ACQUIRED CARDIOVASCULAR DISEASE

ORIGINAL ARTICLE

Electron Microscopic Comparison of Radial Artery Grafts in Non-Diabetic and Diabetic Coronary Bypass Patients

Mete Gürsoy, M.D.,* Elif Güzel, M.D.,† Pelin Ertürküner, M.D.,† Idil Çakır, M.S.,‡ Egemen Duygu, M.D.,§ Füsun Gülcan, M.D.,¶ and A.Faruk Hökenek, M.D.*

**Department of Cardiovascular Surgery, Acibadem International Hospital, Istanbul, Turkey; †Cerrahpasa Faculty of Medicine, Department of Histology and Embryology, Istanbul University, Istanbul, Turkey; ‡Division of Health Management, Vocational School of Health Services, Acibadem University, Istanbul, Turkey; §Department of Cardiology, Acibadem International Hospital, Istanbul, Turkey; and ¶ Department of Anesthesiology, Acibadem International Hospital, Istanbul, Turkey*

ABSTRACT ***Objective:*** **We compared electron microscopic histologic changes of the radial artery grafts in non-diabetic and diabetic patients.** ***Methods:*** **Thirty-six patients were divided into three groups according to their diabetic status (Group I had no diabetes mellitus [DM], Group II had type two DM and HbA1c levels were <7.5%, and Group III had type 2 DM but HbA1c levels were >7.5%). Distal parts of radial artery grafts were evaluated with scanning electron microscopy in a blind fashion by two histologists. Electron microscopic scores were compared among the groups.** ***Results:*** **Radial artery electron microscopic scores were significantly different between group 1, 2 and 1, 3 and 2, 3 ($p = 0.028$, $p < 0.001$, and $p < 0.001$). In linear regression analysis, duration of DM ($p = 0.027$) and fasting plasma glucose ($p = 0.001$) were found as independent risk factors for histologic changes of radial artery grafts.** ***Conclusion:*** **Duration of DM and poor glycemic control were found to be associated with radial artery electron microscopic changes.** doi: 10.1111/jocs.12761 *(J Card Surg 2016;31:410–415)*

Coronary artery bypass grafting is still the preferred treatment in diabetic patients with multivessel disease, and recent studies showed that increased number of arterial grafts is associated with improved long-term outcomes following coronary surgery.[1–3]

The radial artery (RA) has been considered as the second arterial graft after the right internal thoracic artery (RIMA) since its reintroduction in the early 1990s.[4–6] In recent studies, the RA graft has been used in diabetic patients with severe proximal stenosis of the coronary arteries.[7–9] However, hyperglycemia may cause increased endothelial permeability, adhesion molecules release, and mononuclear cell migration to the subendothelial region and promote atherogenesis and has been a potential clinical predictor of postoperative saphenous vein (SV) and RA graft failure.[10–12] Increased HbA1c levels have been found to be associated with atherosclerotic changes in internal thoracic artery grafts with electron microscopy.[13]

In the present study, we compared histologic changes of the radial artery grafts with scanning electron microscopy in non-diabetic and diabetic patients who were divided into subgroups according to HbA1c levels.

MATERIALS AND METHODS

Institutional Ethics Committee approval and signed informed consents were obtained (ACU 2015/17). The study was designed in accordance with the CONSORT Statement.[14] Elective coronary artery bypass patients were subdivided into groups according to their HbA1c levels; Group I had no diabetes mellitus (DM), Group II had type 2 DM and HbA1c levels were <7.5%, and Group III had type 2 DM but HbA1c levels were >7.5%. The radial artery was used in non-left anterior descending arteries (non-LAD) with at least a 90% proximal lesion in all cases. In the preoperative examination, non-

Conflict of interest: The authors acknowledge no conflict of interest in the submission.

Address for correspondence: Mete Gürsoy, M.D., Acibadem International Hospital, Istanbul Cad. No: 82 34149, Bakirkoy, Istanbul Turkey. Fax: +902126633010; e-mail: metegursoy35@gmail.com

dominant hand circulation was assessed with an ''Allen's test'' in each patient. Duplex ultrasound was not performed preoperatively and all of the radial artery grafts were considered appropriate to be used for grafting. Exclusion criteria were as follows: emergency operation, presence of renal failure, peripheral arterial disease, absence of non-LAD coronary lesion >90%, and preoperative catheterization using the radial artery.

Plasma HbA1c levels were measured with the turbidimetric technique by COBAS® INTEGRA 400 plus (Roche Diagnostics, Indianapolis, IN, USA). The radial artery was harvested with a pedicle using the monopolar electro-scalpel (Valleylab Force Fx, Tyco Healthcare, Princeton, CO, USA) at settings of 20 W from the distal wrist to the perforating branch of the radial artery. Each patient received an intravenous nitrate infusion but not Ca++ channel blockers during the radial artery harvesting. Patients received intravenous bolus insulin if required, insulin drips were not used routinely. Following systemic heparinization (400 U/kg) the distal 1.5 cm of the artery was transected with a #11 surgical blade for electron microscopic evaluation.

Ultrastructural analysis

Tissue samples were incubated in 2.5% glutaraldehyde in Millonig's buffer (pH 7.4) for 4 hours in room temperature for fixation, then rinsed. The samples were further post-fixed in 1% osmium tetroxide in Millonig's buffer (pH 7.4) for 30 minutes in room temperature, then rinsed. They were dehydrated in a graded series of ethanol to absolute ethanol in preparation for embedding in araldite (Sigma-G4901, St. Louis, MO, USA). Semi-thin sections from the polymerized blocks were stained with toluidine blue. Ultra-thin sections (50–60 nm) were cut by an ultramicrotome (Reichert UM 3, Wien, Austria), placed on copper grids and stained with uranyl acetate and lead citrate. Sections were analyzed and photographed using a transmission electron microscope using an accelerating voltage of 80 kV (Jeol-1011, Tokyo, Japan) with an attached digital camera (Olympus-Veleta TEM Camera, Tokyo, Japan).

Sections of each artery were analyzed by two histologists in a blind fashion with a semi-quantitative scoring system described previously (Table 1).[13] Parameters of the semi-quantitative scoring system were as follows: (a) ultrastructural changes in endothelial cells: normal thickness; 0, decreased endothelial cell thickness; 1, disturbed endothelial wall integrity; 2, totally damaged endothelial layer; 3, (b) ultrastructural changes in arterial wall: normal arterial wall; 0, presence of intraendothelial vacuole; 1, presence of intraendothelial vacuole and subendothelial edema; 2, presence of intraendothelial vacuole and subendothelial edema and presence of vacuoles and intercellular edema in tunica media and tunica adventitia; 3, (c) ultrastructural changes in endothelial mitochondria: normal mitochondria; 0, mild swelling; 1, prominent swelling; 2, amorphic material deposition in mitochondria; 3. Each slide was evaluated and total scores were calculated as the total of endothelial, arterial wall, and mitochondrial scores. In case of dispute regarding scores between two histologists, an average score was calculated.

Statistical analysis

Data were analyzed with SPSS release 17.0 (Statistical Package for the Social Sciences, Inc., Chicago, IL, USA). Sample size calculation was done according to 1-U difference between ultrastructural endothelial score with 80% power and $\alpha = 0.05$, and at least eight patients were planned to be involved in each group. While categorical variables were expressed as numeric and percentile, the continuous variables were defined as mean ± standard deviation. Normality of parameters was assessed with Shapiro–Wilk test. Categorical variables were compared with χ^2 test. Continuous variables of groups were compared with one way analysis of variance (ANOVA) and Kruskal–Wallis test. Tamhane and Tukey tests were used as posthoc test. Multivariate logistic regression analysis was performed. Statistical significance was accepted when the p-value was less than 0.05.

RESULTS

Thirty-six patients were included in the study with 12 patients in each group. All diabetic patients were Type 2 and on oral antidiabetic treatment. Patients received enoxaparine and beta blockers preoperatively.

TABLE 1
Preoperative Characteristics of Patients

	Group 1	Group 2	Group 3	p (1–2)	p (1–3)	p (2–3)
n	12	12	12	NS	NS	NS
Age	60.5 ± 7.6	53.0 ± 3.4	66.6 ± 4.3	0.021	0.076	<0.001
FPG (mg/dL)	95.7 ± 2.3	117.3 ± 10.9	166.2 ± 7.1	<0.001	<0.001	<0.001
Hemoglobin A1c (%)	5.3 ± 0.4	6.1 ± 0.6	8.2 ± 0.8	<0.001	<0.001	<0.001
Duration of DM	—	9.0 ± 3.4	18.6 ± 6.0	—	—	<0.001
LDL (mg/dL)	156.0 ± 66.6	141.4 ± 25.4	150.3 ± 6.6	0.858	0.987	0.591
CRP (mg/dL)	0.16 ± 0	0.6 ± 0.4	1.3 ± 0.9	0.001	<0.001	0.021
Creatinine (mg/dL)	0.9 ± 0.3	0.8 ± 0.11	1.0 ± 0.3	0.794	0.112	0.098

FPG, fasting plasma glucose; LDL, low-density lipoprotein; CRP, C-reactive protein; NS, nonsignificant; DM, diabetes mellitus.

Patient's mean age was 60.50 ± 7.62, 53.00 ± 3.41, and 66.67 ± 4.37 in groups 1, 2, and 3, respectively and was significantly different between groups 1, 2, and 2, 3 ($p = 0.021$ and <0.001). Hypertension, hyperlipidemia, and smoking history was similar between groups. Mean fasting plasma glucose (FPG) (95.75 ± 2.37, 117.33 ± 10.96, and 166.24 ± 10; $p < 0.001$ for groups 1 and 2, 1 and 3, 2 and 3), HbA1c (5.36 ± 0.46, 6.16 ± 0.61, and 8.29 ± 0.82; $p < 0.001$ for groups 1 and 2, 1 and 3, 2 and 3), and CRP (0.16 ± 0.06, 0.60 ± 0.41, and 1.37 ± 0.92; $p = 0.001$ for groups 1 and 2, $p < 0.001$ for groups 1 and 3, $p = 0.021$ for groups 2 and 3) were found to be significantly different. Serum creatinine and LDL values were similar (Table 2). Duration of DM was significantly longer in Group 3 than Group 2 (18.67 ± 6.05 vs 9.00 ± 3.44 years $p < 0.001$).

Patient's blood glucose levels were measured with arterial blood gases at the beginning of the operation. Mean glucose level was found to be significantly different between groups 1 and 3, and between 2 and 3 (94.50 ± 6.96, 114.83 ± 15.73, 147 ± 25.71, respectively; $p < 0.001$ for groups 1 and 3, $p = 0.002$ for groups 2 and 3). These values correlated significantly with the radial artery electron microscopic scores ($p < 0.001$).

In the electron microscopic evaluation, specimens of the non-DM patients showed normal arterial wall structure. Endothelial cells were normal, with a nucleus containing diffuse chromatin; they possessed abundant rough endoplasmic reticulum and multiple mitochondria with distinct mitochondrial cristae. The subendothelial layer was thin. Tunica media smooth muscle cells were spindle shaped, with an elliptical nucleus. Organelles were located in the perinuclear area, myofilaments and dense bodies could be visualized in the cytoplasm of the smooth muscle cells (Fig. 1A and B).

In segments of the RA of the regulated-DM patients, the endothelial layer was intact and had normal thickness; however, mild swelling of mitochondria and slightly expanded endoplasmic reticulum were observed in the endothelial cells. The subendothelial layer was thickened with the presence of subendothelial edema. A tendency for smooth muscle cells to move to the tunica intima was observed. Smooth muscle cells in the tunica media had vacuoles containing cell debris. No abnormality was present in the ultrastructural evaluation of the tunica adventitia (Fig. 1C and D).

The poorly regulated DM patients endothelial cells showed significant histologic changes. The mitochondria of the cells were swollen and had a vacuolar appearance, with disrupted cristae structures. The subendothelial layer was thickened due to the presence of subendothelial edema; myofibroblasts were seen in the thickened subendothelial layer. Smooth muscle cells had vacuoles containing cell debris as in the regulated-DM group (Fig. 1E and F).

The results of the ultrastructural evaluation are shown in Table 2. Total scores of the specimens of the dysregulated-DM patients were higher compared to those of both non-DM patients and regulated-DM patients ($p < 0.001$ for both comparison). Moreover, total scores of regulated DM group were significantly higher than the total scores of non-DM group ($p < 0.028$).

HbA1c, FPG, duration of DM ($p < 0.001$), and CRP ($p = 0.001$) were correlated with RA electron microscopic scores. These factors were included in the linear regression analysis. Duration of DM ($p = 0.027$) and FPG ($p = 0.001$) was found to be independent risk factors for atherosclerotic changes of the radial artery grafts.

Postoperative course was uneventful for each patient. No preoperative myocardial infarction or ischemic event was observed. Superficial wound complications in the radial artery incision occurred in two cases and were treated with dressing.

DISCUSSION

In the present study, we analyzed the effects of Type II DM on morphology of the RA graft. We found a strong correlation between atherosclerotic changes and diabetes mellitus, which was more significant in the patients who have longer duration of DM, higher fasting plasma glucose, and HbA1c levels. This result indicated that duration of DM, high fasting plasma glucose, and HbA1c values may affect graft morphology.

The Radial Artery Patency Study evaluated angiographic results of 440 RA grafts and 440 SVG. In this study, the RA was found to be protective against occlusion up to 12 months, especially in female patients. After one year, angiographic results revealed the RA use had better outcomes versus SVG use especially in diabetics, which was an independent predictor of graft occlusion.[9] Lin et al.[15] found a significant survival benefit with the RA at 5- and 12-year follow-up in comparison with SVG use in diabetic patients. This benefit was not significant in a non-diabetic subgroup.

TABLE 2
Comparison of Radial Artery Grafts Electron Microscopic Scores

	Group 1	Group 2	Group 3	p(1–2)	p(1–3)	p(2–3)
Score 1	1.0 ± 1.0	0.6 ± 0.9	3.0 ± 0	0.591	<0.001	<0.001
Score 2	1.7 ± 1.1	3.0 ± 0	3.0 ± 0	<0.001	<0.001	1.0
Score 3	1.0 ± 0.7	1.3 ± 0.4	2.3 ± 0.6	0.356	<0.001	<0.001
Total score	3.7 ± 1.5	5.0 ± 1.4	8.3 ± 0.4	0.028	<0.001	<0.001

Score 1: Ultrastructural changes in endothelial layer. Score 2: Ultrastructural changes in arterial wall. Score 3: Ultrastructural changes in endothelial mitochondria.

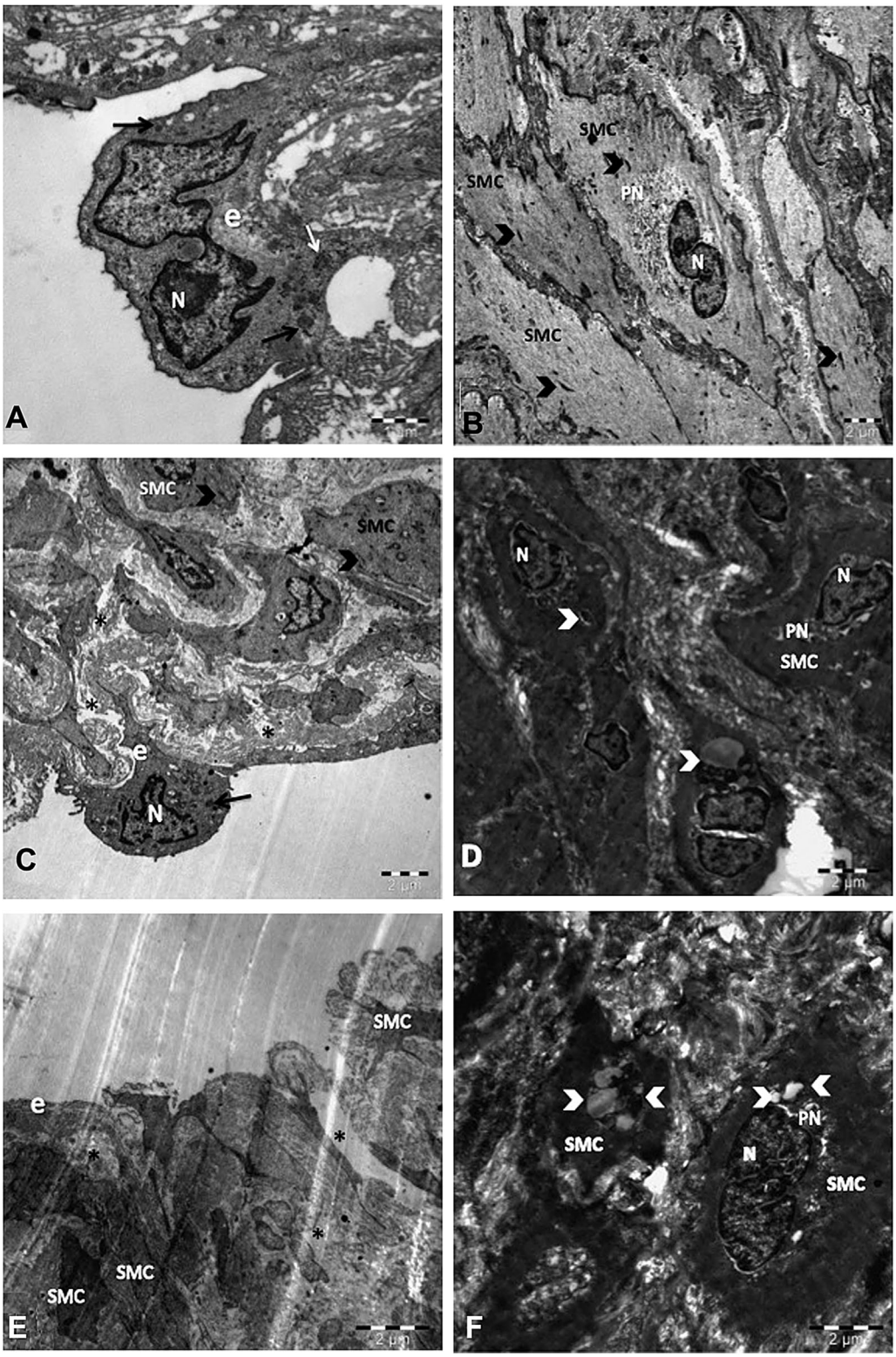

Figure 1. *Figure shows electron microscopic view of the radial artery grafts. (A and B) Representative electron micrographs of radial artery specimens of non-diabetic (A and B), regulated-diabetic (C and D), and dysregulated-DM (E and F) patients. (A) Normal flat endothelial cell is observed, 20,000k×. (B) Transverse section of smooth muscle cells with eliptical nucleus in the tunica media, 6000k×. (C) Intact endothelial cell and its mitochondria with mild swelling, subendothelial edema, and some migrated smooth muscle cells are observed, 7500k×. (D) Some vacuoles in the smooth muscle cells of tunica media are noted, 10.000k×. (E) Damaged endothelial layer and thicker subendothelial layer than the other groups with the presence of edema and migrated smooth muscle cells are seen, 12.000k×. (F) The vacuoles containing cell debris are observed at the perinuclear area of smooth muscle cells in the tunica media, 12.000k×. N, nucleus; e, endothelial cell; black arrow, mithocondria; white arrow, rough endoplasmic reticulum. *, Subendothelial edema; SMC, smooth muscle cell; PN, perinuclear area of smooth muscle cell; black arrow head, dense body; white arrow head, vacuoles containing cell debris.*

DM effects the various components of the vascular system including endothelial cells, vascular smooth muscle cells, and monocyte-derived macrophages. Increased reactive oxygen species, decreased nitric oxide bioavailability, increased toxic metabolites, increased glycation, synthesis of advanced glucose end products (AGEs), impairment of endothelial dependent vasorelaxation have all been considered as potential mechanisms of endothelial damage. Increased levels of IL1-B, IL6, CD36, MCP-1, and activation of protein kinase C in monocyte-derived macrophages trigger vascular injury. Additionally, matrix degradation, proliferation, reactive oxygen species, and non-enzymatic collagen glycation are increased in vascular smooth muscle cells. Matrix components, such as chondroitin sulphate and dermatansulphate, are found to be altered.[16–19] Hyperglycemia also activates transcription factors such as nuclear factor kappa B, which is a key mediator regulating multiple pro-inflammatory and atherosclerotic target genes in endothelial cells, vascular smooth muscle cells, and macrophages.[17,19,20]

In the present study, we found a significant correlation between duration of DM, fasting plasma glucose, HbA1c values, and electron microscopic radial artery electron microscopic scores. The radial artery scores were also significantly higher in patients with regulated DM than those without diabetes. El-Osta et al.[21] showed that transient hyperglycemia may induce atherogenic effects during normoglycemia by various mechanisms including; changes in chromatin remodeling, recruitment of the histone methyltransferase set 7, and increased H3K4 monomethylation in the proximal nuclear factor kappa B promoter, causing increased expression of p65, MCP-1, and VCAM-1.[21] These affects may explain diabetic vascular complications in patients with normoglycemia and normal HbA1c values. This condition is known as ''hyperglycemic memory'' and may explain diabetic vascular pathologies in patients with normal HbA1c.

Previous studies have shown a significant correlation of vascular atherosclerotic changes and duration of diabetes.[22,23] Shah et al.[24] reported that each 1% increase in HbA1c or each year of duration of diabetes is associated with approximately 30% increased odds of a thicker carotid intima media thickness. Zou et al.[25] found that intimal thickness and intima media ratio are increased in diabetic patient's radial artery grafts in comparison with non diabetics using electron microscopy. Chowdury et al.[26] also found increased medial calcification and atherosclerosis of the radial artery grafts in diabetics. Our study demonstrated a strong correlation of electron microscopic atherosclerotic disease and duration of DM. In linear logistic regression analysis, longer duration of DM appeared as an independent determinant of endothelial changes.

This study has several limitations including a small sample size, lack of preoperative duplex ultrasonography, clinical correlation, postoperative graft angiography, and long-term follow-up. Additional follow-up studies are planned to determine whether these EM changes will affect RA graft patency and result in an increased incidence of recurrent ischemic events.

Acknowledgment: *We thank Prof. Dr. Nurhan Ince for her contribution in statistical analysis.*

REFERENCES

1. Raza S, Sabik JF, 3rd, Masabni K, et al: Surgical revascularization techniques that minimize surgical risk and maximize late survival after coronary artery bypass grafting in patients with diabetes mellitus. J Thorac Cardiovasc Surg 2014;148(4):1257–1266.e9.
2. Schwann TA, Zacharias A, Riordan CJ, et al: Sequential radial artery grafts for multivessel coronary artery bypass graft surgery: 10-year survival and angiography results. Ann Thorac Surg 2009;88(1):31–39.
3. Locker C, Schaff HV, Dearani JA, et al: Multiple arterial grafts improve late survival of patients undergoing coronary artery bypass graft surgery: Analysis of 8622 patients with multivessel disease. Circulation 2012;126 (9):1023–1030.
4. Gaudino M, Crea F, Cammertoni F, et al: Morpho-functional features of the radial artery implications for use as a coronary bypass conduit. Ann Thorac Surg 2014;98 (5):1875–1879. doi: 10.1016/j.athoracsur.2014.06.101.
5. Deb S, Singh SK, Moussa F, et al: The long-term impact of diabetes on graft patency after coronary artery bypass grafting surgery: A substudy of the multicenter Radial Artery Patency Study. J Thorac Cardiovasc Surg 2014;148 (4):1246–1253.
6. Schwann TA, Al-Shaar L, Engoren M, et al: Late effects of radial artery vs saphenous vein grafting for multivessel coronary bypass surgery in diabetics: A propensity-matched analysis. Eur J Cardiothorac Surg 2013;44 (4):701–710.
7. Cao C, Manganas C, Horton M, et al: Angiographic outcomes of radial artery versus saphenous vein in coronary artery bypass graft surgery: A meta-analysis of randomized controlled trials. J Thorac Cardiovasc Surg 2013;146(2):255–261.
8. Athanasiou T, Saso S, Rao C, et al: Radial artery versus saphenous vein conduits for coronary artery bypass surgery: Forty years of competition—Which conduit offers better patency? A systematic review and meta-analysis. Eur J Cardiothorac Surg 2011;40(1):208–220.
9. Singh SK, Desai ND, Petroff SD, et al: The impact of diabetic status on coronary artery bypass graft patency: Insights from the radial artery patency study. Circulation 2008;118(14 Suppl):S222–S225.
10. Yanagawa B, Algarni KD, Singh SK, et al: Clinical, biochemical, and genetic predictors of coronary artery bypass graft failure. J Thorac Cardiovasc Surg 2014;148 (2):515–520.e2.
11. Fontbonne A, Eschwede E, Cambien F, et al: Hypertriglyceridaemia as a risk factor of coronary heart disease mortality in subjects with impaired glucose tolerance or diabetes. Results from the 11-year follow-up of the Paris Prospective Study. Diabetologia 1989;32:300–304.
12. Halkos ME, Lattouf OM, Puskas JD, et al: Elevated preoperative hemoglobin A1c level is associated with reduced long-term survival after coronary artery bypass surgery. Ann Thorac Surg 2008;86:1431–1437.
13. Bakuy V, Unal O, Gursoy M, et al: Electron microscopic evaluation of internal thoracic artery endothelial morphology in diabetic coronary bypass patients. Ann Thorac Surg 2014;97(3):851–857.
14. Schulz KF, Altman DG, Moher D, CONSORT Group: CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. PLoS Med 2010;7(3):e000251.

15. Lin J, Cheng W, Czer LS, et al: Coronary artery bypass graft surgery using the radial artery as a secondary conduit improves patient survival. J Am Heart Assoc 2013;2(4): e000266.
16. Alserius T, Anderson RE, Hammar N, et al: Elevated glycosylated haemoglobin (HbA1c) is a risk marker in coronary artery bypass surgery. Scand Cardiovasc J 2008;42:392–398.
17. Paneni F, Beckman JA, Creager MA, et al: Diabetes and vascular disease: Pathophysiology, clinical consequences, and medical therapy: Part I. Eur Heart J 2013;34 (31):2436–2443.
18. Sena CM, Pereira AM, Seiça R: Endothelial dysfunction—A major mediator of diabetic vascular disease. Biochim Biophys Acta 1832;2013(12):2216–2231.
19. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414(6865): 813–820.
20. Brownlee M: The pathobiology of diabetic complications: A unifying mechanism. Diabetes 2005;54(6): 1615–1625.
21. El-Osta A, Brasacchio D, Yao D, et al: Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med 2008;205(10):2409–2417.
22. Yavuz T, Akçay A, Omeroğlu RE, et al: Ultrasonic evaluation of early atherosclerosis in children and adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 15:1131–1136.
23. Liu YP, Zhan WW, Zhang YF, et al: Carotid intima-media thickness and stiffness in relation to type 2 diabetes in Chinese. Endocrine 31:289–293.
24. Shah AS, Dolan LM, Kimball TR, et al: Influence of duration of diabetes, glycemic control, and traditional cardiovascular risk factors on early atherosclerotic vascular changes in adolescents and young adults with type 2 diabetes mellitus. J Clin Endocrinol Metab 2009;94(10):3740–3745.
25. Zou L, Chen X, Chen W, et al: Comparative study on the histomorphology and molecular biology of radial artery conduits in patients with diabetes mellitus who underwent coronary bypass surgery. Diab Vasc Dis Res 2013;10(3):208–215.
26. Chowdhury UK, Airan B, Mishra PK, et al: Histopathology and morphometry of radial artery conduits: Basic study and clinical application. Ann Thorac Surg 2004;78(5): 1614–1621.