Original article

The relationship between fibroblast growth factor 21 with biochemical parameters, anthropometric measurements and dietary intake in type 2 diabetic patients

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Abstract. *Background:* Fibroblast growth factor-21 **(**FGF-21), as a novel cytokine, plays a vital role in improving glucose and lipid metabolism via different metabolic pathways. Therefore, this study aims to investigate the relationship between FGF-21 and metabolic profile parameters in patients with type 2 diabetes mellitus (T2DM) compared to healthy controls. *Methods:* In this cross-sectional study, 85 patients with T2DM and 79 healthy subjects were recruited. Anthropometric measurements, dietary intake, and biochemical measurements were assessed for all participants. *Results:* Serum levels of FGF-21 were negatively associated with hip circumference (HC) (β= -0.267, p= 0.038) and dietary proteins (β = -0.273, p= 0.005) in healthy subjects. Moreover, dietary carbohydrate intake was inversely associated with FGF-21 (β= -0.183, p=0.041) in the total population. Also, serum levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and fasting blood sugar (FBS) in diabetic patients were significantly higher than those in healthy subjects (p<0.05). However, surprisingly, TG) and fasting insulin levels were significantly higher in healthy subjects (p<0.05). Tumor necrosis factor-α (TNF- α) was positively associated with LDL-C in healthy subjects and FBS and hemoglobin A1c (HbA1c) in all participants. Serum TNF-α levels had a significant positive association with body mass index (BMI) (β= 0.303, p= 0.004) and HC (β= 0.294, p= 0.012) in patients with T2DM. *Conclusions:* Our findings revealed that dietary intake of protein and carbohydrates were inversely associated with serum levels of FGF-21. Therefore, more detailed studies are needed to reach a robust conclusion.

Key words: type 2 diabetes mellitus; dietary intake; fgf-21; interleukin-6; tnf-α

Introduction

Diabetes is one of the most prevalent metabolic diseases, and its complications are the major cause of mortality in adults worldwide (1). It also imposes many costs on the healthcare systems (2). According to the American Diabetes Association (ADA), diabetes is defined as an increase in fasting blood sugar (FBS) ≥126 mg/dl and glycated hemoglobin A1c (HbA1c) ≥6.5% (3). Diabetes causes various complications and disabilities, such as cardiovascular disease, stroke, renal failure, and micro and macro-vascular events. Type 2 diabetes mellitus (T2DM), the most common form of diabetes, is recognized by insulin resistance (3). Excess body weight, physical inactivity, and poor nutrition are considered the most common risk factors for T2DM (1).

Fibroblast growth factor (FGF) belongs to a protein family comprising 23 members (4). This protein family has many biological functions, including cell

growth regulation and differentiation, metabolism, angiogenesis, and wound healing (5). FGF-21 is an important cytokine that the liver is the major site for its expression and function. It is also expressed in adipocytes and muscle tissue in response to exercise and nutritional factors (6,7). FGF-21 plays an important role in the improvement of glucose and lipid metabolism via different pathways (6). It has also been suggested that FGF-21 may play a pivotal role in the signaling of metabolic pathways in diabetes (8).

Elevated serum FGF-21 levels could be considered a predictor of T2DM (9). It has been shown that FGF-21 significantly improves insulin sensitivity in adults with obesity (10). Also, FGF-21 induces the expression of glucose transportes-1 (GLUT1) through the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and increases glucose uptake. On the other hand, evidence suggests a positive association between serum FGF21 level with serum triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and adipocyte-*fatty acid binding protein* levels (11,12). Given the controversial results on the role of FGF21 in metabolic regulation and insulin homeostasis (13-16); therefore, this study aimed to investigate the relationship between FGF-21 and metabolic profiles, dietary intake, and anthropometric measurements in patients with T2DM compared to healthy controls.

Methods

Eligibility criteria

The cross-sectional study was carried out on 164 subjects. All participants were enrolled through the installed announcement in healthcare and centers clinics

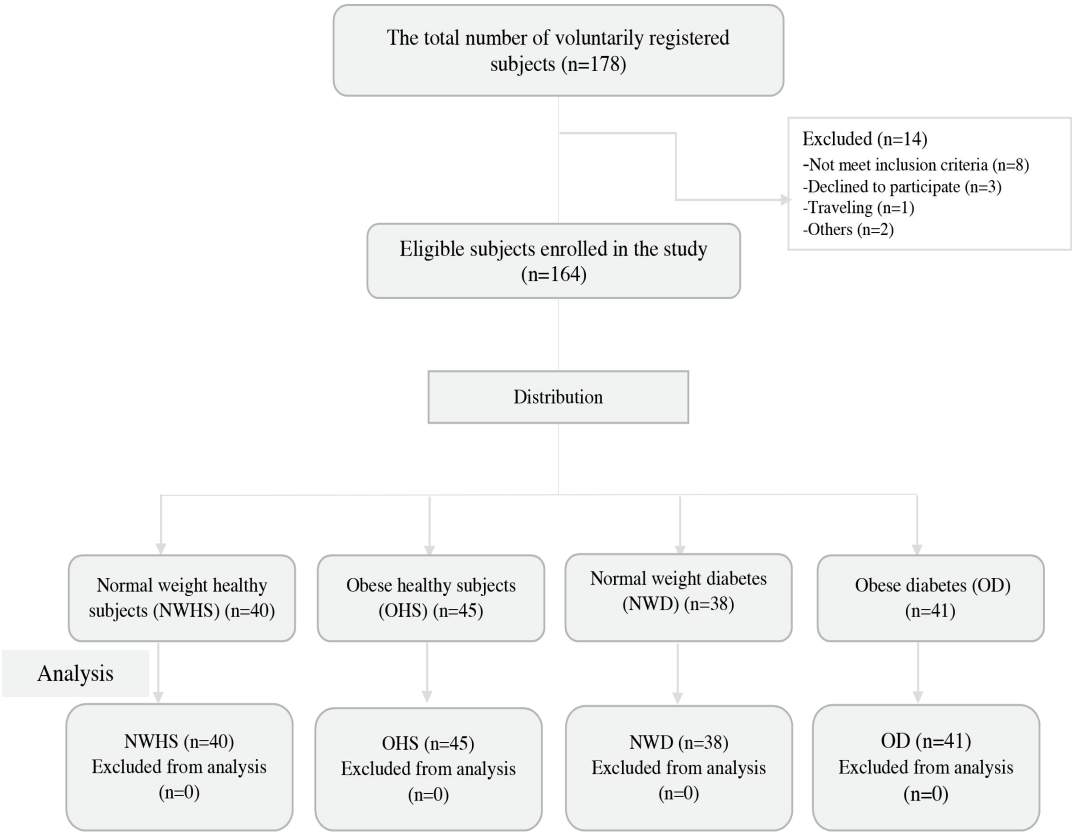

Figure 1. The flowchart of the study from baseline until the end of study.

of the Tabriz University of Medical Science. The Ethic Committee of Tabriz University of Medical Sciences approved the study protocol (No: IR.TBZMED.REC.1398.1253). Before the commencement of the study, written informed consent was obtained from all participants after a detailed explanation of the study design. According to inclusion criteria, eligible subjects (n=164) were randomly divided into four groups: normal-weight healthy subjects (NWHS), healthy subjects with obesity (OHS), normal-weight subjects with T2DM (NWD), and subjects with obesity and T2DM (OD). The subjects were included in the first two healthy groups [normal body mass index (BMI) (18.5-25 kg/m^2) or obesity (BMI ≥ 30 kg/m^2)] if they had no endocrine, gastrointestinal, renal, cardiovascular, rheumatic or autoimmune diseases, hematological disorders, and malignancies. The newly diagnosed patients with T2DM were the next two groups were referred to the Nutrition Research Center by the endocrinologist research collaborator. They were included in the same BMI category as the participants in the two healthy groups. Pregnancy, smoking, dietary supplements consumption (e.g. prebiotic & probiotic supplements, vitamins, minerals, and omega-3 fatty acids during last 3 months), taking chemical and herbal medications (except blood glucose-lowering drugs), and being on modified diet during the past month were considered the study exclusion criteria. Figure 1 shows the flowchart of the study from baseline until the end of study.

Anthropometric and demographic assessments

General characteristics of the patients, including gender, age, marital status, education level, occupation, a history of tobacco smoking, and medical history, were reordered by a standard questionnaire. A Seca™ wall-mounted stadiometer (with an accuracy of 0.1 cm) and a Seca™ portable scale (with an accuracy of 0.1 kg) was used for measuring both height and weight (in light clothing), respectively. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters) or Kg/m^2. Waist circumference (WC) was measured by an inelastic tape from the middle of the lowest rib and iliac crest. Hip circumference (HC) measurement was made using the same instrument in the largest part above the thigh. In a seated and relaxed position, the systolic (SBP) and diastolic (DBP) blood pressures were measured by a mercury sphygmomanometer twice ten minutes apart, and the average number was recorded as blood pressure. Participants' physical activity level was calculated using International Physical Activity Questionnaire (IPAQ) and reported at three levels (Low, medium, and high). Also, dietary intake (Calorie, Macronutrients, and Micronutrients) was assessed using a 3-day oral food record questionnaire (two ordinary days and one weekend) and analyzed using Nutritionist 4 software (Version7; N-squared computing, USA). According to the main variable of Gomez-Ambrosi et al study (17) (waist circumference, r =0.39), α=0.05 and power= 90%, the minumum sample size was calculated as 128 people (32 in each group) by using PASS software.

Biochemical analyses

Biochemical variables were measured, including serum levels of FGF-21, TNF-α, FBS, insulin, and lipid profile. 8-12 hours after overnight fasting, vein blood samples (5 ml) were obtained from all participants. The serum samples were centrifuged at 2500 rpm for 10 minutes (Beckman Avanti J-25; Beckman Coulter, Brea, CA, USA) at room temperature. TG, TC, and high density lipoprotein-cholesterol (HDL-C) serum levels were measured enzymatically using standard methods with an auto-analyzer SA1000. The Friedwald equation calculated LDL-C as: $LDL\text{-}C = TC - (HDL\text{-}C + \frac{TG}{5})$. Using commercial kit, the serum FGF-21 level was determined by enzyme-linked immunosorbent assay (ELISA) (Invitrogen Co Ltd., USA). The serum concentration of TNF-α was measured using commercial ELISA kits (Bioassay Technology Laboratory, Shanghi, Crystal Day Biotech Co Ltd., Shanghi, China). Serum FBS level was measured by the enzymatic colorimetric method using glucose oxidase (Pars Azmoon kit, Tehran, Iran) and HbA1c was analyzed by the latex immunoturbidimetric method. Insulin concentration was also assessed by the ELISA method (GmbH, Mannheim, Germany). A bioelectrical impedance

instrument assessed body fat percent, fat, and fat-free mass (TANITA, Japan). Also, the homeostatic model assessment for insulin resistance (HOMA-IR) and the quantitative insulin-sensitivity check index (QUICKI) were calculated using the following formulas:

- HOMA1-IR = (I× G)/450
- QUICKI = 1/ [log (I_0) + log (G_1)]
- I= fasting insulin (μU/ml), G= FBS (mg/dl)

Statistical analyses

Quantitative and qualitative variables were reported as mean and standard deviation (SD) or frequency, respectively. The Kolmogorov-Smirnov test was used to assay the normal distribution of variables. One-way analysis of variance (ANOVA) and the Tukey posthoc test was used to evaluate the between-group comparisons. Fisher's exact test was used to compare the categorical variables between four study groups. The Kruskal-Wallis test was used to compare non-normally distributed variables in groups. Moreover, linear regression was used to assess the association

Table 1. The demographic, anthropometric and dietary related variables across the study BMI sub-groups.

Variable		Groups				P
		NWHS (n= 40)	OHS (n= 45)	NWD (n= 38)	OD (n= 41)	
Age (year)		47.56± 5.10	46.82± 5.60	45.21± 7.07	45.17± 6.85	0.195
BMR (Kcal)		1711.11± 351.89	1832.84± 490.80	1631.86± 346.83	1790.51± 334.61	0.109
BMI (kg/m^2)		24.76± 2.01	33.23± 3.46*	24.45± 1.73	33.57± 4.14*	<0.001
WC (cm)		92.20± 9.18	106.87± 9.65*	89.29± 10.45	102.12± 8.78*	<0.001
HC (cm)		102.93± 6.82	115.78± 8.55*	103.53± 7.49	118.27± 10.74*	<0.001
Weight (kg)		69.44± 8.67	89.68± 13.18*	67.84± 8.63	86.65± 9.97*	<0.001
Height (cm)		167.33± 9.14	163.93± 9.10	166.55± 10.25	160.95± 7.57**	0.008
Body fat (%)		27.82± 9.01	27.10± 9.06	24.88± 8.62	33.80± 6.61**	<0.001
FM (kg)		24.44± 12.90	22.63± 10.86	18.80± 7.30	29.33± 8.00**	<0.001
FFM (kg)		60.21± 11.89	57.85± 8.45	54.20± 8.97	56.44± 9.09	0.052
Energy (Kcal)		2788.5± 1147.1	2732.1± 941.1	2772.4± 912.9	2836.8± 1014.6	0.972
Protein (gr)		105.66± 35.19	98.55± 36.28	96.54± 36.36	99.00± 36.72	0.953
CHO (gr)		457.03± 163.24	420.97± 161.24	423.88± 151.62	428.16±163.11	0.728
Fat (gr)		94.88± 36.79	83.41±30.33	86.92± 40.39	93.55± 39.76	0.437
Sex	Male	24 (60)	27 (60)	20 (52.6)	11 (26.8)	0.002
	Female	16 (40)	17 (40)	18 (47.4)	30 (73.2)	
Education	Primary	8 (20)	11 (24.4)	5 (13.2)	11 (26.8)	0.506
	High school	19 (47.5)	20 (44.4)	18 (47.4)	19 (46.3)	
	University	13 (32.5)	14 (31.1)	15 (39.5)	11 (26.8)	
PA	Low	10 (25)	9 (20)	11 (28.9)	10 (24.4)	0.493
	Medium	16 (40)	15 (33.33)	15 (39.5)	13 (31.7)	
	High	14 (35)	21 (46.7)	12 (31.6)	18 (43.9)	

Data analysis was done by ANOVA and Tukey Post-hoc test for quantitative variables and Chi-Square test for qualitative variables. Quantitative and qualitative data were summarized as mean± SD and frequency (percent), respectively. Abbreviations: NWHS: Normal Weight Healthy Subjects; OHS: Obese Healthy Subjects; NWD: Normal Weight Diabetics; OD: Obese Diabetics; BMI: Body Mass Index; BMR: Basal Metabolic Rate; WC: Waist Circumference; HC: Hip Circumference; FM: Fat Mass; FFM: Fat Free Mass; CHO: Carbohydrate; PA: Physical Activity. * P less than 0.05 vs. Normal weight groups; ** P less than 0.05 vs. all other groups.

Table 2. The differences in metabolic variables across the study BMI subgroups.

Variable	Groups				P
	NWHS (n= 40)	OHS (n= 45)	NWD (n= 38)	OD (n= 41)	
TC (mg/dl) ØØ	130.0 (103.75-189.75)[c]	130.0 (99.5-154.00)[c]	164.0 (136.0-198.0)	154.0 (126.5-186.0)[c]	**0.001**
TG (mg/dl) Ø	143.70± 70.49	147.82± 61.56	179.66± 39.24*	189.73± 47.92*	< **0.001**
LDL (mg/dl) ØØ	61.30 (37.50-99.75)	50.20 (26.1-89.00)[c]	78.6 (64.9-112.2)	85.7 (64.1-111.6)[c]	<**0.001**
HDL (mg/dl) ØØ	46.50 (39.00-58.25)	48.0 (42.0-55.5)	47.0 (41.0-54.0)	43.5 (39.25-51.75)	0.455
FBS (mg/dl) ØØ	84.50 (78.00-91.00)	83.0 (77.0-92.0)*	132.0 (115.5-168.5)	126.0 (99.0-152.25)	<**0.001**
Fasting insulin (pmol/L) ØØ	17.65 (10.97-24.40)	17.40- 10.10-20.70)[a]	11.6 (7.05-15.8)[a]	10.55 (7.15-15.07)[a]	<**0.001**
HbA1C (%)ØØ	5.00 (4.57-5.40)	4.90 (4.65-5.55)*	7.0 (6.5-8.0)	7.05 (6.50-7.95)	<**0.001**
HOMA-IR ØØ	3.55 (1.99-4.14)	3.11 (2.12-4.13)	3.61 (1.95-5.18)	2.99 (1.77-4.25)	0.725
QUICKI ØØ	0.28 (0.24-0.50)	0.32 (0.24-0.47)	0.27 (0.19- 0.51)	0.33 (0.23-0.56)	0.653

Data analysis was done by ANOVA and Tukey Post-hoc test or Kruskal-Wallis test. Data were summarized as mean± SD or Median (25th-75th) for normal Ø and non-normal ØØ distribution data, respectively. Abbreviations: NWHS: Normal Weight Healthy Subjects; OHS: Obese Healthy Subjects; NWD: Normal Weight Diabetics; OD: Obese Diabetics; TC: Total Cholesterol; TG: Triglyceride; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; FBS: Fasting Blood Sugar; HbA1C: Hemoglobin A1C; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; QUICKI: Quantitative insulin sensitivity check index.* P less than 0.05 vs. Healthy subgroups; a: P less than 0.05 vs. NWHS group; c: P less than 0.05 vs. NWD group.

of serum FGF-21 and TNF-α levels with lipid profile, and anthropometric and dietary variables. All statistical analyses were performed using SPSS software **(**SPSS Inc., Chicago, IL, USA). p-value less than 0.05 was considered statistically significant.

Results

79 healthy subjects and 85 patients with T2DM were enrolled in this study. Of the 79 healthy subjects and 85 patients with T2DM, 39.2 and 60 percent were males, respectively (p= 0.006). Dividing the study subjects into 4 sub-groups, including normal-weight healthy subjects (NWHS; n= 40), healthy subjects with obesity (OHS; n= 45), normal-weight subjects with T2DM (NWD; n= 38), and subjects with obesity and T2DM (OD; n= 41) revealed that body fat percent and fat mass in OD were significantly higher than those in other sub-groups (p<0.05). There was no significant difference between the study sub-groups in terms of age. Demographic, anthropometric, and dietary intakes -related variables are summarized in Table 1. Significant differences were observed between the study groups for age and gender (p<0.05). The mean age of healthy subjects and patients with T2DM was 45.19± 6.19 and 47.21± 5.35 years, respectively (p= 0.039).

Statistical analysis across the BMI subgroups showed that serum TC and LDL-C levels in NWD were lower than those in other groups. Also, TG and HbA1C levels were higher and lower in all patients with T2DM sub-groups than healthy ones (p<0.05). Serum levels of fasting insulin in NWHS were significantly lower than those in other BMI subgroups. Data regarding lipid profile and glycemic indices are summarized in Table 2. Serum levels of TC, LDL-C, and FBS in patients with T2DM were significantly higher than those in healthy subjects, While TG and fasting insulin were significantly higher in healthy subjects (p<0.05). There were no significant differences between the study groups regarding HDL-C, HOMA-IR, and QUICKI (p>0.05).

The serum levels of FGF-21 and TNF-α were illustrated in Figures 2 and 3, respectively.

Serum levels of FGF-21 in healthy subjects and patients were 239.29± 22.36 and 251.27± 21.91 pg/ml, respectively. There was no significant difference

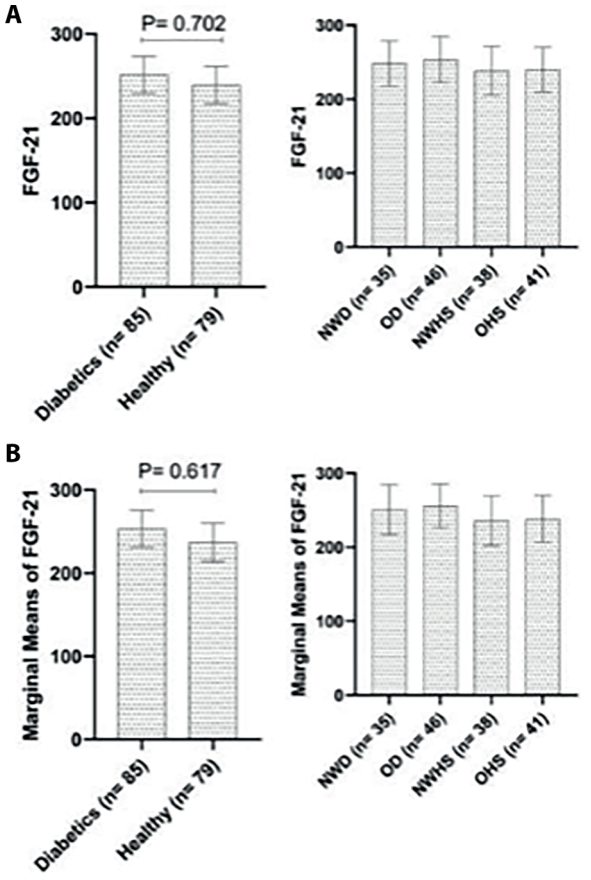

Figure 2. The serum levels of FGF-21 in diabetic patients and healthy people (n=164).

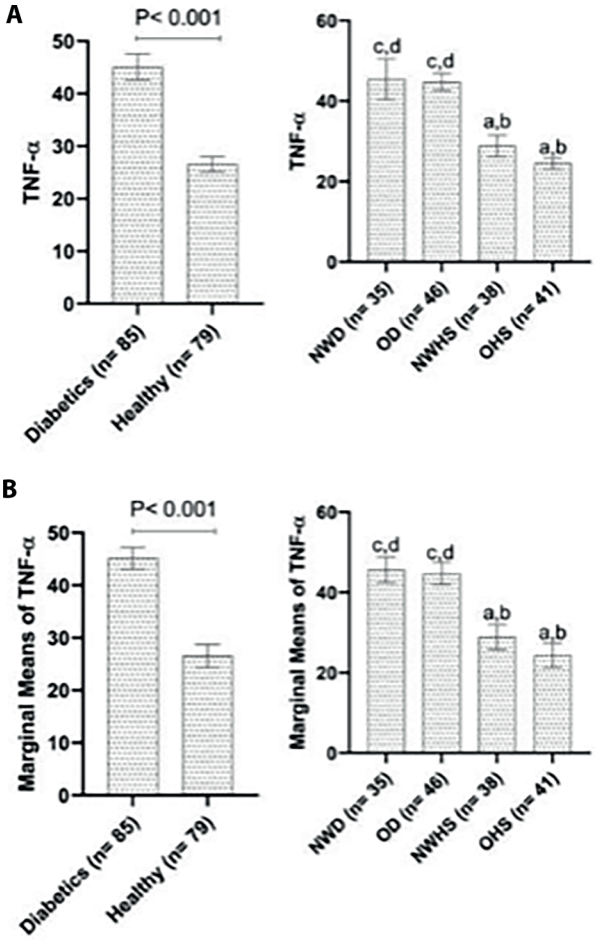

Figure 3. The serum levels of TNF-α in diabetic patients and healthy people (n=164).

between the groups in terms of FGF-21 (p= 0.702). The difference remained insignificant after adjustment for sex and age (p= 0.617). Serum levels of TNF-α were significantly higher in patients (p<0.001). After adjustment for sex and age, this difference remained significant (p<0.001).

The associations of lipid profile and glycemic indices with FGF-21 and TNF-α levels in healthy patients with T2DM are shown in Table 3. No significant association was seen between the mentioned variables and FGF-21. At the same time, TNF-α level was positively associated with LDL-C in healthy subjects (β= 0.281; P= 0.035) and FBS (β= 0.297; P< 0.001) and HbA1C levels (β= 0.305; P< 0.001) in the total population . Assessment of the associations across the BMI sub-groups revealed that the positive association of TNF-α with LDL-C was limited to OHS (β= 0.421; P= 0.039; Table 3).

The associations of anthropometric and dietary related variables with FGF-21 and TNF-α levels in healthy subjects and patients with T2DM are summarized in Table 4. The results showed that BMI (β= 0.303; P= 0.004) and HC levels (β= 0.294; P= 0.030) were significantly positively correlated with TNF-α level in patients with T2DM (p<0.05). On the other hand, HC level and dietary protein intake were negatively associated with FGF-21 levels in healthy subjects (β=-0.267; P= 0.038 and β= -0.265; P= 0.001, respectively) and the total population (β=-0.201; P= 0.012 and β= -0.273; P= 0.005, respectively). Also, the total

Table 3. The association of lipid profile and glycemic indices with FGF-21 and TNF-α across BMI sub-groups.

Variables		FGF-21					TNF-α				
		NWHS (n= 40)	OHS (n= 45)	NWD (n= 38)	OD (n= 41)	Total (n= 164)	NWHS (n= 40)	OHS (n= 45)	NWD (n= 38)	OD (n= 41)	Total (n= 164)
TC	β	-0.093	-0.119	*0.104*	-0.119	0.112	0.010	0.001	-0.097	-0.064	0.119
	P	0.415	0.277	*0.185*	0.277	0.561	0.902	0.990	0.387	0.562	0.097
TG	β	-0.140	0.065	*-0.147*	0.065	-0.127	0.025	-0.111	-0.110	0.018	-0.152
	P	0.219	0.555	*0.059*	0.555	0.278	0.750	0.330	0.315	0.868	0.032
LDL	β	-0.107	-0.117	*0.143*	-0.117	0.012	*0.238*	**0.421**	-0.088	-0.042	0.126
	P	0.349	0.287	*0.067*	0.287	0.638	*0.083*	**0.039**	0.423	0.700	0.081
HDL	β	-0.191	-0.093	*-0.090*	*0.039*	-0.137	*-0.104*	-0.099	0.129	-0.068	-0.134
	P	0.092	0.415	-0.081	*0.622*	0.560	*0.186*	0.383	0.257	0.539	0.461
FBS	β	-0.006	-0.140	0.459	*-0.104*	-0.064	*0.006*	0.099	0.057	0.162	**0.297**
	P	0.936	0.219	-0.032	*0.186*	0.449	*0.937*	0.387	0.619	0.138	<**0.001**
HbA1C	β	-0.016	-0.107	0.772	*0.006*	0.036	*-0.099*	0.099	0.055	0.106	**0.305**
	P	0.839	0.349	-0.118	*0.937*	0.821	*0.205*	0.386	0.628	0.334	<**0.001**
HOMA-IR	β	-0.028	-0.191	0.282	-0.108	-0.045	*-0.034*	0.081	*-0.022*	0.067	0.098
	P	0.726	0.092	0.078	0.344	0.571	*0.667*	0.461	*0.782*	0.544	0.341
QUICKI	β	-0.123	-0.149	0.128	-0.149	-0.109	-0.068	-0.095	*-0.016*	-0.143	-0.091
	P	0.118	0.189	0.940	0.189	0.701	0.539	0.387	*0.839*	0.192	0.281
Insulin	β	0.025	-0.171	0.086	-0.171	-0.023	0.162	0.152	*-0.099*	-0.002	-0.091
	P	0.750	0.132	0.435	0.132	0.781	0.138	0.165	*0.205*	0.989	0.291

Data analysis was done by Linear Regression. Abbreviations: TC: Total Cholesterol; TG: Triglyceride; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; FBS: Fasting Blood Sugar; HbA1C: Hemoglobin A1C; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index.

population had a negative association between dietary carbohydrate intake and FGF-21 level (β= -0.183; P= 0.041). After dividing the study subjects into BMI sub-groups, the association of the dietary protein with FGF-21 in NWHS (β= -0.342; P= 0.021) and BMI with TNF-α in OD (β= 0.670; P= 0.001) remained unchanged.

Discussion

FGF-21, an important and novel cytokine, is mainly expressed in the liver and plays a crucial role in improving glucose and lipid metabolism via different metabolic pathways (6). It has been suggested that FGF-21 may play a pivotal role in signaling metabolic pathways in diabetes (8). In the present study, results revealed no statistically significant difference in serum levels of FGF-21 in patients with T2DM compared to the healthy subjects; however, the FGF-21 levels were higher in T2DM subjects.

Panahi et al., in a case-control study, examined serum levels of FGF-21 in patients with diabetes (18). The results showed the serum levels of FGF-21 in patients with T2DM were higher than those in healthy subjects. In a similar study, Cheng et al. showed that serum levels of FGF-21 in newly-diagnosed and long-term diagnosed patients with T2DM were higher compared to healthy subjects. At the same time, the difference between the two diabetic groups was not considerable (19). FGF-21 is produced preferentially in hepatocytes and has been identified as an

Table 4. The association of dietary and anthropometric variables with FGF-21 and TNF-α across BMI sub-groups.

Variables		FGF-21					TNF-α				
		NWHS (n= 40)	OHS (n= 45)	NWD (n= 38)	OD (n= 41)	Total (n= 164)	NWHS (n= 40)	OHS (n= 45)	NWD (n= 38)	OD (n= 41)	Total (n= 164)
BMI	β	-0.055	-0.127	-0.135	0.067	-0.126	0.066	0.078	-0.071	**0.303**	0.067
	P	0.491	0.264	0.346	0.392	0.108	0.546	0.480	0.435	**0.004**	0.392
WC	β	0.024	-0.060	-0.112	0.146	-0.105	-0.106	0.074	-0.087	0.078	0.146
	P	0.983	0.598	0.412	0.062	0.180	0.334	0.499	0.231	0.943	0.062
HC	β	**-0.267**	-0.213	0.091	0.115	**-0.267**	0.078	0.025	-0.102	**0.294**	0.115
	P	**0.038**	0.067	0.843	0.141	**0.038**	0.480	0.820	0.321	**0.030**	0.141
Body fat	β	-0.076	0.099	0.137	-0.006	-0.006	0.074	0.050	0.231	-0.131	-0.038
	P	0.614	0.386	0.631	0.936	0.936	0.499	0.651	0.321	0.451	0.629
FM	β	*-0.104*	0.081	0.071	-0.016	-0.016	0.025	0.084	0.063	0.100	0.010
	P	*0.186*	0.461	0.238	0.839	0.839	0.820	0.447	0.713	0.124	0.902
FFM	β	*0.006*	-0.095	-0.091	-0.028	-0.028	-0.119	-0.024	0.092	0.0.92	0.025
	P	*0.937*	0.387	0.642	0.726	0.726	0.277	0.826	0.371	0.301	0.750
Energy	β	-0.108	0.152	0.163	-0.038	-0.123	0.065	0.282	*-0.003*	0.085	-0.011
	P	0.344	0.165	0.126	0.629	0.118	0.555	0.078	*0.741*	0.491	0.893
Protein	β	**-0.265**	-0.125	0.086	0.010	**-0.273**	-0.117	0.128	*-0.061*	-0.132	0.031
	P	**0.001**	0.081	0.741	0.902	**0.005**	0.287	0.940	*0.932*	0.147	0.695
CHO	β	-0.171	0.084	0.201	0.025	**-0.183**	0.050	0.086	*-0.142*	-0.004	0.021
	P	0.132	0.447	0.120	0.750	**0.041**	0.651	0.435	*0.300*	0.872	0.791
Fat	β	-0.038	-0.024	0.173	-0.123	-0.121	0.084	0.025	0.153	0.231	-0.077
	P	0.629	0.826	0.083	0.118	0.124	0.447	0.750	0.561	0.763	0.327

Data analysis was done by Linear Regression. Abbreviations: BMI: Body Mass Index; WC: Waist Circumference; HC: Hip Circumference; FM: Fat Mass; FFM: Fat Free Mass; CHO: Carbohydrate.

endocrine and metabolic hormone because of its potential effect on lipid and glucose metabolism, insulin sensitivity, and energy balance. The elevation in serum levels of FGF-21 is a compensatory factor for improving the hemodynamic status in patients with diabetes (20-22). It has been suggested that increased TNF-α levels in diabetes and inflammatory status lead to the disruption in glucose intake by cells (23). This situation causes insulin resistance in T2DM. In this situation, FGF-21 induces gene expression of inflammatory factors. This leads to glucose metabolism improvement in animal models (24). Also, FGF-21 ameliorates gene expression of various inflammatory factors, including C-reactive protein (CRP), interleukin 6 (IL-6), and IL-1β (23). In fact, FGF-21 simultaneously alleviates inflammatory situations and improves inflammation-induced insulin resistance.

In the present study, serum levels of TC, LDL-C, FBS, TG, and TNF-α were considerably higher in patients with T2DM compared to healthy subjects; however, serum insulin levels were significantly higher in the control group. The higher levels of TC, LDL-C, FBS, TG, and TNF-α in patients with T2DM are logical, as similar studies reported the same results (25-27). The interruption in macronutrient metabolism due to the lack of insulin and/or insulin action is the key mechanism for dyslipidemia, leading to elevated FBS and inflammation among these patients

(28, 29). However, the inflammatory status in T2DM is not directly associated with insulin resistance. In this regard, factors like body fat percentage have a more effective role in the inflammatory status of T2DM. However, inflammation, obesity, and T2DM are strongly interrelated disorders. The literature widely talked about dyslipidemia, inflammation, and glycemia (30). Discussing the achieved results regarding TG and insulin is so important. In the present study, serum levels of TG and insulin in healthy subjects were higher than those in patients with T2DM. It is worth noting that in our study, the enrolled patients were not newly diagnosed. The current study did not gather a detailed history of T2DM diagnosis and time to start medications. The medications used in T2DM patients and subclinical dyslipidemia in healthy subjects are the possible reason for the obtained results about serum TG in this study (31, 32). In the pathogenesis of diabetes, increased serum insulin in the early phases and reduced serum insulin in the late phases are proven (33). Therefore, it seems that the enrolled patients have had diabetes for a long time.

Some studies have been conducted recently to elucidate the association of FGF-21 serum levels with metabolic syndrome. Zhang et al., in a case-control study, revealed that the serum level of FGF-21 is directly associated with metabolic syndrome and its components (21). On the other hand, Lin et al. showed that serum FGF-21 level is positively associated with the severity of diabetic retinopathy. In contrast, our results showed no meaningful association between lipid profile and FGF-21 levels. Also, anthropometric variables had no considerable association with FGF-21 level, except for HC. Reinehr et al., in a prospective study, revealed a considerable correlation between serum levels of FGF-21 and HC in lean and obese children (34). To the best of our knowledge, no observational study assesses the association between dietary intake and serum FGF-21 levels. Our results showed the inverse correlation between dietary protein and carbohydrate intake and serum FGF-21 levels. Chalvo-Demersay et al., in an experimental study, revealed that low and high protein intake induced and reduced FGF-21 production in male C57BL/6 mice hepatocytes, respectively (35). On the other hand, Lundsgaard et al., in an interventional study, demonstrated that short-term over-feeding of carbohydrates increased serum levels of FGF-21 in healthy men (36). Therefore, more detailed studies will be conducted about dietary intake's effect on FGF-21 serum level and its gene expression in T2DM.

Some points need to be explained about the limitations of the present study. First of all, detailed history of T2DM diagnosis and time to start medications were not gathered; this point was our work's most important limitation. Also, the criteria for being healthy in the control group was based on self-report. For the enrollment of subjects in the control group, a more precise criterion needs to be established. Finally, the small sample size was another limitation of the present study, which could affect the results.

Conclusions

The results showed that dietary protein and carbohydrate intake was inversely correlated with serum levels of FGF-21. On the other hand, no significant difference was observed between healthy subjects and patients with T2DM in terms of FGF-21 levels. Considering the importance of FGF-21 in improving the hemodynamic status in diabetic patients, large-scale studies with more sample sizes and prospective designs need to confirm our findings in this regard.

Ethics Approval and Consent to Participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethic Committee of Tabriz University of Medical Sciences (No: IR.TBZMED.REC.1398.1253).

Consent for Publication: Written informed consent was obtained from all participants.

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests: The authors declare that they have no competing interests.

Authors Contributions: ZG and LP formulated the research questions and designed the study. EM, HR and MA carried it out the interviews and conducted all the biochemistry tests. LP analyzed the data and EM, HR and MA contributed to writing the article. All authors approved the final version of the manuscript.

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Abbreviations: ADA: American Diabetes Association; ANOVA: One-way analysis of variance; BMI: Body mass index; CRP: C-reactive protein; DBP: Diastolic blood pressure; ELISA: enzyme-linked immunosorbent assay; ERK1/2: Extracellular signal-regulated kinase ½; FBS: Fasting blood sugar; FGF-21: Fibroblast growth factor-21; GLUT1: Glucose transportes-1; HbA1c: Hemoglobin A1c; HC: Hip circumference; HDL-C: High density lipoprotein-cholesterol; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; IL: Interleukin; IPAQ: International Physical Activity Questionnaire; LDL-C: Low density lipoprotein-cholesterol; NIV: Nutritionist 4; NWD: Normal weight subject with T2DM; NWHS: normal weight healthy subjects; OHS: Healthy subjects with obesity; OD: Subjects with obesity and T2DM; QUICKI: Quantitative Insulin Sensitivity Check Index; SBP: Systolic blood pressure; SD: Standard Deviation; SPSS: Statistical Package for the Social Sciences; T2DM: Type 2 diabetes mellitus; TC: Total cholesterol; TG: Triglyceride; TNF- α: Tumor necrosis factor-α; WC: Waist circumference.

References

1. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018 Feb;14(2):88-98. doi: 10.1038/nrendo.2017.151.
2. American Diabetes Association. Economic Costs of Diabetes in the U.S. in 2017. Diabetes Care. 2018 May;41(5):917-928. doi: 10.2337/dci18-0007.
3. Diabetes Canada Clinical Practice Guidelines Expert Committee; Punthakee Z, Goldenberg R, Katz P. Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. Can J Diabetes. 2018 Apr;42 Suppl 1:S10-S15. doi: 10.1016/j.jcjd.2017.10.003.
4. Latko M, Czyrek A, Porębska N, Kucińska M, Otlewski J, Zakrzewska M, Opaliński Ł. Cross-Talk between Fibroblast Growth Factor Receptors and Other Cell Surface Proteins. Cells. 2019 May 14;8(5):455. doi: 10.3390/cells8050455.
5. Ornitz DM, Itoh N. The Fibroblast Growth Factor signaling pathway. Wiley Interdiscip Rev Dev Biol. 2015 May-Jun;4(3):215-66. doi: 10.1002/wdev.
6. Lin X, Liu YB, Hu H. Metabolic role of fibroblast growth factor 21 in liver, adipose and nervous system tissues. Biomed Rep. 2017 May;6(5):495-502. doi: 10.3892/br.2017.890.
7. Davoodi SH, Hajimiresmaiel SJ, Ajami M, et al. Caffeine treatment prevented from weight regain after calorie shifting diet induced weight loss. Iran J Pharm Res. 2014 Spring;13(2):707-18. PMID: 25237367.
8. Fisher FM, Maratos-Flier E. Understanding the Physiology of FGF21. Annu Rev Physiol. 2016;78:223-41. doi: 10.1146/annurev-physiol-021115-105339.
9. Woo YC, Lee CH, Fong CH, et al. Serum fibroblast growth factor 21 is a superior biomarker to other adipokines in predicting incident diabetes. Clin Endocrinol (Oxf). 2017 Jan;86(1):37-43. doi: 10.1111/cen.13229.
10. Li H, Wu G, Fang Q, et al. Fibroblast growth factor 21 increases insulin sensitivity through specific expansion of subcutaneous fat. Nat Commun. 2018 Jan 18;9(1):272. doi: 10.1038/s41467-017-02677-9.
11. Novotny D, Vaverkova H, Karasek D, et al. Evaluation of total adiponectin, adipocyte fatty acid binding protein and fibroblast growth factor 21 levels in individuals with metabolic syndrome. Physiol Res. 2014;63(2):219-28. doi: 10.33549/physiolres.932602.
12. Gao RY, Hsu BG, Wu DA, Hou JS, Chen MC. Serum Fibroblast Growth Factor 21 Levels Are Positively Associated with Metabolic Syndrome in Patients with Type 2 Diabetes. Int J Endocrinol. 2019 Sep 10;2019:5163245. doi: 10.1155/2019/5163245.
13. Salminen A, Kauppinen A, Kaarniranta K. FGF21 activates AMPK signaling: impact on metabolic regulation and the aging process. J Mol Med (Berl). 2017 Feb;95(2):123-131. doi: 10.1007/s00109-016-1477-1.
14. Strowski MZ. Impact of FGF21 on glycemic control. Horm Mol Biol Clin Investig. 2017 Jun 3;30(2): /j/hmbci.2017.30.issue-2/hmbci-2017-0001/hmbci-2017-0001.xml. doi: 10.1515/hmbci-2017-0001.
15. Geng L, Lam KSL, Xu A. The therapeutic potential of FGF21 in metabolic diseases: from bench to clinic. Nat Rev Endocrinol. 2020 Nov;16(11):654-667. doi: 10.1038/s41574-020-0386-0.
16. Ayatollahi SA, Ajami M, Reyhanfard H, Asadi Y, Nassiri-Kashani M, Rashighi Firoozabadi M, Davoodi SH, Habibi E, Pazoki-Toroudi H. BCL-2 and Bax Expression in Skin Flaps Treated with Finasteride or Azelaic Acid. Iran J Pharm Res. 2012 Fall;11(4):1285-90. PMID: 24250563.
17. Gómez-Ambrosi J, Pastor C, Salvador J, et al. Influence of waist circumference on the metabolic risk associated with impaired fasting glucose: effect of weight loss after gastric bypass. Obes Surg. 2007 May;17(5):585-91. doi:

10.1007/s11695-007-9101-7. Erratum in: Obes Surg. 2007 Jul;17(7):996.
18. Panahi Y, Bonakdaran S, Yaghoubi MA, Keramati MR, Haratian M, Sahebkar A. Serum levels of fibroblast growth factor 21 in type 2 diabetic patients. Acta Endocrinol (Buchar). 2016 Jul-Sep;12(3):257-261. doi: 10.4183/aeb.2016.257.
19. Cheng X, Zhu B, Jiang F, Fan H. Serum FGF-21 levels in type 2 diabetic patients. Endocr Res. 2011;36(4):142-8. doi: 10.3109/07435800.2011.558550.
20. Jin QR, Bando Y, Miyawaki K, et al. Correlation of fibroblast growth factor 21 serum levels with metabolic parameters in Japanese subjects. J Med Invest. 2014;61(1-2):28-34. doi: 10.2152/jmi.61.28.
21. Zhang X, Yeung DC, Karpisek M, et al. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes. 2008 May;57(5):1246-53. doi: 10.2337/db07-1476. Epub 2008 Feb 5. Erratum in: Diabetes. 2019 Jan;68(1):235.
22. Zhang X, Yang L, Xu X, et al. A review of fibroblast growth factor 21 in diabetic cardiomyopathy. Heart Fail Rev. 2019 Nov;24(6):1005-1017. doi: 10.1007/s10741-019-09809-x.
23. Wang N, Xu TY, Zhang X, et al. Improving hyperglycemic effect of FGF-21 is associated with alleviating inflammatory state in diabetes. Int Immunopharmacol. 2018 Mar;56: 301-309. doi: 10.1016/j.intimp.2018.01.048.
24. Lee MS, Choi SE, Ha ES, et al. Fibroblast growth factor-21 protects human skeletal muscle myotubes from palmitate-induced insulin resistance by inhibiting stress kinase and NF-κB. Metabolism. 2012 Aug;61(8):1142-51. doi: 10.1016/j.metabol.2012.01.012.
25. Bhowmik B, Siddiquee T, Mujumder A, et al. Serum lipid profile and its association with diabetes and prediabetes in a rural Bangladeshi population. Int J Environ Res Public Health. 2018 Sep 6;15(9):1944. doi: 10.3390 /ijerph15091944.
26. Massing MW, Henley NS, Carter-Edwards L, Schenck AP, Simpson RJ Jr. Lipid testing among patients with diabetes who receive diabetes care from primary care physicians. Diabetes Care. 2003 May;26(5):1369-73. doi: 10.2337 /diacare.26.5.1369.
27. Jabbari M, Barati M, Fathollahi A, et al. Can oral tolerance explain the inconsistencies associated with total dietary diversity and colon cancer? A mechanistic systematic review. Nutr Cancer. 2021;73(11-12):2101-2112. doi: 10.1080/01635581.2020.1819349.
28. Eller-Vainicher C, Cairoli E, Grassi G, et al. Pathophysiology and management of type 2 diabetes mellitus bone fragility. J Diabetes Res. 2020 May 22;2020:7608964. doi: 10.1155/2020/7608964.
29. Barati M, Jabbari M, Nickho H, et al. Regulatory T cells in bioactive peptides-induced oral tolerance; a two-edged sword related to the risk of chronic diseases: a systematic review. Nutr Cancer. 2021;73(6):956-967. doi: 10.1080/01635581.2020.1784442.
30. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res Clin Pract. 2014 Aug;105(2):141-50. doi: 10.1016/j.diabres.2014.04.006.
31. Nimkuntod P, Tongdee P. Association between subclinical atherosclerosis among hyperlipidemia and healthy subjects. J Med Assoc Thai. 2015 May;98 Suppl 4:S51-7. PMID: 26201134.
32. Augustemak de Lima LR, Petroski EL, Moreno YMF, et al. Dyslipidemia, chronic inflammation, and subclinical atherosclerosis in children and adolescents infected with HIV: The PositHIVe Health Study. PLoS One. 2018 Jan 10;13(1):e0190785. doi: 10.1371/journal. pone.0190785.
33. Zaccardi F, Webb DR, Yates T, Davies MJ. Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective. Postgrad Med J. 2016 Feb;92(1084):63-9. doi: 10.1136/ postgradmedj-2015-133281.
34. Reinehr T, Woelfle J, Wunsch R, Roth CL. Fibroblast growth factor 21 (FGF-21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: a longitudinal analysis. J Clin Endocrinol Metab. 2012 Jun;97(6):2143-50. doi: 10.1210/jc.2012-1221.
35. Chalvon-Demersay T, Even PC, Tomé D, et al. Low-protein diet induces, whereas high-protein diet reduces hepatic FGF21 production in mice, but glucose and not amino acids up-regulate FGF21 in cultured hepatocytes. J Nutr Biochem. 2016 Oct;36:60-67. doi: 10.1016/j.jnutbio.2016.07.002.
36. Lundsgaard AM, Fritzen AM, Sjøberg KA, et al. Circulating FGF21 in humans is potently induced by short term overfeeding of carbohydrates. Mol Metab. 2016 Nov 16;6(1):22-29. doi: 10.1016/j.molmet.2016.11.001.

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