



# Impact of Fibroblast Growth Factor-23 on Peripheral Arterial Disease in Type 2 Diabetes Mellitus: A Comparative Cross-Sectional Pilot Study

## Tip 2 Diyabetes Mellitusta Fibroblast Büyüme Faktörü-23'ün Periferik Arter Hastalığı Üzerine Etkisi: Bir Karşılaştırmalı Kesitsel Pilot Çalışma

Esra Hatipoğlu, Nurgül Özgür\*, Mutlu Niyazoğlu\*\*, Atilla Süleyman Dikici\*\*\*, Özlem Balci Ekmekçi\*\*\*\*, Serkan Yalın\*\*\*\*, Zeynep Osar Siva\*, Hasan İlkova\*

Edirne Government Hospital, Clinic of Endocrinology, Edirne, Turkey

\*İstanbul University Faculty of Cerrahpaşa, Department of Internal Medicine, Division of Endocrinology and Metabolism, İstanbul, Turkey

\*\*İstanbul Research Hospital, Clinic of Endocrinology and Metabolism, İstanbul, Turkey

\*\*\*İstanbul University Faculty of Cerrahpaşa, Department of Radiology, İstanbul, Turkey

\*\*\*\*İstanbul University Faculty of Cerrahpaşa, Department of Biochemistry, İstanbul, Turkey

\*\*\*\*\*İstanbul University Faculty of Cerrahpaşa, Department of Internal Medicine, Division of Nephrology, İstanbul, Turkey

### Abstract

**Purpose:** To evaluate whether fibroblast growth factor-23 (FGF-23) has a common role in the pathogenesis of peripheral arterial disease (PAD) and chronic kidney disease (CKD) in type-2 diabetes mellitus (DM).

**Material and Method:** Twenty-one patients with diabetic nephropathy composed the diabetic nephropathy (DM-NP) group and 20 subjects with DM but without NP constituted the DM group. The control group was comprised of 10 age- and gender-matched non-diabetic individuals with CKD.

**Results:** FGF-23 levels were similar in DM-NP and CKD groups ( $p=0.5$ ). Both groups had higher FGF-23 levels compared to DM group ( $p<0.001$  and  $p=0.007$ ). PAD was more common in DM than in CKD ( $p=0.03$ ). In all cases involving DM (e.g., both the DM-NP and DM-groups), FGF-23 levels did not vary with arterial wall changes recorded via Doppler ultrasonography ( $p=0.5$ ).

**Discussion:** NP and PAD may be independent complications of DM. In DM, FGF-23 may be a marker of NP but not of PAD.

**Keywords:** Diabetes mellitus, fibroblast growth factor-23, nephropathy, peripheral arterial disease, atherosclerosis, arterial calcification

### Öz

**Amaç:** Fibroblast büyüme faktörü-23'ün (FGF-23) tip 2 diyabetes mellituslu (DM) olgularda periferik arter hastalığı (PAH) ile kronik böbrek yetmezliği (KBY) patogenezinde ortak bir rolü olup olmadığını değerlendirmeyi amaçladık.

**Gereç ve Yöntem:** Diyabetik nefropatisi (NP) olan 21 olgu DM-NP grubunu, DM olan ancak NP bulunmayan 20 olgu DM-grubunu oluşturdu. Diyabetik olmayan, yaş ve cinsiyet uyumlu 10 KBY'li olgu da kontrol grubunu oluşturdu.

**Bulgular:** DM-NP ve KBY gruplarında FGF-23 düzeyleri benzer idi ( $p=0,5$ ). Her iki grupta da FGF-23 düzeyleri DM grubuna göre daha yüksekti ( $p<0,001$  ve  $p=0,007$ ). PAH DM'de KBY'ye göre daha sık idi ( $p=0,03$ ). DM bulunan tüm olgularda (DM ve DM-NP grupları) FGF-23 düzeyleri Doppler ultrasonografi ile belirlenen periferik arter değişiklikleri ile ilişkili değildi ( $p=0,5$ ).

**Tartışma:** NP ve PAH DM'nin birbirinden bağımsız komplikasyonları olabilir. DM'de FGF-23 nefropati belirteci olabilir.

**Anahtar kelimeler:** Diyabetes mellitus, fibroblast büyüme faktörü-23, nefropati, periferik arter hastalığı, ateroskleroz, arteriyel kalsifikasyon

### Introduction

Diabetes mellitus (DM) is a chronic disease associated with widespread complications; one of the major complications of which is peripheral arterial disease (PAD). If PAD is not recognized and treated promptly, it results in major amputations and increasing DM-associated morbidity and mortality (1,2).

Individuals with DM are prone to develop PAD insidiously at an early stage and once PAD develops, it may progress more rapidly than expected (2,3). Therefore, prevention of PAD in those with DM is of the utmost importance. Recognition of the underlying mechanisms and associated risk factors for development of PAD may facilitate prevention.

DM is also a leading cause of end-stage renal disease and, a considerable majority of the patients with DM ultimately develop chronic kidney disease (CKD) (4,5). Nephropathy (NP) in DM is also a chronic and insidious condition. Both CKD and cardiovascular system pathologies have common underlying mechanisms and risk factors (6) in DM, regardless of whether additional factors in CKD are also involved in the pathogenesis of vascular pathologies, namely PAD. Understanding the common pathogeneses and risk factors may contribute to early recognition of PAD in cases with DM even before it is clinically significant.

Fibroblast growth factor-23 (FGF-23) is a phosphaturic hormone derived from osteocytes whose levels increase in CKD. It is associated with increased mortality and poor cardiovascular outcomes in CKD (7,8). However, the results of previous studies on the impact of FGF-23 on atherosclerosis (AS) and PAD are controversial (8,9,10,11). There are also scarce data on the impact of FGF-23 on PAD in patients with DM (9,12). Herein, we aimed to evaluate whether FGF-23 has a common role in the pathogenesis of PAD and CKD, as well as to determine whether or not FGF-23 can be used as an early marker of PAD in cases with type-2 DM.

## Materials and Methods

This consecutive cross-sectional study was conducted in the endocrinology, metabolism and diabetes, and radiology departments at İstanbul University Faculty of Medicine Cerrahpaşa. The study protocol was approved by the Local Ethics Committee of Cerrahpaşa Faculty of Medicine and informed consent was obtained from all the participants. All the tenets of the Helsinki Declaration were adhered to.

The subject group of the study consisted of 41 patients with type-2 DM who were followed up and treated at the Cerrahpaşa Faculty of Medicine, Endocrinology and Metabolism Outpatient Clinic. Presence of DM was determined by criteria defined by the American Diabetes Association in 2013: HbA1c  $\geq 6.5\%$ , fasting blood glucose (FBG)  $\geq 126$  mg/dl after no caloric intake for at least 8 hours, 2-h plasma glucose  $\geq 200$  mg/dl during 75 g oral glucose tolerance test, all of which were confirmed by repeat testing, as well as the classic symptoms of hyperglycemia or hyperglycemic crisis with a random plasma glucose  $\geq 200$  mg/dl (13).

The group with DM was also subdivided into 2 subgroups based on the presence or absence of diabetic NP (DM-NP). A total of 21 cases with DM-NP composed the DM-NP group, whereas 20 cases with DM only constituted the DM group.

The mean duration of DM in DM-NP and DM groups was 11 (Interquartile range (IQR): 4-23) and 5 (IQR: 2-12.3) years, respectively ( $p=0.06$ ). In the DM-NP group, 2 cases (10%) were on diet only, 4 (19%) were on oral antidiabetic agents only, 7 (33%) were on basal insulin and 8 (38%) on intensive insulin therapy; in contrast, in the DM group, 5 cases (25%) were on diet only, 7 (35%) were on oral antidiabetic agents only, 3 (15%) were on basal insulin and 5 (25%) on intensive insulin therapy ( $p=0.2$ ).

The control group (CKD group) was composed of 10 age- and gender-matched non-diabetic subjects with CKD who were also matched for creatinine and creatinine clearance (CCl) with the DM-NP group. Individuals on hemodialysis, active vitamin D or

biphosphonate treatment, and/or using any estrogen-containing agents were not included in any of the groups.

Demographic data and smoking history were obtained for each subject. Systolic and diastolic blood pressure was measured in a sitting position after a 5-min rest, twice with a 3-min interval, and the average measurements were recorded. Height and weight were used to calculate body mass index (BMI). Laboratory results of each case were obtained concurrently with physical examination. FBG (normal: 70-110 mg/dl), HbA1c (4.8-6%), creatinine (0.6-1.2 mg/dl), CCl (ml/min) and microalbuminuria (MAU) (0-30 mg/24 hour) in a 24-hour urine sample, Ca (8.9-10.3 mg/dl), P (2.7-4.5 mg/dl), albumin (3.5-5 g/dl), parathyroid hormone (PTH) 12-7 pg/ml, 25(OH)D ( $\geq 30$  ug/l), total cholesterol (50-200 mg/dl), low density lipoprotein (LDL) (0-130 mg/dl), high density lipoprotein (HDL) (28-61 mg/dl), and TG (50-150 mg/dl) levels were evaluated for each participants. Corrected calcium levels were calculated by using serum Ca and albumin levels (corrected calcium (mg/dL)=measured total Ca (mg/dL)+0.8 (4.0-serum albumin (g/dL)) and were used for the statistics. The product of Ca and P was also calculated for each case.

Serum samples of each group were obtained during the study period and preserved at  $-80$  °C. The enzyme-linked immunosorbent assay procedure was used to determine 1,25-dihydroxyvitamin D3 and FGF-23 levels (EIAAB, China, Catalog Number: EO467Ge and EIAAB, China, Catalog Number: EO746h; respectively). All the measurements were performed according to instructions provided by the manufacturer. At the final step of the procedure, the levels of absorbance were measured in a microplate reader at 450 nm with the absorption spectrophotometer.

To determine the presence of AS, bilateral colored Doppler ultrasonography (USG) was performed using a 5-12 MHz linear probe (Logiq 9; GE, Wauwatosa, WI, USA). In all subjects, Doppler USG was carried out by the same radiologist at the same room temperature. Common femoral arteries, superficial femoral arteries, deep femoral arteries, popliteal arteries, anterior tibial arteries, dorsalis pedis arteries, and posterior tibial arteries of both right and left lower extremities were evaluated. All but the popliteal arteries were evaluated at the supine position. Popliteal arteries were viewed with the patients at the prone position. At first, arterial wall calcifications and plaques were evaluated with grey-scale USG, and the intensity of the calcifications and size of the plaques, when present, were noted. Calcifications were classified as minimal, diffuse and atherosclerotic, based on their quantity. Grades of the stenosis caused by the plaques were determined by color flow Doppler USG.

Statistical analysis was performed using the SPSS 17.0. The Chi-square test was used for categorical variables. Sample distribution was evaluated with the Kolmogorov-Smirnov test. Continuous variables with normal distribution were compared between the 3 groups by using one-way ANOVA test. Post-hoc analysis was also performed on results having significant difference. Continuous variables with non-normal distributions were compared by using the Kruskal-Wallis test. A  $p$  value of less than 0.05 was considered statistically significant. The variables

with significance were then evaluated by the Mann-Whitney U test to investigate the difference between the groups. The results are presented as median and IQR; a Bonferroni adjusted alpha level of 0.017 was used when the results were considered separately. The Spearman's correlation coefficient was used for calculation of associations between variables, where a p value of less than 0.05 was considered statistically significant.

## Results

In the DM-NP, DM and the CKD groups, the mean age was 57.1±4.9, 54.6±6.4 and 53.7±5.7 years, respectively (p=0.2). Female/male distribution was 7/14 in the DM-NP, 8/12 in DM and 6/4 in the CKD groups (p=0.4). Additional demographic data of the cases are shown in Table 1.

A comparison of the three groups on the basis of laboratory values and physical examination is presented in Table 2. FBG and HbA1c levels were similar in diabetic cases with and without NP (p=0.3 and 0.5). However, the levels of FBG and HbA1c were lower in CKD group than in DM-NP and DM groups (CKD vs. DM-NP: p=0.009 and p=0.001, CKD vs. DM: p=0.003 and p=0.005). In DM-NP group, creatinine and MAU levels were higher and CCI was lower than in DM-only group (p<0.001, p<0.001 and p<0.001). CKD group also had higher creatinine and MAU levels and lower CCI than DM group (p<0.001, p=0.006 and p<0.001). Creatinine, MAU and CCI levels were similar in DM-NP and CKD groups (p=0.6, p=0.3 and p=0.6). Serum albumin levels were lower in DM-NP group than in DM group (p=0.001). The levels of serum albumin in CKD group were similar to that in both DM and DM-NP groups (p=0.6 and p=0.02). There was no significant difference in PTH levels between CKD and DM-NP groups (p=0.4), however, they were lower in DM group than in DM-NP and CKD groups (p<0.001 and p=0.001). Additionally, DM-NP and CKD groups had similar FGF-23 levels (p=0.5). Both groups had FGF-23 levels higher than DM-only group (p<0.001 and p=0.007).

In the entire cohort, FGF-23 was positively correlated with creatinine, MAU and PTH levels (r=+0.5, p<0.001, r=+0.5, p<0.001 and, r=+0.4, p=0.003), whereas it was negatively correlated with CCI and serum albumin levels (r=-0.3, p=0.02 and r=-0.3, p=0.02). When only DM group was taken into consideration, FGF-

23 had tended to be associated with higher FBG levels, although the correlation was statistically insignificant (r=+0.3, p=0.07). FGF-23 was also positively correlated with creatinine, MAU and PTH levels (r=+0.5, p=0.001, r=+0.6, p<0.001 and, r=+0.4, p=0.004), while it was negatively correlated with CCI and serum albumin levels (r=-0.4, p=0.01 and r=-0.4, p=0.004). Additionally, there was a negative correlation between HDL levels and FGF-23 (r=-0.4, p=0.007). On the other hand, total cholesterol, LDL and TG levels were not related to FGF-23 (p=0.9, p=0.9 and p=0.6). Also, HbA1c and disease duration of DM was not correlated with FGF-23 levels (p=0.3 and p=0.6).

Based on Doppler USG, 4 cases with DM-NP, 10 cases with DM and 7 cases with CKD had minimal arterial wall calcification; 4 of the cases with DM-NP, 1 case with DM and none of the cases with CKD had diffuse arterial wall calcification; 12 cases with DM-NP, 8 cases with DM and 1 case with CKD had atherosclerotic changes and, 1 case with DM-NP, 1 case with DM and 2 cases with CKD did not have any changes (p=0.03). Percentages of USG findings in all groups are shown in Figure 1.

In all cases with DM (cases with DM-NP and cases with DM only), there was no association between FGF-23 levels and arterial wall changes as determined by Doppler USG (p=0.5).

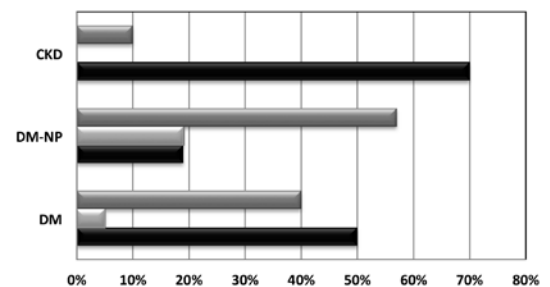
## Discussion

In the presented study, cases with CKD with and without DM had similar but higher FGF-23 levels than the diabetic cases without NP. In the entire cohort, there was a positive association between FGF-23 values and creatinine, MAU and parathormone levels. Furthermore, they increased as the CCI and serum albumin levels decreased. When only cases with diabetes were taken into consideration, FGF-23 tended to be higher in cases having higher FBG levels. However, the correlation was not statistically significant. On the other hand, there was a statistically significant correlation between FGF-23 levels and creatinine, MAU and parathormone levels. The increase in FGF-23 level paralleled with that in creatinine, MAU and parathormone. In subjects with DM, FGF-23 was negatively correlated with CCI and serum albumin levels, meaning that FGF-23 increased when the CCI

**Table 1. Additional characteristics of the cases with diabetes only, diabetic nephropathy and chronic kidney disease**

	DM-NP (n=21)	DM (n=20)	CKD (n=10)	p
Age (years)	57.1±4.9	54.6±6.4	53.7±5.7	0.2
Gender (female/male)	7/14	8/12	6/4	0.4
Smoking history (n, %)	2 (10)	6 (30)	1 (10)	0.2
Comorbidities (n, %)				
Hypertension	17 (81)	15 (75)	10 (100)	0.2
Hyperlipidemia	17 (81)	14 (70)	7 (70)	0.7
Coronary artery disease	8 (38)	1 (5)	0 (0)	0.006*

\*Statistically significant p values, DM: Diabetes mellitus, CKD: Chronic kidney disease, NP: Nephropathy



	DM	DM-NP	CKD
Atherosclerotic Changes	40%	57%	10%
Diffuse Calcification	5%	19%	0%
Minimal Calcification	50%	19%	70%

**Figure 1.** Arterial wall changes in cases with diabetes only, diabetic nephropathy and chronic kidney disease based on lower extremity arterial Doppler ultrasonography

**Table 2. Comparison of the laboratory values and physical examination findings between cases with diabetes only, diabetic nephropathy and chronic kidney disease. Laboratory values and physical examination findings in cases with diabetic nephropathy, diabetes mellitus and chronic kidney disease**

	DM-NP (n=21)	DM (n=20)	CKD (n=10)	p
FBG (mg/dl)	136 (98-197)	118.5 (96.5-146.5)	94.5 (86.3-102)	0.009*
HbA1c (%)	7.3 (6.7-8)	7.1 (5.9-8.2)	5.5 (5.4-6.5)	0.005*
Creatinin (mg/dl)	1.7 (1.4-2.2)	0.8 (0.7-0.9)	1.7 (1.4-2.5)	<0.001*
CCl (ml/dk)	49 (37.2-68)	116 (85.3-131.5)	38.7 (26-59.3)	<0.001*
MAU (mg/24 hour)	159.5 (29.8-874)	15.7 (8-24.8)	415.5 (116.8-1108.3)	<0.001*
Albumin (mg/dl)	3.9 (3.6-4.4)	4.5 (4.3-4.7)	4.5 (4.1-4.6)	0.001*
Ca (mg/dl)	9.3 (8.9-9.4)	9.3 (8.8-9.4)	9 (8.8-9.4)	0.7
P (mg/dl)	3.3 (3.2-3.9)	3.3 (3-3.7)	3.7 (3.2-4.2)	0.3
CaxP	32.5 (28.5-36.7)	30.8 (28.2-32.5)	33.6 (28.7-37.5)	0.3
PTH (pg/ml)	99.5 (46.3-149.5)	35.6 (30.3-46.2)	127.5 (52.7-211.8)	<0.001*
25(OH)Dvit (ug/l)	27 (22.3-34.8)	11.8 (5.2-16.7)	91.8 (59.7-157.5)	0.5
1,25 (OH) <sub>2</sub> Dvit (ug/l)	113.2 (80.9-198)	115.2 (93.9-172.9)	91.8 (59.7-157.5)	0.7
FGF-23 (pg/ml)	16.6 (13.2-28.9)	10.4 (6.4-12.7)	16.1 (12.4-19.6)	<0.001*
Total cholesterol (mg/dl)	181 (153-209.5)	175 (162.3-222.3)	194.5 (156-224.3)	0.8
LDL (mg/dl)	113 (88-127)	106 (92-126)	132.5 (94-152.8)	0.6
HDL (mg/dl)	48 (35-53)	44 (37-61.5)	49 (40-70)	0.4
TG (mg/dl)	132 (86-178.5)	155 (121.8-218.3)	110.5 (74.3-159)	0.3
BMI (kg/m <sup>2</sup> )	29.1 (26-34.4)	30.9 (27.8-33.1)	29.4 (23-35.9)	0.7
Systolic Blood Pressure mmHg	130 (110-140)	125 (112.5-138.8)	130 (125-150)	0.6
Diastolic Blood Pressure (mmHg)	80 (67.5-87.5)	80 (76.3-90)	85 (77.5-100)	0.1

Data was expressed as median and IQR. \*statistically significant p values, FBG: Fasting blood glucose, MAU: Microalbuminuria, FGF-23: Fibroblast growth factor-23, BMI: Body mass index, DM-NP: Diabetic nephropathy, DM: Diabetes mellitus, CKD: Chronic kidney disease, LDL: Low density lipoprotein, HDL: High density lipoprotein, PTH: Parathyroid hormone

and serum albumin levels decreased. AS was more common in cases with DM than in those with CKD. However, FGF-23 was not related to atherosclerotic changes in cases with DM.

FGF-23 is a hormone derived by osteocytes and promotes phosphate excretion by the kidney (14). Moreover, it inhibits 1- $\alpha$ -hydroxylase, thus, it contributes to vitamin D metabolism (14). In patients with CKD, there is a compensatory increase in FGF-23 levels which leads to impaired cardiovascular outcomes and increased mortality (15,16,17,18). FGF-23 contributes to calcium and phosphorus homeostasis which is involved in arterial calcification. Therefore, the question of whether FGF-23 is directly involved in the pathogenesis of PAD has been the subject of research (19). Previous studies on the association between FGF-23 and peripheral arterial changes have generated controversial results (9,10,11,12,19).

In the current study, the subjects with DM-NP and CKD had similar renal functions. Creatinine, CCl, MAU, and parathormone levels were similar in the two groups. On the other hand, cases with DM only had better renal functions, reflected by creatinine, CCl, and MAU. Parathormone levels were also lower in subjects

with DM only, probably as a result of better renal functions. Serum albumin levels were significantly lower in cases with DM-NP than they were in DM, and also tended to be lower than that in CKD group. The difference in serum albumin levels between the DM-NP and CKD groups may be attributed to a difference in diet, which was not specifically asked about in the study. Moreover, Ca, P, CaxP, 25(OH)D, and 1,25(OH)<sub>2</sub>D, each of which may be a confounding factor in interpretation of FGF-23 levels, were also similar between the three groups. Since CCl of the cases was not below 30 ml/min, renal osteodystrophy-related parameters involving Ca, P, CaxP and vitamin D levels were not different between the groups. DM-NP and CKD groups had similar FGF-23 levels. However, DM group had FGF-23 levels strikingly lower than DM-NP and CKD groups. This result confirms previous studies showing that increased FGF-23 may be associated with deteriorated renal functions in DM and it may be a marker of DM-NP (20). Moreover, considering the similar levels of osteodystrophy-related parameters, we may conclude that FGF-23 begins to increase at an earlier stage before renal osteodystrophy develops.

Both in the entire cohort and in all cases with DM, FGF-23 levels increased when creatinine, MAU and PTH levels increased and when CCI and serum albumin levels decreased. Although FGF-23 levels in cases with DM tended to increase with higher FBG levels, the correlation was not statistically significant. Moreover, FGF-23 was not associated with HbA1c or disease duration in patients with DM. This also shows that FGF-23 may reflect the presence of NP in DM, irrespective of disease duration and/or disease control. Interestingly, FGF-23 was also positively correlated with serum albumin levels both in the entire cohort and in cases with DM, and despite this correlation, cases with DM-NP had still high FGF-23 levels, albeit low serum albumin levels. Based on the findings of the current study, FGF-23 levels increased when HDL levels decreased. This may be a clue for cardiovascular effects of FGF-23, however, FGF-23 was not related to total cholesterol, LDL or TG levels. Therefore, it may not be appropriate to draw a definitive conclusion on cardiovascular protection by lower FGF-23 levels.

In the present study, AS was more frequent in individuals with DM, both in those with and without NP, compared to those with chronic renal disease. Moreover, in subjects with DM, FGF-23 levels were similar to that in those with different patterns of arterial changes detected by USG. This means that FGF-23 may not have an impact on calcification patterns and atherosclerotic changes in arterial wall of lower extremities. Therefore, we may speculate that PAD in cases with DM has a more complex mechanism and its pathogenesis includes factors other than NP or NP-associated increased FGF-23.

In conclusion, NP and PAD may be independent complications of DM. Moreover, PAD in DM is a complex process involving additional mechanisms. In cases with DM, FGF-23 may be used as a marker of NP but not for PAD.

### Authorship Contributions

*Ethics Committee Approval: The study protocol was approved by the Local Ethics Committee of Cerrahpaşa Faculty of Medicine, Informed Consent: Consent form was filled out by all participants, Concept: Esra Hatipoğlu, Hasan İlkova, Design: Esra Hatipoğlu, Hasan İlkova, Atilla Süleyman Dikici, Fatih Kantarcı, Data Collection or Processing: Esra Hatipoğlu, Nurgül Özgür, Mutlu Niyazoğlu, Atilla Süleyman Dikici, Özlem Balcı Ekmekci, Serkan Yalın, Fatih Kantarcı, Analysis or Interpretation: Esra Hatipoğlu, Hasan İlkova, Zeynep Osar Siva, İsmail Mihmanlı, Literature Search: Esra Hatipoğlu, Nurgül Özgür, Mutlu Niyazoğlu, Writing: Esra Hatipoğlu, Peer-review: External and Internal peer-reviewed, Conflict of Interest: No conflict of interest was declared by the authors, Financial Disclosure: This research was supported by the Turkish Association for The Study of Diabetes and Obesity.*

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