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Differences in myocardial PTEN expression and Akt signaling in type 2 diabetic and non-diabetic patients undergoing coronary bypass surgery

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Summary

Objective—Patients with diabetes experience increased cardiovascular complications after cardiac surgery. Hyperglycaemia predicts increased mortality after myocardial infarction and may influence cardiovascular risk in humans. Impaired prosurvival phosphatase and tensin homologue on chromosome 10 (PTEN)-Akt signaling could be an important feature of the diabetic heart rendering it resistant to preconditioning. This study was designed to evaluate for differences and relationships of myocardial PTEN-Akt-related signaling and baseline glycaemic control marker in type 2 diabetic and non-diabetic patients undergoing coronary artery bypass surgery.

Methods—Right atrial biopsies and coronary sinus blood were obtained from 18 type 2 diabetic and 18 non-diabetic patients intraoperatively. Expression and phosphorylation of Akt, eNOS, Bcl-2 and PTEN were evaluated by western blot. Plasma 15-F_{2t}-isoprostane concentrations were evaluated by liquid chromatography-mass spectrometry.

Results—PTEN expression and 15-F_{2t}-isoprostane concentrations were significantly higher in diabetic patients. Increased fasting blood glucose levels correlated with increased coronary sinus plasma 15-F2t-isoprostane concentrations. Increased cardiac 15-F_{2t}-isoprostane generation was highly correlated with myocardial PTEN expression. Bcl-2 expression and eNOS phosphorylation were significantly lower in diabetic compared to non-diabetic patients. Akt phosphorylation tended to be lower in diabetic patients, however this tendency failed to reach statistical significance.

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Disclosures

We declare that we have no conflict of interest.

Conclusion—The current results suggest that prosurvival PTEN-Akt signaling is impaired in the diseased diabetic myocardium. Hyperglycaemia and increased oxidative stress may contribute to this phenomenon. These findings strengthen the understanding of the underlying biologic mechanisms of cardiac injury in diabetic patients, which could facilitate development of new treatments to prevent cardiovascular complications in this high-risk population.

Keywords

Type 2 diabetes; Coronary bypass surgery; Ischemia-reperfusion injury; PTEN; Akt signaling

Introduction

Patients with diabetes mellitus are up to five times more likely to develop cardiovascular disease[1] and account for nearly thirty percent of patients undergoing cardiac surgery[2]. Diabetes mellitus is an independent risk factor for increased early and late postoperative morbidity and mortality associated with myocardial injury[3]. This includes an elevated risk for low cardiac output syndrome that can quadruple the overall mortality rate for coronary artery bypass surgery[4].

Major efforts have focused on increasing the myocardial tolerance to ischemia. Unfortunately, the diabetic myocardium is resistant to physical or pharmacologic preconditioning stimulus[5]. Experimental studies have detected corrupted protective signal transduction pathways and enhanced mitochondrial permeability transition, which could explain the increased susceptibility to injury in ischemia-reperfused diabetic myocardium[4, 6]. However, the in vivo characteristics that could place patients at risk during surgery have yet to be fully delineated.

Glycaemic control (Fasting blood glucose, FBG) and glycosylated haemoglobin (blood haemoglobin A1c, HbA1c) have been used as markers of diabetic control[7, 8]. Hyperglycaemia predicts increased mortality after myocardial infarction and may influence cardiovascular risk in humans{Duncan, #46}. More recent interest has focused on the glycaemic control as both marker and mediator of adverse postoperative surgical outcomes{Duncan, #46;Halkos, 2008 #40;Nesto, 2008 #44}. The underlying mechanism of the association is unclear.

The phosphoinositide-3 kinase (PI3K)/Akt prosurvival pathway is central to physical and pharmacologic preconditioning and salvaging ischemia-reperfused myocardium. Once activated, Akt may induce a cytoprotective effect by activating end-effectors including endothelial nitric oxide synthase (eNOS) and anti-apoptotic Bcl-2 to antagonize mitochondrial directed cell death[10, 11]. The diabetic myocardium appears resistant to the effects of physical preconditioning on Akt activation[12, 13]. The principle negative regulator of the PI3K/Akt pathway is phosphatase and tensin homologue on chromosome 10 (PTEN)[14]. Hyperglycaemia induced reactive oxygen or nitrogen species generation could upregulate PTEN antagonism of Akt[15, 16].

To date, no therapeutic approach has been demonstrated clinically effective against cardiac injury in the diabetic population. Strengthening our understanding of the underlying biologic

mechanisms is a research priority that could facilitate development of new treatments to prevent cardiovascular complications in this high-risk patient population. The aims of our study were 1) to evaluate for differences in the regulation of the PTEN and myocardial Akt-related signaling, and 2) to determine its relationship with baseline biochemical markers of glycaemic control in vivo, in type 2 diabetic and non-diabetic patients presenting for coronary artery bypass surgery.

Subjects and Methods

Patients

This investigation conforms to the principles outlined in the Declaration of Helsinki. Following approval of the University of British Columbia Clinical Research Ethics Board and informed patient consent, 36 patients (18 patients with or without type 2 diabetes) undergoing primary coronary artery bypass graft (CABG) surgery randomized in the PROTECT II study were included. Details of the study have been recently described[4].

Study inclusion criteria were: 1) a preoperative systolic blood pressure > 90 mmHg in the absence of inotropic or mechanical support; 2) scheduled for revascularization of two or more coronary vessels. Patients were excluded who: 1) were less than 18 or greater than 80 years old; 2) had co-existing valvular heart disease; 3) had an acute or evolving myocardial infarction; or 4) took nonsteroidal anti-inflammatory drugs, or vitamin C or E within 5 days surgery. Patients stopped taking oral hypoglycemic agents 24 hours before surgery.

Type 2 diabetics were defined by an established history and diagnosis of adult-onset diabetes mellitus treated by diet alone or in combination with oral hypoglycemic agents (regardless of insulin use). Insulin use was initiated when adequate glucose control could not be achieved with oral hypoglycemic agents. Clinically, no patients with microvascular complications or evidence of renal dysfunction (creatinine > 135 μmol/l) were enrolled.

All patients received the same anesthetic induction agent (thiopental) and anesthetic maintenance with moderate dose intravenous narcotic, fentanyl and inhalational agent, isoflurane (0.5% to 1% end tidal), prior to atrial biopsy and coronary sinus sampling and subsequent blinded PRO-TECT II experimental intervention according to study protocol[4].

Myocardial and coronary sinus blood sampling

Tissue biopsies were collected from the right atrial appendage immediately prior to cardiopulmonary bypass. Biopsies were immediately flash frozen in liquid nitrogen and stored at −80°C for subsequent western blotting analysis. Coronary sinus blood was sampled through a retrograde coronary sinus cannula immediately prior to cardiopulmonary bypass. Blood samples were centrifuged at 1300 g for 10 minutes, and the plasma was stored at −80°C in 1.25 ml aliquots in the presence of 0.005% of butylated hydroxytoluene for subsequent liquid chromatography-mass spectrometry (LC-MS) analysis.

LC-MS analysis

15-F_{2t}-isoprostane analysis was performed using immunoaffinity purification followed by LC-MS analysis. In brief, 500 μl of plasma was pretreated, spiked with 15-F_{2t}-isoprostane-

D_4 internal standard and applied to an 8-isoprostane affinity column (Cayman Chemical, Ann Arbor, MI, USA) as previously described[17]. The eluent was dried in a speedvac, and resuspended in water containing 2% acetonitrile and 0.01% NH_4OH for subsequent LC-MS analysis. LC was performed using a 12 minute gradient, from 5% to 20% B, with the following mobile phase composition: A) 0.01% NH_4OH in water. B) 0.01% NH_4OH in acetonitrile. Quantitative analysis was accomplished using an ion trap mass spectrometer (Bruckner Daltonics, Leipzig, Germany), comparing the ratio of the intensity of the signal at m/z = 353.1 and m/z = 357.1.

Western Blotting

Atrial biopsies were homogenized using a tissue grinder in ice-cold radioimmunoprecipitation assay buffer (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) containing 20 mm Tris–HCl (pH 7.4), 150 mm NaCl, 1% Nonider P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.004% sodium azide, 1% phenylmethylsulfonyl fluoride, 1% sodium orthovanadate and 1% protease inhibitor cocktail at 4°C. The lysate was centrifuged at 10,000 g at 4°C for 30 minutes to remove the insoluble material. Supernatants were collected. The protein concentration was measured by Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Then equal amounts of protein (50 μg) from each sample was separated by 12% SDS–PAGE and transferred to nitrocellulose membrane. The membranes were blocked for 1 hour in 5% skim milk and then incubated overnight at 4°C with primary antibody, independently, against the following proteins: PTEN, Akt, phospho-Akt, eNOS, phosphorylated-eNOS, anti-apoptotic Bcl-2, and β-actin (All antibodies purchased from Cell Signaling Technology, Inc., Danvers, MA, USA). Following a 30-minute wash, the membranes were incubated with secondary antibody conjugated to horseradish peroxidase for 1 hour at room temperature. The membranes were then washed for 30 minutes and visualized by enhanced chemiluminescence. Protein expression levels were derived using FluorChem® Q image analyzer (Alpha Innotech, San Leandro, CA, USA), detecting signals from nitrocellulose membranes.

Statistical analysis

All analyses were performed using GraphPad Prism version 4.0 for Mac (GraphPad Software, San Diego California USA, www.graphpad.com). A value of $P < 0.05$ was considered statistically significant. Differences in categorical variables between diabetic and non-diabetic subjects were compared using Fisher's exact test (Table 1). All continuous variables were subject to a Shapiro-Wilk normality test and presented as mean and standard deviation (mean ± SD). Normally distributed data derived from diabetic patients was compared with that of non-diabetic counterparts using an unpaired two-tailed t-test with Welch's correction (Table 1). Non-normally distributed data were similarly compared using a Mann-Whitney test (Figure 1a, c and Figure 2b~h). Correlation analyses were performed using linear regression with a two-tailed Pearson test where the *P*-value is indicative of a non-zero slope, which is described according to its 95% confidence interval (CI) (Figure 1b, d and Figure 3a, b).

Results

Patients

The preoperative characteristics of non-diabetic and diabetic patients are described in Table 1. There were no significant differences between diabetic and non-diabetic groups with respect to gender, age, height, left ventricular ejection fraction, cardiac risk factors (hypertension, current smoker or dyslipidemia), or preoperative medication use (β-blocker, angiotensin-converting enzyme inhibitor, digoxin, or statin). However, weight ($P = 0.02$), body surface area ($P = 0.046$) and calcium-channel blocker use ($P = 0.007$) were higher in diabetic group. Diabetic subjects also had a significant higher fasting blood glucose level ($P = 0.003$).

A profile of diabetic patients included in the study is listed in Table 2. Diabetic patients had a blood HbA1c level of 6.89 ± 1.16% (range from 4.7% to 9.5%). Diet alone (1 of 18 subjects) or in combination with insulin (3 of 18 subjects) or oral hypoglycemic agents (Metformin, Glyburide, Gliclazide, Rosiglatazone or Thiazolidenedione) were used in diabetic subjects to treat type 2 diabetes.

15-F_{2t}-isoprostane levels

In order to investigate whether diabetic patients in the current study had increased reactive oxygen species activity, and to find evidence in support of its relationship with fasting blood glucose levels, we measured plasma free 15-F_{2t}-isoprostane levels in coronary sinus blood. The concentration of 15-F_{2t}-isoprostane in this study was significantly greater in coronary sinus plasma from diabetic patients (100.50 ± 57.26 pg/ml) compared to non-diabetic patients (53.44 ± 35.14 pg/ml) ($P < 0.001$, Mann-Whitney test; Figure 1a).

Correlation analysis between 15-F_{2t}-isoprostane and fasting blood glucose levels was performed using linear regression. This analysis revealed a significant correlation ($P = 0.02$, r = 0.3869) with a slope of 9.06 (95% CI: 1.41 to 16.72) (Figure 1b).

PTEN expression

In order to investigate whether diabetic patients presenting for CABG surgery had increased PTEN level, and to find evidence of its relationship with plasma 15-F_{2t}-isoprostane levels, we evaluated PTEN expression by western blot and performed a correlation analysis between PTEN and the 15-F_{2t}-isoprostane levels using linear regression.

As shown in Figure 1c, biopsies from diabetic patients had significantly higher PTEN protein expression than those from non-diabetic patients (non-diabetic: 0.55 ± 0.50, diabetic: 1.01 ± 0.64, $P = 0.005$, Mann-Whitney test). In addition, the linear regression analysis revealed a significant correlation between PTEN/β-actin and 15-F_{2t}-isoprostane levels ($P = 0.008$, r = 0.4445) with a slope of 0.007 (95% CI: 0.002 to 0.011) (Figure 1d).

Akt expression and phosphorylation

Atrial biopsies from diabetic and non-diabetic patients exhibited similar levels of total Akt protein expression (non-diabetic: 1.27 ± 1.43, diabetic: 1.05 ± 0.66, $P = 0.41$, Mann-

Whitney test; Figure 2a, b). The extent of Akt phosphorylation (phospho-Akt/Akt) tended to be lower in atrial tissue biopsies from diabetic patients (1.26 ± 0.73) than from non-diabetic patients (1.81 ± 1.64), however this tendency was not statistically significant ($P = 0.06$, Mann-Whitney test; Figure 2a, d).

PTEN is the main negative modulator of PI3K/Akt signaling pathway. In order to find evidence of the relationship of Akt phosphorylation with PTEN expression, we performed a correlation analysis between PTEN and the extent of Akt phosphorylation using linear regression. The linear regression analysis revealed a significant negative correlation between PTEN/β-actin and phospho-Akt/Akt ($P = 0.048$, r = 0.3324) with a slope of -0.70 (95% CI: −1.39 to −0.01) (Figure 3a).

Expression and activation of Akt targets: Bcl-2 and eNOS

In order to further investigate the extent of Akt signaling, we evaluated the expression and activation of two key downstream signaling intermediates Bcl-2 and eNOS.

The eNOS protein levels tended to be higher in biopsies from diabetic patients (1.18 ± 0.68) than those from non-diabetic patients (1.13 ± 1.54), however this tendency was not statistically significant ($P = 0.06$, Mann-Whitney test; Figure 2a, f). By contrast, biopsies from diabetic patients had significantly lower extents of eNOS phosphorylation (phospho-eNOS/eNOS) than those from non-diabetic patients (non-diabetic: 2.10 ± 2.67, diabetic: 0.55 ± 0.44, $P = 0.03$, Mann-Whitney test; Figure 2a, h).

Biopsies from diabetic patients had significantly lower levels of Bcl-2 than those from non-diabetic patients (non-diabetic: 1.04 ± 0.54, diabetic: 0.57 ± 0.34, $P = 0.005$, Mann-Whitney test; Figure 2a, e). In addition, a linear regression analysis revealed a significant negative correlation between Bcl-2/β-actin and fasting blood glucose levels ($P = 0.008$, r = 0.4339) with a slope of −0.13 (95% CI: −0.22 to −0.03) (Figure 3b).

Discussion

The aim of the present study was to evaluate for baseline differences and relationships of myocardial PTEN-Akt-related signaling to biochemical makers of glycaemic control in type 2 diabetic and non-diabetic patients undergoing CABG surgery as part of the PRO-TECT II study[4]. We have made three observations that pertain to patients with type 2 diabetes; 1) increased 15-F_{2t}-isoprostane concentrations in coronary sinus plasma, which was significantly correlated with fasting blood glucose levels; 2) increased myocardial PTEN expression; 3) reduced myocardial Akt signaling, as shown by the combination of decreased eNOS phosphorylation, decreased Bcl-2 expression and a tendency towards lower Akt phosphorylation in the myocardium. These findings strengthen the understanding of the underlying biologic mechanisms of cardiac injury in diabetic patients, which could facilitate development of new treatments to prevent cardiovascular complications in this high-risk patient population.

Cardiovascular diseases are the leading cause of morbidity and mortality in the insulin resistant patient population[1]. Thirty percent of patients presenting for coronary bypass

surgery are insulin resistant[2]. Diabetes mellitus in general and perioperative hyperglycaemia in particular has been associated with marked increase in postoperative complications including death{Duncan, #46;Nesto, 2008 #44}. The molecular defects in response to type 2 diabetes and hyperglycaemia contributing to poor operative outcomes have not been well defined. Experimental studies suggest that the diabetic heart is resistant to both ischemic and pharmacological preconditioning[5, 18]. However, the in vivo characteristics that could place patients at risk during surgery have yet to be fully delineated.

Oxidative stress is a hallmark of type 2 diabetes. 15-F_{2t}-isoprostanes are surrogate markers of oxidative stress in type 2 diabetes[19], whose levels in coronary sinus blood reflect the amount of reactive oxygen species in coronary circulation. In this study, the baseline level of plasma 15-F_{2t}-isoprostane in coronary sinus blood was significantly higher in patients presenting with type 2 diabetes (Figure 1a). Acute hyperglycaemia stimulates isoprostane generation and decreases plasma antioxidant defenses[20, 21]. The level of isoprostane is reported to vary with the degree of glucose fluctuation, even in the absence of an increase in HbA1c in healthy patients or those with type 2 diabetes[22]. This is consistent with our present findings, which demonstrated a significant correlation between fasting blood glucose and coronary15-F_{2t}-isoprostane (Figure 1b).

PTEN is a dual protein lipid phosphatase that dephosphorylates the secondary messenger produced by PI3K, and interrupts the downstream activation of Akt. It plays a significant role in regulating the balance between survival and death in many cell types, including cardiomyocytes. Indeed, PTEN is associated with a reduction in preconditioning efficacy [23], and its inactivation may increase myocardial survival following an ischemic episode and ischemia-reperfusion injury[24]. Mocanu et al reported an increased PTEN level in the Goto Kakizaki rat heart model and suggested diabetes may be associated with increased PTEN level, at least in the myocardium[23]. We detected PTEN expression was significantly higher in atrial tissue from patients with type 2 diabetes (Figure 1c), supporting their finding.

Chronic pathological reactive oxygen species production induces PTEN expression[14, 15]. In our patient series, increased fasting blood glucose levels correlated with increased levels of 15-F_{2t}-isoprostane in coronary sinus blood (Figure 1b). Increased cardiac 15-F_{2t}-isoprostane generation was highly correlated with PTEN in human diabetic myocardium (Figure 1d), indicative of isoprostane's potential role in its generation.

The activation of Akt signaling is central to cardioprotective signaling pathways that reduce myocardial ischemia-reperfusion injury[11]. Accordingly, ischemic and pharmacological pre- and post-conditioning strategies exert their cardioprotective effects by recruiting the Akt signaling pathway[10]. To protect the diabetic myocardium, it appears necessary to sufficiently recruit these preconditioning stimuli. The resistance of the diabetic myocardium to preconditioning implies that a higher signaling threshold and a critical level of Akt phosphorylation must be attained to mediate myocardial protection. Indeed, Tsang *et al* reported a reduced level of Akt phosphorylation in a Goto-Kakizaki rat type 2 diabetic model[12]. By contrast, Huisamen reported that Akt was hyperphosphorylated in a Zucker obese rat model, but under-phosphorylated in a streptozotocin-induced type 2 diabetic rat model[25]. In the current study, the extent of Akt phosphorylation tended to be lower in the

diabetic group, but this trend failed to reach statistical significance ($P = 0.06$; Figure 2a, d). This tendency is in agreement with other clinical studies reporting significant reductions in Akt phosphorylation among diabetic patients[13, 26]. The preoperative use of insulin, an Akt activator, may have confounded our results since this trend becomes significant in subanalysis of patients treated with oral hypoglycemic agents alone (see Table 2; $P = 0.01$ for phospho/total Akt if excluding patients who received preoperative insulin, data not shown).

Phosphorylation of eNOS and upregulation of Bcl-2 represent two important pro-survival end-effectors downstream of Akt signaling. Akt directly activates eNOS by phosphorylation at Serine-1177 and Serine-615, and induces Bcl-2 protein expression through cyclic AMP-response element-binding protein and nuclear transcription factor-κB [27, 28]. Our data show that both the extent of eNOS phosphorylation, and the expression of Bcl-2 expression are significantly lower in atrial tissue from diabetic patients. The combination of lower eNOS phosphorylation and Bcl-2 expression may further reflect impaired Akt signaling.

We observed a trend of increased total eNOS expression in patients with type 2 diabetes (Figure 1a, f). Since the increased total eNOS expression was accompanied by a significantly lower phospho-eNOS/eNOS ratio (Figure 1a, h), our results may reflect a compensation mechanism whereby reduced eNOS activation induces eNOS expression in insulin resistance. We acknowledge that these results contrast with several previous reports[26], and further investigation is warranted.

We examined for a potential association between PTEN and Akt phosphorylation in our study. Our results revealed a significant negative correlation between PTEN and the extent of Akt phosphorylation (Figure 3a), suggesting that the decrease in the level of Akt phosphorylation results from an increased level of PTEN in myocardial tissue from insulin resistant patients.

Sulphonylureas, biguanides, thiazolidinediones and meglitinides have antioxidant effects[29]. Metformin treatment has been associated with nitric oxide generation in response to increases in AMP-activated protein kinase mediated activation of eNOS[30]. Thiazolidinediones are insulin sensitizers that improve the actions of insulin in endothelium. Despite recent reports of the cardioprotective properties of the acute administration of metformin in the setting of myocardial ischemia-reperfusion[30], we observed increased baseline levels of PTEN and reductions in baseline Akt and eNOS in patients on chronic therapy, a pattern consistent with underlying insulin resistance. In our clinical practice, we do not substitute insulin therapy when withholding oral hypoglycemic therapies prior to surgery. Therefore, these baseline derangements may explain the increased resistance to ischemic preconditioning stimuli previously described in *ex vivo* atrial tissues from patients with type 2 diabetes as previously described by Sivaraman et al[13]. These investigators detected reduced levels of Akt activation in human diabetic myocardium, but levels of PTEN, an upstream regulator (reactive oxygen species), and putative downstream effectors of Akt were not measured, as in our study.

Previous study identified the relationship of blood glucose and HgmA1c to downregulation of vascular antiapoptotic Bcl2 in skin[31]. We were able to identify such a direct correlation in human diabetic myocardium in our study (Figure 1b). Given the importance of Bcl2 in the prevention of mitochondrial directed cell death, this finding may be indicative of an underlying mitochondrial defect in diabetic cardiac tissue, rendering it susceptible to ischemia-reperfusion injury.

Study limitation

This study describes baseline characteristics. The observational nature reflects association and cannot test for, or directly imply, causation. Inter-patient biologic variability, while not unexpected, might influence and explain overlapping results. Detailed analysis of the potential confounding effects of preoperative drug therapies on our findings in blood and tissue is beyond the scope of this study. Protein expression in the atrium tissue is a surrogate measure of activity and expression in ventricular tissue. However, previous authors have reported a lack of significant differences between chambers, and have established the use of atrial biopsies in *ex vivo* experiments[32].

PTEN negatively regulates insulin signaling via action on Akt. Suppression of PTEN has been shown to restore glycaemic control[33]. PTEN is modulated during myocardial ischemia and reperfusion and its loss is involved in the induction of the ischemic preconditioning stimulus, via the transient upregulation of Akt at reperfusion[34]. Chronic Akt activation may be maladaptive and associated with cardiac dysfunction and failure, but its acute enhancement is associated with cyto- and cardioprotection, and improved contractility[35]. Based on our findings, the inhibition of PTEN or activation of Akt signaling to enhance insulin signaling, simultaneously targeting perioperative glycometrics and cardiac tissues, may prove to be an effective treatment alternative for the cardioprotection of surgical patients presenting with type 2 diabetes. The response of myocardial PTEN and Akt signaling to an intravenous anesthetic with antioxidant potential that inhibits mitochondrial permeability transition or pharmacologic preconditioning with volatile anesthesia is currently being evaluated in patients with type 2 diabetes presenting for cardiac surgery (the PRO-TECT II Study)[4]. The clinical efficacy of such an approach could improve outcomes and prognosis. This has yet to be determined.

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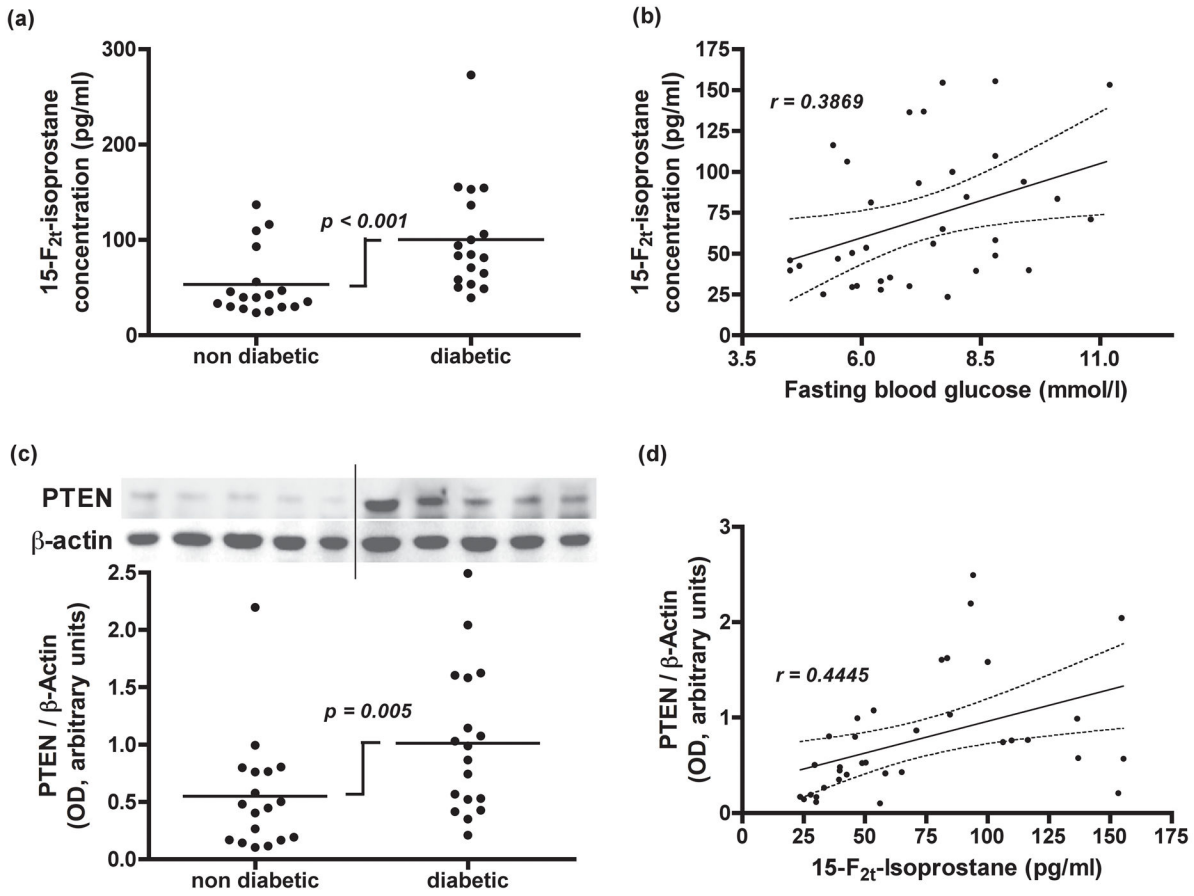

Figure 1.

(a) Dot plot representing plasma free15-F_{2t}-isoprostane concentrations in coronary sinus blood from coronary bypass patients with or without type 2 diabetes. P-values refer to differences between the groups as determined by Mann-Whitney test. (b) Correlation analysis between 15-F_{2t}-isoprostane concentrations and fasting blood glucose levels (slope = 9.06, 95% CI: 1.41 to 16.72; $P = 0.02$) by using linear regression with a two-tailed Pearson test. (c) Representative western blot for PTEN expression in atrial tissue biopsies taken from patients with or without type 2 diabetes. Dot plot represents PTEN expression normalized to β-actin expression. P-values refer to differences between the groups as determined by Mann-Whitney test. (d) Correlation analysis between atrial tissue PTEN/β-actin expression and coronary sinus plasma free 15-F_{2t}-isoprostane concentrations (slope = 0.007, 95% CI: 0.002 to 0.011; $P = 0.008$) by linear regression with a two-tailed Pearson test.

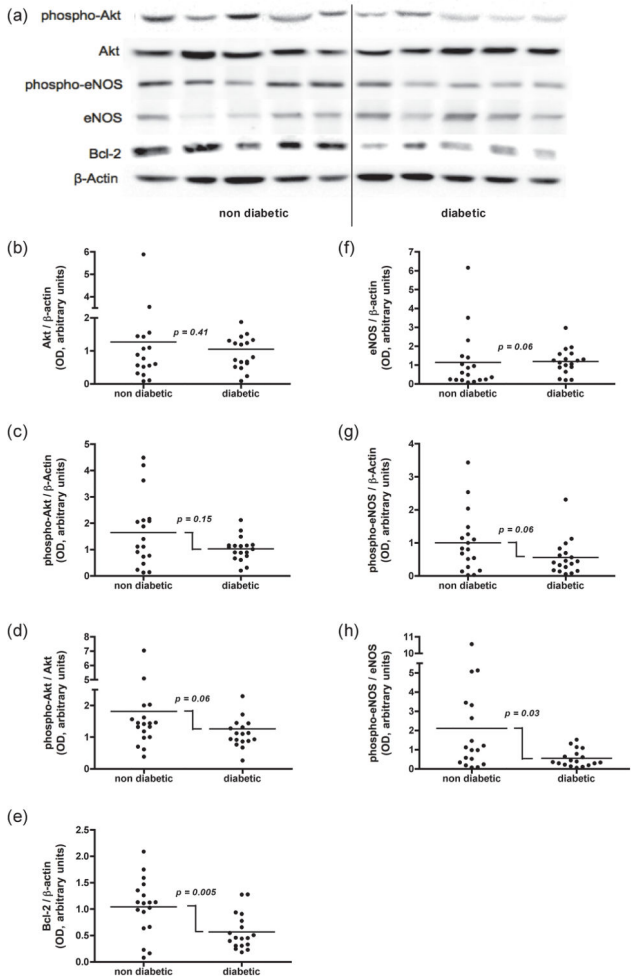

Figure 2.

Western blot comparison of Akt signaling in coronary bypass patients with or without type 2 diabetes. (a) Representative blots for phospho-Akt, Akt, phospho-endothelial nitric oxide (eNOS), eNOS, Bcl-2, and β-actin in non-diabetic and diabetic patients, respectively. Dot plot depictions of (b) Akt/β-actin, (c) Phospho-Akt/β-actin, (d) Phospho-Akt/Akt, (e) Bcl-2/β-actin, (f) eNOS/β-actin, (g) phospho-eNOS/β-actin, (h) phospho-eNOS/eNOS expression in atrial tissue biopsies from patients with or without type 2 diabetes. P-values refer to differences between the groups as determined by Mann-Whitney test.

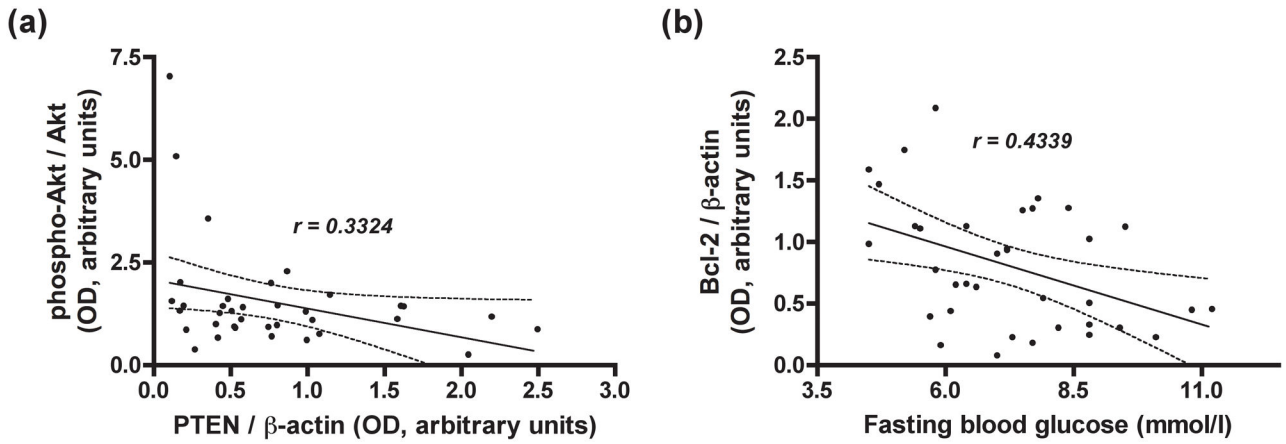

Figure 3.

Correlation analysis between (a) atrial tissue phospho-Akt/Akt and PTEN/β-actin expression (slope = -0.70, 95% CI: −1.39 to −0.01; $P = 0.048$), and (b) atrial tissue Bcl-2/β-actin expression and fasting blood glucose levels (slope = −0.13, 95% CI: −0.22 to −0.03; $P = 0.008$) from 36 patients undergoing by pass surgery. The analyses were performed using linear regression with a two-tailed Pearson test.

Table 1

Preoperative patient characteristics

Characteristic	Non Diabetic (n = 18)	Diabetics (n = 18)	P-value
Gender, male/female	14/4	16/2	0.66
Age, year	62.5 ± 8.5	65.7 ± 7.3	0.23
Weight, kilogram	79.57 ± 16.42	92.34 ± 14.22	0.02
Height, centimeter	171.3 ± 9.90	172.9 ± 5.64	0.55
Body surface area, meter^2	1.92 ± 0.24	2.06 ± 0.17	0.046
Left ventricular ejection fraction, %	47.71 ± 10.5	56 ± 14.25	0.06
Fasting blood glucose level, mmol/L	6.44 ± 1.42	8.1 ± 1.63	0.003
Cardiac risk factors, *n* (%)			
Hypertension	11 (61)	15 (83)	0.26
Current smoker	5 (28)	4 (22)	> 0.99
Dyslipidemia	17 (94)	17 (94)	> 0.99
Preoperative medication use, *n* (%)			
β-blocker	14 (77)	14 (77)	> 0.99
Calcium-channel blocker	1 (6)	9 (50)	0.007
Angiotensin-converting enzyme inhibitor	10 (56)	11 (61)	> 0.99
Digoxin	1 (6)	2 (11)	> 0.99
Statin	18 (100)	17 (94)	> 0.99

Values are presented as mean and standard deviation (mean ± SD), unless otherwise indicated. P-values refer to differences between the groups as determined by the unpaired two-tailed t-test with Welch's correction for continuous variables or Fisher's exact test for categorical variables.

Table 2

Profile of diabetic patients included in the study

No.	Age	Sex	FBG (mmol/l)	HbA1c (%)	Insulin and/or Oral hypoglycaemic or Diet	15-F_{2t}-isoprostane (pg/ml)	Western blot densitometry analysis	
							PTEN/β-actin	Phospho-Akt/Akt
1	74	M	7.9	6.2	Metformin	100.02	1.58	1.13
2	64	M	6.2	6	Metformin	81.26	1.61	1.45
3	55	M	8.2	8.1	Rosiglatazone	84.63	1.03	1.10
4	72	M	5.8	6.8	Metformin/Glyburide	50.35	0.53	0.91
5	50	M	7.2	6.3	Thiazolidenedione	272.95	1.14	1.71
6	74	M	7	7.5	Metformin	136.50	0.99	1.31
7	66	M	10.8	8.2	Insulin	70.90	0.87	2.29
8	61	M	10.1	na	Metformin	83.51	1.62	1.43
9	65	M	8.8	6.3	Metformin	48.85	0.52	0.94
10	66	M	5.7	5.5	Diet	106.24	0.74	0.93
11	68	M	6.1	6.4	Metformin/Glyburide	53.55	1.08	0.77
12	70	F	9.4	7.1	Metformin	94.05	2.49	0.88
13	72	M	7.7	6.7	Metformin/Gliclazide	154.53	2.04	0.26
14	71	M	7.7	6.3	Insulin/Metformin	65.03	0.43	1.27
15	60	F	8.4	8.7	Insulin/Metformin	39.47	0.35	3.57
16	64	M	11.2	9.5	Metformin/Glyburide	153.20	0.21	0.86
17	55	M	8.8	4.7	Metformin/Glyburide	58.32	0.42	0.67
18	76	M	8.8	6.9	Metformin	155.33	0.57	1.12

Abbreviations: FBG, Fasting blood glucose; HbA1c, Haemoglobin A1c, PTEN, phosphatase and tensin homologue on chromosome 10; M, Male; F, Femal; na, not available.