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Serum adropin and miR-21 expression as predictors of endothelial dysfunction in type 2 diabetes mellitus and vascular complications

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Adropin is a peptide involved in the regulation of glycolipid metabolism, contributing to improved glucose homeostasis and the mitigation of dyslipidemia. The objective of this study is to ascertain whether there is a discrepancy in the expression of microRNA-21 (miRNA-21) and adropin levels in Type 2 diabetes mellitus (T2DM) patients who also exhibit macro- and micro-vascular complications (nephropathy, neuropathy, retinopathy) were also observed to uncomplicated diabetes patients and healthy individuals; to explore the relationship between serum adropin and miR-21, endothelial dysfunction, and carotid intima-media thickness (CIMT). The present study comprised 89 patients with T2DM (microvascular *n* = 24, macrovascular *n* = 20, uncomplicated type 2 *n* = 45) and 19 non-diabetic coronary artery disease (CAD). The control group was composed of 20 healthy individuals. Expression of miRNA-21 in all diabetic patients was significantly higher than control group, while adropin levels were found to be significantly lower. No significant difference was observed between the diabetic patient groups with microvascular complications and those without complications regarding miRNA-21 and adropin levels. The miR-21 expression and adropin levels of the non-complicated diabetic group and only the coronary disease group were significantly higher and lower than the control group. CIMT was significantly higher in patients with macrovascular complications and non-diabetic CAD than in the other groups. A positive correlation was found between miR-21 and CIMT, whereas a moderate negative correlation was detected between miR-21 and adropin levels. The present study indicated that adropin and miR-21 can be equally good markers both in separating diabetic patients with macrovascular complications from the healthy group. In the meantime, the endothelial cell is an important target, and endothelial dysfunction is important in diabetic vasculature. Increased miR-21 expression and decreased adropin levels can be explained by the damage that hyperglycemia causes to the endothelium in diabetic patients.

Keywords Type 2 diabetes mellitus, Macrovascular complications, Microvascular complications, MiR-21, Adropin

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Type 2 diabetes mellitus (T2DM) is a complex chronic disease that requires continuous management and multifactorial risk reduction strategies. Its pathogenesis involves both genetic and environmental factors, often leading to progressive β-cell dysfunction and insulin resistance[1,2]. According to the World Health Organization (WHO), over 422 million people are affected globally, highlighting its near-epidemic status and significant burden on healthcare systems[3]. T2DM complications range from acute hyperglycemia to chronic conditions such as retinopathy, neuropathy, and cardiovascular disease. Despite therapeutic advancements, individualized treatment remains challenging due to variations in disease progression, complications, and comorbidities. A deeper understanding of genetic and molecular mechanisms is crucial for developing personalized interventions[4].

Adropin, a 76-amino acid peptide identified in 2008, plays a key role in energy homeostasis and lipid metabolism. Studies have linked adropin to insulin resistance, carbohydrate-lipid metabolism, endothelial function, and cardiovascular health[5,6]. Its involvement in these pathways suggests potential as a therapeutic target, particularly in metabolic disorders such as T2DM.

MicroRNAs (miRNAs) are ~ 22-nucleotide, single-stranded RNAs that regulate gene expression by targeting mRNAs for degradation or translational repression[7]. Numerous miRNAs have been linked to diabetes and its complications[8–11]. While miR-21 expression is downregulated in diabetic and insulin-resistant mice[12], other studies report its upregulation in patients with acute heart failure and diabetes, suggesting its potential as a biomarker[13]. Given their regulatory roles, miRNAs are key to understanding the molecular mechanisms of diabetes.

The complex nature of T2DM highlights the need to understand its genetic and biochemical basis for early detection and personalized treatment. Although current research is promising, the specific roles of molecules like miRNAs and adropin in T2DM pathophysiology remain unclear[12]. Zhou et al.[13] demonstrated that miR-21 targets and suppresses PPARα in endothelial cells, creating a feedback loop that enhances flow-induced inflammation. This miR-21–PPARα interaction promotes vascular inflammation and contributes to endothelial dysfunction.

The primary aim of this study is to assess whether serum adropin levels and miR-21 expression significantly differ among T2DM patients with and without vascular complications compared to healthy controls and patients with non-diabetic coronary artery disease (CAD). The secondary aim is to investigate the association between serum adropin and miR-21 levels with endothelial dysfunction, measured by carotid intima-media thickness (CIMT), and to evaluate their potential as predictive markers of macrovascular complications.

Materials and methods

Ethical approval

Ethical approval was obtained from the Clinical Research Ethics Committee of Istanbul University, Cerrahpaşa Medical Faculty (Approval Date-Number: 21/06/2016-227647). Written informed consent was obtained from all participants before their inclusion in the study, ensuring that they were fully informed about the study's purpose, procedures, and their rights.

Subjects

This observational, cross-sectional study was conducted between July 2016 and October 2017. Patients diagnosed with T2DM were included in the study according to the diagnostic criteria established by the American Diabetes Association (ADA).

Classification of vascular complications

T2DM patients were grouped based on the predominant type of vascular complication. Microvascular complications included diabetic retinopathy (ETDRS-based diagnosis), neuropathy (confirmed by ENMG), and nephropathy (based on albuminuria and/or reduced eGFR). Macrovascular complications included CAD, peripheral artery disease, or cerebrovascular events, confirmed by clinical and imaging findings. In patients with both types, classification was based on the dominant clinical presentation. This stratification aimed to explore whether adropin and miR-21 levels differ according to the primary vascular involvement.

We divided the study cohort into five distinct groups[1]: Diabetic Group [DM] (n= 45), including patients without complications, mean age: 52.9 ± 9.5 years; females: 30, males: 15[2]; Diabetic Group with Microvascular Complications (n= 24) with mean age: 51.8 ± 6.4 years; females: 17, males: 7[3]; Diabetic Group with Macrovascular Complications (n= 20) with mean age: 55.7 ± 3.4 years; females: 8, males: 12[4]; Coronary Artery Disease without Diabetes Mellitus (n = 19) with mean age: 51.8 ± 7.4 years; females: 11, males: 8; and[5] Healthy Control Group [HC] (n = 20), including persons without any diagnosed endocrine, cardiovascular, and inflammatory disorders with mean age: 47.8 ± 4.7 years; females: 9, males: 11. As this was an observational study, no randomization or blinding was applied (Fig. 1).

In the study, patients with T2DM were categorized into three subgroups. The uncomplicated diabetes group included patients without any diabetes-related complications at the time of inclusion. The microvascular diabetes group comprised patients with at least one microvascular complication, while the macrovascular diabetes group consisted of patients with at least one macrovascular complication. Individuals were excluded if they had acute or chronic metabolic, systemic, autoimmune, or inflammatory conditions; malignancies; chronic alcohol use; or were currently on GLP-1 receptor agonists, hepatotoxic drugs, or oral contraceptives.

The primary outcome for the calculation was the between-group difference in mean miR-21 levels. Based on preliminary data (pilot mean difference $\Delta = 20$ units, pooled SD $\sigma = 15$), we used the formula for one-way ANOVA sample size (approximated by pairwise two-group comparisons):

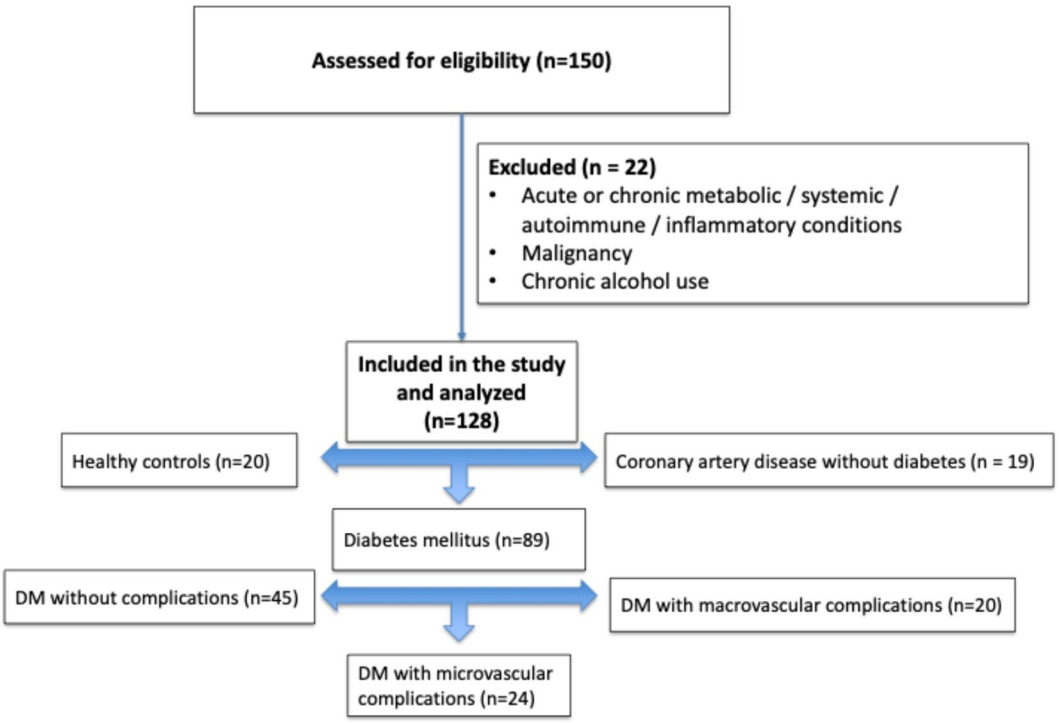


Fig. 1. Flow diagram of participant selection and grouping.

$$n = \frac{2(\left(Z_{1-\frac{\alpha}{2}}\right) + (Z_{1-\beta}))^2 \sigma^2}{\Delta^2}$$

where $\alpha = 0.05$ (two-tailed), $1-\beta = 0.80$ (power), $Z_{1-\alpha/2}$=1.96, $Z_{1-\beta}$ =0.84. Substituting these values yields ≈ 17 subjects per group.

Allowing for a 15% dropout rate, we planned to recruit at least 20 participants in each group. The final sample sizes (n = 19–45 per group) thus met or exceeded this requirement.

Ophthalmological evaluation

Exclusion criteria encompassed individuals with known ocular diseases other than diabetic retinopathy (DR), a history of ocular surgery, or inflammatory ocular conditions. Participants using topical ocular medications or having other systemic diseases aside from diabetes were also excluded. A thorough ophthalmological examination was performed, including assessments of refractive error, best-corrected visual acuity, slit-lamp examination, intraocular pressure measurement using pneumotonometry, and dilated fundus examination. The severity of diabetic retinopathy was determined using the Early Treatment Diabetic Retinopathy Study (ETDRS) classification.

The evaluation of polyneuropathy

The patients were evaluated for diabetic polyneuropathy by a neurologist using ENMG. According to the reports obtained from ENMG, the patients were found to have polyneuropathy.

Diabetic polyneuropathy was evaluated using electroneuromyography (ENMG). Nerve conduction studies (NCS) were performed bilaterally on the upper and lower limbs while subjects were in a supine position. The Nihon Kohden Neuropack S1 MEB-940 EMG-ER system was used. The median, ulnar, tibial, peroneal, sural, and superficial peroneal nerves were examined for compound muscle action potentials (CMAP) and sensory nerve action potentials (SNAP). Parameters such as onset latency, peak-to-peak amplitude, and conduction velocity were measured and interpreted according to reference values[14].

Measurements of carotid intima-media thickness (CIMT)

The extracranial carotid arteries were examined using a standardized protocol by the same radiologist. CIMT was measured using a Siemens Acuson S3000 ultrasound device using a 9L4 (4.0–9.0 MHz) linear transducer. The subjects' carotid system was evaluated in B-mode, pulsed Doppler mode, and color mode, with the subject in the supine position, with their head slightly turned to the contralateral side of the carotid artery being examined. Carotid IMT is measured by calculating the space between the intimal-luminal and medial-adventitial interfaces of the carotid artery.

Laboratory analyses

Blood samples were obtained at least 24 h before the administration of the drugs, and standardized procedures were followed. Blood specimens were collected following an overnight fast of 10 to 12 h. The serum and plasma samples obtained after centrifugation were stored at −80 °C until adropin analysis.

Levels of serum adropin were assayed by the ELISA kit (Human AD (Adropin), Cat. No. E-EL-H5307, ARP American Research Products, USA). Results were expressed as pg per ml of serum (pg/mL). The sensitivity of this kit was 7.5 ng/L. Intra- and inter-CV were 6.3% and 7.5%, respectively.

Biochemical markers, including glucose, cholesterol, triglycerides, HDL-C, and LDL-C, were assessed using enzymatic techniques on the Roche Cobas Integra 400 analyzer (Germany). Insulin concentrations were determined by electrochemiluminescence immunoassay (ECLIA) using the Roche-Hitachi E170 system. C-reactive protein (CRP) was quantified using nephelometry (Beckman Coulter, Germany), while HbA1c levels were measured by high-performance liquid chromatography (Bio-Rad, Variant Turbo 2, USA). Albumin excretion in 24-hour urine collections was quantified on the Roche Hitachi P800 analyzer. Glomerular filtration rate (GFR) was estimated utilizing the CKD-EPI formula. Insulin resistance was assessed using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), which is calculated from fasting glucose and insulin concentrations according to the following formula:

$$HOMA-IR = \frac{\text{glucose}\left[\frac{\text{mg}}{\text{dL}}\right] \times \text{insulin}\left[\frac{\mu\,\text{U}}{mL}\right]}{405}$$

MiRNA analysis

The total RNA extraction process, inclusive of small RNA from serum, was conducted utilizing the mirVana RNA Isolation Kit (miRNeasy Kit, Qiagen, CA). All isolation procedures were carried out in strict adherence to the manufacturer's guidelines, with no deviations or modifications made to the standard. The synthesis of complementary DNA (cDNA) was conducted from the total RNA isolated from the serum of all subjects using the miScript Reverse Transcription Kit (Qiagen, Valencia, CA). The concentration and quality of the nucleic acids were determined using the Qubit assay and Qubit fluorometer. The expression levels of hsa-mir-21 (hsa: Homo sapiens) and RNU44 were further quantified using the miScript SYBR® Green PCR Kit (Qiagen, Valencia, CA) in conjunction with miScript-specific primers on the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA). RNU44 was chosen as the endogenous control. The relative expression levels of miR-21 were subsequently determined using the $2^{-\Delta\Delta CT}$ method, with each sample being analyzed in triplicate.

Statistical analysis

SPSS version 22.0 (IBM) was used for statistical evaluations of all data obtained. The normality of continuous variables was assessed using the Shapiro–Wilk test. Variables with a normal distribution are presented as mean ± standard deviation (SD), and those without a normal distribution as median (interquartile range, IQR). Between-group comparisons for normally distributed variables were performed using one-way ANOVA with Bonferroni-adjusted Tukey post-hoc tests; for non-normally distributed variables, the Kruskal–Wallis test was used, with pairwise comparisons by the Mann–Whitney U test. Categorical variables were compared by the χ^2 test or Fisher's exact test as appropriate. Correlations were analyzed using Pearson's or Spearman's correlation coefficients, depending on normality. To adjust for potential confounders, we constructed multiple linear regression models with circulating miR-21 and adropin levels as dependent variables. As covariates, we included age, sex, body mass index (BMI), hypertension status, smoking status, total cholesterol, and triglyceride levels—factors known to influence vascular biomarkers. We entered all covariates simultaneously ("enter" method) to estimate the independent association of each variable. Before model fitting, we evaluated multicollinearity among independent variables by calculating variance inflation factors (VIFs). All VIFs were < 2.0, indicating no substantial multicollinearity. Receiver operating characteristic (ROC) curve analyses were conducted to determine sensitivity, specificity, area under the curve (AUC), and optimal cut-off values. A two-tailed p-value < 0.05 was considered statistically significant.

Results

Demographic characteristics of study groups were presented in Table 1. The diabetic patient group was divided into three subclasses: diabetic group without complications ($n = 45$), diabetic group with microvascular complications ($n = 24$), and diabetic group with macrovascular complications ($n = 20$). In addition, as a control group, there were healthy control groups ($n = 20$) and a coronary artery disease without diabetes mellitus group ($n = 19$). A comparison of the demographic data in these groups was conducted, and statistically significant differences were identified between BMI ($p < 0.001$), the frequencies of hypertension ($p = 0.03$), the drugs used in treatment (Calcium channel blockers, $p = 0.015$; ACE inhibitors, $p = 0.028$; Beta blockers, $p < 0.001$; ASA, $p < 0.001$; Insulin, $p < 0.001$; Metformin, $p < 0.001$; DPP-4 inhibitors, $p < 0.001$; Statins, $p < 0.001$ and Fibrate, $p = 0.012$), Systolic blood pressure ($p < 0.001$) and diastolic blood pressure ($p < 0.001$).

The laboratory parameters and carotid intima–media thickness (CIMT) measurements for all study groups are summarized in Table 2. Fasting blood glucose and HbA_1c were markedly elevated in all diabetic groups compared with healthy controls (both $p < 0.001$ by one-way ANOVA with Tukey post-hoc). Post-hoc analyses showed that each diabetic subgroup (without complications, with microvascular complications, and with macrovascular complications) had significantly higher fasting glucose and HbA_1c than controls (all $p < 0.001$). Moreover, the CAD-without-DM group also differed from controls (fasting glucose $p < 0.001$; HbA_1c $p < 0.001$) and from the diabetic groups ($p < 0.001$ for each comparison). Total cholesterol was higher in the diabetic-without-complications and diabetic-microvascular groups versus controls (205 ± 42 and 186 ± 46 vs. 172 ± 15

	Healthy Control ($n=20$)	Diabetic Group without complications ($n=45$)	Diabetic Group with Microvascular complications ($n=24$)	Diabetic Group with Macrovascular complications ($n=20$)	CAD without DM ($n=19$)	*P*
Age (years)	47.8 ± 4.7	52.9 ± 9.5	51.8 ± 6.4	55.7 ± 3.4	51.8 ± 7.4	0.055
Gender (F/M)	9/11	30/15	17/7	8/12	11/8	0.168
Body mass index (kg/m^2)	23.3 ± 1.6	33.9 ± 6.1	35.1 ± 7.7	30.9 ± 3.3	32.6 ± 4.2	**< 0.001**
Smoking (%)	7 (35)	17 (37.8)	10 (41.6)	8 (40)	7 (36.8)	0.765
Hypertension (%)	0	22 (48.9)	18 (75)	15 (75)	14 (73.7)	**0.03**
Hyperlipidemia (%)	0	19 (42.2)	13 (54.2)	12 (60)	9 (47.4)	0.056
Treatment (%)						
Calcium channel blockers	0	5 (11.1)	3 (12.5)	5 (25)	2 (10.5)	**0.015**
ACE inhibitors	0	3 (6.7)	5 (20.8)	8 (40)	7 (36.8)	**0.028**
Angiotensin receptor blockers	0	15 (33.3)	13 (54.2)	9 (45)	5 (26.3)	0.108
Diuretics	0	15 (33.3)	11 (45.8)	10 (50)	5 (26.3)	0.262
Beta blockers	0	6 (13.3)	3 (12.5)	12 (60)	14 (73.7)	**< 0.001**
ASA	0	10 (22.2)	9 (37.5)	18 (90)	16 (84.2)	**< 0.001**
Insulin	0	19 (42.2)	15 (62.5)	15 (75)	0	**< 0.001**
Metformin	0	35 (77.7)	23 (95.8)	16 (80)	0	**< 0.001**
DPP-4 inhibitors	0	15 (33.3)	13 (54.2)	8 (40)	0	**< 0.001**
Statins	0	19 (42.2)	12 (50)	16 (80)	15 (78.9)	**< 0.001**
Fibrate	0	1 (2.2)	1 (4.2)	3 (15)	4 (21)	**0.012**
Systolic blood pressure (mm Hg)	111 ± 19	136 ± 17	140 ± 18	145 ± 22	140 ± 17	**< 0.001**
Diastolic blood pressure (mm Hg)	72 ± 6	80 ± 9	78 ± 8	79 ± 16	84 ± 13	**< 0.001**

Table 1. Demographic characteristics of study groups. Abbreviations: F = Female; M = Male; BMI = Body Mass Index; CAD = Coronary Artery Disease; ACE = Angiotensin-Converting Enzyme; ASA = Acetylsalicylic Acid. Statistical tests: Continuous variables were compared by one-way ANOVA (with Bonferroni-adjusted Tukey post-hoc); categorical variables by χ^2 or Fisher's exact test. A two-tailed $p < 0.05$ was considered significant.

	Healthy Control ($n=20$)	Diabetic Group without complications ($n=45$)	Diabetic Group with Microvascular complications ($n=24$)	Diabetic Group with Macrovascular complications ($n=20$)	CAD without DM ($n=19$)
Fasting Blood Glucose (mg/dL)	89 ± 7	163 ± 72 a***	164 ± 73 a***	164 ± 49 a***	96 ± 6 b***, c***, d***
HbA1c (%)	5.6 ± 0.4	7.6 ± 1.9 a***	7.8 ± 1.5 a***	7.5 ± 1.2 a***	5.6 ± 0.4 b***, c***, d***
HOMA-IR	2.3 ± 0.8	6.5 ± 5.9	6.4 ± 4.6	5.3 ± 3.3	13.8 ± 36.3
Total cholesterol (mg/dL)	172 ± 15	205 ± 42 a*	186 ± 46 a*	177 ± 38 b*	194 ± 33 a*
HDL (mg/dL)	52 ± 13	48 ± 14	49 ± 13	44 ± 11	47 ± 10
LDL (mg/dL)	107 ± 17	121 ± 39	105 ± 35	101 ± 31	113 ± 31
Triglyceride (mg/dL)+	81 (65–98)	193 (140–245) a***	134 (90–180) a***, b**	172 (120–210) a***, b*,c*	153 (110–195) a***, b*
Creatinine (mg/dl)	0.9 ± 0.1	0.9 ± 0.2	1.2 ± 1.1	0.9 ± 0.3	1 ± 0.1
Creatinine clearance (mL/min)	132 ± 22	129 ± 34	126 ± 45	106 ± 32 a*,b*,c*	108 ± 19 a*,b*
Albumin/creatinine ratio+	4 (3–5)	14 (8–30)	242 (50–400)	418 (200–700)	5 (4–6)
CRP (mg/L) +	0.9 (0.6–1.2)	6 (3–12)	9.6 (4–15)	2.9 (2.0–4.0)	2.6 (1.8–3.5)
Adropin (ng/L) +	180 ± 39	95 ± 27 a***	85 ± 18 a***	75 ± 15 a***, b*	53 ± 8 a***, b***, c***
miR-21+	15 ± 10	54 ± 13 a***	74 ± 21 a***, b***	105 ± 23 a***, b***, c***	165 ± 22 a***, b***, c***, d***
CIMT (mm)	0.61 ± 0.11	0.95 ± 0.21	1.01 ± 0.26	1 ± 0.2	1.08 ± 0.22

Table 2. Laboratory findings and CIMT values of study groups. Abbreviations: CIMT = Carotid Intima-Media Thickness; CAD = Coronary Artery Disease; CRP = C-Reactive Protein; HbA_1c = Hemoglobin A_1c. Statistical tests: Normality was assessed by the Shapiro–Wilk test. Continuous variables with a normal distribution were compared by one-way ANOVA (with Bonferroni-adjusted Tukey post-hoc tests); non-normally distributed variables were compared by the Kruskal–Wallis test and are indicated by an asterisk (+). Categorical variables were compared by the χ^2 test. A two-tailed $p < 0.05$ was considered significant. a: vs. Healthy Control, b: vs. Diabetic Group without complications, c: vs. Diabetic Group with Microvascular complications, d: vs. Diabetic Group with Macrovascular complications, *:$p < 0.05$, **:$p < 0.01$, ***:$p < 0.001$.

mg/dL; both $p < 0.05$), and in the CAD-without-DM group (194 ± 33 mg/dL; $p < 0.05$). HDL and LDL cholesterol levels did not differ significantly among groups ($p = 0.473$ and $p = 0.195$, respectively). Triglycerides, which were non-normally distributed, were substantially increased in all diabetic groups compared with controls (median [IQR] 193 [140–245], 134 [90–180], and 172 [120–210] vs. 81 [65–98] mg/dL; $p = 0.001$, Kruskal–Wallis), with each diabetic subgroup differing from controls (all $p < 0.001$) and the macrovascular group also differing from the uncomplicated group ($p < 0.05$). Serum creatinine was similar across groups ($p = 0.464$), but creatinine clearance was lower in the macrovascular complication group compared with controls (106 ± 32 vs. 132 ± 22 mL/min; $p < 0.05$) and versus the other diabetic subgroups (all $p < 0.05$). Albumin/creatinine ratio and CRP did not differ significantly ($p = 0.063$ and $p = 0.136$, respectively). Plasma fibrinogen concentrations were significantly elevated in all patient groups compared with healthy controls ($p < 0.001$). Tukey post-hoc testing confirmed higher fibrinogen in each diabetic subgroup and in the CAD-without-DM group versus controls (all $p < 0.001$). Adropin levels, which were non-normal, were progressively lower across the spectrum from healthy controls (median 180 ng/L [150–210]) to diabetic without complications (95 [80–110]) to diabetic with microvascular (85 [70–100]) and macrovascular complications (75 [60–90]), reaching their lowest in CAD-without-DM (53 [45–60]; $p < 0.001$). All diabetic groups differed from controls ($p < 0.001$), the macrovascular group was lower than the uncomplicated group ($p < 0.05$), and CAD-without-DM was lower than the microvascular group ($p < 0.001$). Conversely, miR-21 expression increased stepwise from controls (15 [10–20]) through the diabetic groups (54 [45–65], 74 [60–85], and 105 [90–120]; all $p < 0.001$ vs. control) to CAD-without-DM (165 [150–180]; $p < 0.001$ vs. all diabetic subgroups). CIMT was significantly greater in each patient group compared with healthy controls (controls 0.61 ± 0.11 mm vs. 0.95 ± 0.21, 1.01 ± 0.26, 1.00 ± 0.20, and 1.08 ± 0.22 mm; all $p < 0.001$ by ANOVA with post-hoc).

As shown in Table 3, a correlation was observed between the expression levels of adropin and miR-21, as well as their relationships with demographic data, laboratory results, and CIMT values. The analysis revealed several statistically significant associations. A very weak positive correlation was found between miR-21 and BMI ($r = 0.230$, $p = 0.012$), while creatinine clearance exhibited a very weak negative correlation with miR-21 ($r = -0.265$, $p = 0.004$). Furthermore, a weak positive correlation was observed between miR-21 and CIMT ($r = 0.438$, $p < 0.001$), and a moderate negative correlation was identified between miR-21 and adropin levels ($r = -0.667$, $p < 0.001$). Adropin levels also showed a weak negative correlation with BMI ($r = -0.339$, $p < 0.001$). HbA1c and triglyceride levels demonstrated very weak negative correlations ($r = -0.182$, $p = 0.047$ and $r = -0.181$, $p = 0.049$, respectively). Furthermore, a moderate negative relationship was identified between CIMT and adropin levels (r: −0.585, $p < 0.001$).

Table 4; Fig. 2 present the diagnostic utility of adropin, miR-21, HbA1c, and CIMT in distinguishing between groups based on ROC curve analyses. In differentiating uncomplicated diabetes patients from the healthy control group, miR-21 demonstrated the highest sensitivity, specificity, and AUC values (cut-off: 32.7, AUC = 0.990, sensitivity = 95.6%, specificity = 95%). The corresponding values for other parameters were as follows: adropin (cut-off: 133, AUC = 0.971, sensitivity = 90%, specificity = 88.9%), HbA1c (cut-off: 6.1, AUC = 0.921, sensitivity = 71.1%, specificity = 95%), and CIMT (cut-off: 0.75, AUC = 0.956, sensitivity = 95.6%, specificity = 90%).

Similarly, in distinguishing patients with microvascular complications from healthy controls, miR-21 was again the most effective parameter (cut-off: 38.9, AUC = 1.000, sensitivity = 100%, specificity = 100%). The performance of other parameters was as follows: adropin (cut-off: 116, AUC = 0.990, sensitivity = 95%,

	miR-21		**Adropin**	
	r	***p***	***r***	***p***
Age (years)	0.171	0.064	−0.146	0.114
Body mass index (kg/m^2)	**0.230**	**0.012**	**−0.399**	**< 0.001**
Fasting Blood Glucose (mg/dL)	−0.088	0.340	−0.165	0.074
HbA1c (%)	−0.143	0.121	**−0.182**	**0.047**
Total cholesterol (mg/dL)	0.029	0.754	−0.130	0.159
HDL (mg/dL)	−0.142	0.124	0.106	0.251
LDL (mg/dL)	−0.038	0.680	−0.025	0.784
Triglyceride (mg/dL) *	0.091	0.327	**−0.181**	**0.049**
Creatinine (mg/dL)	−0.033	0.721	−0.020	0.832
Creatinine clearance (mL/min)	**−0.265**	**0.004**	0.171	0.064
Albumin: creatinine ratio*	0.126	0.242	−0.145	0.176
CRP (mg/L) *	−0.010	0.910	−0.085	0.355
Adropin (ng/L) *	**−0.667**	**< 0.001**		
CIMT (mm)	**0.438**	**< 0.001**	**−0.585**	**< 0.001**

Table 3. Relationship between miR-21 and Adropin levels in the study groups ($n = 128$). Abbreviations: CIMT = Carotid Intima-Media Thickness. All correlations were calculated across all participants, including healthy controls and patient groups. Statistical tests: Correlations were assessed using Pearson's correlation coefficient for normally distributed data or Spearman's rank correlation for non-normal data and are indicated by an asterisk (*). A two-tailed $p < 0.05$ was considered significant.

Healthy control vs. diabetic group without complications group					
	Sensitivity (%)	Specifity (%)	AUC	Cut-off	*p*
Adropin (ng/L)	90	88.9	0.971	133	**< 0.001**
miR-21	95.6	95	0.990	32.7	**< 0.001**
HbA1c (%)	71.1	95	0.921	6.1	**< 0.001**
CIMT (mm)	95.6	90	0.956	0.75	**< 0.001**
Healthy control vs. diabetic group with microvascular complications group					
	Sensitivity (%)	**Specifity (%)**	**AUC**	**Cut-off**	**p**
Adropin (ng/L)	95	95.8	0.990	116	**< 0.001**
miR-21	100	100	1.000	38.9	**< 0.001**
HbA1c (%)	87.5	95	1.000	6.1	**< 0.001**
CIMT (mm)	100	100	0.966	0.9	**< 0.001**
Healthy control vs. diabetic group with macrovascular complications group					
	Sensitivity (%)	**Specifity (%)**	**AUC**	**Cut-off**	**p**
Adropin (ng/L)	100	100	1.00	92.8	**< 0.001**
miR-21	100	100	1.00	50.3	**< 0.001**
HbA1c (%)	70	100	1.00	6.6	**< 0.001**
CIMT (mm)	100	100	0.918	0.9	**< 0.001**
Healthy control vs. coronary artery disease without diabetes mellitus group					
	Sensitivity (%)	**Specifity (%)**	**AUC**	**Cut-off**	**p**
Adropin (ng/L)	100	100	1.00	84.8	**< 0.001**
miR-21	100	100	1.00	78.8	**< 0.001**
CIMT (mm)	94.7	100	0.997	0.9	**< 0.001**

Table 4. Sensitivity, specificity, AUC, cut-off and asymptotic significance of parameters in study groups. Abbreviations: CIMT = Carotid Intima-Media Thickness; AUC = Area Under the Curve. Statistical tests: Receiver operating characteristic (ROC) curve analyses were performed to determine sensitivity, specificity, AUC, and optimal cut-off values. Asymptotic significance (p-value) was calculated for each ROC; $p < 0.05$ was considered significant.

specificity = 95.8%), HbA1c (cut-off: 6.1, AUC = 1.000, sensitivity = 87.5%, specificity = 95%), and CIMT (cut-off: 0.9, AUC = 0.966, sensitivity = 100%, specificity = 100%).

Adropin and miR-21 were identified as equally effective biomarkers for distinguishing diabetic patients with macrovascular complications from the healthy control group, both achieving perfect diagnostic accuracy with a cut-off of 92.8 for adropin and 50.3 for miR-21. In comparison, HbA1c and CIMT also demonstrated significant diagnostic value, with cut-off values of 6.6 for HbA1c and 0.9 for CIMT, but with slightly lower performance. Similarly, adropin and miR-21 demonstrated excellent performance in distinguishing coronary artery disease (CAD) patients without diabetes from the healthy control group. Both markers exhibited perfect diagnostic accuracy, with a cut-off of 84.8 for adropin and 78.8 for miR-21. CIMT, while still effective, showed slightly lower discriminatory power, with a cut-off of 0.9.

Discussion

In recent years, research focused on endothelial dysfunction and preservation of endothelial function has identified new molecules that are predicted to have an important effect on achieving vascular hemostasis. In human-oriented studies, hypotheses were tested that adropin may be an independent diagnostic factor in diseases related to endothelial dysfunction. In the current study, the coronary artery disease (CAD) without DM group has the lowest adropin levels and the highest miRNA-21 expression. In the macrovascular group, adropin levels were significantly lower in comparison to those in the microvascular group, whereas miR-21 expression was significantly elevated. A positive correlation was observed between miR-21 and carotid intima-media thickness (CIMT), alongside a moderately negative correlation between miR-21 and adropin levels. These results indicate that reduced adropin levels and elevated miR-21 expression may serve as predictive markers for atherosclerosis associated with endothelial dysfunction. Additionally, CIMT appears to hold potential as a clinically valuable predictor of vascular risk in diabetic patients with CAD.

The protective role of adropin in endothelial function has been extensively documented[15]. Diabetic retinopathy (DR) is a critical microvascular complication of T2DM. Li et al.[16] identified a negative correlation between serum and vitreous adropin levels and the presence of diabetic retinopathy (DR), suggesting a potential role for adropin in the pathophysiology of this condition. Similarly, Topuz et al.[17] showed that adropin levels were significantly diminished in T2DM patients with endothelial dysfunction (ED) compared to those without ED, identifying adropin as a novel and reliable noninvasive marker for evaluating endothelial function. Furthermore, Celik et al.[18] found lower maternal and neonatal serum adropin levels in women with gestational diabetes mellitus

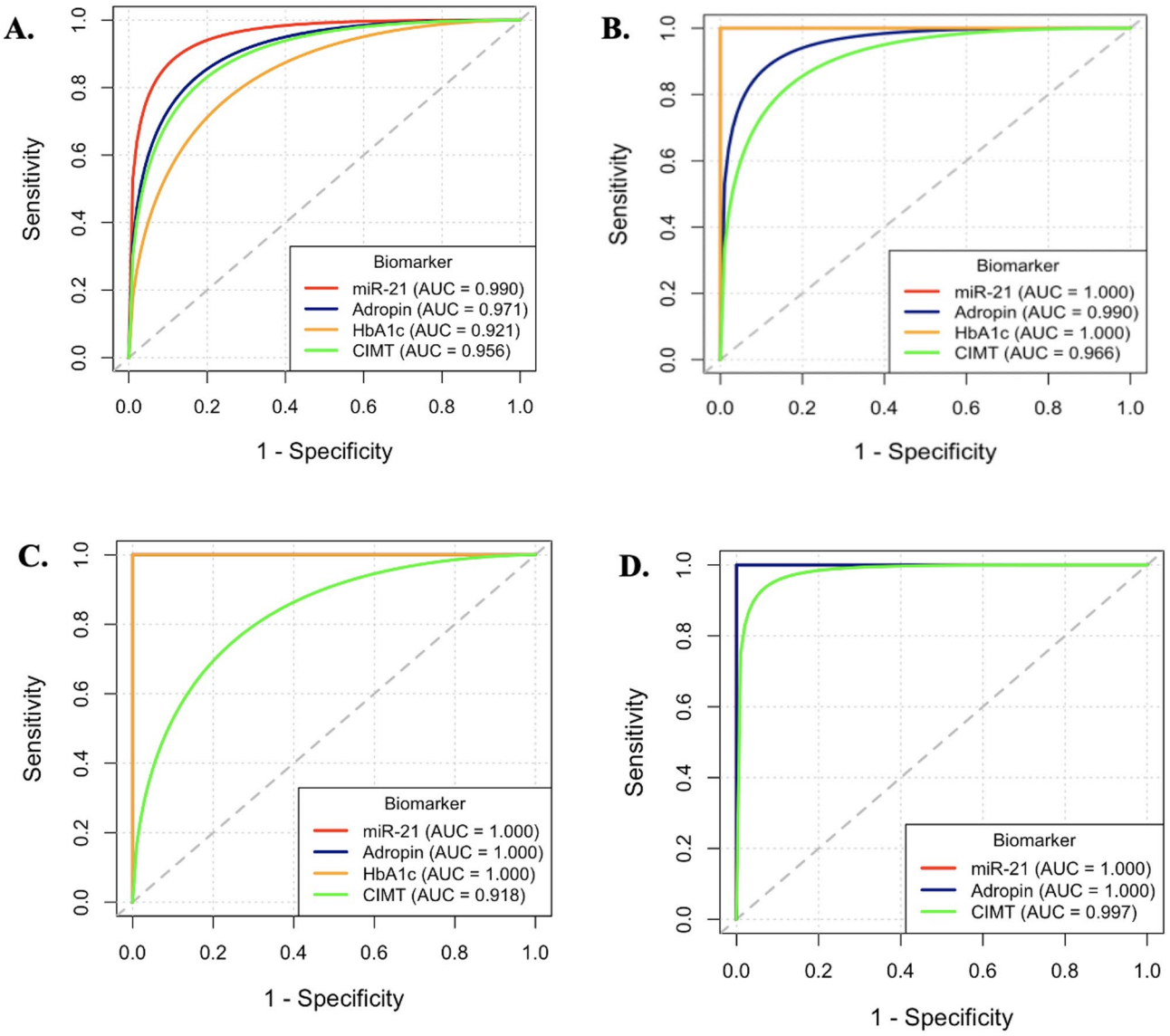


Fig. 2. ROC curves comparing healthy controls with: (**A**) diabetic patients without complications, (**B**) diabetic patients with microvascular complications, (**C**) diabetic patients with macrovascular complications, and (**D**) patients with coronary artery disease without diabetes mellitus.

(GDM). These findings suggest that diminished adropin levels may be associated with GDM or that chronic insulin resistance induced by hyperglycemia could suppress adropin expression[5,18]. Additionally, reduced serum adropin levels have been associated with the angiographic severity of coronary atherosclerosis, with evidence indicating that reduced circulating adropin may promote CAD in both T2DM and non-diabetic individuals[19].

However, studies investigating adropin in diabetes have produced inconsistent results[16–24]. For instance, a study conducted in Iran found higher adropin levels in T2DM patients and linked these levels to an increased risk of developing T2DM, particularly in individuals with rs7903146T/T and rs7903146C/T genotypes. The authors hypothesized that elevated adropin levels might reflect an adaptive response to endothelial dysfunction, influenced by the disease duration, which was longer in their cohort (42 to 50 months versus 19.32 ± 3.14 years). In another study, Berezina et al.[25] reported that lower adropin levels in T2DM patients with chronic congestive heart failure (CH) could independently predict chronic kidney disease (CKD) at stages 1–3. Additionally, Mansour et al.[26] proposed that serum adropin level assessment could serve as a potential risk indicator for ischemic heart disease development in individuals with T2DM.

The present study demonstrated that diabetic groups with complications exhibited significantly higher adropin levels in comparison with those without complications, while the CAD without DM group exhibited the lowest levels. A weak negative correlation between adropin levels and BMI, a very weak negative correlation between HbA1c and triglycerides, and a moderate negative correlation with CIMT were statistically significant. These findings imply that adropin, recognized for its protective effects on the endothelium, could act as a novel regulator of endothelial function.

Previous studies offer varying insights into adropin's role. While some researchers reported no correlation between adropin levels and fasting blood glucose, triglycerides, cholesterol, LDL, or age in diabetes[17,21,24], others identified a significant relationship between adropin and HbA1c[24]. Akcılar et al.[27] demonstrated that intraperitoneal administration of adropin improved lipid metabolism, reduced insulin resistance, and inhibited hepatic inflammation in hyperlipidemic rats, highlighting its anti-hyperlipidemic properties. Additionally, adropin appears critical in glucose metabolism regulation, as evidenced by studies involving T2DM, T1DM, and GDM patients[26–28].

Currently, no reliable soluble biomarkers for endothelial dysfunction in cardiovascular diseases exist. However, miR-21, a conserved noncoding RNA involved in vascular biology, has been linked to endothelial dysfunction and vascular remodeling[29–31]. Li et al.[32] reported elevated hsa-miR-21 expression in atherosclerotic lesions, with upregulation observed across arterial beds compared to controls. miR-21 regulates key genes involved in atherosclerosis, as corroborated by Raitoharju et al.[33], who highlighted its critical role in vascular remodeling. Sustained miR-21 induction contributes to proinflammatory responses in endothelial cells through a positive feedback loop targeting peroxisome proliferator-activated receptor-α (PPAR-α)[34]. Additionally, miR-21 deficiency has been linked to decreased aortic elastin content, increased media layer thickness, and heightened aortic stiffness[29].

Studies on miR-21 in diabetes have yielded conflicting results[35–40]. Zampetaki et al.[36] found lower plasma levels of miR-21 in diabetic subjects compared to controls. Diabetes mellitus (DM) negatively impacts the quantity and functionality of circulating endothelial progenitor cells (EPCs), with miR-21 downregulated in EPCs derived from T2DM patients[37]. However, miR-21 is overexpressed in endothelial cells under diabetic conditions, promoting survival by preventing apoptosis[38]. Zeng et al.[38] also observed elevated miR-21 levels in diabetic endothelial cells compared to healthy donors, suggesting a role in mitigating reactive oxygen species (ROS)-induced damage.

In contrast, an investigation by Feddersen et al.[41] revealed no substantial correlation between newly detected subclinical atrial fibrillation and miR-21-5p levels in patients with hypertension and DM. Despite such inconsistencies, evidence suggests that miR-21 inhibition could serve as a novel therapeutic approach to protect against glucose variability-induced oxidative damage in diabetes[42]. Inhibition of miR-21 has been shown to enhance high glucose-induced apoptosis and inhibit endothelial cell proliferation, underscoring its therapeutic potential.

Limited information exists regarding miR-21 in diabetic complications. In the current study, the highest miR-21 expression was observed in the diabetic group with macrovascular complications among diabetic groups. A notable finding was that patients with CAD without diabetes exhibited significantly elevated levels of miR-21 compared to those with diabetes who experienced macrovascular complications. A statistically significant weak positive correlation was found between miR-21 and CIMT, while a moderately negative correlation was observed with adropin. miR-21 exhibits high sensitivity and specificity, potentially serving as a marker to differentiate between diabetic patients with macrovascular or microvascular complications and CAD patients without diabetes, and healthy individuals. It plays a key role in diabetic disorders, regulated by various metabolic stimuli, including glucose. miR-21 expression is also upregulated in diabetic nephropathy (DN)[35]. Mazzeo et al.[43] identified miR-21-3p from circulating extracellular vehicles (EVs) as a potential prognostic biomarker for diabetic retinopathy (DR), highlighting its association with the hypoxic conditions of this complication. Similarly, Zhong et al.[44] demonstrated increased miR-21 levels in db/db mice over a 20-week period, correlating with worsening hyperglycemia and microalbuminuria. Knockdown of miR-21 delayed DN progression, suggesting its therapeutic potential in preventing diabetic kidney damage.

These findings, along with ours, underscore the potential of anti-miR-21 therapy to mitigate diabetic complications and improve endothelial dysfunction. The development of innovative biomarkers remains critical for the effective management of endothelial dysfunction in T2DM complications.

Limitations of the study

While our findings suggest potential therapeutic opportunities, such as adropin-based treatments or anti-miR-21 strategies for atherosclerotic complications, several limitations should be acknowledged. First, the relatively small sample size in some subgroups may limit the generalizability of our results. Additionally, focusing solely on miR-21 without assessing other relevant miRNAs could narrow the scope of molecular insights. The cross-sectional and single-center design of the study inherently restricts the ability to establish causal relationships and limits the broader applicability of the findings. Although we controlled several confounding factors through strict exclusion criteria, residual confounders related to lifestyle, medication use, or other variables cannot be entirely ruled out. One limitation of this study is the potential influence of medications (e.g., insulin, statins, ACE inhibitors) on biomarker levels, which could not be fully analyzed due to the limited sample size. Finally, biomarker levels were measured at a single time point, which may not reflect their dynamic changes over the course of disease progression. These limitations highlight the need for larger, longitudinal, and multicenter studies to further validate and expand upon our findings.

Conclusion

In this study, we observed decreased serum adropin levels and increased miR-21 expression in diabetic patients with vascular complications compared to healthy controls. These findings suggest that both adropin and miR-21 may have potential as biomarkers associated with macrovascular complications in T2DM. Endothelial dysfunction, a key factor in diabetic vasculature, may be linked to these molecular changes; however, the cross-sectional design of our study limits causal interpretation. The observed associations may reflect underlying endothelial injury due to chronic hyperglycemia, but further research is needed to confirm this relationship. Larger, longitudinal, and multicenter studies including a broader panel of miRNAs are necessary to better

understand the complex interplay between endothelial function, adropin, miR-21, and vascular complications in diabetes, and to evaluate their potential therapeutic relevance.

Data availability

The data underlying this article are available within the publication. If you have any further questions, please email the corresponding author at [omurtabak@yahoo.com.tr](mailto: omurtabak@yahoo.com.tr).

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References

1. Introduction Standards of medical care in Diabetes-2020. *Diabetes Care*. **43**, S1–S2 (2020).
2. Association, A. D. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes 2019. *Diabetes Care*. **42**, S13–S28 (2019).
3. GLOBAL REPORT ON DIABETES WHO Library Cataloguing-in-. Publication Data Global report on diabetes, (2016). http://www.who.int/about/licensing/copyright_form/index.html accessed 13 April 2020).
4. Fradkin, J. E. & Rodgers, G. P. Diabetes research: A perspective from the National Institute of diabetes and digestive and kidney diseases. *Diabetes* **62**, 320–326 (2013).
5. Kumar, K. G. et al. Identification of Adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. *Cell. Metab.* **8**, 468–481 (2008).
6. Marczuk, N. et al. Adropin-physiological and pathophysiological role Adropina-rola Fizjologiczna i Patofizjologiczna. *Postep Hig Med. Dosw*. **70**, 981–988 (2016).
7. Bartel, D. P. & MicroRNAs Genomics, Biogenesis, Mechanism, and function. *Cell* **116**, 281–297 (2004).
8. Pandey, A. K. et al. MicroRNAs in diabetes: tiny players in big disease. *Cell. Physiol. Biochem*. **23**, 221–232 (2009).
9. Luo, M. et al. Platelet-derived miR-103b as a novel biomarker for the early diagnosis of type 2 diabetes. *Acta Diabetol.* **52**, 943–949 (2015).
10. Zhou, H. et al. MicroRNA expression profiling based on microarray approach in human diabetic retinopathy: A systematic review and Meta-Analysis. *DNA Cell. Biol.* **39**, 441–450 (2020).
11. Park, S. et al. Mir2diabetes: A literature-curated database of Microrna expression patterns, in diabetic microvascular complications. Genes (Basel); 10. Epub ahead of print 1 October 2019. https://doi.org/10.3390/genes10100784
12. Zhao, X. Y. & Shao, K. Roles of MicroRNA-21 in the pathogenesis of insulin resistance and diabetic mellitus-induced non-alcoholic fatty liver disease. *Acta Acad. Med. Sin*. **38**, 144–149 (2016).
13. Zhou, J. et al. MicroRNA-21 targets peroxisome proliferators-activated receptor-alpha in an autoregulatory loop to modulate flow-induced endothelial inflammation. *Proc. Natl. Acad. Sci. U S A*. **108** (25), 10355–10360 (2011).
14. Kimura, J. *Electrodiagnosis in Diseases of Nerve and Muscles* 4th edn (Oxford University Press, 2013).
15. Lovren, F. et al. Adropin is a novel regulator of endothelial function. *Circulation* **122** (11 Suppl), S185–S192 (2010).
16. Li, S. et al. The association of serum and vitreous Adropin concentrations with diabetic retinopathy. *Ann. Clin. Biochem*. **56**, 253–258 (2019).
17. Topuz, M. et al. Plasma Adropin levels predict endothelial dysfunction like flow-mediated dilatation in patients with type 2 diabetes mellitus. *J. Investig Med.* **61** (8), 1161–1164 (2013).
18. Celik, E. et al. Maternal and fetal Adropin levels in gestational diabetes mellitus. *J. Perinat. Med.* **41** (4), 375–380 (2013).
19. Wu, L. et al. Low serum Adropin is associated with coronary atherosclerosis in type 2 diabetic and non-diabetic patients. *Clin. Chem. Lab. Med.* **52** (5), 751–758 (2014).
20. Zang, H., Jiang, F., Cheng, X., Xu, H. & Hu, X. Serum Adropin levels are decreased in Chinese type 2 diabetic patients and negatively correlated with body mass index. *Endocr. J.* **65** (7), 685–691 (2018).
21. Beigi, A. et al. Association between serum Adropin levels and gestational diabetes mellitus; a case-control study. *Gynecol. Endocrinol.* **31** (12), 939–941 (2015).
22. Dąbrowski, F. A. et al. First and third trimester serum concentrations of Adropin and copeptin in gestational diabetes mellitus and normal pregnancy. *Ginekol. Pol.* **87** (9), 629–634 (2016).
23. Hu, W. & Chen, L. Association of serum Adropin concentrations with diabetic nephropathy. *Mediators Inflamm.* **2016**, 6038261 (2016).
24. Palizban, A. A., Yazdani, A. H. & Jahanbani-Ardakani, H. Role of rs7903146 polymorphism and Adropin serum level in patients with diabetes mellitus; a case-control study from Isfahan, Iran. *Arch. Physiol. Biochem.* **128** (2), 378–381 (2022).
25. Berezina, T. A. et al. Adropin predicts chronic kidney disease in type 2 diabetes mellitus patients with chronic heart failure. *J. Clin. Med.* **12** (6), 2231 (2023).
26. Mansour, H. K., Makboul, K., Elhalawany, S. H., Ibrahim, B. E. & Marawan, D. A. A study of the assessment of serum adropın level as a rısk factor of ıschaemıc heart dısease ın type 2 dıabetes mellıtus cases. *Georgian Med. News* ;(328–329):115–117. (2022).
27. Akcılar, R. et al. The effect of Adropin on lipid and glucose metabolism in rats with hyperlipidemia. *Iran. J. Basic. Med. Sci.* **19** (3), 245–251 (2016).
28. Polkowska, A., Pasierowska, I. E., Pasławska, M., Pawluczuk, E. & Bossowski, A. Assessment of Serum Concentrations of Adropin, Afamin, and Neudesin in Children with Type 1 Diabetes. Biomed Res Int. ; 2019: 6128410. (2019).
29. Ji, R. et al. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. *Circ. Res.* **100** (11), 1579–1588 (2007).
30. Guduric-Fuchs, J. et al. Deep sequencing reveals predominant expression of miR-21 amongst the small non-coding RNAs in retinal microvascular endothelial cells. *J. Cell. Biochem.* **113** (6), 2098–2111 (2012).
31. Zhang, X. Y. et al. Induction of thoracic aortic remodeling by endothelial-specific deletion of microRNA-21 in mice. *PLoS One*. **8** (3), e59002 (2013).
32. Li, T. et al. Identification of miR-130a, miR-27b and miR-210 as serum biomarkers for atherosclerosis obliterans. *Clin. Chim. Acta*. **412** (1–2), 66–70 (2011).
33. Raitoharju, E. et al. miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere vascular study. *Atherosclerosis* **219** (1), 211–217 (2011).
34. Zhou, J. et al. MicroRNA-21 targets peroxisome proliferators-activated receptor-α in an autoregulatory loop to modulate flow-induced endothelial inflammation. *Proc. Natl. Acad. Sci. USA*. **108** (25), 10355–10360 (2011).
35. Lan, H. Y. Transforming growth factor-β/Smad signalling in diabetic nephropathy. *Clin. Exp. Pharmacol. Physiol.* **39** (8), 731–738 (2012).
36. Zampetaki, A. et al. Plasma MicroRNA profiling reveals loss of endothelial miR-126 and other MicroRNAs in type 2 diabetes. *Circ. Res.* **107** (6), 810–817 (2010).
37. Meng, S. et al. Downregulation of microRNA-126 in endothelial progenitor cells from diabetes patients impairs their functional properties, via target gene Spred-1. *J. Mol. Cell. Cardiol.* **53** (1), 64–72 (2012).

38. Urbich, C., Kuehbacher, A. & Dimmeler, S. Role of MicroRNAs in vascular diseases, inflammation, and angiogenesis. *Cardiovasc. Res.* **79** (4), 581–588 (2008).
39. Ling, H. Y. et al. MiRNA-21 reverses high glucose and high insulin induced insulin resistance in 3T3-L1 adipocytes through targeting phosphatase and tensin homologue. *Exp. Clin. Endocrinol. Diabetes*. **120** (9), 553–559 (2012).
40. Zeng, J. et al. MiR-21 is overexpressed in response to high glucose and protects endothelial cells from apoptosis. *Exp. Clin. Endocrinol. Diabetes*. **121** (7), 425–430 (2013).
41. Feddersen, S. et al. No detectable differences in MicroRNA plasma levels between diabetic hypertensive patients with and without incident subclinical atrial fibrillation. *J. Clin. Med.* **13** (9), 2554 (2024).
42. La Sala, L., Mrakic-Sposta, S., Micheloni, S., Prattichizzo, F. & Ceriello, A. Glucose-sensing microRNA-21 disrupts ROS homeostasis and impairs antioxidant responses in cellular glucose variability. *Cardiovasc. Diabetol.* **17** (1), 105 (2018).
43. Mazzeo, A. et al. Functional analysis of miR-21-3p, miR-30b-5p and miR-150-5p shuttled by extracellular vesicles from diabetic subjects reveals their association with diabetic retinopathy. *Exp. Eye Res.* **184**, 56–63 (2019).
44. Zhong, X. et al. miR-21 is a key therapeutic target for renal injury in a mouse model of type 2 diabetes. *Diabetologia* **56** (3), 663–674 (2013).

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O.T., S.D. and H.U. wrote the main manuscript text and S.D and A.C. prepared Figs. 1 and 2. All authors reviewed the manuscript.

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Declarations

Competing interests
The authors declare that there are no competing interests associated with this study.

Informed consent statement
Informed consent was obtained from all participants involved in the study.

Additional information
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