

The TSR1 domain is found in extracellular and membrane-bound proteins and plays a pivotal role in various biological functions, including cell-cell and cell-matrix interactions, cell proliferation, migration, adhesion, apoptosis, neuronal development, and immunity. Comprising 60 amino acid residues, TSR1 features a distinctive structure with a reverse three-stranded β-sheet at its core, characterized by conserved amino acids such as tryptophan (W), arginine (R), and cysteine (C) (44).

Within ISM1, the TSR1 domain exhibits several motifs. Notably, the CSVTCG motif within TSR1 facilitates interactions with CD36, triggering anti-angiogenic activity by inducing apoptosis in ECs. ISM1's TSR1 domain also contains a DGE motif at positions 218 to 220, known as an alpha- 2- beta 1 (α2β1) ligand sequence, a critical collagen receptor on platelets. It is also a key co-stimulating pathway in effector T cells, potentially linked to arthritis pathogenesis. This α2β1 integrin is expressed in human T helper cells and enhances the survival of effector cells by inhibiting Fas- induced apoptosis. In an avian model, α2β1 integrin facilitates interactions between embryonic retinal cells and collagen. Additionally, the TSR1 domain of ISM2 features an EPQ motif, which determines carbohydrate binding specificity (44, 45).

On the other hand, The AMOP domain is exclusively found in extracellular-domain-containing proteins, primarily associated with cell adhesion, indicating its extracellular localization. This domain spans approximately 100 amino acid residues and is characterized by eight invariant (C) residues (44, 45).

AMOP-containing proteins share conserved (C) residues, with ISM1 and ISM2's AMOP domains featuring a KGD motif. This motif binds to integrin alpha- 11- beta- 3 (α11β3), which is involved in the inhibition of platelet aggregation and plays a crucial role in integrin-mediated cellular adhesion and tumor metastasis (45).

Furthermore, the AMOP domain in ISM1 contains an RKD motif, known for its involvement in integrin- dependent cell adhesion. The RKD motif also exhibits selectivity in binding to the extracellular surface of αvβ5 integrins, which are implicated in vascular permeability and cell migration. Additionally, the AMOP domain in ISM2 includes a WSRL motif, recognized for its role in autophagy induction (45).

2.2.3. The Functions of ISM1

Angiogenesis can be defined as the process of which new blood vessels are formed (from existing capillaries or post capillary veins) and providing the way for the cells to get their metabolic needs like oxygen and nutrients. For cancer cells to get its metabolic needs it also requires undergoing angiogenesis. Therefore, anti-angiogenesis is a pivotal pathway in the prevention of malignant tumors. The ISM1 AMOP domain contains "RKD" which can diminish platelet's function and binds with αvβ5 leading to obstruction in the vascular endothelial growth factor (VEGF), suggesting that ISM1 may be associated with cell adhesion and angiogenesis (12, 45, 48). The binding of full- length ISM1 protein to αvβ5 causes inhibition of capillary angiogenesis in EC and that may affect cell migration, adhesion, and apoptosis with time. As a result, ISM1 contributes to the progression of tumorigenesis as well as works as regulator of tumor cells' migration and invasion, especially in solid tumors (44, 45).

ISM1 plays an important role in activating intracellular apoptosis through caspases (12). This adipokine has 14 invariant (C) residues, of which the TRS1 structural domain contains 6 (C) residues, while the AMOP structural domain has 8 (C) residues. These residues are responsible for the apoptotic function of ISM1. ISM1 reacts to αvβ5 and gives rise to apoptosis via recruitment and activation of caspase-3. ISM1 enters ECs through binding to GRP78; interacts with the inner mitochondrial membrane protein (ACC); interferes with adenosine diphosphate (ADP)/ATP exchange, suppresses ATP levels in cytoplasm, and induces apoptosis. In addition, the proapoptotic cyclic peptide BC71 in the AMOP domain interacts with GRP78, activates the tumor suppressor protein P53 and caspase-8 signaling, and causes apoptosis (48, 49). A study indicated that ISM1 can exert its proapoptotic activity on lobular breast cancer through obstructing angiogenesis and promoting apoptosis, so it can be considered as a biomarker for predicting the prognosis of breast cancer (50).

Additionally, studies showed that ISM1 is correlated with the hematopoietic functions. Hematopoietic stem cells (HSC) differentiate into progenitor cells that give rise to various blood cells. The knockdown of ISM1 results in suppressing the generation of HSC and progenitor cells; thus, ISM1 deletion resulted in a reduction of bone marrow cells, including erythrocytes, leukocytes, and macrophages (48, 51).

In hypertensive individuals, ISM1 levels were directly associated with systolic (SBP) and diastolic blood pressure (DBP); however, they showed negative correlations with nocturnal urine Na^+ concentration and nocturnal urine Na^+ excretion. This suggests that ISM1 is a protective adipokine against hypertension and renal inflammation (52).

On the other hand, ISM2 is primarily associated with supporting the activation of mesenchymal and stromal cells through the transforming growth factor beta signaling pathway and adjusting the secretion of inflammatory cytokines via the nuclear factor kappa (NF-κB) signaling pathway, thus promoting epidermal-mesenchymal transition (53). Conversely, strong expressions of ISM1 were detected in patients with gestational hypertension and preeclampsia, while reduced serum ISM2 levels were revealed in preeclampsia patients (45).

2.2.3.1. The Role of ISM1 in Metabolism

Adipose tissues, the liver and skeletal muscles play an essential role in maintaining systemic energy homeostasis. In obesity conditions, excessive energy intake can give rise to subcutaneous fat spillage, accumulate ectopic lipids in the liver, skeletal muscles and other tissues, decrease glucose tolerance and lead to inflammatory reactions. Finally, a variety of metabolic diseases may occur. Insulin is the only hypoglycemic hormone in the body, promotes glucose uptake and lipid synthesis, which in the case of hyperinsulinemia may contribute to obesity or alcoholic FLD. Recent studies have defined that ISM1 plays an endogenous role in glucose regulation by activating the P13K-AKT signaling pathway of insulin and insulin-like growth factor receptors, leading to increased adipose glucose uptake and suppressed hepatic lipid synthesis, encouraging hepatocytes to shift from lipogenic to protein synthesis pathways. Consequently, the ablation of ISM1 results in impaired glucose tolerance and decreased adipose glucose uptake (12-14, 54, 55).

ISM1 shows positive relationship with obesity (12). A study found that administration of ISM1 effectively relieves diabetes in diet-induced obese mice and improves liver steatosis in a diet-induced fatty liver mouse model (56). One study revealed that ISM1 contributes to the progression effects of metformin in newly diagnosed type 2 diabetic mellitus (T2DM) patients, it increases glucose tolerance and shows negative correlations with LDL-C levels (12). Also, another study by Alshawaf et al. (2025) (57) done on Kuwaiti adults has affirmed the protective role of ISM1 against diabetes, IR, and

metabolic diseases, thus, it can be utilized as a biomarker for obesity-related metabolic diseases (57).

In 2021, high expression of ISM1 in mature adipocytes (especially brown adipocytes) was detected. This reflects that ISM1 may be closely related to the function of mature adipose tissues (15, 56). GLUT4 is regulated mainly by ATP or insulin in skeletal muscle and adipocytes (56). *In vitro* study has demonstrated that ISM1 promotes GLUT4 translocation to the plasma membrane from the cytoplasm, in turn triggering endogenous phosphorylation of the energy metabolism factor protein kinase (AktS473) and increasing cellular glucose uptake (48).

ISM1 was shown to induce AktS473 phosphorylation levels in various mature adipocytes and in human primary skeletal muscle cells (14). Treatment with PI3K inhibitors completely block the glucose uptake mechanism induced by ISM1, suggesting that ISM1 stimulates PI3K to promote glucose uptake in adipocytes (48).

The mammalian target of rapamycin (mTOR) (mTORC1 and mTORC2) is activated by insulin, thus regulates insulin sensitivity. Additionally, ISM1 also regulates glucose uptake via mTORC2-PI3K-Akt in conjunction with insulin- INSR/insulin-like growth factor receptor-PI3K-Akt. Notably, although ISM1 and insulin have identical regulatory effects on glucose, ISM1 doesn't act on INSR, but rather binds to a unique receptor to activate the PI3K-Akt pathway and promotes glucose uptake by adipocytes (12, 48).

In the context of adipose tissues, ISM1 shows enriched expression in mature brown fats compared to murine inguinal white adipose tissues (iWat) and epidymal white adipose tissues (eWat) tissues, particularly exhibiting higher levels in brown adipose tissues relative to iWat. Notably, RNA and protein analyses of isolated mature adipocytes demonstrate that ISM1 is predominantly expressed in mature fat cells, with negligible ISM1 expression observed in the stromal vascular fraction of adipose tissues (15). ISM1 exhibits an essential function in modulating lipid metabolism in the liver, although its specific receptor in hepatocytes remains unidentified. It significantly curbs the lipogenesis pathway through reducing the expression of sterol regulatory element-binding protein 1c (SREBP-1c) and its target genes, which include ACC, FAS, and LDL receptors. Additionally, ISM1 restrains the expression of peroxisome proliferator-activated receptor γ coactivator 1β (PGC1β) and carbohydrate response element binding protein (ChREBPβ)

(13, 48). Meanwhile, ISM1 plays a critical role in regulating protein metabolism by increasing protein synthesis in the liver and skeletal muscle (16). This process occurs through the phosphorylation of Akt-mTORC1-S6 signaling pathway. Phosphorylated Akt activates mTORC1, which in turn activates ribosomal protein $SD^{S235/S236}$, thus enhancing protein synthesis (12, 13, 56, 58). In fact, ISM1 has been shown to strengthen the therapeutic mechanisms of Hydroxytyrosol against liver damage, hyperlipidemia, and IR (13). In contrast, Insulin favors the activation of both protein and lipid synthesis in target tissues. These findings highlight the importance of ISM1 in the development of potential therapeutic interventions for metabolic disorders (13, 48).

3. MATERIALS and METHODS

3.1. Materials

The list of devices, tools, and materials used for this study and their manufacturers are listed in Table 1 and the commercial kits are available in Table 2.

Table 1. Devices, tools and materials used in the laboratories, and their manufacturers

Name	Manufacturer
Analytical Balance	Mettler Toledo AB 204-S (Sweden)
Autoanalyzer	Beckman Coulter brand AU 5800 (USA)
Automatic Pipettes	Thermo Labsystems, SOCOREX
Blood Pressure Monitor	Automatic Wrist Monitor (Sanitas SBM06, Lithuania)
Body weight scale	Comedones BSK-1(Türkiye)
Centrifuge	Beckman Coulter Allerga ™ 64R (Ireland)
Centrifuge	Eppendorf, Centrifuge 5810 (Germany)
Deep Freezer (-80 °C)	Thermo Electron Corporation (USA)
Heated orbital shaker	Nüve SL350 (Türkiye)
Magnetic Stirrer	IKA – RH basic 2, Termal (Türkiye)
Microcentrifuge	Thermo IEC Micromax (USA)
Microcentrifuge Tube	Iso Lab, Lot No: MTPPN7015002 (Germany)
Microplate Reader	Bio Rad, iMark Microplate Reader 19839 (Japan)
Microplate Reader	Molecular Devices, SpectraMax Paradigm Multi-Mode Detection Platform (USA)
Microplate Reader	Thermoscientific Multiskan Sky High Microplate Spectrophotometer (Singapore)
Microplate Washer	BioTek ELx50 (USA)
pH-meter	Hanna Instruments, HI 9321(UK)
Pure Water Purifier	PURELAB Classic
Refrigerated Centrifuge	Beckman-Coulter (USA)
Refrigerator (+4 °C)	Arçelik, Vestel (Türkiye)
Shaker Incubator	ShelLab/Sheldon S14-2 (USA)
Vortex	IKA Vortex 30950, Genius 3 (Türkiye)
1000 µL Pipette tip	ISOLAB (Germany)
200 µL Pipette tip	ISOLAB (Germany)

Table 2. Commercial kits

Kits	Trademark, Product code, Lot No, City, Country
Human ApoB100, ELISA	Elabscience, E-EL-H0476, UX51T44B1784, Texas,USA
Human ISM1, ELISA	Cloud-Clone Corp USCN, SEQ515Hu, L231115768, Houston, USA
Human MTTP, ELISA	BT-LAB, E3826Hu, 202312011, Shanghai, China
Human NEFFA, colorimetric	Elabscience, E-BC-K013-S, WX07B6J85565, Texas,USA

3.2. Methods

This study was conducted by Karadeniz Technical University, Department of Medical Biochemistry. Approval for the conduct of the study was obtained from Karadeniz Technical University, Faculty of Medicine, Scientific Research Ethics Committee with protocol number (No: 2023/116, date 12.06.2023). Within the scope of the study, Postgraduate Thesis Project (BAP06) support was received from Karadeniz Technical University, Scientific Research Projects Coordination Unit with the project code No: TYL-2023-10907.

3.2.1. Study Design

Considering the results obtained using the G Power (G*Power 3.1.9.2, Kiel University, Kiel, Germany) program; when the effect size (large) is accepted as 0.70, it was calculated that at a minimum of 35 volunteers should be included in each group for the study to reach 80% power with 5% type 1 error. Based on this value, it was decided that the number of individuals in each group would be 40.

The study group represented the volunteers who applied to Farabi Hospital of Karadeniz Technical University. Our study group consisted of 80 individuals in total, 40 healthy controls and 40 HTG patients. Within the framework of our study, active infections, cancer, acute-chronic renal and hepatic failure, endocrine and metabolic disorders, lipid-lowering drug use, digestive-absorption disorders, chronic alcohol use, and pregnancy were taken as exclusion criteria. Our study did not have age restrictions; all people aged 18 and above were included in the research. Individuals with TG levels below 150 mg/dL and cholesterol levels under 200 mg/dL were included in control group. People whose TG levels were ≥150 mg/dL were considered hypertriglyceridemic group. However, then we divided individuals with HTG into two subgroups, HTNC=20 and HTHC =20.

Before blood collection was done, weight, height, waist circumference (WC), hip circumference (HC), SBP and DBP measurements were taken from the individuals who accepted to participate and be recorded in the study. Following a one-night (12-hour) fast, venous blood samples were withdrawn from the patient's forearms between 8:30 and 9:00 in the morning. They were collected in a 5 mL serum separator gel biochemistry tube for serum, and an anticoagulant tube containing ethylene diamine tetra acetic acid (EDTA) was utilized for plasma collection. After keeping the blood samples for 30 min, they centrifuged at 3000 rpm for 10 min at 4˚C. The serum and plasma samples obtained were aliquoted and stored in 1.5 mL capped tubes at -80˚C until biochemical measurements were performed.

3.2.2. Biochemical Analysis

3.2.2.1. Parameters Determined by the AutoAnalyzer

The biochemical parameters used in the study [TGs, total cholesterol (TC), HDL-C, LDL-C, glucose, insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), albumin, blood urea nitrogen (BUN), and creatinine] were measured in the Karadeniz Technical University Farabi Hospital Medical Biochemistry Laboratory. Beckman Coulter brand AU 5800 (USA) model clinical chemistry autoanalyzer was available in the laboratory and was used for measuring the biochemical parameters. The autoanalyzer was subjected to daily quality control procedures. The name of the method and the principle of measuring each parameter are outlined below:

Glucose concentration was analyzed by using enzymatic (hexokinase/glucose-6-p dehydrogenase) UV test. In this method, glucose is phosphorylated to glucose-6-phosphate by hexokinase enzyme in the presence of ATP and magnesium ions. Then in the second reaction, the released glucose-6-phosphate oxidizes in the presence of glucose-6-phosphate dehydrogenase enzyme to produce gluconate-6-phosphate, and nicotinamide adenine dinucleotide (NAD^+) is simultaneously reduced to nicotinamide adenine dinucleotide hydrogen (NADH). The absorbance was measured at 340 nm which is proportional to the glucose concentration in the sample.

TGs levels were measured using the enzymatic colorimetric (cholesterol oxidase peroxidase) test. This procedure is based on a series of multiple enzymatic reactions. Firstly, TG in the sample is hydrolyzed by microbial lipase to produce glycerol and fatty

acids. Then, glycerol kinase works to phosphorylate glycerol by ATP to yield G3P. In the presence of glycerol phosphate oxidase, G3P is oxidized to produce hydrogen peroxide (H_2O_2) and dihydroxy acetone phosphate. The reaction of H_2O_2 with 4-aminophenazone N, N-bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) by peroxidase enzyme produces a chromophore read at 660/800nm. This absorbance is proportional to the TG content in the sample.

TC concentration was determined using an enzymatic colorimetric (cholesterol oxidase-peroxidase) test. CEase hydrolyze CE to form free cholesterol. The released cholesterol oxidizes to cholesten-3-one by the cholesterol oxidase, and H_2O_2 simultaneously releases. Then, H_2O_2 in the presence of peroxidase reacts with 4-aminoantipyrine and phenol, oxidatively, to produce the chromophore. The produced red quinonimine dye is measured spectrophotometrically at 540/600 nm.

LDL-C concentration was evaluated using an enzymatic colorimetric (cholesterol oxidase peroxidase) test. CEase and cholesterol oxidase degrade all other non-LDL lipoproteins (HDL, VLDL, CM). The H_2O_2 released by this reaction is degraded by catalase. In the second step, the protective agent is separated from LDL, and catalase becomes inactive by sodium azide. Ultimately, this process releases blue dye, which indicates the quantity of LDL present in the sample. The CHO/PAP system can then be used to quantify the amount of LDL in the sample.

HDL-C concentration was estimated with enzymatic colorimetric (cholesterol oxidase peroxidase) test. In this method, an anti-human-β-lipoprotein antibody binds to lipoproteins other than HDL, (LDL, VLDL, and CM). The resulting antigen-antibody complexes suppresses enzyme reactions when the second reaction is catalyzed by CEase and cholesterol oxidase, which converts HDL-C with water (H_2O) and oxygen (O_2) into cholest-4-en-3-one, fatty acids, and H_2O_2 is introduced. Later, H_2O_2 in the presence of peroxidase reacts with 4-amino antipyrine, producing blue dye. The amount of HDL-C is measured using this enzyme-chromogen system.

Insulin concentration was quantified by chemiluminescence assay method. In this assay, specific antibodies recognize and bind to insulin molecules in the sample. This complex is then exposed to a chemiluminescent substrate and emits light. The amount of light emitted is proportional to the quantity of insulin in the sample.

ALT concentration was estimated with kinetic-UV, pyridoxal- 5'- free phosphate method. ALT facilitates the transfer of amino groups from alanine to 2-oxoglutarate forming pyruvate and glutamate. Then, LDH catalyzes the reaction of pyruvate with NADH, which leads to the formation of lactate and NAD^+. The absorbance is measured at a wavelength of 340 nm; this absorbance reflects the ALT concentration in the sample.

AST concentration was measured with kinetic-UV, pyridoxal- 5'- free phosphate method. In this method the AST catalyzes the transamination of aspartate and 2-oxoglutarate, leading to the production of L-glutamate and oxalacetate. Subsequently, malate dehydrogenase catalyzes the reduction of oxalacetate to L-malate, while simultaneously NADH is converted to NAD^+. The absorbance measured at 340 nm is proportional to the AST activity in the sample.

LDH activity was evaluated with kinetic UV test. In this procedure, LDH catalyzes the oxidation of lactate to pyruvate, accompanied by the reduction of NAD^+ to NADH. The increase in NADH measured at 340 nm reflects the enzyme activity in the sample.

Albumin level was determined by employing the photometric colorimetric method. Bromcresol green reacts with albumin to form the green-colored complex (albumin-bromcresol green). By using the absorbance measured at 600/800 nm, the albumin concentration is calculated.

BUN concentration was assessed via kinetic UV (urease/glutamate dehydrogenase) method. Urea is hydrolyzed by urease enzyme in the presence of H_2O, resulting in the production of ammonia (NH_3) and carbon dioxide (CO_2). The generated NH_3 combines with 2-oxoglutarate and NADH to form glutamate and NAD^+. This reaction is facilitated by the catalytic activity of glutamate-dehydrogenase. The decrease in NADH absorbance is proportional to the amount of urea in the sample.

Creatinine concentration was measured using kinetic colorimetric Jaffe test. Creatinine reacts with picric acid in an alkaline medium to form a yellow-orange compound known as the creatinine-picrate complex. Its absorbance is measured at 520/800 nm, which is proportional to the concentration of creatinine in the sample.

3.2.2.2. Determination of ISM1 Levels

The concentration of human ISM1 that was taken from the serum sample was measured using an ISM1 sandwich-ELISA kit provided in Table 2. The measurements

were done according to the steps found in the instruction manual. Absorbance was read at 450 nm using a microplate reader. The sample concentrations were calculated in ng/mL according to the standard graph shown in Figure 1A.

3.2.2.3. Determination of Apo B100 Lipoprotein Levels

Human Apo B100 blood serum concentration was determined using an ApoB100 sandwich-ELISA commercial kit that was mentioned in Table 2. The serum ApoB100 measurements were performed according to the protocols provided in the kit. Absorbance was read at 450 nm using a microplate reader.

Apo B100 concentrations were calculated according to the graph provided in Figure 1B. Results were expressed in ng/mL.

3.2.2.4. Determination of MTTP Levels

Human MTTP blood serum concentration was measured using the MTTP sandwich-ELISA kit listed in Table 2. The measurements were performed following the outlined steps given in the manual instruction kit. Absorbance was read at 450 nm using a microplate reader. The measurements were represented in ng/mL based on the standard chart in Figure 1C.

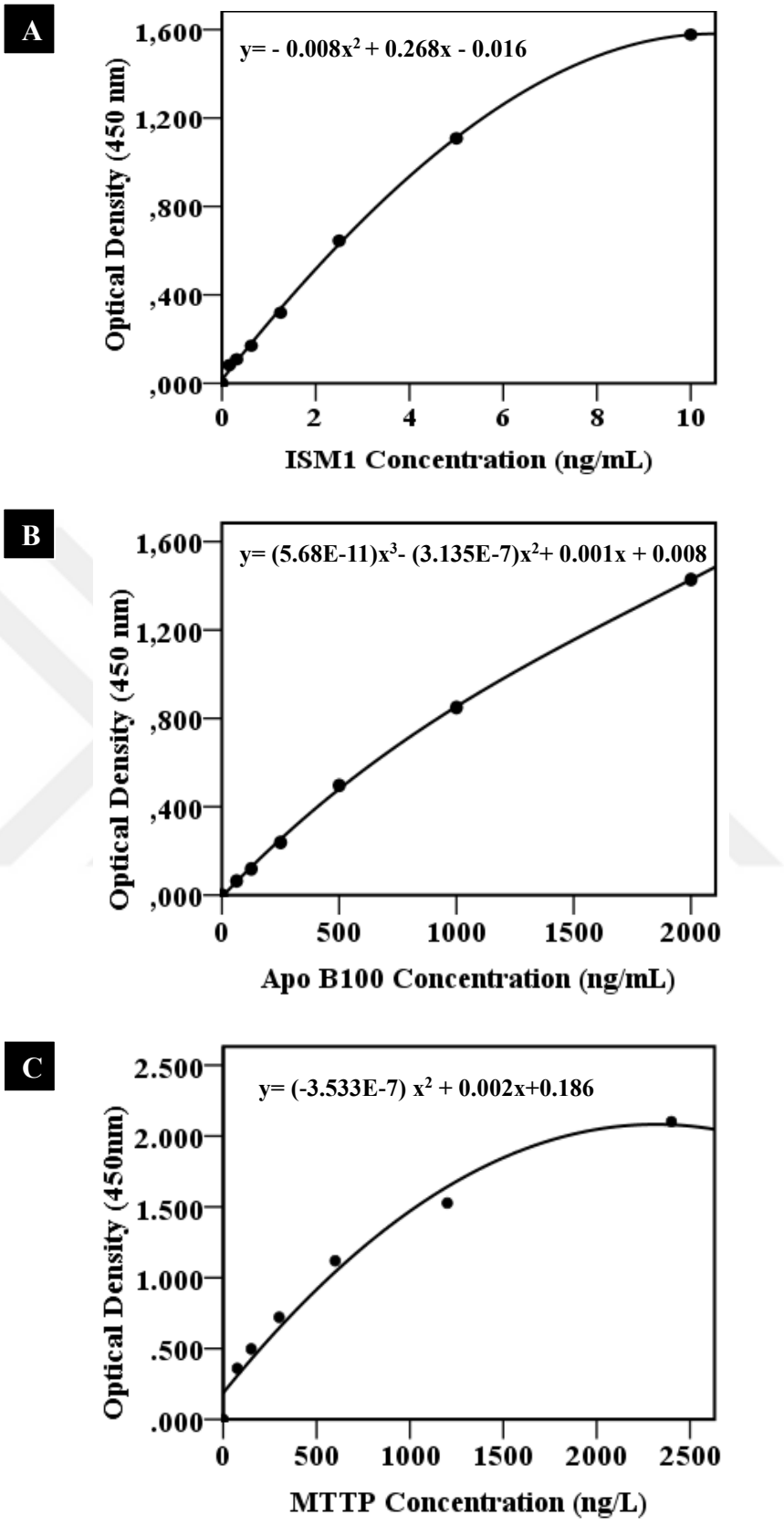


Figure 1. Standard curve for ISM1 (A), Apo B100 (B) and MTTP (C) concentrations

3.2.2.5. Determination of NEFFA Levels

Human NEFFA in blood serum samples was measured using the commercial colorimetric assay kit mentioned in Table 2. This kit's detection principle was based on the reaction of NEFFA with nantokite in weak acidity conditions, generating copper soap with an absorption peak at 715 nm. The concentration of the sample can be calculated according to the "Equation 1" and depending on the optical density (OD) value of the sample.

The standard curve is y= ax+b.

$$NEFFA\ (mmol/L) = (\Delta A_{715}\ -b) \div a \times \frac{V1}{V2} \times F \qquad \text{(Equation 1)}$$

ΔA_{715} = $OD_{sample} - OD_{control.}$

b= The intercept of the standard curve.

a= The slope of the standard curve.

$V_{1=}$ The volume of the extraction solution (R1=600 µL).

$V_{2=}$ The volume of the serum (50 µL).

F= Dilution factor (the dilution factor of the human serum is 1).

By using the NEEFA standard graph (Figure 2), the concentration of the NEEFA in serum sample was calculated as (mmol/L).

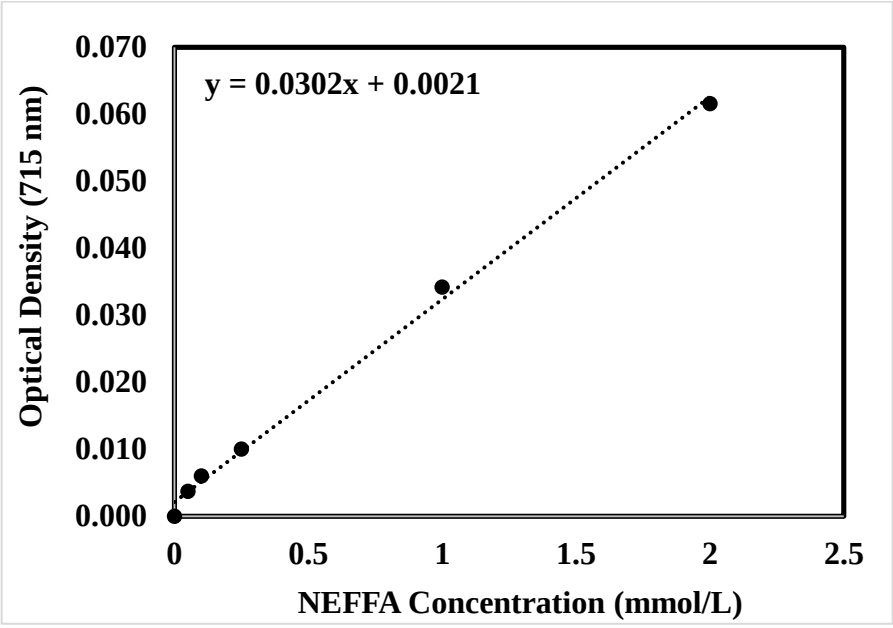


Figure 2. Standard curve for NEEFA concentration

3.2.3. Mathematical Equations Used in the Study

The equations for computational parameters in the study are shown in Table 3. These formulas aid in understanding IR, diabetes risk, and atherosclerosis and its relation to ISM1.

Table 3. Calculated parameters

Parameter	Formula	Ref
Mean arterial pressure (MAP)	$\frac{DBP + (SBP - DBP)}{3}$	59
Body mass index (BMI)	$\frac{Weight\ (kg)}{Height^{2}\ (m^{2})}$	60
Triglyceride index (TyGi)	$Ln\ \frac{(TG\ [mg/dL] \times glucose\ [mg/dL]}{2}$	61
Non-HDL-C	Total cholesterol – HDL-C	62
Atherogenic index of plasma (AIP)	Log (TG [mmol/L] / HDL-C [mmol/L])	63
Metabolic score for insulin resistance (METS-IR)	$\frac{Ln\ (2 \times Glucose\ [mg/dL] + TG\ [mg/dL]) \times BMI)}{Ln\ (HDL\text{-}C\ [mg/dL])}$	64
Homeostatic model assessment for insulin resistance (HOMA-IR)	$\frac{Insulin\ (\mu U/mL)] \times Glucose\ (mg/dL)}{405}$	64

3.2.4. Statistical Analysis

The data were statistically evaluated in the "SPSS (IBM SPSS Statistics 23)" program and "MedCalc (version 23.0.9)" program for receiver-operating characteristic curve "ROC" analysis. The conformity of the parameters to the normal distribution was understood using “Shapiro-Wilk” test. The "Mann-Whitney U" test was used to compare two variables that did not fit the normal distribution, and the "Student's t" test was used for binary variables that fit the normal distribution. Additionally, "Kruskal–Wallis" test was used to compare more than two variables that did not fit the normal distribution, on the

other hand, “One-Way ANOVA” test was employed in the comparison of more than two variables that fit the normal distribution. The "Tukey" test was used for post hoc evaluations. Non-parametric variables were given as “Median (interquartile range (IQR)) and 25th-75th percentiles”, however, the parametric variables were expressed as "Mean ± Standard deviation (SD)". Furthermore, the “Spearman" test was applied for correlating variables that do not fit the normal distribution, whereas "Pearson" test was used for comprehending the correlation between variables that fit the normal distribution. Finally, ROC curve was estimated to determine the diagnostic ability of binary groups. The closer the area under curve (AUC) is to value 1, the stronger the relationship between the groups and the studied variables. $p < 0.05$ was considered statistically significant.

4. RESULTS

The results and the analyses of the individuals participating in the study are shown below.

4.1. Comparison of Demographic Data According to Main Groups, Subgroups and Gender

As can be concluded from Table 4, individuals with HTG exhibited older ages, higher body weight, BMI, WC, HC, WC/HC, blood pressure, and MAP compared to healthy individuals and these differences proved to be significant ($p < 0.05$). In terms of comparing subgroups, the demographic data showed that HTHC and HTNC individuals displayed higher body weight, WC, and WC/HC compared to the control group ($p < 0.05$).

Table 4. Comparisons of demographic data between the main study groups

	Control (n=40)	**HTG (n=40)**	**p**
Genders (F/M)	26/14	14/26	
Age (years)	41 ± 9	46 ± 10	0.013
Weight (kg)	74 ± 11	84 ± 11	0.001
Height (m)	1.67 ± 0.09	1.69± 0.09	0.112
BMI (kg/cm^2)	26.48 ± 4.04	29.09 ± 3.55	0.003
WC (cm)	88 ± 11	98 ± 9	0.001
HC (cm)	104 [13] (96 -109)	108 [11] (102 -113)	0.033
WC/HC	0.85 ± 0.10	0.91 ± 0.10	0.001
SBP (mmHg)	120 [14] (110 - 124)	125 [25] (112 - 137)	0.019
DBP (mmHg)	80 [10] (71 - 81)	80 [10] (80 - 90)	0.028
MAP (mmHg)	93 [12] (85 – 97)	97 [12] (93 - 105)	0.007

Parametric results were given as "[Mean ± SD]" and p values were given according to Student t-test. Non-parametric results were given as "Median [IQR] and 25th -75th percentiles" and p values according to 'Mann Whitney U' test. $p < 0.05$ was accepted statistically significant. F, female; M, male.

Additionally, HTHC patients showed older ages, higher BMI, and SBP compared to the control group ($p < 0.05$). Additionally, HTHC patients exhibited higher DBP and MAP compared to both HTNC and control groups, and this difference is significant ($p < 0.05$), as shown in Table 6. The demographic data of total females and total males are elucidated in Table 7. As can be concluded from the table, there are significant differences between total males and total females in terms of body weight, height, WC, and WC/HC ($p < 0.05$). Although a critical increase in MAP ratio is seen in males, this increase is insignificant; the "p value" shows a borderline as seen in Table 7.

4.2. Serum ISM1, Lipids, Lipoprotein, and Biochemical Parameters in Main Groups, Subgroups and Gender

In our comparison of patients across the main groups, we observed that HTG individuals exhibited considerable differences in the levels of serum ISM1, TGs, TyGi, AIP, METS-IR, MTTP, TC, LDL-C, non-HDL-C, Apo B100, insulin, HOMA-IR, ALT, AST and albumin compared to control patients ($p < 0.05$). On the other hand, there were no significant differences in terms of serum glucose level, NEFFA, HDL-C, LDH, BUN, and creatinine among the two groups ($p > 0.05$), as seen in Table 5. The comparison of ISM1 levels between control and HTG patients is shown in Figure 3A.

According to Table 6, the biochemical tests revealed that HTHC and HTNC patients showed increased levels of serum TG, TyGi, AIP, METS-IR, ApoB100, ALT, AST, and albumin compared to the control group; this elevation was shown to be significant ($p < 0.05$). Moreover, HTHC patients showed higher concentrations of serum MTTP and insulin compared to the control group ($p < 0.05$). Notably, (HTHC) patients demonstrated higher levels of serum ISM1, TC, LDL-C, non-HDL-C, and HOMA-IR compared to HTNC patients and the control group, and these differences are shown to be significant ($p < 0.05$). Individuals with HTNC exhibited higher serum ISM1, TC, LDL-C, HDL-C, and non-HDL-C levels compared to the control group ($p < 0.05$). No significant variations in serum glucose, NEFFA, LDH, BUN, and creatinine levels ($p > 0.05$). The comparison of ISM1 levels between control and HTNC and HTHC patients is demonstrated in Figure 3B.

Table 5. Comparisons of the results of biochemical parameters between study groups

	Control (n=40)	HTG (n=40)	p
Glucose (mg/dL)	89 [13] (82 - 95)	90 [10] (86 - 96)	0.491
TG (mg/dL)	86 [51] (64-115)	210 [58] (179 - 237)	0.001
TC (mg/dL)	166 [34] (152 -186)	221 [56] (195 - 251)	0.001
LDL-C (mg/dL)	112 ± 21	156 ± 36	0.001
HDL-C (mg/dL)	50 [12] (44 - 56)	51 [13] (42 - 55)	0.722
Non-HDL-C (mg/dL)	115 ± 22	176 ± 36	0.001
Apo B100 (ng/mL)	3380 [1919] (2575 - 4494)	4369 [1450] (3619 - 5069)	0.003
MTTP (ng/L)	261 [249] (181 - 430)	430 [522] (252 - 774)	0.006
NEFFA (mmol/L)	4.29 ± 0.6	4.30 ± 0.7	0.903
AIP	0.21 ± 0.21	0.65 ± 0.12	0.001
Insulin (µIU/mL)	6.63 [6.37] (3.47 - 9.84)	10.80 [9.74] (6.14 - 15.88)	0.001
HOMA-IR	1.40 [1.40] (0.70 - 2.10)	2.51 [2.08] (1.38 - 3.46)	0.001
METS-IR	7.46 ± 0.24	7.99 ± 0.18	0.001
TyGi	8.17 ± 0.39	9.16 ± 0.27	0.001
ISM1 (ng/mL)	0.87 [0.19] (0.77 - 0.96)	1.66 [0.85] (1.23 - 2.08)	0.001
Albumin (g/dL)	44 ± 2.10	46 ± 2.25	0.001
BUN (mg/dL)	12 ± 3.29	13 ± 2.96	0.356
Creatinine (mg/dL)	0.78 ± 0.17	0.85 ± 0.19	0.065
ALT (U/L)	16 [13] (12 - 25)	28 [17] (19 - 36)	0.001
AST (U/L)	19 [6] (16 - 22)	24 [7] (20 - 27)	0.001
LDH (U/L)	160 [29] (148 - 177)	169 [45] (145 - 190)	0.376

Parametric results were given as "[Mean ± SD]" and p values were given according to 'Student t-test. Non-parametric results were given as "Median [IQR] and 25th -75th percentiles" and p values according to 'Mann Whitney U' test. $p < 0.05$ was accepted statistically significant.

Table 6. Comparisons of demographic data and biochemical parameters between the study subgroups

	Control (n= 40)	HTNC (n=20)	HTHC (n=20)	p
Genders (F/M)	(26/14)	(5/15)	(9/11)	
Age (years)	41 ± 9	43 ± 9	50 ± 9 [a, b]	0.003
Weight (kg)	74 ± 11	83 ± 10 [a]	85 ± 13 [a]	0.001
Height (m)	1.67 ± 0.09	1.70 ± 0.10	1.69 ± 0.08	0.280
BMI (kg/cm^2)	26.48 ± 4.04	28.8 ± 3.6	29.4 ± 3.6 [a]	0.011
WC (cm)	88 ± 11	97 ± 8 [a]	99 ± 11 [a]	0.001
HC (cm)	104 [13] (96 - 109)	107 [13] (102 - 115)	108 [10] (102 - 112)	0.089
WC/HC	0.85 ± 0.10	0.90 ± 0.06 [a]	0.93 ± 0.08 [a]	0.001
SBP (mmHg)	120 [14] (110 - 124)	122 [21] (110 - 131)	130 [20] (120 - 140) [a]	0.021
DBP (mmHg)	80 [10] (71- 81)	80 [6] (79 - 85)	88 [13] (80- 93) [a, b]	0.015
MAP (mmHg)	93 [12] (85 – 97)	96 [12] (88 - 100)	100 [16] (93 - 109) [a, b]	0.005
Glucose (mg/dL)	89 [13] (82- 95)	89 [13] (79 - 92)	90 [12] (87- 99)	0.253
TG (mg/dL)	86 [51] (64 - 115)	203 [55] (175 - 230) [a]	225 [66] (180 - 246) [a]	0.001
TC (mg/dL)	166 [34] (152 - 186)	195 [17] (183 - 200) [a]	249 [41] (235- 276) [a, b]	0.001
LDL-C (mg/dL)	112 ± 21	128 ± 20 [a]	184 ± 25 [a, b]	0.001
HDL-C (mg/dL)	50 [12] (44 - 56)	46 [10] (41- 51) [a]	52 [9] (50 - 59) [b]	0.011
Non-HDL-C (mg/dL)	120 [36] (98 - 134)	147 [24] (136 – 160) [a]	199 [27] (185 – 212) [a, b]	0.001
Apo B100 (ng/mL)	3380 [1919] (2575 - 4494)	4425 [1552] (3442 - 4994) [a]	4309 [1698] (3632 - 5330) [a]	0.013
MTTP (ng/L)	261 [249] (181 - 430)	349 [473] (208 – 681)	558 [759] (313 - 1072) [a]	0.004
NEFFA (mmol/L)	4.15 [0.66] (3.92 - 4.58)	4.20 [1.14] (3.91 - 5.05)	4.22 [0.49] (4.00 - 4.49)	0.846
AIP	0.21 ± 0.21	0.68 ± 0.12 [a]	0.61 ± 0.11 [a]	0.001
Insulin (µIU/mL)	6.63 [6.37] (3.47- 9.84)	7.28 [9.60] (5.10- 14.70)	11.40 [6.75] (9.55- 16.30) [a]	0.001
HOMA-IR	1.40 [1.40] (0.70- 2.10)	1.60 [1.71] (1.13- 2.84)	2.77 [1.71] (2.10- 3.81) [a, b]	0.001
METS-IR	7.46 ± 0.24	7.98 ± 0.15 [a]	7.99 ± 0.20 [a]	0.001
TyGi	8.17 ± 0.39	9.11 ± 0.30 [a]	9.21 ± 0.24 [a]	0.001
ISM1 (ng/mL)	0.87 [0.19] (0.77- 0.96)	1.28 [0.7] (1.20 - 1.90) [a]	1.94 [0.9] (1.55- 2.45) [a, b]	0.001
Albumin (g/dL)	44 ± 2.10	46 ± 2.01 [a]	46 ± 2.52 [a]	0.003
BUN (mg/dL)	12 ± 3.29	12 ± 2.72	13 ± 3.17	0.394
Creatinine (mg/dL)	0.78 ± 0.17	0.88 ± 0.22	0.83 ± 0.16	0.131
ALT (U/L)	16 [13] (12- 25)	27 [16] (20- 36) [a]	30 [21] (17- 38) [a]	0.001
AST (U/L)	19 [6] (16 - 22)	24 [6] (20 - 26) [a]	24 [8] (20 - 28) [a]	0.001
LDH (U/L)	164 ± 24	162 ± 28	181 ± 41	0.062

Parametric tests results were given as "[Mean ± SD]" and p values were given according to 'One Way ANOVA. Non-parametric tests results were given as "Median [IQR] and 25th -75th percentiles" and p values according to 'Kruskal Wallis Test'. $p < 0.05$ was accepted statistically significant with respect to [a]: control and [b]: HTNC. F, female; M, male.

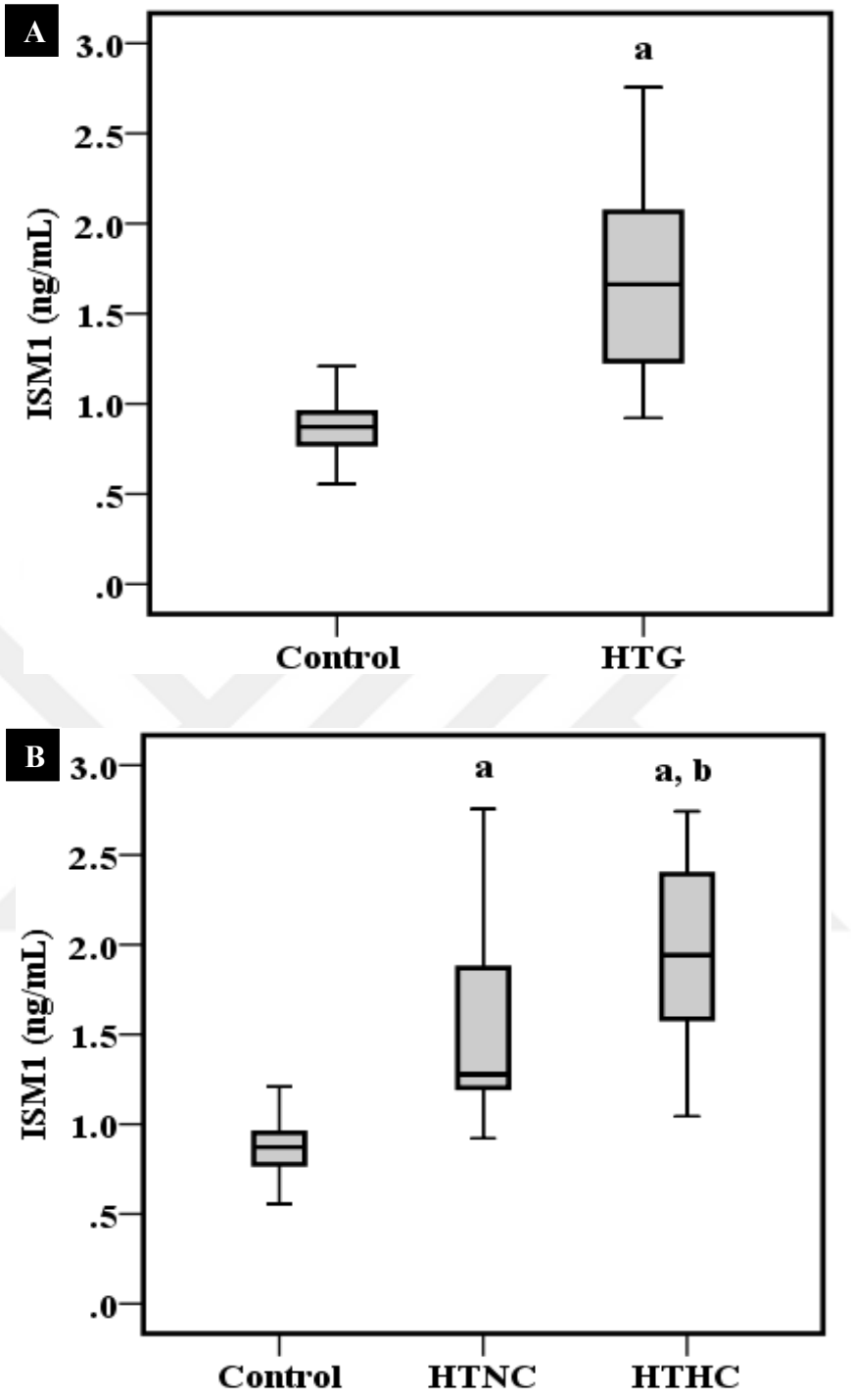


Figure 3. Comparisons of ISM1 levels A) between main groups (Control and HTG), and B) among subgroups (Control, HTNC and HTHC). $p < 0.05$ was accepted statistically significant with respect to [a]: control and [b]: HTNC

Table 7. Comparisons of demographic data and biochemical parameters between females and males

	Females (n=40)	Males (n=40)	p
Age (years)	44 [13] (37- 50)	45 [9] (40 - 49)	0.950
Weight (kg)	74 ± 12	84 ± 11	0.001
Height (m)	1.62 ± 0.05	1.75 ± 0.08	0.001
BMI (kg/cm^2)	28.13 ± 4.54	27.44 ± 3.40	0.444
WC (cm)	90 ± 13	96 ± 9	0.017
HC (cm)	106 [14] (97 – 111)	105 [9] (101 – 110)	0.935
WC/HC	0.85 ± 0.08	0.91 ± 0.07	0.001
SBP (mmHg)	120 [20] (110 – 130)	121 [21] (110 – 131)	0.273
DBP (mmHg)	80 [17] (71 – 88)	80 [10] (80 – 90)	0.171
MAP (mmHg)	93 ± 10	98 ± 10	0.058
Glucose (mg/dL)	90 [11] (84 - 95)	89 [14] (83 - 97)	0.839
TG (mg/dL)	117 [111] (73 – 184)	177 [131] (101 - 232)	0.004
TC (mg/dL)	190 [36] (163 - 199)	191 [66] (164 - 230)	0.648
LDL-C (mg/dL)	128 [30] (108 - 138)	134 [51] (110 - 161)	0.353
HDL-C (mg/dL)	53 ± 9	47 ± 8	0.001
Non-HDL-C (mg/dL)	135 [37] (115 – 152)	147 [63] (119 – 182)	0.147
Apo B100 (ng/mL)	3652 [2562] (2839 - 5401)	4166 [1718] (2997 - 4715)	0.402
MTTP (ng/L)	285 [276] (216 – 492)	359 [577] (197 – 774)	0.644
NEFFA (mmol/L)	4.27 ± 0.64	4.33 ± 0.62	0.698
AIP	0.37 [0.40] (0.11 - 0.51)	0.59 [0.35] (0.38 - 0.73)	0.001
Insulin (µIU/mL)	6.86 [6.27] (4.38 - 10.65)	9.47 [9.18] (5.07 - 14.25)	0.204
HOMA-IR	1.51 [1.54] (0.95 - 2.49)	1.93 [1.91] (1.13 - 3.04)	0.294
METS-IR	7.63 [0.65] (7.33 - 7.98)	7.88 [0.53] (7.54 - 8.07)	0.026
TyG	8.48 ± 0.55	8.85 ± 0.59	0.005
ISM1 (ng/mL)	0.95 [0.82] (0.85 - 1.67)	1.23 [0.74] (0.93 - 1.67)	0.073
Albumin (g/dL)	44 [3] (42 - 45)	46 [3] (44 - 47)	0.001
BUN (mg/dL)	11 [3] (10 – 13)	13 [5] (11 – 16)	0.003
Creatinine (mg/dL)	0.69 ± 0.11	0.94 ± 0.15	0.001
ALT (U/L)	15 [9] (11 - 20)	28 [13] (22 – 35)	0.001
AST (U/L)	19 [7] (16 - 23)	23 [8] (19 - 27)	0.003
LDH (U/L)	168 [39] (152 - 191)	160 [39] (141 - 180)	0.223

Parametric results were given as "[Mean ± SD]" and p values were given according to 'Student t-test. Non-parametric results were given as "Median [IQR] and 25th -75th percentiles" and p values according to 'Mann Whitney U' test. $p < 0.05$ was accepted statistically significant.

The biochemical tests of total females and total males are described in Table 7. As can be perceived from the table, there are significant differences between total males and total females in terms of serum TG, TyGi, AIP, METS-IR, HDL-C, ALT, AST, albumin, BUN, and creatinine ($p < 0.05$). Although a critical increase in serum ISM1 levels is seen in males, this increase is not significant; “p value” shows a borderline. On the other hand, there were no significant differences between females and males in terms of the other remaining biochemical parameters ($p > 0.05$).

4.3. Correlation of ISM1 Levels with the Other Parameters

The relationship of ISM1 with demographic data, lipid, and biochemical parameters in the total study groups (n=80) was studied. It was found that there were significant positive correlations between ISM1 and the age of the patients, weight, BMI, WC, HC, WC/HC, SBP, DBP, MAP, TG, TC, LDL-C, non-HDL-C, ApoB100, MTTP, AIP, insulin, HOMA-IR, METS-IR, TyGi, BUN, ALT and AST ($p < 0.05$). The significant correlations of ISM1 with demographic and biochemical parameters are shown in Table 8. Additionally, the significant correlations of ISM1 with lipid parameters and insulin are depicted in Figure 4. On the other hand, ISM1 didn't show any significant relationship with body height, serum glucose, HDL-C, NEFFA, albumin, creatinine and LDH ($p > 0.05$). Moreover, the total study group exhibited significant elevations in serum MTTP levels with TG, TC, NEFFA, ISM1 and albumin ($r = 0.22$, $p = 0.048$; $r = 0.23$, $p = 0.038$; $r = -0.24$, $p = 0.032$; $r = 0.25$, $p = 0.022$; $r = 0.22$, $p = 0.047$ respectively).

4.4. Analysis of ROC Curves for ISM1 and Insulin in the Main Groups and Subgroups

The ROC analysis demonstrated that the ISM1 curve had higher sensitivity and specificity compared to the insulin curve across both the main groups and subgroups. The cut-off value was determined to be greater than 1.1562, with a significant level of $p=0.0001$. Notably, the ISM1 curve in control vs HTG, control vs HTNC, and control vs HTHC displayed AUC values of (0.965, 0.953, and 0.978, respectively), as illustrated in diagrams 5(A), 5 (B), and 5 (C).

Table 8. Correlations of serum ISM1 concentrations with the other parameters in all participants (n=80)

	r	p
Age (years)	0.29	0.009
Weight (kg)	0.39	0.001
Height (cm)	0.19	0.082
BMI (kg/cm^2)	0.32	0.003
WC (cm)	0.47	0.001
HC (cm)	0.32	0.003
WC/HC	0.44	0.001
SBP (mmHg)	0.34	0.002
DBP (mmHg)	0.26	0.017
MAP (mmHg)	0.35	0.001
Glucose (mg/dL)	0.14	0.210
TG (mg/dL)	0.75	0.001
TC (mg/dL)	0.62	0.001
LDL-C (mg/dL)	0.53	0.001
HDL-C (mg/dL)	-0.08	0.501
Non-HDL-C (mg/dL)	0.69	0.001
Apo B100 (ng/mL)	0.23	0.044
MTTP (ng/L)	0.25	0.022
NEFFA (mmol/L)	-0.15	0.157
AIP	0.73	0.001
Insulin (µIU/mL)	0.39	0.001
Homa-IR	0.39	0.001
METS-IR	0.72	0.001
TyGi	0.77	0.001
Albumin (g/dL)	0.18	0.107
BUN (mg/dL)	0.23	0.042
Creatinine (mg/dL)	0.19	0.092
ALT (U/L)	0.32	0.004
AST (U/L)	0.25	0.025
LDH (U/L)	0.09	0.401

p values for Spearman correlation

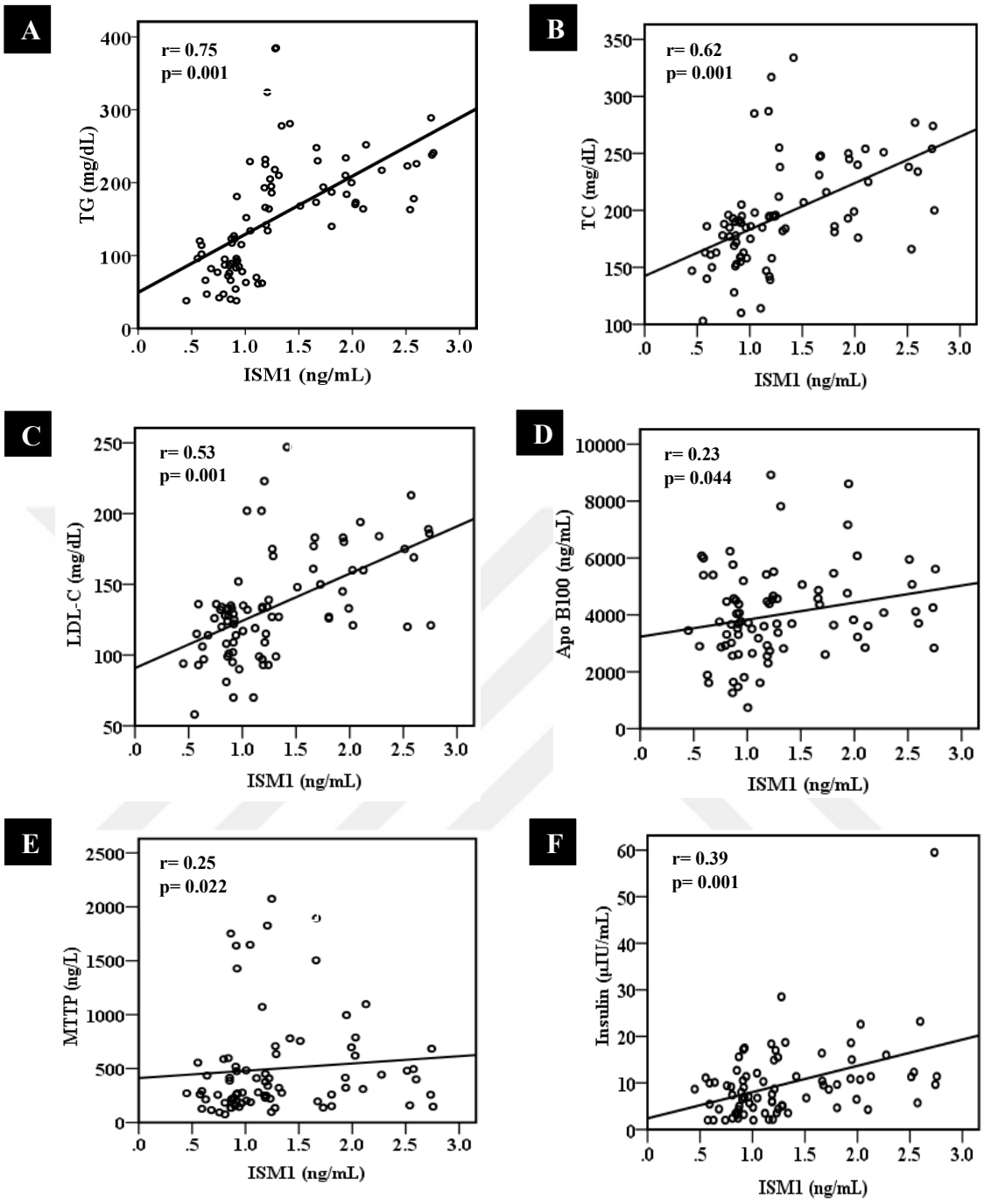
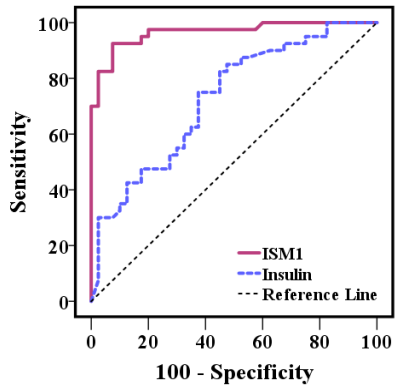


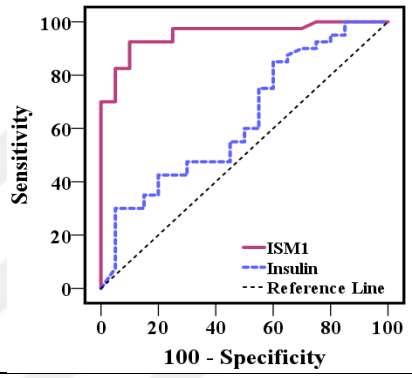
Figure 4. The correlations of ISM1 levels with A) TG, B) TC, C) LDL-C, D) Apo B100, E) MTTP, and F) insulin levels in all participants

A



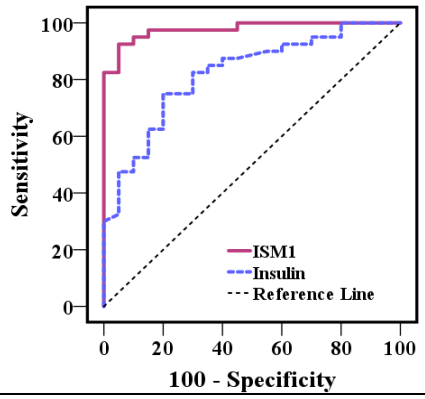
Control vs HTG	AUC (95 % CI)	Sensitivity	Specificity	Cutoff	P
ISM1	0.965 (0.898-0.993)	92.5 (79.6-98.4)	92.5 (79.6 - 98.4)	>1.1562	0.0001
Insulin	0.727 (0.616-0.821)	62.5 (45.8-77.3)	75.0 (58.8-87.3)	>9.41	0.0001

B



Control vs HTNC	AUC (95 % CI)	Sensitivity	Specificity	Cutoff	p
ISM1	0.953 (0.865-0.991)	90.0 (68.3-98.8)	92.5 (79.6-98.4)	>1.1562	0.0001
Insulin	0.696 (0.564-0.808)	50.0 (27.2-72.8)	85.0 (70.2-94.3)	>10.5	0.0102

C



Control vs HTHC	AUC (95 % CI)	Sensitivity	Specificity	Cutoff	P
ISM1	0.978 (0.901-0.999)	95.0 (75.1-99.9)	92.5 (79.6-98.4)	>1.1562	0.0001
Insulin	0.821 (0.700-0.908)	80.0 (56.3-94.3)	75.0 (58.8-87.3)	>9.41	0.0001

Figure 5. Results of ROC analysis for comparison between ISM1 and insulin parameters in A) control vs HTG, B) control vs HTNC groups, and C) control vs HTHC

5. DISCUSSION

HTG is a form of dyslipidemia characterized by elevated levels of TG in the blood, exceeding 150 mg/dL (1.7 mmol/L). The underlying cause of HTG is either genetic or related to external factors such as metabolic disorders, obesity, diabetes and alcohol abuse (65, 66). On the other hand, ISM1 is a newly identified insulin-like adipokine secreted by mature fat cells, exhibiting endocrine functions and demonstrating its significance in managing metabolic disturbances by regulating glucose, lipid, and protein metabolism (55, 57).

Our research focuses on evaluating ISM1 levels in HTG conditions and identifying the relationship between ISM1 and insulin resistance. We assessed the levels of ISM1 in patients with normoglycemic HTG, both with and without hypercholesterolemia. Then, we examined the relationship of ISM1 with the metabolic indices, the demographic values and the biochemical parameters in all participants. Finally, a comparison was made of ISM1 levels according to gender. The primary study findings were as follows: (1) ISM1 levels were significantly higher in HTG, HTNC and HTHC patients compared to control group; (2) While ISM1 levels were higher in males than in females, this difference was deemed to be of negligible significance due to the borderline value ($p = 0.073$); (3) ISM1 showed significant associations with the majority of the demographic and biochemical parameters.

As seen in Figure 3, it was found that ISM1 levels increased in HTG, HTNC, and HTHC individuals compared to the control group. Additionally, a rise in MTTP and Apo B100 levels was also noticed within these groups (Tables 5 and 6). These results suggest that the adipokine ISM1 has an influence on the DNL pathway and lipid parameters in the liver, thus it can trigger hepatic TG synthesis and the release of liver- derived VLDL, leading to the accumulation of TRLs in the bloodstream. Therefore, our study shows that ISM1 is linked to body fat and elevated in people with high TG levels. Persistent elevation of TRL levels has the potential to increase the risk of developing metabolic problems such as dyslipidemia.

Research established by Lei et al. (67) has determined that ISM1 levels increased in patients with metabolic dysfunction-associated FLD, T2DM and metabolic syndromes. Higher levels of ISM1 have also been observed in individuals with obesity (55, 67). In contrast, Alshawaf et al. (57) reported that ISM1 values were low in T2DM and obese individuals. Moreover, in patients diagnosed with neuropathy associated with T2DM,

ISM1 levels were found to be low, although this finding did not reach statistical significance (68). In contrast to our findings, a study demonstrated that ISM1 plays a crucial role in increasing blood glucose uptake and suppressing lipogenesis, preventing IR, and combating HTG and hyperglycemia (56). Research exhibited that ISM1 restrains DNL in the hepatocytes by repressing insulin-induced expression of SREBP-1c and ChREBPβ, which are typically involved in the conversion of carbohydrates into fatty acids (13, 56, 58). Another study conducted by Reghupaty et al. (69) found that the pharmacological administration of recombinant ISM1 protein to mice decreased the hepatic synthesis of TG, DAG, and ceramide, thereby reducing the potential risk of lipid disturbances and hepatic steatosis. Additionally, Qu et al. (70) have delineated in their study on Carp fish that ISM1 showed the potential to increase glucose uptake by promoting the expression of glycolysis-related mRNAs, including glucose kinase, hexokinase, and phosphofructokinase, whilst concomitantly downregulate the expression of mRNAs associated with gluconeogenesis such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the hepatocytes. Recent studies found that ISM1 performs a pivotal function in protein anabolism and catabolism by shifting the lipogenesis state to a protein synthesis state through stimulating the AKT-mTORC1-S6 pathway in the liver and muscle cells (48).

Higher serum ISM1 levels were detected in males compared to females in all participants; however, this increase was not statistically significant ($p = 0.073$) (Table 7). Similarly, a study has pointed out that ISM1 levels increased in pubertal obese boys compared to girls, yet this difference was not deemed significant (55). Another study by Lei et al. (67) exhibited no significant differences in ISM1 levels between females and males. It was reported that the function of β-cells in males with diabetes was less efficient compared to those of diabetic females, and that ISM1 levels in males were higher than in females and were inversely correlated with β-cell function (71). Conversely, a study by Liao et al. (72) demonstrated higher serum levels of ISM1 in lean females with T2DM compared to obese females and males with T2DM.

In our study, we found that there were a series of significant positive correlations between serum ISM1 and most of the parameters (Table 8). Weight, BMI, WC, HC, and WC/HC are demographic parameters that indicate the risk of being overweight or obese. In our study, there was a direct relationship between ISM1 and the above-mentioned indicators (Table 8), suggesting that ISM1 plays a role in fat accumulation and obesity.

Similarly, a study revealed positive correlations between ISM1 and BMI or maternal WC in gestational diabetic mellitus (GDM) patients (73). A relationship of ISM1 with BMI was also indicated by Lei et al. (67). It has been observed that ISM1 levels were correlated with subcutaneous fat area/ visceral fat area and important in the prognosis of HTG and obesity-related health risks (74). On the other hand, a study reported no correlation between ISM1 and BMI (72). Indirect correlations were also verified between ISM1 and HC or BMI values (57).

SBP, DBP, MAP, and AIP are parameters indicating vascular efficiency and serve as signals for detecting the risk of hypertension and vascular impairment (75). We speculate that the positive correlation of ISM1 levels with SBP, DBP, MAP and AIP (Table 8) may indicate the role of ISM1 in endothelial cells and the body's endeavor to modulate vascular integrity. Our findings are endorsed by the observations of Deng et al. (52), who displayed elevated serum ISM1 levels in cases with hypertension and noted a positive correlation between these levels and both SBP and DBP. On contrast, Liao et al. (72) found no correlation between ISM1 and SBP or DBP in T2DM patients. Some studies have proven the prominent role of ISM1 on ECs, this protein can bind to the endothelial membrane of the cancer cell via the avβ5 and GRP78 receptors and causes cancer cell death, thus reduces the risk of cancer cell development (45, 48, 50, 76). Another study indicates that ISM1 contributes to ECs apoptosis and anti-angiogenesis through acting on the aforementioned receptors and causing disruptions in the ADP/ATP exchange between mitochondria and cytosol, thus leading to ECs apoptosis (72, 77).

It was hypothesized that the high levels of ISM1, in conjunction with its association with HOMA-IR (Table 8), may serve as a marker playing a role in the development of IR. Supportingly, a study done by Şentürk and her colleagues (73) has stated that ISM1 was positively correlated with HOMA-IR in pregnant women with GDM (73). A supporting study conducted positive correlations between ISM1 levels and insulin or HOMA-IR in a diverse study group comprising patients with metabolic-associated FLD, T2DM, and healthy participants (67). Recent studies exhibited that ISM1 plays a role in the uptake of sugar from the blood by activating PI3K/Akt in an insulin-independent pathway, preventing IR and hepatic steatosis (12, 13). HOMA-IR, METS-IR, TyGi and high insulin levels typically indicate pancreatic β-cell dysfunction, along with the risk of diabetes, IR and pancreatic diseases (78). In contrast, Alshawaf et al. (57) reported that ISM1 values

were low in T2DM and obese individuals. Moreover, in patients diagnosed with neuropathy associated with T2DM, ISM1 levels were found to be low, although this finding did not reach statistical significance (68).

Positive correlations were observed between ISM1 and ALT, AST, as well as other lipid parameters (Table 8). This suggests a potential link between ISM1 and the risk of obesity and liver disorders. Supporting this finding, a study conducted by Lei et al. (67) also reported positive correlations between ISM1 levels and ALT, AST, TG, TC, and LDL-C in a total study group including patients with T2DM, metabolic-associated FLD and healthy participants.

BUN is a by-product of protein metabolism produced by the liver and excreted by the kidneys; thus, it is considered when assessing kidney function. In the current study, we observed a positive correlation between ISM1 levels and BUN levels (Table 8). In contrast to our findings, a study reported that there were no correlations between ISM1 and BUN in T2DM patients (72). However, a study demonstrated that ISM1 plays a crucial role in kidney development; it binds to alpha- 8- beta- 1 (α8β1) and activates glial cell-derived neurotrophic factor/receptor tyrosine kinase signaling, ultimately causing mesenchymal condensation and ureteral epithelial branching morphogenesis. Additionally, ISM1 interacts with two other important receptors located within the mesenchyme cells of the kidney, namely Ephrin-β1 and Plexin-β2. This interaction enhances cytoskeleton rearrangement, cell adhesion, and cell migration (79). A study revealed that ISMI levels were increased in T2DM patients who experienced a low estimated glomerular filtration rate (eGFR) when compared to those with high values of eGFR (80). Another study was conducted on T2DM and non-diabetic sensorimotor peripheral neuropathy (non-DSPN) patients found that serum ISM1 levels were higher in T2DM and non-DSPN compared to DSPN patients and healthy controls, however, the increase in ISM1 levels in non-DSPN compared to DSPN patients wasn't significant (72).

The limitation of our study was that the selected number of subjects with HTG was insufficient, therefore, this caused the number of subjects in the subgroups to be small. The limited sample size may have contributed to the inability to identify some significant relationships of ISM1 with the other parameters.

As a result, we suggested that ISM1 may play a significant role in the development of TRLs, and therefore of HTG. It may be an indicator of IR-related disorders. Further

studies are necessary to investigate the relationship between ISM1 and lipid metabolism, and related - pathological conditions.

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APPENDICES

APPX 1. (Continue)

KTÜ TIP FAKÜLTESİ BİLİMSEL ARAŞTIRMALAR ETİK KURULU KARAR FORMU

BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	"Hipertrigliseridemik Bireylerde Serum İstmin-1 Protein Düzeyleri ve Endojen Lipid Metabolizmasıyla İlişkisinin İncelenmesi"		
	ARAŞTIRMANIN PROTOKOL/PLAN KODU	2023/116		
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI UNVANI/ADI/SOYADI	Prof. Dr. Birgül KURAL		
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ UZMANLIK ALANI	Tıbbi Biyokimya		
	TEZ SAHİBİ/DİĞER ARAŞTIRICILAR, ÜNVANI/ADI/SOYADI	Yük.Lis.Öğr.Kawther A.M.S. ALEDRESI, Prof.Dr.Asım ÖREM, Prof.Dr.Cihan ÖREM, Dr.Öğr.Üyesi Fulya BALABAN YÜCESAN, Dr.Serap ÖZER YAMAN, Arş.Gör.Dr.Kevser AYAR, Yük.Lis.Öğr.Feride Mehtap YÜCEL		
	DESTEKLEYİCİ			
	ARAŞTIRMANIN NİTELİĞİ			
	ARAŞTIRMANIN TÜRÜ	TEZ ☒	AKADEMİK AMAÇLI ☐	
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ☒	ÇOK MERKEZLİ ☐	ULUSAL ☐

	Belge Adı	Tarihi	Versiyon Numarası	Dili
DEĞERLENDİRİLEN BELGELER	ARAŞTIRMA PROTOKOLÜ/PLANI			Türkçe ☒ İngilizce ☐ Diğer ☐
	BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU			Türkçe ☐ İngilizce ☐ Diğer ☐
	OLGU RAPOR FORMU			Türkçe ☐ İngilizce ☐ Diğer ☐

	Belge Adı		Açıklama
DEĞERLENDİRİLEN DİĞER BELGELER	TÜRKÇE ETİKET ÖRNEĞİ	☐	
	SİGORTA	☐	
	ARAŞTIRMA BÜTÇESİ	☐	
	BİYOLOJİK MATERYEL TRANSFER FORMU	☐	
	İLAN	☐	
	YILLIK BİLDİRİM	☐	
	SONUÇ RAPORU	☐	
	GÜVENLİLİK BİLDİRİMLERİ	☐	
	DİĞER:	☐	

APPX 2. (Continue)

	TIP FAKÜLTESİ DEKANLIĞI ASGARİ BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU ÖRNEĞİ (HASTA GÖNÜLLÜ)			KARADENİZ TEKNİK ÜNİVERSİTESİ Tıp Fakültesi TIPF
Dok. Kodu: KKY. FR. 29	Yayın Tarihi: 24.05.2022	Revizyon No: 01	Revizyon Tarihi: 25.11.2022	Sayfa Sayısı: 02

Araştırmaya katılımın isteğe bağlı olduğu ve istediğim zaman, herhangi bir cezaya veya yaptırıma maruz kalmaksızın, hiçbir hakkımı kaybetmeksizin araştırmaya katılmayı reddedebileceğim ve istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğim belirtildi.

Yukarıda yer alan ve araştırmaya başlanmadan önce gönüllüye verilmesi gereken bilgileri gösteren 2 sayfalık metni okudum ve sözlü olarak dinledim. Aklıma gelen tüm soruları araştırıcıya sordum, yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı.

Çalışmaya katılmayı isteyip istemediğime karar vermem için bana yeterli zaman tanındı.

Bu açıklamaları anladım ve gönüllülükle bu onamı verdim. Bana ait tıbbi bilgilerin gözden geçirilmesi, transfer edilmesi ve işlenmesi konusunda araştırma yürütücüsüne yetki veriyor ve söz konusu araştırmaya ilişkin bana yapılan katılım davetini hiçbir zorlama ve baskı olmaksızın hiçbir baskı ve zorlama olmaksızın kendi rızamla gönüllü olarak katılmayı kabul ediyorum.. Bu formu imzalamakla yerel yasaların bana sağladığı hakları kaybetmeyeceğimi biliyorum.

''**Hipertrigliseridemik bireylerde serum istmin-1 protein düzeyleri ve endojen lipid metabolizmasıyla ilişkisinin incelenmesi**'' başlıklı araştırma kapsamında alınan biyolojik örneklerimin (kan, idrar vb.);

☐ Sadece yukarıda bahsi geçen araştırmada kullanılmasına izin veriyorum.

☐ İleride yapılması planlanan tüm araştırmalarda kullanılmasına izin veriyorum.

☐ Hiçbir koşulda kullanılmasına izin vermiyorum.

Bu formun imzalı ve tarihli bir kopyası bana verildi.

GÖNÜLLÜNÜN		**İMZASI**
ADI & SOYADI		
ADRESİ		
TEL. & FAKS		
TARİH		
VELAYET VEYA VESAYET ALTINDA BULUNANLAR İÇİN VELİ VEYA VASİNİN		**İMZASI**
ADI & SOYADI		
ADRESİ		
TEL. & FAKS		
TARİH		
AYDINLATAN HEKİM		**İMZASI**
ADI & SOYADI		
TARİH		
GEREKTİĞİ DURUMLARDA TANIK		**İMZASI**
ADI & SOYADI		
GÖREVİ		
TARİH		

APPX 3. (Continue)

	TIP FAKÜLTESİ DEKANLIĞI		KARADENİZ TEKNİK ÜNİVERSİTESİ Tıp Fakültesi TIPF
	ASGARİ BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU ÖRNEĞİ (SAĞLIKLI GÖNÜLLÜ)		
Dok. Kodu: KKY. FR. 29	Yayın Tarihi: 24.05.2022	Revizyon No: 01	Revizyon Tarihi: 25.11.2022	Sayfa Sayısı: 02

Araştırmaya katılımın isteğe bağlı olduğu ve istediğim zaman, herhangi bir cezaya veya yaptırıma maruz kalmaksızın, hiçbir hakkımı kaybetmeksizin araştırmaya katılmayı reddedebileceğim ve istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğim belirtildi.

Yukarıda yer alan ve araştırmaya başlanmadan önce gönüllüye verilmesi gereken bilgileri gösteren 2 sayfalık metni okudum ve sözlü olarak dinledim. Aklıma gelen tüm soruları araştırıcıya sordum, yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı.

Çalışmaya katılmayı isteyip istemediğime karar vermem için bana yeterli zaman tanındı.

Bu açıklamaları anladım ve gönüllülükle bu onamı verdim. Bana ait tıbbi bilgilerin gözden geçirilmesi, transfer edilmesi ve işlenmesi konusunda araştırma yürütücüsüne yetki veriyor ve söz konusu araştırmaya ilişkin bana yapılan katılım davetini hiçbir zorlama ve baskı olmaksızın hiçbir baskı ve zorlama olmaksızın kendi rızamla gönüllü olarak katılmayı kabul ediyorum.. Bu formu imzalamakla yerel yasaların bana sağladığı hakları kaybetmeyeceğimi biliyorum.

''**Hipertrigliseridemik bireylerde serum istmin-1 protein düzeyleri ve endojen lipid metabolizmasıyla ilişkisinin incelenmesi**'' başlıklı araştırma kapsamında alınan biyolojik örneklerimin (kan, idrar vb.);

☐ Sadece yukarıda bahsi geçen araştırmada kullanılmasına izin veriyorum.

☐ İleride yapılması planlanan tüm araştırmalarda kullanılmasına izin veriyorum.

☐ Hiçbir koşulda kullanılmasına izin vermiyorum.

Bu formun imzalı ve tarihli bir kopyası bana verildi.

GÖNÜLLÜNÜN		**İMZASI**
ADI & SOYADI		
ADRESİ		
TEL. & FAKS		
TARİH		
VELAYET VEYA VESAYET ALTINDA BULUNANLAR İÇİN VELİ VEYA VASİNİN		**İMZASI**
ADI & SOYADI		
ADRESİ		
TEL. & FAKS		
TARİH		
AYDINLATAN HEKİM		**İMZASI**
ADI & SOYADI		
TARİH		
GEREKTİĞİ DURUMLARDA TANIK		**İMZASI**
ADI & SOYADI		
GÖREVİ		
TARİH		

CURRICULUM VITAE

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LANGUAGES

1. Arabic
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PUBLICATIONS

1. Lucchi C, Marcucci M, **Aledresi KAMS**, Costa AM, Cannazza G, Biagini G (2024). Subthreshold cannabidiol potentiates levetiracetam in the kainic acid model of temporal lobe epilepsy: A Pilot Study. Pharmaceuticals (Basel) 17(9):1187.
2. **Ameen Muhammed Saeed Aledresi K**, Kural B, Kör S (2024). An important adipokine: isthmin-1. Farabi Med J 4(1):12-16.
3. **Aledresi KAMS**, Kural B (2024). International studies and evaluations in the field of

health sciences. Serüven Publishing, Ankara, Türkiye; Page: 129-149.

4. Anwer S, **Muhammad K,** Ali Z, Esmail E, Shekh A (2020). Microiıological assessment of tissue paper before and after remaining in toilets. International Health Sciences Conference (INESEG); Page: 717-728.

REWARDS / INCENTIVES SCHOLARSHIPS

1. Full funded scholarship for studying Master's degree by YTB (Presidency for Turks Abroad and Related Communities)
2. A reward form the governor of Erbil (Frsat Sofy), Higher Education and scientific research, Ministry of Health, and the University presidency for being top Student of Class of 2020- Department of Medical Biochemistry/ College of Health Sciences/ Hawler Medical University/ Erbil/ Iraq.

HOBBIES

1. Playing the Kemençe