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Original Article

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Does Irisin Have an Effect on the Development of Type 2 Diabetes Mellitus and the Development of Atherosclerosis in Diabetic Patients?

Bengisu Akyol Tulgar[1], Ayten Üstündağ[1], Nermin Tunçbilek[2], Gülşah Akcelep Burgazdere[2], Necdet Süt[3], İlhan Kurultak[4], Sedat Üstündağ[4]

[1]Department of Internal Medicine, Trakya University Faculty of Medicine, Edirne, Türkiye
[2]Department of Radiology, Trakya University Faculty of Medicine, Edirne, Türkiye
[3]Department of Biostatistics, Trakya University Faculty of Medicine, Edirne, Türkiye
[4]Department of Nephrology, Trakya University Faculty of Medicine, Edirne, Türkiye

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a prevalent public health problem with high mortality rate due to the complications it causes. However, recent data indicate that irisin may play an important role in preventing the development of T2DM and reducing the occurrence of atherosclerosis. The purpose of our study was to investigate the effect of irisin on the development of T2DM and carotid artery intima–media thickness (C-IMT), an early indicator of atherosclerosis in diabetic subjects without known cardiovascular disease (CVD).

Methods: Our study included 41 healthy volunteers and 93 patients who were followed up with a diagnosis of T2DM. Irisin levels were determined by the sandwich enzyme-linked immunosorbent assay method using the Elabscience® Human Irisin Elisa Kit H6120. C-IMT measurement was performed using a Toshiba Aplio 500 brand ultrasonography device, a B-Mode USG, and a 7.5-18 MHz linear probe. In the T2DM group, patients were divided into those with or without subclinical atherosclerosis, in addition to those with low, medium, and high irisin levels, and the results were compared.

Results: We found that patients with T2DM had significantly lower irisin levels than the healthy control group ($P < .001$). In our patient group, each 10-year increase in patient age increased C-IMT by 0.06 mm (95% confidence interval (CI): 0.03-0.08) mm, and being male increased C-IMT by 0.117 mm (95% CI: 0.068-0.166). A negative linear relationship was found between C-IMT and irisin levels, which did not reach statistical significance ($r = -0.145$, $P = .165$). Compared to patients with high irisin levels, patients with low irisin levels had 0.113 mm thicker C-IMT values (0.734 ± 0.129 mm and 0.621 ± 0.140 mm, respectively, $P = .004$).

Conclusion: Our findings indicate that low irisin levels facilitate the development of T2DM and increase diabetic complications such as subclinical atherosclerosis and diabetic nephropathy.

Keywords: Diabetes mellitus, irisin, atherosclerosis, carotid artery intima–media thickness

Corresponding author: Ayten Ustundag ✉ aytenustundag@trakya.edu.tr

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INTRODUCTION

Type 2 diabetes mellitus (T2DM), which occurs as a result of insulin deficiency or ineffectiveness, is a widespread and severe public health issue with a high mortality rate.[1,2] Unfortunately, the prevalence of T2DM is gradually rising due to decreased physical activity and obesity.[1] While there were 215 million diabetics worldwide in 2000, and it was predicted that this figure would rise to 440 million by 2030, an analysis conducted by the International Diabetes Federation in 2015 revealed that the total number of people with diabetes worldwide had reached 415 million.[3] In 2019, this number was projected to be 463 million (9.3% of adults).[4] The most likely scenario is that the prevalence will be 570 million people in 2030 and 740 million in 2045.[1] This condition suggests that diabetes will become a much more serious public health concern in the coming years if preventive measures are not taken.

In addition to metabolic complications, people with diabetes develop damage to various organs, especially the cardiovascular system and kidneys, depending on the duration and severity of the disease.[5] In previous decades, the high mortality rate due to metabolic complications from the earliest stages of diabetes has decreased over the years due to the discovery of more effective treatment agents and improved clinical practices, and the life expectancy of diabetic patients has been extended. However, as diabetes incidence has increased and patient lifespans have lengthened, micro- and macrovascular complications associated with diabetes-accelerated atherosclerosis have become increasingly common.[1-4] Approximately, 5 million people die every year due to diabetes complications worldwide.[3] Research has demonstrated that endothelial dysfunction, which leads to early-accelerated atherosclerosis in diabetics, is a critical factor in the development of micro-macrovascular complications. However, the pathogenetic mechanisms underlying accelerated atherosclerosis and associated increased mortality seen in diabetic individuals are unclear.[6] Therefore, it is crucial for public health to identify the factors that contribute to the development of diabetes and its complications, and to develop treatments for them.

Irisin is a proteolytic product of the transmembrane protein fibronectin type III domain 5 found in skeletal muscle and to a lesser extent, white adipose tissue. Recent data claims that irisin may play a role in the early and accelerated development of atherosclerosis.[7] In experimental studies, it has been demonstrated that irisin application reduces the formation of mature adipocytes, increases cellular glucose uptake by increasing the expression of glucose transporter type 4 (GLUT4), increases glycolysis, and reduces adipocyte size. Furthermore, these studies have found that irisin improves glucose homeostasis. The fact that it positively affects insulin resistance and lipid profile suggests that irisin may influence the development of diabetes and related complications.[8-12]

MAIN POINTS

- Diabetes mellitus is a widespread public health problem with a high mortality due to the atherosclerotic complications it causes.
- Irisin, which stimulates exercise and cold-induced conversion of white adipose tissue cells to brown adipose tissue, has been shown to inhibit the development of type 2 diabetes mellitus and atherosclerosis, but the findings are contradictory.
- Our study is the first to investigate the relationship between irisin levels and carotid artery intima–media thickness in type 2 diabetic subjects.
- Our results showed that irisin level was significantly lower in the diabetic group compared to healthy subjects, and carotid artery intima–media thickness was significantly higher in diabetics with low irisin level compared to those with high irisin level. These findings suggest that irisin may have a protective effect against the development of type 2 diabetes and reduces the risk of atherosclerosis in diabetic patients.

Research on the function of irisin in the pathogenesis of T2DM in humans has yielded conflicting results. Despite the widespread belief that low irisin levels promote the onset of diabetes,[13-17] other research has discovered that irisin has no effect on the disease's development and that, over a 2.6-year follow-up period, cases with high basal irisin levels have a higher risk of developing T2DM.[18-20] The research into the relationship between irisin levels and the development of atherosclerotic disease in people with T2DM is much more limited, and the findings are incompatible. We did not find any study investigating the relationship between carotid artery intima–media thickness (C-IMT), which is a non-invasive and early indicator of subclinical atherosclerosis, and irisin in patients with T2DM in the literature review.[21,22]

Thus, we aimed to investigate 3 issues in the present study: (i) whether there is a difference in serum irisin levels between healthy individuals and patients with T2DM; (ii) the factors associated with C-IMT, and (iii) the effect of irisin on C-IMT in patients with T2DM.

MATERIAL AND METHODS

Study Population

Our study was conducted with the approval of the Ethics Committee of Trakya University Faculty of Medicine, numbered 2020/302, dated August 24, 2020. The study included 93 patients diagnosed with T2DM who presented to the General Internal Medicine, Nephrology, Endocrinology and Metabolism Diseases, Obesity, and Diabetes Outpatient Clinics of Trakya University Faculty of Medicine between August 2020 and August 2021, who were over 18 years of age, had written consent for the study, and had sufficient cooperation and orientation. Forty-one healthy volunteers with no history of acute/chronic disease were included. Pregnant or breastfeeding women, patients with acute diseases, known cardio-cerebrovascular diseases, patients with a history of malignancy, and patients with inadequate cooperation or orientation were not included in the study.

Clinical and Laboratory Examinations

The patient's height, weight, waist circumference, systolic blood pressure (SBP) and diastolic blood pressure (DBP), duration of diabetes, family history of diabetes, presence of diabetic retinopathy (DRp), smoking, medications history and fasting blood glucose (FBS) for the last month was recorded. Estimated glomerular filtration rate (eGFR) calculated with the CKD-EPI formula, serum sodium (Na^+) and potassium (K^+), uric acid, triglycerides (TG), total cholesterol (TC), low/high-density lipoprotein cholesterol (LDL/HDL-C), glycosylated hemoglobin (HbA1c), 24-hour urine protein excretion (UPE), and 24-hour urine albumin excretion (UAE) amounts were also recorded. Body mass index (BMI) was calculated using the formula [weight(kg)/height$(m)^2$].

In addition, after a 12-hour fasting interval, blood samples were taken and were centrifuged at a speed of 3000 rpm for 5 minutes.

At least 500 microliters of serum were then taken by pipette, transferred to the Eppendorf tube, and stored in a −70°C refrigerator until the working day. The serum samples taken were removed from the freezer 1 day before the laboratory study and allowed to thaw at room temperature for 24 hours. Serum irisin levels were measured by the sandwich enzyme-linked immunosorbent assay method using the Elabscience® Human Irisin Elisa Kit H6120 in the Department of Biochemistry laboratory. The results were evaluated using the Biotek Quant Microplate Reader. Irisin values for all individuals were studied twice, and the average of the 2 measurements was used in statistical evaluations.

Carotid Intima–Media Thickness Measurement

The C-IMT measurement of each case was performed using a Toshiba Aplio 500 brand device, a B-Mode USG, and a 7.5-18 MHz linear probe. The same radiologist performed all USG examinations in a quiet environment after each individual had rested for approximately 15 minutes. For carotid artery imaging, the neck muscles were relaxed by angling the neck to the opposite side at approximately 20° while the patient was lying in the supine position. Measurements were made from 3 different points, 1 cm distal to the left and right anterior carotid artery, and only the posterior (far) wall was evaluated, and IMT measurements were made. The average of 3 measurements on the right and left was taken, and the thicker measurement was recorded as C-IMT.

Statistical Analysis

All statistical calculations were made using SPSS PC ver.22 (IBM SPSS Corp.; Armonk, NY, USA) software. Descriptive statistics were given as numbers, percentages, and arithmetic mean ± standard deviation. The Kolmogorov–Smirnov test was first investigated during the statistical evaluation phase to determine whether the parametric data fit a normal distribution. If the parametric data were normally distributed, the differences between the parametric data of the 2 groups were examined with the independent Student's *t*-test. If the parametric data were not normally distributed, the difference between the 2 groups was investigated using the nonparametric *t*-test (Mann–Whitney *U*) in independent groups. The chi-square test was used to analyze categorical data, and the Fischer test was used when chi-square test conditions were not met. When the multiple relationships between C-IMT and irisin, and other data in the entire study and DM groups were distributed normally, the Pearson correlation test was used. If parametric data were not normally distributed or categorical data were evaluated, they were investigated with the Spearman correlation analysis. $P < .05$ was accepted as the level of statistical significance.

RESULTS

Comparison of Data from Healthy Volunteers and Type 2 Diabetes Mellitus Patients

When the data of 41 healthy individuals and 93 T2DM individuals were compared, the mean patient age (53.6 ± 9.0 yrs., 53.9 ± 9.66 yrs., respectively, p = .837), gender ratio (65.9% female, 61.3% female, respectively, p = .757), and smoking rate (22%, 31%, p = .376) were found statistically similar. Compared with the healthy control group, patients with T2DM had BMI (25.5 ± 2.2 kg/m^2, 30.2 ± 4.6 kg/m^2, $P < .001$), mean SBP (122 ± 7 mm Hg, 129 ± 11 mm Hg, respectively, $P < .001$), DBP (78 ± 5 mm Hg, 80 ± 6 mm Hg, respectively, $P = .025$), and FBS (99 ± 8, 178 ± 67 mg/dL, respectively, $P < .001$) values were found to be high. There were no individuals using any medication in the healthy control group. T2DM patients had a mean diabetes duration of 10 ± 8 years. In our T2DM patients, 38% had arterial hypertension (HT), while 31% had DRp. Fifty-eight percent of our patients were taking anti-hypertensive medications, 42% were taking anti-lipemic medications, 27% were taking acetylsalicylic acid (ASA), 71% were taking oral antidiabetics, and 52% were on insulin. Diabetics had higher mean C-IMT compared to healthy individuals (0.840 ± 0.090 mm vs. 0.650 ± 0.100 mm; $P < .001$). The mean irisin levels of the healthy control and T2DM groups are shown in Figure 1.

Comparison of Demographic, Clinical, and Laboratory Data of Patients Without Subclinical Atherosclerosis (Carotid Artery Intima–Media Thickness < 0.750 mm) and Patients with Subclinical Atherosclerosis (Carotid Artery Intima–Media Thickness ≥ 0.750 mm) in the Patient Group

Ninety-three patients with T2DM were divided into those with C-IMT values <750 mm (no subclinical atherosclerosis) and those with C-IMT values ≥750 mm (subclinical atherosclerosis). No statistically significant difference was found between the 2 subgroups in terms of age, smoking rate, use of drugs rates except anti-hypertensive and ASA, SBP, and levels ofFBG HbA1c, uric acid, TC, LDL-C, HDL-C, TG, Na^+, K^+, and eGFR.

Patients with subclinical atherosclerosis were mostly males ($P < .001$). Mean DM duration ($P = .018$); DRp occurrence rates ($P = .001$); DBP ($P = .042$); UPE ($P = .027$); and UAE

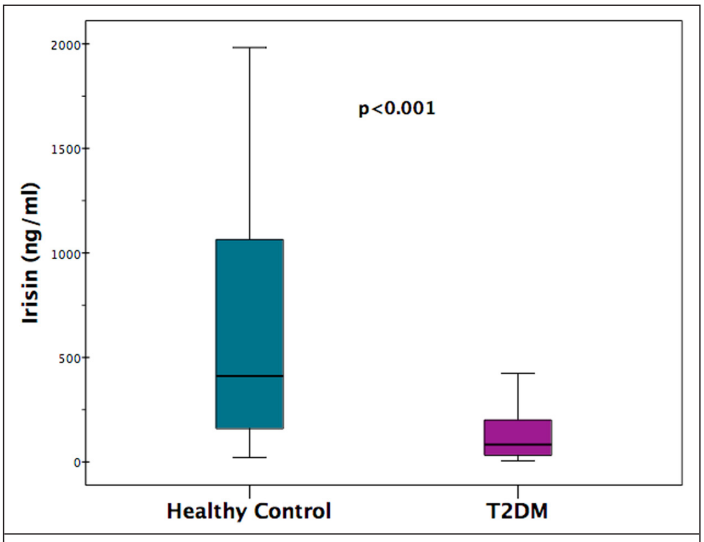

Figure 1. Irisin levels of the study groups. T2DM, type 2 diabetes mellitus.

Table 1. Demographic, Clinical, and Laboratory Data of Type 2 Diabetic Patients With or Without Subclinical Atherosclerosis

Parameter	*P*	Subclinical Atherosclerosis (+)	Subclinical Atherosclerosis (−)	Parameter	*P*	Subclinical Atherosclerosis (+)	Subclinical Atherosclerosis (−)
Age +/− SD (years)*	.118	52.7 ± 10.0	55.9 ± 8.9	FBS (mg/dL)#	.457	174 ± 68	185 ± 70
Female n (%)	< .001	44 (72.%2)	13 (36.1%)	HbA1c (%)*	.862	8.6 ± 1.9	8.7 ± 2.4
Duration of DM +/− SD (years)*	.018	8.5 ± 7.1	12.5 ± 8.7	Uric acid (mg/dL)*	.869	5.5 ± 1.8	5.5 ± 1.6
Smoking n (%)	.083	14 (24.6%)	15 (41.7%)	TC (mg/dL)*	.483	195 ± 63	186 ± 47
Hypertension n (%)	.496	34 (59.6%)	24 (66.7%)	LDL-C (mg/dL)*	.449	129 ± 47	122 ± 39
Diabetic retinopathy n (%)	< .001	10 (17.5%)	19 (52.8%)	HDL-C (mg/dL)*	.626	48.8 ± 11.2	47.6 ± 13.3
Anti-hypertensive n (%)	.182	30 (52.6%)	24 (66.7%)	TG (mg/dL)*	.541	160 ± 79	148 ± 109
Anti-lipid n (%)	.328	26 (46.4%)	13 (36.1%)	Na^+ (mmol/L)#	.811	139 ± 3	140 ± 3
ASA n (%)	.111	12 (21.1%)	13 (36.1%)	K^+ (mmol/L)*	.596	4.5 ± 0.4	4.5 ± 0.5
OAD n (%)	.648	42 (73.7%)	24 (66.7%)	eGFR (mL/min/1.73 m^2)#	.562	92 ± 29	83 ± 35
Insulin n (%)	.545	28 (49.1%)	20 (56.6%)	UPE (mg/day)#	.027	466 ± 949	1478 ± 2604
SBP +/− SD (mm Hg)*	.265	128 ± 12	131 ± 9	UAE (mg/day)#	.005	178 ± 750	711 ± 1591
DBP +/− SD (mm Hg)*	.042	79.4 ± 7.4	82.0 ± 4.8	Irisin (ng/mL)#	.280	178 ± 209	144 ± 186
BMI +/− SD (kg/m^2)#	.137	30.8 ± 4.9	29.3 ± 4.00	C-IMT (mm)*	< .001	0.647 ± 0.102	0.842 ± 0.098

Subclinical atherosclerosis: Caotid artery intima–media thickness ≥ 0.750 mm
ASA, acetylsalicylic acid; BMI, body mass index; C-IMT, carotid artery intima–media thickness; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; K+, potassium; LDL-C, low density lipoprotein cholesterol; Na+, sodium; OAD, oral antidiabetic; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; UAE, albumin excretion in 24-hour urine; UPE, protein excretion in 24-hour urine.
Statistical evaluation: *Independent samples *t*-test.
**Mann–Whitney *U*-test.

levels were higher in patients with subclinical atherosclerosis than in patients without subclinical atherosclerosis. Although not reaching statistical significance, the patient's age (*P* =.018), BMI (*P* =.137), anti-hypertensive drugs (*P* =.189), and ASA (*P* =.111) use rates were clinically significantly higher in patients with subclinical atherosclerosis. The irisin levels of patients with subclinical atherosclerosis were 35 ng/mL lower than those without subclinical atherosclerosis (144 ± 186 ng/mL; 178 ± 208 ng/mL, respectively, *P* =.280). Demographic, clinical, and laboratory data of patients with and without subclinical atherosclerosis are shown in Table 1, and the irisin levels of the 2 groups are shown in Figure 2.

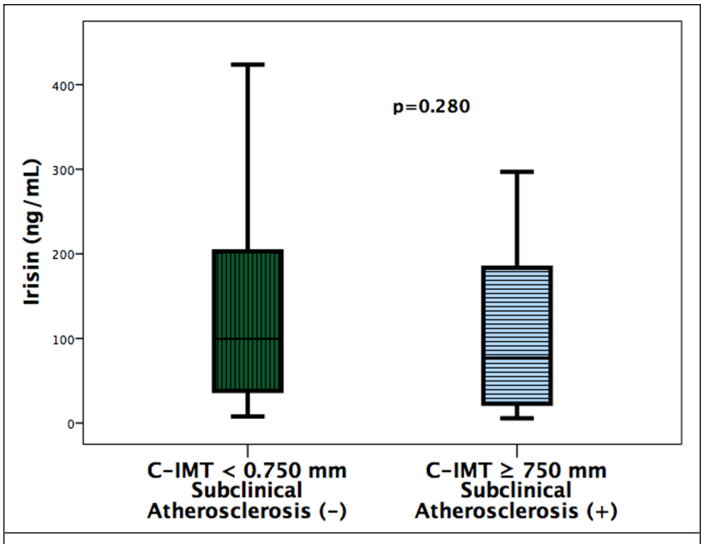

Figure 2. Carotid artery intima–media thickness of the type 2 diabetic patients with or without subclinical atherosclerosis. C-IMT, carotid artery intima–media thickness.

Evaluation of Multiple Correlations Between Carotid Artery Intima–Media Thickness and Other Data in Patients with Type 2 Diabetes Mellitus

In our study group, a positive linear relationship was found between C-IMT and patient age ($r = 0.393$, $P < .001$), male gender ($r = 0.375$, $P < .001$), presence of DRp ($r = 0.369$, $P < .001$), UAE

Table 2. Evaluation of the Determinants of the Carotid Artery Intima–Media Thickness

Parameter	*r*	*P*	Parameter	*r*	*P*
Age (years)[p]	0.393	<.001	Irisin (ng/mL)[s]	−0.145	.165
Gender (male)[s]	0.375	<.001	DBP (mm Hg)[s]	0.123	.239
DRp (+)[s]	0.389	<.001	eGFR (mL/min/1.73 m²)[s]	−0.081	.425
Duration of DM (years)[s]	0.286	.005	HDL-C (mg/dL)[p]	−0.083	.428
UAE (mg/D)[s]	0.291	.005	Anti-lipid (+)[s]	−0.080	.451
UPE (mg/D)[s]	0.215	.039	OAD (+)[s]	−0.061	.559
ASA (+)[s]	0.199	.056	K^+ (mmol/L)[s]	0.059	.572
Anti-hypertensive (+)[s]	0.193	.064	TG (mg/dL)[p]	−0.056	.594
Smoking (+)[s]	0.185	.076	LDL-C (mg/dL)[p]	−0.029	.782
SBP (mm Hg)[p]	0.171	.102	Na^+ (mmol/L)[s]	−0.029	.782
FBS (mg/dl)[p]	0.170	.104	TC (mg/dL)[p]	−0.025	.812
Hypertension (+)[s]	0.163	.119	HbA1c (%)[p]	0.014	.894
BMI (kg/m²)[p]	−0.148	.158			

ASA, acetylsalicylic acid; BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; DRp, diabetic retinopathy; eGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; K+, potassium; LDL-C, low density lipoprotein cholesterol; Na+, sodium; OAD, oral antidiabetic; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; UAE, albumin excretion in 24-hour urine; UPE, protein excretion in 24-hour urine.
Statistical evaluation: [p]Pearson correlation test.
[r]Spearman correlation test.

(*r* = 0.291, *P* = .005), and UPE (*r* = 0.215, *P* = .039). A negative linear relationship was found between C-IMT and irisin levels, which did not reach statistical significance (*r* = −0.145, *P* = .165). The multiple correlations between C-IMT and other data in the study group are shown in Table 2, and the relationship between C-IMT and irisin are shown in Figure 3.

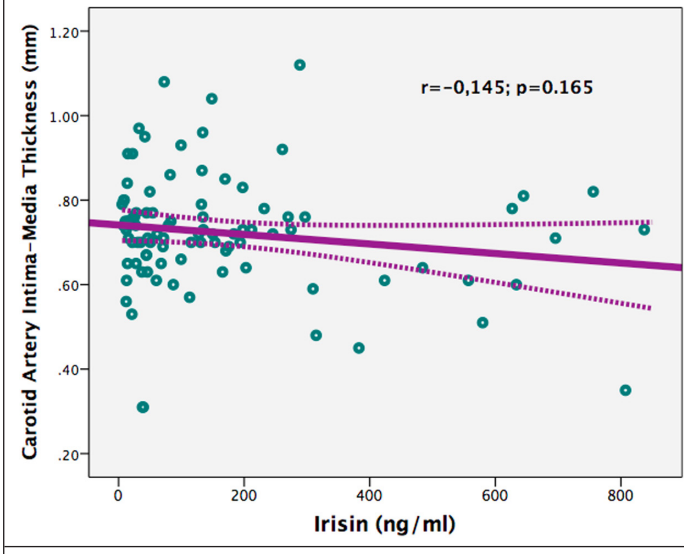

Figure 3. The relationship between carotid artery intima–media thickness and irisin levels in type 2 diabetic patients.

Identifying Independent Predictors of Carotid Artery Intima–Media Thickness in Type 2 Diabetes Mellitus Patients

In our patient group a multivariate linear regression test (model stepwise) was performed by taking all of the factors shown in Table 2 that were found to be associated with C-IMT in order to reveal the independent determinant of C-IMT, adjusted for the effect of all other factors. Patient age (*P* = .000001) and male gender (*P* = .000027) were identified as independent factors associated with an increase in C-IMT (*P* < .00001) for the model, R^2 = 0.334. Independent of all other factors, each 10-year increase in patient age increased C-IMT by 0.06 (CI 95%: 0.03-0.08) mm, while being male increased C-IMT by 0.117 mm (CI 95%: 0.068-0.166). There was no independent relationship between irisin levels and C-IMT.

Evaluation of Demographic, Clinical, and Laboratory Data of Patients with Type 2 Diabetes Mellitus According to Irisin Levels

Since the irisin levels of our patients with T2DM did not conform to normal distribution, they were divided into 3 groups: low (irisin <200 ng/mL, n = 70), median (irisin ≥200-<300 ng/mL, n = 9), and high (irisin ≥300 ng/mL, n = 14) irisin groups according to histogram evaluation of the data, and the data of the patients with low and high irisin were compared statistically. The mean irisin values were 72 ± 58 (5.56-198) ng/mL in the low irisin group, 254 ± 33 (203-297) ng/mL in the median irisin group, and 575 ± 193 (310-837) ng/mL in the high irisin group. Fasting blood

Table 3. Demographic, Clinical, and Laboratory Data of Type 2 Diabetic Patients with Low Irisin Levels or High Irisin Levels

Parameter	*P*	Low Irisin Group	High Irisin Group	Parameter	*P*	Low Irisin Group	High Irisin Group
Age +/–SD (years)*	.087	55.2 ± 8.6	48.7 ± 13.0	FBS (mg/dL)*	.077	174 ± 56	144 ± 58
Female n (%)	.296	18 (42%)	4 (27%)	HbA1c (%)*	.002	8.5 ± 1.9	7.2 ± 1.1
Duration of DM +/–SD (years)#	.172	9.8 ± 7.5	7.9 ± 8.5	Uric Acid (mg/dL)*	.655	5.9 ± 1.6	5.6 ± 1.8
Smoking n (%)	.188	15 (35%)	2 (13%)	TC (mg/dL)*	.134	190 ± 58	165 ± 45
Hypertension n (%)	.174	13 (30%)	2 (13%)	LDL-C (mg/dL)*	.088	124 ± 39	104 ± 34
Diabetic retinopathy n (%)	.848	27 (63%)	9 (60%)	HDL-C (mg/dL)*	.934	49.4 ± 12.5	49.1 ± 11.8
Anti-hypertensive n (%)	.611	20 (47%)	6 (40%)	TG (mg/dL)*	.083	145 ± 72	110 ± 46
Anti-lipid n (%)	.568	11 (25.6%)	5 (33.3%)	Na^+ (mmol/L)*	.131	140 ± 3	141 ± 3
ASA n (%)	.738	32 (74%)	20 (67%)	K^+ (mmol/L)*	.776	5.4 ± 6.5	4.5 ± 7.2
OAD n (%)	.456	29 (50.0%)	6 (40%)	eGFR (mL/min/1.73 m^2)#	.320	82.7 ± 33.3	92.2 ± 35.8
Insulin n (%)	.941	130 ± 11	130 ± 12	UPE (mg/day)#	.008	926 ± 1678	612 ± 1733
SBP +/–SD (mm Hg)*	.432	82 ± 6	80 ± 5	UAE (mg/day)#	.026	437 ± 1098	280 ± 1028
DBP +/–SD (mm Hg)*	.568	11 (25.6%)	5 (33.3%)	Irisin (ng/mL)*	<.001	31.3 ± 18.7	557 ± 181
BMI +/–SD (kg/m^2)*	.610	31.1 ± 5.1	30.4 ± 4.1	C-IMT (mm)*	.042	0.712 ± 0.129	0.630 ± 0.140

ASA, acetylsalicylic acid; BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; K+, potassium; LDL-C, low density lipoprotein cholesterol; Na+, sodium; OAD, oral antidiabetic; SBP, systolic blood pressure; TC, total cholesterol; UAE, albumin excretion in 24-hour urine; UPE, protein excretion in 24-hour urine.
Statistical evaluation: *independent samples *t*-test. **Mann–Whitney *U*-test.
#Mann–Whitney *U*-test.

glucose (*P* = .001), HbA1c (*P* < .001), LDL-C (*P* = .027), UPE (*P* = .006), and UAE (*P* =.037) values were found to be higher in the low-irisin group compared to the high-irisin group. Although not reaching statistical significance, the patient's age (*P* = .092), smoking rates (*P* = .054), and TG values (*P* = .056) of the patients in the low-irisin group were higher than those in the high-irisin group. In addition, the C-IMT values of the patients in the low irisin group were 113 mm thicker than those in the high irisin group (0.734 ± 0.129 mm and 0.621 ± 0.141 mm, respectively, *P* = .004). A comparison of the data of patients with low and high irisin is shown in Table 3, and the C-IMT values of the 2 groups are shown in Figure 4.

DISCUSSION

We found that the levels of irisin in our T2DM patients were significantly lower than those of our healthy control group, even though the 2 groups had similar average age, gender distribution, and smoking rates. This suggests that low irisin levels might contribute to the development of T2DM. In our diabetic patient group, the higher FBG and HbA1c values of patients with low irisin than those with high irisin indicate the important role of irisin in glucose metabolism.[8-12] Experimental, animal, and human studies have shown various positive effects of irisin on glucose homeostasis. After irisin produced in myocytes is secreted, it induces the production of uncoupling protein 1 in adipose tissue, thus ensuring the transformation of white adipose tissue into brown adipose tissue, increasing the inner membrane conductivity of mitochondria in adipocytes, and increasing heat production instead of adenosine triphosphate production, thus increasing energy consumption.[8] It was determined that glucose uptake in human skeletal muscle cells (HSMC) incubated with 50 nM irisin for 1 hour increased by approximately 30%,

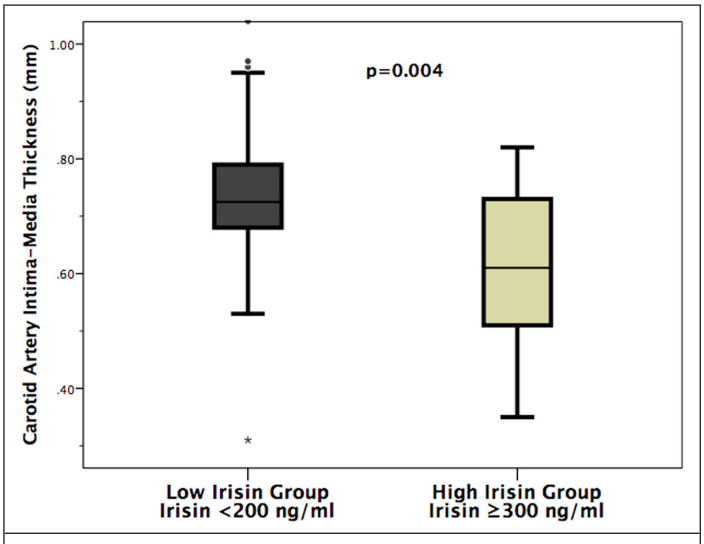

Figure 4. Carotid artery intima–media thickness of the type low irisin group and high irisin group.

and this increase was similar to the incubation of HSMCs with 100 nM insulin.[9] In the same study, it was found that GLUT4, hexokinase 2 is involved in glucose metabolism, and peroxisome proliferator-activated receptor co-activator 1 alpha gene expressions involved in lipid metabolism increased, while glycogen phosphorylase gene expression involved in glycogenolysis decreased in HSMC cells incubated with irisin for 6 hours.[10] It has been shown that incubation of 200 ng/mL irisin in rat cardiomyocyte H9c2 cell culture reduces insulin resistance by inhibiting autophagy through PI3K/Akt pathway activation.[11] In vitro, it was determined that incubation of rat pancreatic β cells with 100 ng/mL irisin for 24 hours increased pancreatic islet cell proliferation through activation of ERK and p38 MAPK signaling pathways, protected the cells from glucose-induced apoptosis, and thus improved pancreatic β cell functions.[12] All of these findings suggest that irisin has a preventative effect on the development of T2DM. On the other hand, the fact that irisin precursor FDCN5 gene expression decreased by 20% in human primary muscle cell cultures of diabetic individuals incubated with 10-20 nM glucose for 6 days compared to non-diabetics suggests that irisin production may be further suppressed in diabetics.[13]

Numerous studies in the literature have found that people with T2DM have lower irisin levels than healthy individuals. Choi et al[14] stated that the irisin levels in diabetic patients were almost 50% lower compared to healthy individuals among the 104 patients with normal glucose tolerance and 104 patients with newly diagnosed T2DM in their study. Another study by Xuan et al[15] conducted on 71 T2DM and 71 healthy volunteers aged ≥60 years showed that the diabetic group had statistically significantly lower irisin levels by approximately 100 ng/mL. In a study by Kurdiova et al[13] on 99 men with T2DM, it was found that irisin levels in overweight/obese and impaired glucose tolerance test subjects were not different from healthy individuals. However, irisin levels in T2DM subjects were 40% lower than in normal-weight healthy individuals. Li et al[15] conducted a study on 362 patients with T2DM and 100 healthy subjects, and found that the levels of irisin were 50% lower in people with T2DM. Song et al,[17] in their meta-analysis, which evaluated the results of 26 studies including 3667 patients, found that irisin levels were, on average, half as low in individuals with T2DM compared to those without. Some studies in the literature present data that are different from those of our study results. Tang et al[18] showed that irisin levels were statistically similar in 68 individuals with normal glucose tolerance (NGT), 63 individuals with impaired glucose tolerance (IGT), and 72 patients with newly diagnosed T2DM. Xie et al[19] also found similar irisin levels in their study group of 50 individuals with newly diagnosed T2DM and 50 with NGT. Huh et al[20] evaluated the basal irisin levels in 4 groups and found that the group with the highest irisin developed T2DM 4.1 times more frequently than the group with the lowest irisin during a follow-up period of 2.6 years.[20] The genetic factors and biological differences of the study populations may have played a role in the difference between our study results and the results of these studies.

It is estimated that approximately 10.7% of deaths worldwide are due to DM and its complications.[5] The results of a systematic review of 4.5 million individuals revealed that up to 50% of mortality in diabetics is due to atherosclerotic cardiovascular disease (CVD).[2] It is critical to completely understand the pathogenetic mechanisms of early and rapidly developing atherosclerosis in diabetic persons, identify potential risk factors, and find effective therapy agents to minimize the disease's high mortality rate. Therefore, the second aim of our study was to investigate the effect of irisin on the development of atherosclerosis in a group of patients without known atherosclerotic disease with a mean age of 54 years, a mean duration of DM of 10 years, approximately 60% women, one-third smokers, 62% hypertensive, and a mean eGFR of 88 mL/min/1.73 m^2. Before the clinical signs of coronary atherosclerosis appear in patients, the arterial wall develops significant functional and structural defects such as endothelial dysfunction and increased intima–media thickness (subclinical atherosclerosis). C-IMT measured by ultrasonographic method is considered an inexpensive, easily applied, reproducible, non-invasive indicator used in epidemiologic, clinical, and observational studies to assess the presence and prevalence of atherosclerosis.[28,29] It was found that each 0.130 mm increase in C-IMT increased the risk of myocardial infarction (MI), mortality, and coronary events by 1.4-fold in individuals who had undergone coronary bypass surgery.[21] In our patients with T2DM, we found that C-IMT was approximately 0.200 mm higher compared to the control group with similar age, sex distribution, and smoking rates. These data suggest that our patients are at high risk for atherosclerosis and related mortality despite having no known CVD. Similar to our study results, Gomez-Marcos et al[23] found that in their study group consisting of 231 healthy individuals, 104 pre-diabetic individuals, and 92 T2DM cases with a mean age of 60 years, 55% of whom were women, with a 10-year risk of developing CVD between 5 and 10% according to the Framingham score, the C-IMT of patients who developed diabetes was 0.050 mm higher than the mean of the other 2 groups. Brohall et al,[24] in their meta-analysis, evaluated the results of 23 studies, including with T2DM, with IGT, and healthy individuals found that people with diabetes had an average C-IMT thickness of 0.130 mm. Additionally, they reported that diabetics had a 40% increased risk of MI and stroke.

When we examined the relationship between C-IMT and other data in our study group, we found a positive linear relationship between C-IMT and patient age, male gender, presence of DRp, duration of DM, daily urinary albumin, and protein excretion. We found a positive linear correlation between C-IMT and smoking, the presence of HT, and high SBP, and a negative linear correlation between BMI and irisin levels, although this correlation was close to the limit of statistical significance for all parameters ($P < .200$). It has been reported that C-IMT of ≥0.750 mm had 90% specificity and 73% sensitivity in detecting coronary artery disease.[22] Therefore, when we classified our patients with T2DM as C-IMT <0.750 mm (without subclinical atherosclerosis) and C-IMT ≥0.750 (with subclinical atherosclerosis), we found that

36% of our patients without known CVD had subclinical atherosclerosis. We found that male gender, long duration of diabetes, presence of DRp, elevated DBP, elevated UPE, and elevated UAE were factors that increased the development of subclinical atherosclerosis at the level of statistical significance, while increasing patient age and increasing BMI were factors that increased the development of subclinical atherosclerosis, although at the limit of statistical significance ($P < .200$). In the logistic regression analysis, we performed to reveal the independent determinants of C-IMT increase, we found that each 10-year increase in patient age increased C-IMT by 0.06 mm, and being of male gender increased C-IMT by 0.117 mm, independent of the effect of all other factors. Similar to our findings, Tripolt et al[25] showed that patient age, diabetes duration, and SBP positively correlated with C-IMT. Wang et al[26] found that patient age, SBP increase, duration of diabetes, LDL-C, smoking history, and family history of HT were positively associated; low 25-OH vitamin D3 and low calcium were negatively associated with C-IMT in their study group of 314 patients with T2DM. The study conducted by Momeni et al[27] involving 154 T2DM patients revealed a significant positive correlation between C-IMT and patient age, diabetes duration, SBP, and UPE. Saif et al,[28] found a positive linear relationship between C-IMT and duration of diabetes, SBP, DBP, FBG, postprandial blood glucose, HbA1c, TC, TG, and the presence of DNp in 140 normotensive diabetic patients with DRp. The authors also showed that increasing patient age, PPBG, and TG levels were independent predictors of increased C-IMT.[28]

The demonstration that irisin inhibits endothelial progenitor cell proliferation, reduces inflammation, reduces oxidative stress, reduces endothelial cell transformation into mesenchymal cells, reduces vascular smooth muscle cell proliferation and transformation into osteoblasts suggests that irisin may reduce atherosclerosis development and its non-invasive marker C-IMT increase.[7] The positive effects of irisin on glucose metabolism and insulin resistance, which we have previously summarized, also reveal its potential to prevent C-IMT increases and atherosclerotic disease development.[8-12] In our literature review, we did not find any study examining the relationship between irisin levels and C-IMT in T2DM patients. When evaluated in terms of chronic diseases other than T2DM, Ismail et al. reported a negative linear relationship between irisin and C-IMT levels in patient groups consisting of 50 Behçet's disease patients and 50 controls;[29] and Lee et al[30] found a negative linear relationship between irisin levels and C-IMT in patient groups consisting of 102 patients on peritoneal dialysis for end-stage kidney disease. When we divided our patients with T2DM into 3 groups based on irisin levels, we found that C-IMT was 0.113 mm thicker in the group with lower irisin levels than in the group with higher irisin levels. On the other hand, although it did not reach statistical significance, we found that there was a negative linear relationship between irisin levels and C-IMT, and that irisin levels were 35 ng/mL higher in patients with subclinical atherosclerosis than in patients without subclinical atherosclerosis. All these findings suggest that having low irisin increases the risk of developing subclinical atherosclerosis.

Another significant result of our study was that UPE and UAE showed a positive linear relationship with C-IMT; UAE was 0.5 g/day higher, and UPE was 1 g/day higher in patients with subclinical atherosclerosis compared to patients without subclinical atherosclerosis ($P < .05$ for both). We also observed a positive linear correlation between the presence of DRp and C-IMT ($P < .001$). We found that approximately 35% more patients with subclinical atherosclerosis developed DRp compared to patients without subclinical atherosclerosis ($P < .001$). Similar to our findings, studies have shown a significant association between UAE, UPE, and C-IMT.[5,29] Data show a positive linear relationship between DRp development and C-IMT.[29,30] We divided our patients into 3 groups based on their irisin levels and found that UPE was 31% higher and UAE was 25% higher in the low irisin group than in the high group ($P < .05$ for both). These findings suggest that low irisin levels may facilitate the development of DNp, the most common complication of diabetes in our patient group.

The findings of our study are important because it is the first study investigating the relationship between irisin and subclinical atherosclerosis development in individuals with T2DM. Nonetheless, our findings must be interpreted with caution and a number of limitations of the present study should be borne in mind. Our study was observational, and our findings do not indicate a definitive causal relationship. Although the number of patients included in the study was determined by power analysis, it is relatively small to decide all diabetics. The individuals participating in our study have a single ethnicity.

In our study, we found that low irisin levels play a role in developing type 2 diabetes. The fact that the C-IMT in our patients with low irisin levels was 0.113 mm thicker than in patients with high irisin levels suggests that low irisin levels could contribute to atherosclerosis. Patients with low irisin levels had higher daily urinary protein/albumin excretion, implying that low irisin levels could be linked to the development of diabetic nephropathy. Our findings suggest that developing treatment agents that increase irisin levels will contribute to reducing high morbidity and mortality in diabetic patients.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethics committee approval was received from the Scientific Research Ethics Committee of Trakya University Medical Faculty (approval number: TUTF-BAEK 2020/302; date: August 24, 2020).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Author Contributions: Concept – S.Ü., B.A.T.; Design – S.Ü., B.A.T., A.Ü., N.S.; Supervision – S.Ü.; Resources – S.Ü., B.A.T.; Materials – N.T., B.A.T., G.A.B.; Data Collection and/or Processing – B.A.T., G.A.B, N.T.; Analysis and/or Interpretation – S.Ü., B.A.T, A.Ü., N.S.; Literature Search – S.Ü., B.A.T., İ.K.; Writing Manuscript – A.Ü., B.A.T., S.Ü.; Critical Review – S.Ü., A.Ü., İ.K.

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