Effect of family history of type-2 diabetes on coronary flow reserve and it's relationship with insulin resistance: an observational study

Tip 2 diyabetes aile öyküsünün koroner akım rezervi üzerine etkisi ve onun insülin direnci ile ilişkisi: Bir gözlemsel çalışma

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ABSTRACT

Objective: Coronary microvascular function among offspring of patients with diabetes mellitus might be compromised when compared to persons with no first-degree relative with diabetes mellitus. The aim of the study was to evaluate effect of family history of type-2 diabetes on coronary flow reserve.

Methods: In this observational study, we evaluated coronary flow reserve (CFR) via echocardiography of 95 subjects having a biological parent with type-2 diabetes and 34 healthy volunteers without any biological parent with type-2 diabetes. We have analyzed possible association with CFR and homeostasis model assessment - insulin resistance (HOMA-IR). Comparison analyses were made using independent samples t test, Chi-square test and one-way ANOVA. Association of independent variables with CFR was obtained by correlation analysis and stepwise linear regression model including potential confounders.

Results: CFR was significantly lower in the positive family history group than in the controls. Moreover, when compared with controls, the subgroup of insulin-sensitive subjects in the positive family history group also had significantly reduced CFR (2.67±0.28 vs. 2.83±0.19; p=0.01). Correlation analysis revealed that CFR was inversely correlated with HOMA-IR, (r=-0.433), fasting glucose (r=-0.331), fasting insulin (r=-0.396), and hemoglobin (Hb)A1c (r=-0.405). When the positive family history group was divided into tertiles of insulin resistance (HOMA-IR <1.3, 1.3-2.6, and >2.6; Groups 1-2, and 3), there was a significant difference in CFR between Groups 1 and 2 and between Groups 1 and 3 (p<0.05 for all). Though statistically not significant, there was also a difference in CFR between Groups 2 and 3. In a linear regression model, only fasting glucose level was independent predictor of CFR (β=-677; p value =0.001, 95% CI: -0.061 and -0.019).

Conclusion: Nondiabetic first-degree relatives of patients with type-2 diabetes are at increased risk of developing coronary microvascular dysfunction. *(Anadolu Kardiyol Derg 2013; 13: 48-56)*

Key words: Coronary flow reserve, diabetes, heritage, regression analysis

ÖZET

Amaç: Diyabetes mellitusun birinci derece yakınlarında koroner mikrovasküler fonksiyonlar, ailesinde tip 2 diyabet olmayan olgulara göre bozulmuş olabilir. Bu çalışmanın amacı; Tip 2 diyabet aile öyküsünün koroner akım rezervi (KAR) üzerine olası etkisinin değerlendirmektir.

Yöntemler: Bu gözlemsel çalışmada biz, ekokardiyografik yöntemle tip 2 diyabetes mellitus aile öyküsü olan 95 olgu ve aile öyküsü olmayan 34 sağlıklı gönüllüyü değerlendirdik. Biz aynı zamanda KAR ile HOMA-IR arasındaki muhtemel ilişkiyi de test ettik. Grupları karşılaştırmak için; Student's t-testi, Ki-kare testi ve tek-yönlü ANOVA testleri kullanıldı. KAR ve diğer değişkenler arasındaki bağımsız ilişki korelasyon ve adımsal çoklu regresyon analizi ile değerlendirildi.

Bulgular: KAR, tip 2 diyabet aile öyküsü olan grupta kontrol grubuna göre anlamlı şekilde daha düşüktü. Bunun da ötesinde kontrol grubu ile kıyaslandığında, tip 2 diyabet aile öyküsü olup, insülin direnci bulunmayan alt grupta da, KAR istatistiksel olarak anlamlı şekilde düşük bulundu (2.67±0.28 vs. 2.83±0.19; p=0.01). Korelasyon analizinde koroner akım rezervinin HOMA-IR (r=-0.433), açlık glikozu (r=-0.331), açlık insülini (r=-0.396) ve hemoglobin (Hb)A1c (r=-0.405) ile anlamlı ve negatif yönde ilişkili olduğu saptandı. Tip 2 diyabet aile öyküsü olan grup insülin direncine göre tertiplendiğinde (HOMA-IR <1.3, 1.3-2.6, ve >2.6; sırasıyla grup 1-2 ve 3), KAR grup 1 ve 2 ve grup 1 ve 3 arasında anlamlı

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şekilde farklı bulundu (tüm grup için p<0.05). İstatistiksel olarak anlamlılığa ulaşmasa da grup 2 ve 3 arasında da KAR açısından fark bulundu. Regresyon analizinde KAR' daki bozulmanın tek bağımsız değişkeni olarak açlık glikozunun olduğu tespit edildi (β=-677; p değeri=0.001, %95 CI: -0.061 ve -0.019).
Sonuç: Tip 2 diyabetin birinci derece sağlıklı yakınlarında, koroner mikrovasküler fonksiyonların bozulma riski artmıştır.
(Anadolu Kardiyol Derg 2013; 13: 48-56)
Anahtar kelimeler: Koroner akım rezervi, diyabet, genetik yatkınlık, regresyon analizi

Introduction

Diabetes mellitus is associated with a 2-to-4-fold increased rate of death from coronary artery disease (1). Moreover, nondiabetic first-degree relatives of patient with type-2 diabetes are at increased risk of developing diabetes and cardiovascular disease (CVD) (2). Irrespective of the etiological process leading to type-2 diabetes, the most common form is characterized by a marked reduction in insulin-mediated glucose uptake, predominantly in skeletal muscle, and a relative insulin deficiency (3). Insulin resistance (IR) has also been demonstrated in healthy first-degree relatives of type-2 diabetic patients, and has therefore been proposed as an early marker of abnormal glucose metabolism (4). Moreover, premature atherosclerosis has been linked to IR. The cardiovascular risk in patients with IR, with or without glucose intolerance, has been described in a recent meta-analysis investigating hyperinsulinemia as a surrogate marker (5).

Endothelial dysfunction, a precursor of atherosclerosis, has been reported in nondiabetic first-degree relatives of persons with type-2 diabetes (6). However, endothelial dysfunction detected in brachial arteries may not reflect the condition of the coronary vasculature, as brachial and coronary circulations differ in terms of the pattern of blood flow, their metabolic regulation, microvascular architecture, and the pathways that are activated to induce hyperemia. Measurement of coronary flow reserve (CFR) is used to assess epicardial coronary arteries and to examine the integrity of the coronary microvascular circulation. Impaired endothelial function and reduced CFR, which reflect coronary microvascular dysfunction, have been shown to be an early manifestation of coronary atherosclerosis. Use of transthoracic second harmonic Doppler echocardiography (TTDE) to evaluate CFR has become popular and its feasibility has been validated (7).

Whether persons with a family history of diabetes mellitus have coronary endothelial dysfunction as a precursor of coronary atherosclerosis is unknown.

We hypothesize that coronary microvascular function among offspring of patients with diabetes mellitus might be compromised when compared to persons with no first-degree relative with diabetes mellitus.

Methods

Study design

This is a cross-sectional observational study.

Study population

The overall study population consisted of 129 subjects: 95 subjects with a positive family history of type 2 diabetes and 34 healthy volunteers with no family history of type-2 diabetes. Their demographic and clinical data are shown in Table 1. This study was performed between August 2009 and January 2012 at the Cardiology and Endocrinology Department of Başkent University Training and Research Center in Konya, Turkey. Study population was selected from patients and their offspring of cardiology and endocrinology outpatient clinic, and our hospital staff.

Offspring of the type 2 diabetes mellitus cohort were divided into tertiles of IR, as follows: Group 1: homeostasis model assessment - insulin resistance index (HOMA-IR) <1.3, Group 2: HOMA-IR 1.3-2.6, and Group 3: HOMA-IR >2.6. The inclusion criteria were: age between 18-45 years, at least one parent with type 2 diabetes mellitus, no coronary artery disease according to HOMA-IR, and no coronary artery disease-related symptoms. Exclusion criteria were: presence of any disease that could cause CFR impairment, such as hemolytic, hepatic and renal diseases, hypertension, diabetes mellitus, and impaired glucose tolerance, family history of coronary artery disease in a first-degree male relative <55 years and in a first-degree female relative <65 years, excessive alcohol consumption (>120 g/d), and morbid obesity [body mass index (BMI) >35 kg/m^2]. Subjects using any vasoactive drug or smoking, and those with ST segment changes or T-wave specific for myocardial ischemia, Q-wave, and incidental left bundle branch block on ECG were excluded. Individuals were also excluded if they had triglyceride levels >4.56 mmol/L (400 mg/dL) or left ventricular mass index (LVMI) ≥126 g/m^2 for males and ≥99 g/m^2 for females.

The study was conducted according to the recommendations set forth by the Declaration of Helsinki on Biomedical Research Involving Human Subjects. Written informed consent was obtained from each subject, and the institutional ethics committee approved the study protocol.

Study protocol and study variables

Subjects were evaluated after an overnight fast and a 24-hour (h) period of abstinence from alcohol and vigorous physical exercise. Following the blood draw, a standard 75-g oral glucose tolerance test was performed with venous blood sampling at 0, 15, 30, 60, 90, and 120 minutes (min) to measure plasma glucose and serum insulin to allow determination of IR using HOMA-IR (8, 9).

The baseline variables included demographic, laboratory and 2-D echocardiographic parameters; predictor variable was defined as presence of positive family history for diabetes mellitus and coronary flow reserve was a primary outcome variable.

Table 1. Baseline characteristics of study groups

Variables	Family history (+) (n=95)	Family history (-) (n=34)	*p
Age, years	34.2±5.0	33.2±4.8	0.33
Male/female, n/n	46/49	15/19	0.671
BMI, kg/m^2	26.5±2.3	26.1±2.8	0.47
Systolic BP, mmHg	117.6±9.8	116.5±12.6	0.64
Diastolic BP, mmHg	74.8±5.5	74.1±8.1	0.61
Heart rate, bpm	71.9±4.5	72.8±4.5	0.36
Fasting glucose, mmol/l, mg/dL	5.2±0.4 (94.3±6.9)	5.1±0.2 (92.7±4.4)	0.24
Total cholesterol, mmol/l, mg/dL	4.81±0.74 (185.1±28.8)	4.63±0.80 (178.3±30.8)	0.26
Triglyceride, mmol/l, mg/dL	1.33±0.52 (116.8±45.9)	1.37±0.63 (120.5±56.0)	0.71
HDL-cholesterol, mmol/l, mg/dL	1.21±0.26 (46.7±9.9)	1.19±0.27 (45.6±10.5)	0.61
LDL-cholesterol, mmol/l, mg/dL	3.01±0.61 (115.8±23.7)	2.82±0.76 (108.6±29.4)	0.17
Hemoglobin, mg/dL	14.1±2.8	14.0±1.2	0.96
hs-CRP, mg/L	2.59±2.00	2.13±2.28	0.32
HbA1c, %	5.2±0.28	5.1±0.25	0.35
Fasting insulin, µU/l	8.1±3.9	7.1±2.6	0.09
HOMA-IR	1.9±0.9	1.6±0.6	0.07
2-h post-challenge glucose, mmol/l, mg/dL	6.2±0.9 (112.8±15.2)	6.3±0.6 (116.0±11.7)	0.22

Data are presented as mean±SD and numbers
*Independent samples t- test and Chi-square test
BMI- body mass index, BP- blood pressure, HDL- high-density lipoprotein, HOMA-IR- homeostasis model assessment - insulin resistance, HbA1c-hemoglobin A1c, hs-CRP- high-sensitivity C-reactive protein LDL- low-density lipoprotein

Family history was defined during the medical interview by participant report of diabetes in either both biological parents (positive family history), or in neither biological parent nor any first-degree relative (negative family history).

Biochemical analyses

Serum glucose was measured by a spectrophotometric method (Aeroset automated analyzer, Abbott Laboratories, Abbott, IL, USA). Fasting insulin was measured by the immunoturbidimetric method. Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride were measured by enzymatic methods. The HOMA-IR was calculated using the formula: fasting serum insulin (micro-units per milliliter) x fasting plasma glucose (FPG) (micromoles per liter/22.5, which has been found to correlate with glucose clamp measurement in nondiabetic, diabetic and hypertensive populations (10). Plasma levels of high sensitivity C-reactive protein (hs-CRP) were measured with a highly sensitive sandwich ELISA technique.

Echocardiographic examination

Each subject was examined using an Acuson Sequoia C256 Echocardiography System equipped with 3V2c and 5V2c high-resolution transducers with second harmonic capability (Acuson, Mountain View, CA, USA). Each subject underwent two-dimensional, M-mode, and subsequent standard and pulsed-tissue Doppler echocardiographic examinations. The echocardiographic images were recorded on VHS videotapes. Diastolic and systolic interventricular septal (IVS) thickness, posterior wall (PW) thickness, left ventricular end-diastolic diameter (LVEDD), and left ventricular end-systolic diameter (LVESD) were measured on the parasternal long-axis views, and LVM was calculated according to the Penn convention (11). All measurements were performed on M-mode images. The pulsed Doppler sample volume was positioned at the mitral leaflet tips. Early diastolic peak flow velocity (E), late diastolic peak flow velocity (A) and E/A ratio, and E-wave deceleration time (DT) were measured from transmitral Doppler spectra. The Doppler tissue imaging (DTI) program was set to the pulsed-wave Doppler mode. Filters were set to exclude high-frequency signals, and the Nyquist limit was adjusted to a velocity range of –15 to 20 cm/s. Gains were minimized to allow for a clear tissue signal with minimal background noise. All DTI recordings were obtained during normal respiration. A 5-mm sample volume was placed at the apical four-chamber view on the lateral corner of the mitral annulus (12). The resulting velocities were recorded for 5 to 10 cardiac cycles at a sweep speed of 100 mm/s, and stored on VHS videotape for later offline analyses. The following measurements were determined as indexes of regional systolic function: time velocity integral of myocardial systolic (*S*m) wave, myocardial early (*E*m) and atrial (*A*m) peak velocities (cm/s) and

*E*m/*A*m ratio. Isovolumic relaxation time (IVRT) was measured as the time interval occurring between the end of *S*m and the onset of *E*m. All diastolic parameters were measured in three consecutive cardiac cycles and averaged. The same blinded investigator performed the echocardiography, and two blinded cardiologists analyzed the echocardiogram recordings.

CFR measurement

Visualization of the distal left anterior descending (LAD) coronary artery was performed using a modified, foreshortened, two-chamber view obtained by sliding the transducer medially from an apical two-chamber view to best align with the interventricular sulcus. Subsequently, coronary flow in the distal LAD was examined by color Doppler flow mapping over the epicardial part of the anterior wall, with the color Doppler velocity range between 8.9 and 24.0 cm/s. The color gain was adjusted for optimal imaging. The acoustic window was placed at approximately the midclavicular line, in the fourth and fifth intercostal spaces, with the patient in a left lateral decubitus position (7). Coronary blood flow in the LAD (middle to distal) was evaluated by color Doppler flow mapping. All patients had Doppler recordings of the LAD with a dipyridamole infusion at a rate of 0.56 mg/kg over 4 minutes (Fig. 1). All patients had continuous heart rate and electrocardiographic monitoring as well as blood pressure recordings performed at baseline, during dipyridamole infusion and during recovery. Echocardiographic images were recorded on VHS videotapes. Two blinded experienced echocardiographers analyzed the recordings. Placing the sample volume on the color signal allowed spectral Doppler waveforms of the LAD to reveal the characteristic biphasic flow pattern with larger diastolic and smaller systolic components. Coronary diastolic peak velocities were measured at baseline and after dipyridamole infusion (0.56 mg/kg over 4 minutes) by averaging the highest three Doppler signals for each measurement. CFR was defined as the ratio of hyperemic to baseline diastolic peak velocities. To test the coefficient of repeatability of the CFR measurement, the measurement was repeated in 10 control subjects two days later. Intra-observer intra-class correlation coefficient for coronary flow measurement was 0.904, and for CFR was 0.911.

Statistical analyses

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, version 9.0, SSPS Inc, Chicago, IL, USA). Data are expressed as means±SD. Comparison analyses were made using independent samples t-test and one-way ANOVA followed by Scheffe test or Kruskal-Wallis test (comparison of a characteristic across the 3 study groups if that characteristic did not have a normal distribution, such as hs-CRP, fasting insulin and HOMA-IR) to compare continuous variables. The Pearson's correlation analysis was used to test the possible associations between CFR and the study variables. Association of independent variables with CFR was obtained by stepwise linear regression model including potential confounders. A $p<0.05$ was considered statistically significant.

Results

The demographic data and clinical parameters of the study population

No subject had diabetes mellitus and/or impaired glucose tolerance. Glycosylated hemoglobin (HbA1c) level was within the normal range in all participants. The demographic and baseline characteristics are summarized in Table 1. The two groups were similar regarding age, BMI, systolic and diastolic blood pressure, fasting and post-challenge glucose, HOMA-IR, hs-CRP, total cholesterol, HDL-cholesterol, and LDL-cholesterol levels.

Analyses of the echocardiographic measurements

IVS and PW thickness, LVEDD, LVESD, left ventricular ejection fraction (EF), and LVMI were similar between patients with positive family history and negative family history. The left atrium diameter was significantly different between groups (p=0.01) (Table 2).

Standard and tissue Doppler echocardiographic analyses

When compared with controls, the positive family history group had significantly greater A-wave velocity (p=0.04). Mitral inflow E-wave velocity and E/A ratio wave velocities ($p<0.001$) were significantly smaller in the positive family history group than controls. The E-wave DT and IVRT were significantly greater in the positive family history group than the negative family history group. Lateral *E*m velocity and lateral IVRT did not differ between the groups, but lateral *E*m/*A*m ratio and E/*E*m ratio significantly lower in positive family history group ($p<0.05$) (Table 2).

Analysis of CFR measurements

Resting and hyperemic diastolic peak flow velocity values were similar between the two groups. However, CFR was sig-

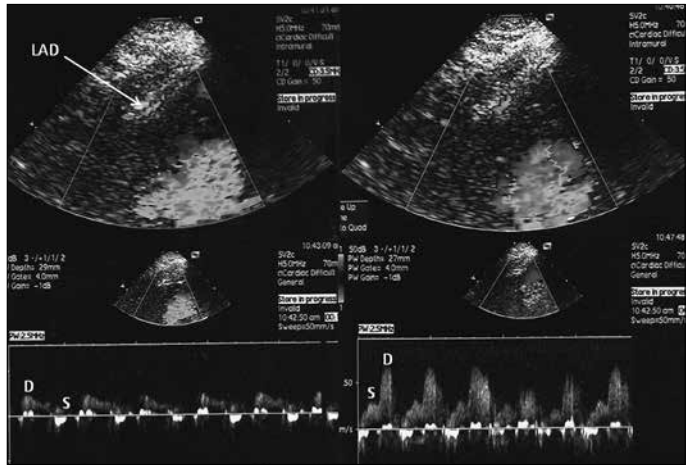

Figure 1. Mid-to-distal segment of the LAD in color-coded transthoracic Doppler echocardiography (arrows) and spectral Doppler coronary blood flow by sampling in mid-to-distal segment of the LAD
D - diastole, LAD - left anterior descending artery, LV - left ventricle, S - systole

Table 2. Conventional echocardiographic and tissue Doppler parameters of the left ventricle study in the groups

Variables	Family history (+) (n=95)	Family history (-) (n=34)	*p
IVS thickness, cm	0.87±0.11	0.89±0.10	0.19
PW thickness, cm	0.84±0.10	0.85±0.09	0.72
LVDD, cm	4.55±0.37	4.47±0.34	0.31
LVSD, cm	2.91±0.60	2.80±0.30	0.30
LAD, cm	3.10±0.36	3.04±0.31	0.01
EF (%)	67.0±4.5	67.0±2.3	0.950
LVMI, g/m^2	75.8±12.8	80.4±12.4	0.140
Mitral E-wave max, cm/s	79.9±14.5	84.0±7.6	0.04
Mitral A-wave max, cm/s	66.6±11.2	62.1±8.2	0.03
E/A ratio	1.21±0.20	1.37±0.18	<0.001
Mitral E-wave deceleration time, ms	194.4±28.1	183.7±22.7	0.032
Isovolumic relaxation time, ms	103.9±14.3	96.5±13.9	0.009
Lateral Em, cm/s	20.8±4.1	20.0.4±4.7	0.34
Lateral Am, cm/s	16.9±3.1	14.7±2.1	<0.001
Lateral IVRT, ms	94.3±17.1	95.2±13.0	0.75
Lateral Em/Am, ratio	1.26±0.26	1.35±0.19	0.03
E/Em ratio	3.93± 0.79	4.38±0.93	0.016
Baseline HR, bpm	74.3±9.1	73.7±5.8	0.73
Peak HR, bpm	100.9±12.2	102.5±8.2	0.49
Peak systolic BP, mmHg	120.1±12.2	121±12.3	0.62
Peak diastolic BP, mmHg	78.2±7.0	77.3±5.9	0.54
Basal DPFV, cm/s	24.7±4.4	23.0±5.2	0.10
Hyperemic DPFV, cm/s	59.8±11.7	64.9±15.1	0.07
CFR ratio	2.44±0.36	2.83±0.19	<0.001

Data are presented as mean±SD values
*Independent samples t- test
Am - atrial peak velocity, BP - blood pressure, CFR - coronary flow reserve, DPFV - diastolic peak flow velocity, EF - ejection fraction, Em-early peak velocity, HR - heart rate, IVRT-isovolumic relaxation time, IVS - interventricular septum, LAD - left atrium diameter, LVDD - left ventricular diastolic diameter, LVSD - left ventricular systolic diameter, LVMI - left ventricular mass index, PW - posterior wall

nificantly lower in the positive family history group than in the negative family history group (Table 2). Moreover, when compared with controls, the insulin-sensitive subgroup among the positive family history group also had significantly reduced CFR (p=0.01) (Table 3).

Associations of CFR and other study variables

Correlation analysis revealed that CFR was inversely correlated with hs-CRP (r=-0.340), systolic (r=-0.341) and diastolic blood pressure (r=-0.296), HOMA-IR (r=-0.433), fasting glucose (r=-0.331), fasting insulin (r=-0.396), HbA1c (r=-0.405), and age (r=-245) in all groups. Though statistical significance was not achieved, triglyceride values tended to negatively correlate with CFR values (r=-0.126). Plasma HDL-cholesterol levels were positively correlated with CFR values (r=0.227). Two-hour post-challenge glucose and BMI did not correlate with CFR values. Mitral E/A ratios, which are representative of left ventricular active relaxation, were positively correlated with CFR (r=0.432; p<0.001). Similarly, DTI-derived mitral lateral annular *E*m/*A*m ratios were significantly and positively correlated with CFR (r=0.307; p<0.001).

When CFR was entered as the dependent variable, and age, HDL-cholesterol, systolic and diastolic blood pressure, HOMA-IR, fasting glucose, fasting insulin, HbA1c, and hs-CRP values were entered as the independent variables in a linear regression model, only fasting glucose level had an independent association with CFR (β=-677; 95% CI:-0.061 and -0.019; p=0.001).

CFR and insulin resistance

To assess the relationship between HOMA-IR and CFR, the positive family history group was divided into tertiles of IR (HOMA-IR <1.3, 1.3-2.6, and >2.6, Groups 1-3, respectively). There was a significant difference in CFR between Groups 1 and 2 and between Groups 1 and 3 (p<0.05). Though statistically not significant, there was also a difference in CFR between Groups 2 and 3 (Tables 3, 4).

Table 3. Echocardiographic findings according to HOMA-IR of family history (+) subjects

Variables	Group 1 HOMA-IR <1.3 (n=30)	Group 2 HOMA-IR 1.3-2.6 (n=40)	Group 3 HOMA-IR >2.6 (n=25)	*F	*p
Systolic BP, mmHg	114.2±10.8	118.8±9.0	119.6±9.4	1.789	0.17
Diastolic BP, mmHg	72.6±5.7	76.1±6.0	75.2±4.7	2.452	0.09
Baseline HR, bpm	71.6±3.6	71.8±5.5	72.3±3.6	0.379	0.68
Peak HR, bpm	103.7±11.7	99.0±12.9	100.7±11.3	1.267	0.28
Peak systolic BP, mmHg	121.5±12.2	120±12.3	119.3±13.1	0.62	0.45
Peak diastolic BP, mmHg	77.2±7.0	76.3±5.9	76.1±6.3	0.75	0.71
Basal DPFV, cm/s	24.2±4.5	24.2±4.6	26.1 ±3.9	1.89	0.15
Hyperemic DPFV, cm/s	64.3±13.1	57.8±10.8	57.5±9.9	3.52	0.03
CFR, ratio	2.67±0.28	2.42±0.35**	2.22 ±0.07***†	13.46	<0.001

Data are presented as mean±SD values
*One-way ANOVA followed by Scheffe test:
**p value: 0.006 group 1 versus 2
†p value <0.001, group 1 versus 3
***p value: 0.07 group 2 versus 3
BP - blood pressure, CFR - coronary flow reserve, DPFV - diastolic peak flow velocity, HOMA-IR - homeostasis model assessment - insulin resistance, HR- heart rate

Discussion

Our investigation revealed that normal and overweight, glucose-tolerant offspring of type-2 diabetic patients have lower CFR than age and BMI-matched control subjects without family history of type-2 diabetes. Our data also demonstrated significant negative correlations between CFR and both serum fasting insulin levels and IR characterized by the HOMA-IR in normoglycemic subjects with normal epicardial coronary arteries. The lower ratio of hyperemic to basal peak diastolic coronary flow velocity (CFR) is associated with higher HOMA-IR. Thus, higher IR is associated with increased coronary resistance. Furthermore, coronary microvascular dysfunction was also demonstrated in the group of the most insulin-sensitive offspring of diabetic parents.

It is well established that the genetic predisposition to type-2 diabetes is associated with higher risk for CVD. Pannacciulli et al. (13) demonstrated that carotid atherosclerosis was significantly increased in the offspring of patients with diabetes mellitus. Goldfine et al. (6) found that in multiple regression analysis, only family history remained a significant determinant for endothelial dysfunction. We also found that coronary microvascular function was impaired in the group of insulin-sensitive offspring of diabetic parents. Our study implicates that increase in cardiovascular risk begins well before the onset of overt diabetes, and coronary microvascular changes accompany metabolic disturbances caused by developing diabetes mellitus even in its very early stages.

In this study, we also found that CFR impairment increases in conjunction with increased IR. Investigating earlier stages of IR, Caballero et al. (14) reported peripheral endothelial dysfunction in normoglycemic relatives of patients with type-2 diabetes. Quinones et al. (15) suggested that IR in healthy subjects is associated with coronary endothelial dysfunction. Prior et al. (16) recently demonstrated that positron emission tomography-derived endothelial- dependent coronary vasoreactivity was significantly diminished in insulin-resistant individuals, as well as in patients with impaired glucose tolerance and normotensive, hypertensive patients with type-2 diabetes mellitus. They also reported an attenuation of cold-stimulated coronary flow in IR in the absence of glucose intolerance (15, 16). We now extend this observation to apparently healthy insulin-sensitive offspring of parents with type-2 diabetes. Although the pleiotropic metabolic disturbances of the prediabetic state may contribute to atherosclerosis progression, our findings suggest that a strong family history of diabetes is associated with diminished CFR and may contribute to cardiovascular risk in advance of overt diabetes. Several previous investigations also found endothelial dysfunction in relatives of type-2 diabetics (14), though this remains controversial (17). In these studies, offspring cohorts differed from control subjects with respect to post-challenge glucose and insulin, cholesterol, severity of IR, BMI, blood pressure, and other factors (14, 17). These confounding differences make it difficult to assess independent effects of family history of diabetes on endothelial function. In a study of Goldfine et al. (6), it was shown that compromised cardiovascular health was observed to be greater in cases when both parents had diabetes mellitus than when only one of the parents had diabetes mellitus. Our results implicate that genetic predisposition to develop diabetes mellitus might result in some metabolic changes that are accompanied by coronary microvascular impairment.

In this study, we also found a negative correlation between fasting glucose level and CFR. Ning et al. (18) and Succurro et al. (19) studied individuals with both FPG and 2-h plasma glucose within the normoglycemic range. They found that subjects whose 2-h plasma glucose concentration did not return to their FPG level after a 75-g oral glucose load were more insulin- resistant and had worse CVD outcomes than those whose 2-h plasma glucose returned to the FPG level. In our study, IR and hs-CRP changes corresponded to those reported by Ning et al. (18) and

Table 4. Baseline characteristics according to HOMA-IR of family history (+) subjects

Variables	Group 1 HOMA-IR <1.3 (n=30)	Group 2 HOMA-IR 1.3-2.6 (n=40)	Group 3 HOMA-IR >2.6 (n=25)	F/Chi-square	p
Age, years	34.5±5.0	33.7±4.9	34.5± 5.2	0.280	0.75
BMI, kg/m^2	26.1±2.3	26.8±2.3	26.7±2.2	1.014	0.36
Systolic BP, mmHg	114.2±10.8	118.8±9.0	119.6±9.4	1.789	0.17
Diastolic BP, mmHg	72.6±5.7	76.1±6.0	75.2±4.7	2.452	0.09
Heart rate, bpm	71.6±3.6	71.8±5.5	72.3±3.6	0.296	0.74
Fasting glucose, mmol/l, mg/dL	5.1± 0.4 (92.6±7.4)	5.2±0.3 (94.8±6.1)	5.2±0.1 (95.4±7.6)	1.317	0.27
Total cholesterol, mmol/l, mg/dL	4.63±0.66 (178.3±25.6)	4.89±0.71 (187.9±27.4)	4.90±0.88 (188.6±33.9)	1.027	0.36
Triglyceride, mmol/l, mg/dL	1.16±0.54 (101.4±47.7)	1.34±0.44 (117.4±38.8)	1.51±0.58 (133.3±50.1)	2.965	0.057
HDL-cholesterol, mmol/l, mg/dL	1.29±0.22 (49.8±8.4)	1.21±0.29(45.5±11.3)	1.13±0.20 (43.3±7.8)	2.516	0.09
LDL-cholesterol, mmol/l, mg/dL	2.82±0.56 (108.3±21.6)	3.08±0.63 (118.3±24.3)	3.12±0.62 (120.2±24.1)	1.796	0.17
Hemoglobin , mg/dL	13.6±1.2	14.3±1.2	14.2±4.8	0.509	0.60
hs-CRP, mg/L	2.01±1.57	2.37±1.62	3.63±2.62*	5.853	0.054
Fasting insulin, µU/l	4.08±1.8	8.1±1.4†	13.7±2.7**	77.68	<0.001
HOMA-IR	0.93±0.3	1.86±0.3†	3.24±0.7**	77.49	<0.001
2-h post-challenge glucose, mmol/l, mg/dL	6.2±0.7 (111.9±12.7)	6.1±0.9 (111.3±15.6)	6.4±0.9 (116.3±17.6)	0.814	0.44

Data are presented as mean±SD values
*One-way ANOVA followed by Scheffe test and Kruskal-Wallis test with posttest:
*: p value<0.05, group 1versus 3
**: p value<0.001 group 1 versus 3; group 2 versus 3
†: groups value <0.001 group 1 versus 2
BMI - body mass index, BP - blood pressure, HDL - high-density lipoprotein, HOMA-IR - homeostasis model assessment -insulin resistance, hs-CRP - high-sensitivity C - reactive protein, LDL - low-density lipoprotein

Succurro et al. (19); however, changes in the CFR levels were not statistically different. This might be due to the smaller sample size of our study. Hyperglycemia may adversely affect vascular function through multiple mechanisms, including increased flux through the polyol pathway, increased oxidative stress, activation of protein kinase C-beta, and function of advanced glycation end-products (20). The most attractive concept, however, refers to a decrease of nitric oxide (NO) bioavailability either due to diminished production, increased inactivation of NO, or both, and may result from several mechanisms (21). Diminished insulin sensitivity, elevation of plasma free fatty acids, and hypertriglyceridemia have been implicated to alter intracellular signaling of NO synthase activity and to reduced NO production (21).

The relation between markers of glucose metabolism and CFR in individuals with genetic predisposition to diabetes mellitus is less clear. This study also shed light on the association of serum HbA1c levels as a long-term marker of glucose metabolism with coronary microvascular function in non-diabetic subjects. We found a significant inverse association between HbA1c value and CFR in subjects with a genetic predisposition to diabetes mellitus. Bobbert et al. (22) demonstrated that HbA1c was the most informative glycemic marker with respect to intima-media thickness (IMT) in normoglycemic subjects. Additionally, Lorbeer et al. (23) demonstrated that higher serum HbA1c levels in non-diabetic subjects are inversely associated with flow-mediated dilation (FMD) in females without antihypertensive medication, but not in males. In addition to the previous data that IMT and FMD are surrogates of vascular atherosclerotic burden, their study implicated that coronary microvascular function is impaired in the offspring of diabetic patients. Our results endorse the current knowledge that subclinical disturbances in glucose metabolism measured by serum HbA1c level are associated with subclinical cardiovascular effects detected by CFR measurement.

We found clinically not important however statistically significant left atrial enlargement in subjects with diabetic parents. This finding is most possibly a reflection of developing diastolic dysfunction, which may cause also impairment in coronary microvascular function.

Subclinical inflammation is associated with IR (24) and CVD (25). In our study, a significantly inverse correlation between CFR and hs-CRP was observed. It may suggest a role for inflammation in the altered CFR. In this respect, a link between inflammation and endothelial dysfunction has been suggested in type-2 diabetes (25).

Study limitations

- In this study, we have performed a delicate work to eliminate confounding factors for CFR. However, we acknowledge that coronary microvascular functions might be affected several factors some of them are not known currently.

- Impairment in CFR in this population was caused by some possible metabolic changes that were still in the subclinical

stage. This study indicates that one possible explanation of CFR impairment in this population is increased CRP. Of course, there may be some additional factors in CFR impairment to be elucidated by additional studies.

- The correlation between CFR and HOMA-IR and CRP values in these subjects implicates developing metabolic changes. Therefore, increase in CRP value is only a reflection of metabolic changes rather than being the cause of CFR impairment.

- We believe that our study has a small sample size, and it is not possible to infer exact conclusions according to our results. Therefore additional studies with larger study populations are needed to elucidate the issue.

Conclusion

Our study shows that coronary microvascular function is abnormal in first-degree relatives of patients with type-2 diabetes mellitus. These findings, when considered together with previous reports of subclinical CVD in first-degree relatives of subjects with diabetes mellitus, strongly support the existence of a link between genetic predisposition to CFR and IR and type-2 diabetes and increased risk of atherosclerotic CVD. As a result, genetically mediated metabolic changes in offspring of patients with type-2 diabetes mellitus is accompanied by impaired CFR, which is a well-established surrogate marker of developing atherosclerosis.

Conflict of interest

We affirm that none of the authors has a personal or financial relationship that has any potential to inappropriately influence (bias) his or her actions or the manuscript. No financial or other potential conflicts of interest regarding the manuscript exist (including involvement with any organization with a direct financial, intellectual, or other interest in the subject of the manuscript). In addition, no grants or sources of financial support related to the topic(s) of the manuscript were received by the authors.

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