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Serum vascular endothelial growth factor level is elevated in patients with impaired glucose tolerance and type 2 diabetes mellitus

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Abstract

Objective: This study was performed to investigate the serum vascular endothelial growth factor (VEGF) levels in Chinese patients with impaired glucose tolerance (IGT) and newly diagnosed type 2 diabetes mellitus (T2DM).

Methods: A total of 189 subjects (41 controls, 40 patients with IGT, and 108 patients with newly diagnosed T2DM) were recruited. Serum VEGF levels were determined by ELISA; other metabolic parameters were assessed by standard laboratory methods.

Results: There were significant differences in serum VEGF levels among the T2DM, IGT, and control groups (T2DM vs. controls: 72.00 [45.40, 98.35] pg/mL vs. 53.10 [36.30, 116.25] pg/mL; IGT vs. controls: 78.17 [55.52, 137.25] pg/mL vs. 53.10 [36.30, 116.25] pg/mL). Moreover, serum VEGF levels were positively associated with the homeostasis model assessment for insulin resistance (HOMA-IR) value. Multiple linear regression analysis indicated that the HOMA-IR value was an independent risk factor for elevated serum VEGF level.

Conclusions: Both IGT and T2DM patients exhibited increased serum VEGF levels, compared with controls; increased serum VEGF level was positively associated with the HOMA-IR value. Therefore, the increased serum VEGF level might partially result from increased insulin resistance in these patients.

Keywords

Type 2 diabetes mellitus, impaired glucose tolerance, vascular endothelial growth factor, insulin resistance, cardiovascular risk, hyperinsulinism, regression analysis, homeostasis

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Introduction

Vascular endothelial growth factor (VEGF) is a growth factor that induces angiogenesis in vascular endothelial cells.[1] In addition, VEGF is a key element in the pathogenesis of cardiovascular diseases (CVDs),[2] and is regarded as a predictor of cardiovascular risk.[3] CVD is the principal cause of disability and death among patients with diabetes mellitus, which exacerbates atherosclerosis. Several studies have demonstrated that serum VEGF levels were elevated in patients with diabetes complications.[4,5] However, to the best of our knowledge, there have been no assessments of the VEGF level involved in impaired glucose tolerance (IGT) or the relationship between VEGF and insulin resistance in Chinese populations. Therefore, our study investigated serum VEGF levels in a Chinese population with newly diagnosed type 2 diabetes mellitus (T2DM) or IGT to elucidate the relationship between serum VEGF and insulin resistance.

Methods

Subjects

Subjects were enrolled from February 2016 to July 2018 by the Department of Endocrinology at Beijing Chao Yang Hospital. An oral glucose tolerance test was performed by administering 75 g of glucose in the morning after overnight fasting. During the oral glucose tolerance test, blood samples were obtained at 0 and 120 minutes. The exclusion criteria were pregnancy or possible pregnancy, CVD, liver and renal function impairment, thyroid disease, infectious disease, cancer, and/or systemic inflammatory diseases. All subjects had received no diabetes medication and/or had not undergone diet therapy before the present study. None of the subjects had been using agents known to influence glucose or lipid metabolism. All subjects received written information about the study and provided written informed consent to participate. The study was approved by the ethics committee of Chao-yang Hospital in Beijing, China, and the study protocol was designed in accordance with the guidelines of the Declaration of Helsinki.

Data collection and laboratory tests

All subjects underwent clinical assessments of age, sex, height, and weight. The total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), uric acid (UA), and homocysteine (HCY) were enzymatically determined (Dade Behring Diagnostics, Deerfield, IL, USA). Fasting serum insulin (FINS) was measured using a chemiluminescence immunoassay with an Access 2 immunoassay system (reference interval: 1.9–23 mIU/mL; Beckman Coulter, Inc., Brea, CA, USA). Serum VEGF levels were determined by ELISA, in accordance with the manufacturer's protocols (R&D Systems, Minneapolis, MN, USA). HbA1c was measured by the HLC-723G7 analyzer (reference interval: 4%–6%; Tosoh Corporation, Tokyo, Japan). Body mass index (BMI) was calculated as weight (kg) divided by the height squared (m^2). The homeostasis model assessment index of insulin resistance (HOMA-IR) and β-cell function (HOMA-β) were calculated using the following equation: HOMA-IR = (fasting insulin × fasting plasma glucose)/22.5; HOMA-β = 20 × fasting insulin/(fasting plasma glucose–3.5).[6]

Statistical analyses. Data were analyzed using IBM SPSS Statistics, version 19.0 (IBM Corp., Armonk, NY, USA). Continuous data (e.g., BMI, age, fasting blood glucose [FBG], HbA1C, TC, LDL-C, and HDL-C), are expressed as mean ± standard deviation. Because some data (i.e., TG, FINS, HOMA-IR, HOMA-β, and serum VEGF level) were

not normally distributed, the values are expressed as median (interquartile range). Variables that were not normally distributed were log-transformed before statistical analysis. Comparisons among groups were performed using analysis of variance with post hoc least significant difference test. Associations between the serum VEGF level and other parameters were examined using Pearson's or Spearman's rank correlation analyses and multiple linear regression analysis. Two-tailed $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics and metabolic parameters of control, IGT, and T2DM subjects

A total of 189 subjects were enrolled in the study. Based on the 2013 American Diabetes Association diagnostic criteria,[7] our study included 108 individuals with newly diagnosed T2DM, 40 individuals with IGT, and 41 individuals with normal glycemic tolerance (controls). The characteristics and parameters of the three groups are summarized in Table 1. The T2DM, IGT, and control groups showed no differences in sex, age, or BMI. The serum levels of HOMA-IR value, FBG, and HbA1c were significantly higher in the T2DM group than in the control group. The HbA1c level and HOMA-IR value were significantly higher in the IGT group than in the control group. Compared with the value in the T2DM group, serum HOMA-β values were significantly higher in the control and IGT groups. Compared with the level in the control group, median serum VEGF levels were significantly higher in the T2DM and IGT groups (T2DM vs. controls: 72.00 [45.40, 98.35] pg/mL vs. 53.10 [36.30, 116.25] pg/mL; $P < 0.05$; IGT vs.

Table 1. Characteristics and metabolic parameters of the study subjects.

	Control (n = 41)	IGT (n = 40)	T2DM (n = 108)
Sex (male/female)	19/22	20/20	68/40
Age	51.6 ± 11.03	50.8 ± 13.33	50.8 ± 11.80
BMI, kg/m^2	24.99 ± 3.85	25.22 ± 3.57	27.20 ± 4.69
FBG, mmol/L	4.95 ± 0.49	5.91 ± 0.88	8.74 ± 3.40*
HbA1c, %	5.22 ± 0.45$^{\#}$	5.98 ± 0.39*	8.06 ± 2.00*$^{\#}$
TG, mmol/L	1.26 [0.91, 1.71]	1.60 [1.18, 1.91]	1.58 [1.12, 2.49]
TC, mmol/L	4.75 ± 0.86	5.09 ± 0.97	4.86 ± 0.93
LDL-C, mmol/L	2.93 ± 0.89	3.08 ± 0.91	2.86 ± 0.80
HDL-C, mmol/L	1.25 ± 0.27	1.24 ± 0.27	1.16 ± 0.24
UA, μmol/L	338.77 ± 78.28	361.69 ± 91.22	345.62 ± 85.96
HCY, μmol/L	14.72 ± 5.30	14.71 ± 7.38	14.42 ± 5.66
FINS, μU/mL	8.6 [5.05, 12.30]	9.30 [6.48, 15.38]	9.9 [6.50, 15.90]
HOMA-IR	1.64 [1.08, 2.49]$^{\#}$	2.50 [1.67, 3.97]*	3.55 [2.36, 5.69]*$^{\#}$
HOMA-β	72.32 [56.50, 140.63]	80.52 [60.96, 134.90]	46.43 [27.33, 46.33]*$^{\#}$
VEGF, pg/mL	53.10 [36.30, 116.25]$^{\#}$	78.17 [55.52, 137.25]*	72.00 [45.40, 98.35]*

Data are mean ± standard deviation, unless indicated otherwise. FINS, HOMA-IR, and VEGF are shown as median [upper limit of the lowest quartile and lower limit of the highest quartile].

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; UA, uric acid; HCY, homocysteine; FINS, fasting serum insulin; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment of β-cell function; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus; VEGF, vascular endothelial growth factor. *$P<0.05$ vs. control group. $^{\#}P<0.05$ vs. IGT group.

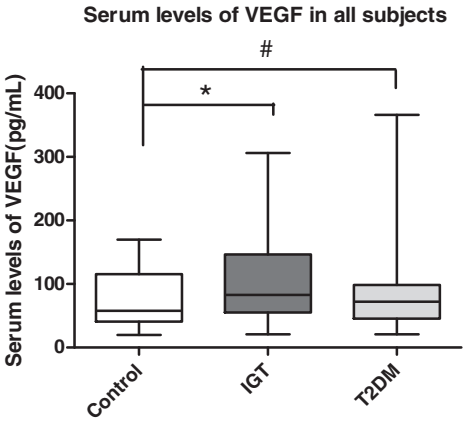

Figure 1. Serum VEGF levels in patients with IGT (40), NGT (41), and newly diagnosed T2DM (108). The serum VEGF level was significantly higher in the IGT group than in the control group ($P < 0.05$), and the serum VEGF level was significantly higher in the T2DM group than in the control group ($P < 0.05$). Abbreviations: VEGF, vascular endothelial growth factor; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus; NGT, normal glycemic tolerance. *: IGT vs. controls, $P < 0.05$, #: T2DM vs. controls, $P < 0.05$.

controls: 78.17 [55.52, 137.25] pg/mL vs. 53.10 [36.30, 116.25] pg/mL; $P < 0.05$) (Figure 1).

Correlations between VEGF and other variables

We assessed the relationships between serum VEGF levels and other variables in all individuals and found that the serum VEGF level was positively associated with the HOMA-IR value ($r = 0.306$, $P < 0.05$) (Table 2). However, we found no significant relationships between VEGF and any of the following factors: sex, age, BMI, HbA1c, FBG, TG, TC, HDL-C, LDL-C, UA, or HCY. The relationships between serum VEGF levels and metabolic parameters were explored within each group. In the T2DM and IGT groups, the serum VEGF level was positively associated with the HOMA-IR value ($r = 0.317$ and 0.579, $P < 0.05$), whereas no significant relationships were found between VEGF and any of the following factors: sex, age, BMI, HbA1c, FBG, TG, TC, HDL-C, LDL-C, UA, or HCY. In the control group, no significant relationships were found between VEGF and any of the following factors: HOMA-IR value, sex, age, BMI, HbA1c, FBG, TG, TC, HDL-C, LDL-C, UA, or HCY.

Table 2. Multivariate linear regression analyses of the relationships between serum VEGF and other characteristics of all study subjects.

Parameters	β	t	*P* value
Age	0.003	−0.026	0.979
Sex	−0.011	−0.096	0.924
BMI, kg/m^2	0.119	1.117	0.245
FBG, μmol/L	−0.07	−0.450	0.654
HbA1c, %	0.61	0.413	0.680
TC, mmol/L	−0.171	−0.404	0.687
HDL-C, mmol/L	0.128	0.833	0.407
TG, mmol/L	0.122	0.438	0.663
LDL-C, mmol/L	0.034	0.096	0.923
UA, μmol/L	0.029	−0.272	0.786
HCY, μmol/L	0.006	0.048	0.901
LgHOMA-IR	0.306	2.662	0.009*

The listed parameters were included in multiple linear regression analysis.
Abbreviations: IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus; BMI, body mass index; FBG, fasting blood glucose; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; HCY, homocysteine; LgHOMA-IR, log transformation of homeostasis model assessment for insulin resistance; VEGF, vascular endothelial growth factor. *$P < 0.05$.

Multivariate linear regression analyses of factors associated with VEGF levels

After adjusting for changes in sex, BMI, age, HbA1c, TG, TC, HDL-C, LDL-C, UA, and HCY, the HOMA-IR value was the only significant predictor of an increased serum VEGF level in all subjects ($\beta = 0.306$, $P < 0.05$) (Table 2), particularly

Table 3. Multivariate linear regression analyses of the relationships between serum VEGF and other characteristics in the control, IGT, and T2DM groups.

	Control			IGT			T2DM		
Parameters	β	t	P value	β	t	P value	β	t	P value
Age	−0.154	−0.452	0.063	0.253	0.744	0.472	−0.221	−1.263	0.213
Sex	0.798	−1.039	0.329	0.239	0.726	0.483	0.096	0.526	0.601
BMI, kg/m^2	0.094	0.261	0.801	−0.011	−0.040	0.969	0.081	0.495	0.623
FBG, μmol/L	−0.223	−0.584	0.575	0.031	0.082	0.936	−0.011	−0.107	0.915
HbA1c, %	0.422	0.974	0.358	−0.022	−0.094	0.927	0.014	0.075	0.940
TC, mmol/L	0.204	0.097	0.925	−0.073	−0.055	0.957	−0.115	−0.197	0.845
HDL-C, mmol/L	0.552	0.805	0.444	−0.348	−0.699	0.499	0.143	0.676	0.502
TG, mmol/L	0.502	1.253	0.282	−0.063	−0.073	0.943	0.076	0.172	0.864
LDL-C, mmol/L	−0.610	−0.341	0.742	−0.232	−0.206	0.840	0.057	0.120	0.905
UA, μmol/L	0.349	0.511	0.623	−0.120	−0.338	0.741	−0.032	−0.212	0.833
HCY, μmol/L	0.201	0.360	0.728	0.148	0.371	0.718	−0.037	−0.242	0.810
LgHOMA-IR	−0.422	−1.039	0.329	0.745	2.498	0.030*	0.342	2.054	0.046*

Parameters were included in multiple linear regression analysis.

Abbreviations: IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus; BMI, body mass index; FBG, fasting blood glucose; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; HCY, homocysteine; LgHOMA-IR, log transformation of homeostasis model assessment for insulin resistance; VEGF, vascular endothelial growth factor. *$P<0.05$

in the T2DM and IGT groups ($\beta = 0.342$ and 0.745, $P < 0.05$) (Table 3).

Discussion

T2DM patients are at high risk of CVD, but it is unclear whether this risk is present for prediabetic patients. The present study is the first to show that serum VEGF levels were increased in both IGT and T2DM patients in a Chinese population. Furthermore, the serum VEGF level was positively associated with the HOMA-IR value. Multiple linear regression analysis revealed that the HOMA-IR was a significant predictor of increased serum VEGF levels in all subjects.

VEGF is a growth factor that can induce angiogenesis in vascular endothelial cells, and is a significant regulator of angiogenesis in both physiological and pathological conditions.[8] VEGF is a key factor in the maintenance of normal endothelial function under physiological conditions; however, abnormally high VEGF concentrations will cause aberrant angiogenesis.[9] Several studies have demonstrated that serum levels of VEGF were higher in patients with coronary artery disease and peripheral vascular disease, compared with normal subjects.[10–13] Additionally, in patients with acute myocardial infarction, serum VEGF levels were significantly higher than those in normal subjects; however, these levels were reduced after heparin administration or percutaneous coronary intervention.[14,15] Data from a study by Zou et al.[16] showed that increased spontaneous production of VEGF-A may induce angiogenesis after acute myocardial infarction by initiating the production of reactive oxygen species and inducing the endoplasmic reticulum stress–autophagy axis in vascular endothelial cells. Data from a study by Celletti et al.[17] demonstrated that recombinant human VEGF could increase atherosclerotic plaque formation and progression. VEGF has been regarded

as a predictor of cardiovascular risk: serum VEGF levels correlated significantly with cerebrovascular accident risk scores and 10-year CVD risk.[3] Our study found that the serum VEGF levels of the T2DM group were significantly higher than those of controls; moreover, serum VEGF levels were higher in IGT patients. Consistent with these findings, a recent study in Finland showed that serum VEGF levels were significantly higher in subjects with prediabetes and diabetes than in normoglycemic subjects.[18] Therefore, we presume that diabetes patients and IGT patients both carry significant cardiovascular risks.

In our study, the serum VEGF level was positively associated with the HOMA-IR value. Multiple linear regression analysis found that the HOMA-IR value was the only significant predictor of increased serum VEGF levels. Insulin resistance is significantly associated with CVD and endothelial dysfunction. Furthermore, insulin has an important role in vasodilatation in the endothelium through the activation of endothelial nitric oxide synthase (eNOS) and subsequent production of nitric oxide (NO).[19] Our previous study revealed that insulin resistance induced endothelial dysfunction through reduced expression of eNOS and impaired NO production.[20] Changes in the production of VEGF by activated proinflammatory cells are related to environmental/ambient oxidative stress.[21] VEGF is reportedly regulated by insulin, inflammatory cytokines, and peroxisome proliferator-activated receptor γ activators.[22,23] Insulin regulates expression of the *VEGF* gene and myocardial vascularization through the activation of the insulin receptor in the PI3K/Akt pathway. When this pathway is selectively inhibited, myocardial VEGF expression and capillary density are reduced in patients with insulin resistance.[24] Several studies have shown that hyperinsulinemia and insulin resistance are independent predictors of ischemic heart disease.[25,26] Kaess et al.[27] reported that the relationship between the risk of CVD events and circulating levels of VEGF exhibits an inverted U shape. In addition, a higher VEGF level is presumed to serve as a compensatory response for atherosclerosis-related ischemia through the promotion of neovascularization. Oduk et al.[28] showed that the administration of VEGF nanoparticles could significantly improve cardiac performance, angiogenesis, infarct size, wall thickness, and myocardial hypertrophy. Notably, VEGF is regarded as an important factor in the neovascularization of proliferative diabetic retinopathy.[4] Clinicians now use intravitreal injections of anti-VEGF biologics to reduce VEGF signaling in the retina.[29,30] In patients with diabetes, circulating VEGF may become an important marker for prediction of the severity of diabetic renal disease;[31] in particular, VEGF-A is reportedly significantly higher in patients who have both diabetes and end-stage renal disease.[5]

Insufficient insulin bioavailability and action in diabetes may trigger VEGF release and neovascularization.[32] Furthermore, overexpression of VEGF could cause a lack of insulin sensitivity.[33] Our study results showed that the serum levels of VEGF in the T2DM and IGT groups were significantly higher than the level in the control group; in addition, the serum level of VEGF was higher in the IGT group than in the T2DM group, but the levels were not significantly different between the two groups. We speculate that the increase in serum VEGF level during the early stage of diabetes might have beneficial compensatory effects in T2DM and IGT subjects.

Our study had three limitations. First, it was a cross-sectional study, so it could not demonstrate a causal relationship between insulin resistance and VEGF level. Second, it was a small-scale study and might have involved sampling errors

that affected the results. Larger clinical trials and animal studies are needed to confirm our findings. Finally, our study only included Chinese individuals; therefore, our research results might not be generalizable to other ethnic groups.

Conclusions

Serum VEGF levels were increased in both IGT and T2DM patients in a Chinese population, and were positively associated with the HOMA-IR values in these patients. Increased insulin resistance might partially explain increased VEGF levels in IGT and T2DM patients, relative to controls.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Leung DW, Cachianes G, Kuang WJ, et al. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989; 246: 1306–1309.
2. Clarkin CE, Mahmoud M, Liu B, et al. Modulation of endoglin expression in islets of langerhans by VEGF reveals a novel regulator of islet endothelial cell function. *BMC Res Notes* 2016; 9: 362.
3. Felmeden DC, Spencer CG, Belgore FM, et al. Endothelial damage and angiogenesis in hypertensive patients: relationship to cardiovascular risk factors and risk factor management. *Am J Hypertension* 2003; 16: 11–20.
4. Witmer AN, Vrensen GF, Van Noorden CJ, et al. Vascular endothelial growth factors and angiogenesis in eye disease. *Progr Retinal Eye Res* 2003; 22: 1–29.
5. Treweeke A, Hall J, Lambie S, et al. Preliminary study of hypoxia-related cardiovascular mediator-markers in patients with end-stage renal disease with and without diabetes and the effects of haemodialysis. *PLoS One* 2017; 12: e0178171.
6. Bermudez V, Cano R, Cano C, et al. Homeostasis model assessment (HOMA) as surrogate insulinization criteria inpatients with type 2 diabetes. *Am J Ther* 2008; 15: 409–416.
7. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2013; 36: S67–S74.
8. Ferrara N, Gerber HP and Le Couter J. The biology of VEGF and its receptors. *Nat Med* 2003; 9: 669–676.
9. Laakkonen JP, Lahteenvuo J, Jauhiainen S, et al. Beyond endothelial cells: vascular endothelial growth factors in heart, vascular anomalies and placenta. *Vasc Pharmacol* 2019; 112: 91–101.
10. Blann AD, Belgore FM, McCollum CN, et al. Vascular endothelial growth factor and its receptor, Flt-1, in the plasma of patients with coronary or peripheral atherosclerosis, or type II diabetes. *Clin Sci (Lond)* 2002; 102: 187–194.
11. Ogawa H, Suefuji H, Soejima H, et al. Increased blood vascular endothelial growth factor levels in patients with acute myocardial infarction. *Cardiology* 2000; 93: 93–99.
12. Roller RE, Renner W, Dorr A, et al. Oxidative stress and increase of vascular endothelial growth factor in plasma of

patients with peripheral arterial occlusive disease. *Thromb Haemost* 2001; 85: 368.
13. Soeki T, Tamura Y, Shinohara H, et al. Serum hepatocyte growth factor predicts ventricular remodeling following myocardial infarction. *Circ J* 2002; 66: 1003–1007.
14. Kawamoto A, Kawata H, Akai Y, et al. Serum levels of VEGF and basic FGF in the subacute phase of myocardial infarction. *Int J Cardiol* 1998; 67: 47–54.
15. Shimokawahara H, Jougasaki M, Setoguchi M, et al. Relationship between vascular endothelial growth factor and left ventricular dimension in patients with acute myocardial infarction. *J Cardiol* 2014; 64: 360–365.
16. Zou J, Fei Q, Xiao H, et al. VEGF-A promotes angiogenesis after acute myocardial infarction through increasing ROS production and enhancing ER stress-mediated autophagy. *J Cell Physiol* 2019; 234: 17690–17703. doi: 10.1002/jcp.28395.
17. Celletti FL, Waugh JM, Amabile PG, et al. Vascular endothelial growth factor enhances atherosclerotic plaque progression. *Nat Med* 2001; 7: 425–429.
18. Saukkonen T and Mutt SJ. Adipokines and inflammatory markers in elderly subjects with high risk of type 2 diabetes and cardiovascular disease. *Sci Rep* 2018; 8: 12816.
19. Montagnani M, Chen H, Barr VA, et al. Insulin-stimulated activation of eNOS is independent of Ca2+ but requires phosphorylation by Akt at Ser(1179). *J Biol Chem* 2001; 276: 30392–30398.
20. Liu J, Xu Y, Zhang H, et al. Coronary flow velocity reserve is impaired in hypertensive patients with hyperhomocysteinemia. *J Hum Hypertens* 2014; 28: 743–747.
21. Earle KA, Zitouni K and Nourooz-Zadeh J. Lipopolysaccharide-induced VEGF production and ambient oxidative stress in type 2 diabetes. *J Clin Endocrinol Metab* 2019; 104: 1–6.
22. Rega G, Kaun C, Demyanets S, et al. Vascular endothelial growth factor is induced by the inflammatory cytokines interleukin-6 and oncostatin m in human adipose tissue in vitro and in murine adipose tissue in vivo. *Arterioscler Thromb Vasc Biol* 2007; 27: 1587–1595.
23. Gealekman O, Burkart A, Chouinard M, et al. Enhanced angiogenesis in obesity and in response to PPARgamma activators through adipocyte VEGF and ANGPTL4 production. *Am J Physiol Endocrinol Metab* 2008; 295: E1056–E1064.
24. He Z, Opland DM, Way KJ, et al. Regulation of vascular endothelial growth factor expression and vascularization in the myocardium by insulin receptor and PI3K/Akt pathways in insulin resistance and ischemia. *Arterioscler Thromb Vasc Biol* 2006; 26: 787–793.
25. Tan C, Sasagawa Y and Mori M. The association between insulin resistance, metabolic syndrome, and ischemic heart disease among Rumoi residents. *J Gen Family Med* 2017; 18: 360–364.
26. Jeppesen J, Hansen TW, Torp-Pedersen C, et al. Relationship between common lipoprotein lipase gene sequence variants, hyperinsulinemia, and risk of ischemic heart disease: a population-based study. *Atherosclerosis* 2010; 211: 506–511.
27. Kaess BM, Preis SR, Beiser A, et al. Circulating vascular endothelial growth factor and the risk of cardiovascular events. *Heart* 2016; 102: 1898–1901.
28. Oduk Y, Zhu W, Kannappan R, et al. VEGF nanoparticles repair the heart after myocardial infarction. *Am J Physiol Heart Circ Physiol* 2018; 314: H278–H284.
29. Simo R, Sundstrom JM and Antonetti DA. Ocular Anti-VEGF therapy for diabetic retinopathy: the role of VEGF in the pathogenesis of diabetic retinopathy. *Diabetes Care* 2014; 37: 893–899.
30. Carroll RM, Yu Y and VanderBeek BL. Predictive factors for patients receiving intravitreal anti-vascular endothelial growth factor for the treatment of diabetic macular edema. *Eur J Ophthalmol* 2019: 1120672119827856.
31. Santilli F, Spagnoli A, Mohn A, et al. Increased vascular endothelial growth factor serum concentrations may help to

identify patients with onset of type 1 diabetes during childhood at risk for developing persistent microalbuminuria. *J Clin Endocrinol Metab* 2001; 86: 3871–3876.
32. Dandona P, Aljada A, Mohanty P, et al. Insulin suppresses plasma concentration of vascular endothelial growth factor and matrix metalloproteinase-9. *Diabetes Care* 2003; 26: 3310–3314.
33. Elias I, Franckhauser S and Bosch F. New insights into adipose tissue VEGF-A actions in the control of obesity and insulin resistance. *Adipocyte* 2013; 2: 109–112.