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Selective PKC Beta Inhibition with Ruboxistaurin and Endothelial Function in Type-2 Diabetes Mellitus

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Abstract

Purpose—Type-2 diabetes mellitus increases risk of atherosclerotic cardiovascular disease. However, the mechanisms linking hyperglycemia and atherosclerosis remain poorly understood. One proposed mechanism involves endothelial dysfunction via activation of protein kinase C beta (PKC beta). Prior studies demonstrate beneficial effects of PKC beta inhibition on microvascular parameters, but, to date, no study has examined the effect on macrovascular atherosclerotic readouts.

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Methods—The goal of this double-masked, placebo-controlled trial in type-2 diabetes was to assess the effect of the PKC beta-specific inhibitor, ruboxistaurin (32 mg/day for 6 weeks) on ultrasound assessed brachial artery flow mediated dilatation (FMD), a surrogate of macro vascular endothelial function, and urinary isoprostanes, indices of oxidant stress.

Results—Compared to placebo, ruboxistaurin tended to improve FMD (difference in 6-week change in FMD, mean±SD millimeter) at one (0.13±0.26 mm, p=0.08) and 5 min (0.12±0.21 mm, p=0.02) after cuff deflation, but had no effect on nitroglycerin-mediated dilatation or urinary isoprostanes.

Conclusions—This proof of concept trial is the first to suggest that specific inhibition of PKC beta may improve macro vascular endothelial function in type-2 diabetes. Larger trials including clinical endpoints are warranted to determine the potential efficacy of PKC beta inhibition in reducing atherosclerotic cardiovascular complications in diabetes mellitus.

Keywords

Type 2 diabetes; Protein kinase C beta; Endothelial function; Oxidant stress; Macro vascular disease

Introduction

Macrovascular atherosclerotic cardiovascular diseases (CVD) continue to be the leading cause of death in patients with type-2 diabetes mellitus [1]. This portends a significant health and societal burden, especially in an aging population with increasing obesity and type-2 diabetes. The risk of CVD in type-2 diabetes is only partly explained by traditional risk factors and current therapies fail to adequately abate CVD. Further, macrovascular, unlike microvascular complications, are only minimally ameliorated by tight glycemic control [2]. Thus, there is a great need for novel therapies that target vascular pathophysiology in the diabetic state.

Protein kinase Cs (PKCs) belong to a family of cytoplasmic serine/threonine kinases that participate in vascular cell signal transduction [3]. In particular, PKC beta (both the beta and $beta_2$ splice variants) is present in cardiovascular tissues and is activated by circulating concentrations of glucose and fatty acids that are present in type-2 diabetes [4, 5]. Indeed, PKC beta has increased activity in both microvascular [6] and macrovascular [7, 8] diabetic tissues and is linked to vascular pathology in rodent diabetic models [9]. PKC beta mediates diverse signaling, including oxidant, inflammatory, mitogenic and angiogenic effects, in diabetic vascular tissues that may promote atherosclerotic CVD [10].

PKC beta inhibitors are a new class of drugs that are effective in attenuating the vascular complications of diabetes in animal models. In particular, PKC beta inhibition blocks hyperglycemia mediated vascular cell dysfunction in vitro while retarding retinal and renal microvascular disease in rodent models [9, 11]. The evidence so far from phase-three clinical trials of ruboxistaurin, a highly selective inhibitor of PKC beta1 and beta2 isoforms, suggests efficacy in reducing the risk of visual loss in moderately severe diabetic retinopathy [12] and attenuating peripheral neuropathy [13, 14] although findings [15] require further validation as only statistical significance for secondary endpoints was achieved. Some have suggested a role for these agents for diabetic microangiopathy [16]. From a pathophysiological standpoint [17], there is evidence that selective inhibition of PKC beta1 and beta2 may ameliorate vascular complications of diabetes [18]. Notably, PKC beta activation in diabetes is found equally in macrovascular and microvascular tissue [7, 8, 11] suggesting a potential benefit of PKC beta inhibition also in reducing diabetic macro

vascular CVD. To date, however, no studies of the effect of PKC beta inhibition on CVD or it surrogates in type-2 diabetes have been reported.

The purpose of this proof of concept, placebo-controlled clinical trial of ruboxistaurin was to test the effect of PKC beta inhibition in patients with type-2 diabetes on urinary isoprostanes, indices of oxidant stress [19] and on brachial artery flow mediated dilatation (FMD), an ultrasound based measurement of macro vascular endothelial function [20–22].

Methods

This was a single-centered, double-masked, placebo-controlled, parallel, randomized clinical trial comparing ruboxistaurin (32 mg daily) with placebo in patients with type-2 diabetes. To be included, patients had to be ≥35 years of age diagnosed with type 2 diabetes mellitus (type 2 diabetes mellitus as diagnosed by their physician (criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus from the American Diabetic Association [23] with fasting low-density lipoprotein (LDL) cholesterol <160 mg/dL, triglycerides <400 mg/dL, resting systolic blood pressure <160 mm Hg, and HbA1c between 7.0% and 11.0%, inclusive. Exclusions included impaired renal function (serum creatinine >2.0 mg/dl, renal transplant, or dialysis); impaired liver function (hepatic enzymes >2× upper normal limit); active use of tobacco products; use of aspirin, non-steroidal anti-inflammatory drugs or antioxidant vitamins within 14 days; use of systemic antibacterial, antifungal, or antiviral drugs or inhibitors and inducers of cytochrome P450 3A4; active infection/inflammation; major surgery (abdominal, thoracic, vascular, or cranial) within 3 months; treatment for cancer within 6 months; investigational drug use within 30 days; and pregnancy, breast feeding, or child-bearing potential without use of a reliable method of birth control. The University of Pennsylvania (Penn) institutional review board approved the study protocol, written informed consent was provided by all participants and study procedures were carried out at the Penn General Clinical Research Center (GCRC).

Of 165 participants undergoing screening, 52 were eligible and randomly assigned to treatment and 49 completed the study (Fig. 1). In our *a priori* design, participants who discontinued prior to the collection of endpoint measurements were replaced. The main outcomes were (a) urinary levels of the isoprostane, 8,12-*iso*-iP$F_{2\alpha}$-VI and (b) flow mediated dilation (FMD) of the brachial artery at ultrasound. Exploratory outcomes included urinary albumin excretion rate and plasma lipoprotein levels. Based on published estimates [24], a sample size of 48 patients should have provided 80% power, at two-sided alpha level of 0.05, to detect a 30% difference between groups in change in urinary isoprostanes from baseline to endpoint.

Protocol and procedures

If eligible at Visit 1 (screening), participants returned within 4 weeks for Visit 2 randomization to ruboxistaurin (32 mg/day) or placebo for a minimum of 6 weeks, but a maximum of 8 weeks, prior to Visit 3, study completion. Dose and time were selected based on a phase 1b trial demonstrating efficacy of this dose on vascular endpoints, including vascular function after 1 week of treatment in healthy volunteers [25] and 28 days of treatment in patients with early diabetic retinopathy [26].

At randomization, medications were recorded, urine (2× 12 h collections), for isoprostanes and albumin, and blood, for plasma lipids, routine chemistry and hematology, were collected. Brachial artery FMD was measured by ultrasound. Participants completed a daily drug diary. At study completion, similar procedures were performed, drug diaries were collected, pill counts and adverse events were recorded.

Brachial artery reactivity

Brachial artery ultrasound evaluation of endothelial function was performed as per published guidelines [20]. Participants, fasting and free from caffeine and alcohol for 12 h, were studied in a quiet, dimmed, temperature-controlled (24°C) laboratory. Female participants were studied during the luteal phase of the menstrual cycle, as FMD varies during the menstrual phase of the cycle. After baseline B mode ultrasound imaging (Acuson Sequoia 256 Cardiac ultrasound system, Siemens Medical) using a 15L8 linear-array transducer, a small blood pressure cuff was positioned proximally in order to occlude arm systolic flow. After occlusion at 200 mmHg of pressure (5 min), the cuff was released and the ultrasound diameter was again measured 1 and 5 min after hyperemia. Fifteen minutes later, a second baseline ultrasound was acquired, sublingual nitroglycerine (0.4 mg) was administered and a final scan was performed 4 min later. The images were analyzed with FDA-approved and validated commercial software (Medical Imaging Applications, Iowa City, Iowa). The diameter of the vessel was measured by a single reader (SA) who was blinded to study status. Intra-reader reliability, verified on 25% of the studies, was internally consistent (spearman r=0.95). In addition, an expert reader (EM) selected 25% of the studies at random to validate measurements, and the inter-reader correlation was high (spearman r=0.98). Brachial artery diameters as well as absolute and percent change in flow- and nitroglycerine-induced dilatation are reported. The overall coefficient of variation for FMD measurements across visits was 1.3%.

Laboratory parameters

Urinary levels (nanograms per milligram creatinine) of the isoprostane 8,12-*iso*-iP$F_{2\alpha}$-VI were measured by liquid chromatography (HyperClone C18-BDS, 5 μm, 150×2 mm column), tandem mass spectrometry (LC/MS/MS) as previously described [27]. Briefly, this method utilizes reverse phase solid phase extraction (SPE) followed by LC/MS/MS using negative ion electrospray, selected reaction monitoring techniques. The transitions monitored are m/z 353>115 for the endogenous iP and m/z 357>m/z 115 for the internal standard, tetradeuterated 8,12-*iso*-iP$F_{2\alpha}$-VI with quantitation by peak area ratio. Urine levels (nanograms per milligram creatinine) of albumin were measured on a Beckman Coulter LX 20 platform using a turbidmetric method. Plasma levels of low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low density lipoprotein (VLDL) cholesterol and triglycerides were measured enzymatically following ultracentrifugation fractionation of serum lipids in Penn's Clinical Lipid Laboratory, which is certified by the Center for Disease Control. Serum biochemistry and blood hematology were performed in a centralized Quest laboratory.

Statistical methods

Normality of continuous variables was assessed using SAS PROC UNIVARIATE and variables with marked departures from normality were log transformed. Summary statistics (mean, standard deviation) for continuous clinical and laboratory variables and change from baseline to endpoint are presented. Categorical variables were examined between treatments using Fisher's exact test. Analysis of endpoints was performed using a modified intention-to-treat (ITT) principle for all subjects with main outcome data (isoprostane and FMD) at visit 3 (i.e. protocol completers) using an analysis of covariance (ANCOVA) model that included a main effect term for treatment and for the baseline efficacy variable. Least-squares means were used to test the effects of ruboxistaurin versus placebo and to obtain the corresponding between treatment p-value. Statistical significance was defined as a p-value<0.05. For safety analyses, all participants who enrolled in the study were included regardless of whether they had measurements of efficacy.

Results

Baseline characteristics, discontinuation and compliance

Baseline characteristics including concomitant medications were similar both in the treatment group and the placebo group (Table 1). Participants were 31% female, 55% African American, 38% Caucasian, and had a mean age of 55 years. Two placebo-treated patients discontinued for personal reasons and one patient on ruboxistaurin was discontinued by study physician due to poor compliance. Compliance, defined as >85% of study drug taken by tablet count, was 96.3% for ruboxistaurin treatment.

Effect of ruboxistaurin on endothelial function

After randomization, but prior to receiving drug, brachial artery diameters at rest, following cuff deflation and after nitroglycerine were similar in ruboxistaurin and placebo groups (Table 2). For example, in the groups randomized to receive ruboxistaurin and placebo, mean diameters were 3.89 mm vs. 3.77 mm (p=0.80) at rest, 4.29 mm vs. 4.14 mm (p=0.77) and 4.14 mm vs. 4.00 mm (p=0.80) at 1 min and 5 min post-cuff diameters respectively, and 4.62 mm vs. 4.53 mm (p=0.90) following NTG. There was high within-subject correlation between pre-cuff (baseline 1) and pre-NTG (baseline 2) diameters (spearman r=0.94). Furthermore, FMD in the ruboxistaurin group at 1 min (mean 0.39 mm±SD 0.21 mm) and 5 min (0.24±0.20) was not statistically different compared to the placebo group at 1 min (0.37±0.22, p=0.64) and 5 min (0.24±0.22, p=0.99) (Table 2 and Fig. 2a). Endothelial function, therefore, was similar in both groups prior to starting therapy.

Following 6 weeks of therapy, pre-cuff brachial artery diameters had excellent within-subject correlation with predrug values (spearman r=0.88). At 6 weeks, mean FMD in the ruboxistaurin group compared to the placebo group at 1 min (mean 0.39 mm±SD 0.24 mm vs. 0.24±0.15; p<0.001) and at 5 min (0.28±0.26 vs. 0.16±0.11; p<0.001) suggested improved FMD with ruboxistaurin treatment. Indeed, the average change in absolute FMD over the 6-weeks of treatment tended to differ (mean±SD mm) between ruboxistaurin and placebo (Table 2) at 1 (0.13± 0.26 mm, p=0.08) and 5 min (0.12±0.21, p=0.02). Furthermore, change in percent FMD over 6 week of treatment (Fig. 2b) at 1 min (11.2±9.9% vs. 6.5±4.4%; p=0.02) suggested improved FMD with ruboxistaurin, but not at 5 min (6.0±10% vs. 4.4±3.3%; p=0.23). In exploratory analyses, we did not find any statistically significant differences in the effect of RBX therapy on FMD across subgroups stratified by median levels of microalbuminuria and HbA1C, or by the presence or absence of statin use (data not shown). However, these exploratory analyses are underpowered and cannot exclude a true differential effect of RBX across strata. No statistically significant difference was observed between treatment groups over 6-weeks in nitroglycerin (0.4 mg) mediated dilatation, a measure of endothelium-independent vasodilatation (Table 2).

Effect of ruboxistaurin on urinary isoprostanes, urinary albumin excretion rate, laboratory parameters and adverse events

Compared to placebo, ruboxistaurin treatment produced no statistically significant effect on urinary levels of 8,12-*iso*-iPF$_{2\alpha}$-VI (p=0.80) or urinary albumin excretion rate (p=0.85) (Table 3). There were no differences in routine chemistries or hematology measures between treatment groups. A statistically significant difference was observed in the change in LDL-C levels (decreased in the placebo arm but not with ruboxistaurin), but not in total cholesterol, HDL-C and triglyceride levels (Table 3). No differences between treatment groups in adverse events were observed (data not shown). No deaths were reported and most adverse events were mild in severity; one participant in the placebo arm suffered a myocardial infarction.

Discussion

We found that specific inhibition of PKC beta appeared to improve brachial artery endothelial vasodilator function in patients with type-2 diabetes. This study provides the first suggestive evidence in patients with type-2 diabetes of a potential benefit for this class of drugs on diabetic macrovascular disease, the leading cause of death in these patients. These preliminary findings may have implications for the potential clinical utility of PKC beta inhibitors since the phase-three clinical trials of ruboxistaurin performed to date suggest that it is well tolerated and may provide efficacy in diabetic retinopathy [12] and peripheral neuropathy [13, 14].

Activation of vascular PKC beta is thought to occur preferentially in type-2 diabetes and insulin resistance through glycemic disturbance, elevation of non-esterified fatty acids and vascular oxidant stress. Each of these abnormalities increases de novo synthesis of vascular diacylglyerol (DAG) [4, 7, 8, 28, 29], and DAG directly activates most PKC isoforms (both classic and novel), including PKC beta, a major isoform in the vasculature. Oxidant signals also activate PKCs indirectly via phospholipase D and hydrolysis of phosphotidylcholine to DAG [29]. Notably, coincident increases in DAG levels and membrane associated PKC beta, a measure of *in situ* PKC activation, are consistently observed in the vasculature of both acute and chronic animal models of diabetes [7]. Hyperglycemia and elevated circulating free fatty acids also appear to induce vascular reactive stress in part through PKC-dependent activation of NAD(P)H oxidase [30, 31]

PKC beta activation has diverse vascular signaling effects that promote diabetes related vascular pathologies via oxidant stress [28], inflammation [32] and endothelial dysfunction [10]. In endothelial cells, PKC beta activation of NAD(P)H [33], modulation of endothelial nitric oxide synthase (eNOS) [34] and induction of adhesion molecules [32] lead to atherogenic endothelial dysfunction. In parallel in vascular smooth muscle cells, PKC beta induces mitogenic growth factors and endothelin-1 while activation of cytosolic phosphoslipase A2 generating vasoactive eicosanoids promoting vascular smooth muscle cell proliferation and hyperplasia [10, 35].

Many of these metabolic atherogenic pathophysiologies can be normalized by treatment with PKC inhibitors in animal models [11]. Indeed, PKC beta inhibition blocks hyperglycemia mediated vascular cell dysfunction *in vitro* [4, 36], retards retinal and renal micro vascular disease in rodent models [11, 37], and recent trials support its potential efficacy in attenuating progression of clinical microvascular complications [12, 14, 38]. Despite these experimental and microvascular effects, there is almost no data on PKC beta inhibition on atherosclerosis related readouts in humans.

PKC beta activation is found equally in large and small arteries in diabetic models [11] raising the possibility of favorable effects of PKC beta inhibition on macrovascular complications of diabetes. The degree of macrovascular endothelial vasodilator dysfunction is believed to reflect the extent of atherogenic oxidant and inflammatory pathophysiology in patients with type-2 diabetes [39]. Consistent with this concept, experimental hyperglycemia or infusion of free fatty acids can impair large vessel endothelial function both *in vitro* and *in vivo* [40, 41]. Remarkably, in a small proof of concept study, Beckman found that hyperglycemia-induced brachial artery endothelial dysfunction in healthy volunteers was completely attenuated by selective inhibition of PKC beta [25].

Ours is the first study to suggest that PKC beta inhibition might improve macrovascular endothelial function in patients with type-2 diabetes. Although, this study was of short duration and used a surrogate measure of vascular function, brachial artery FMD represents a standard for assessment of large vessel endothelial function [42, 43], is strongly correlated

with atherosclerosis, and predicts poor CVD outcomes [21, 22]. Our findings should be interpreted with caution given the small proof of concept design, the small differences between treatments and the tendency for a loss of endothelial function in the placebo arm over the course of the trial. However, similar direction of ruboxistaurin effects on FMD at 1 and 5 min and the high-within subject correlations in pre-cuff diameter readings over the course of the study suggest findings may be valid. In this context, our study provides the basis for next-step trials of longer duration assessing the impact of PKC beta inhibitors on atherosclerosis readouts in type-2 diabetic patients. We did note a decrease in LDL levels in the placebo group compared to the RBX group. Because there were no changes in concomitant medication usage during the study period and other lipid parameters changed little, we believe that this difference may be due chance although dietary improvement in the placebo arm might have contributed. Regardless, a true improvement in LDL cholesterol in the placebo group should bias toward the null rather than produce a spurious finding.

PKC activation, as noted above, is closely related to vascular oxidant stress [28, 44, 45]. Therefore, we hypothesized that PKC beta inhibition would improve macrovascular endothelial function, in part, by reducing oxidant stress. However, we found no effect of ruboxistaurin on urinary excretion of isoprostanes, a validated index of oxidant stress in diabetes, vascular injury and atherosclerosis [24, 46]. Thus, oxidant stress, at least as measured by this integrated measure of non-enzymatic lipid peroxidation, may not be causal in PKC beta related endothelial dysfunction. It is possible however that alternative measures, such as indices of NO biosynthesis, superoxide or vascular NADPH, may provide more appropriate biomarkers of PKC beta related vascular oxidant stress rather than urinary isoprostanes. Alternatively, our study may have been underpowered or of too short a duration to detect effects on isoprostanes. Indeed, the lack of effect on urinary albumin excretion rate is perhaps consistent with the need for larger studies of longer duration given significant reduction in urinary albumin excretion rate by ruboxistaurin in a previous clinical trial [47]. In this context, our study is limited by short duration, relatively small sample size and focus on specific surrogate measures of macro vascular disease. In addition, the specific mechanism(s) by which PKC beta inhibition may improve human macro vascular endothelial function remains to be elucidated.

Conclusion

This is the first trial showing that specific inhibition of PKC beta may improve brachial artery FMD in type-2 diabetes, and therefore might represent a novel therapy for macrovascular complications of diabetes. Larger trials of clinical endpoints are warranted to determine the potential efficacy in diabetic CVD.

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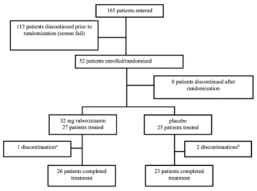

Fig. 1.
Disposition of trial participants

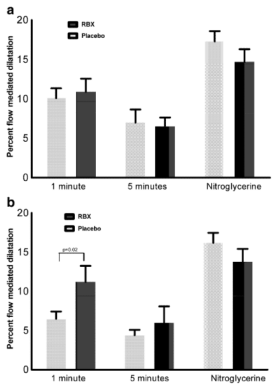

Fig. 2.

Change in flow mediated dilatation (FMD) by treatment group. Percent flow mediated dilatation (FMD) at 1 and 5 min after cuff deflation and percent nitroglycerin induced dilatation are presented **a** at randomization, before treatment and **b** following 6 weeks on treatment. After 6-weeks treatment, percent FMD in the ruboxistaurin group was greater than in placebo group at one min (11.2±9.9% vs. 6.5±4.4%; p=0.02) but at 5 min the difference between ruboxistaurin and control did not reach statistical significant (6.0±10% vs. 4.4±3.3%; p=0.23). The average change in absolute FMD over the 6-weeks of treatment tended to differ (mean±SD millimeter) between ruboxistaurin and placebo (Table 2) at one (0.13±0.26 mm, p=0.08) and 5 min (0.12±0.21, p=0.02)

Table 1

Baseline characteristics of study participants

	Placebo (*N*=23[a]) Median (IQ range); *N* (%)	**RBX 32 mg (*N*=26[a]) Median (IQ range); *N* (%)**
Age (years)	56 (49–64)	52 (45–60)
Race (Caucasian)	12 (46%)	7 (27%)
Male gender	15 (65%)	19 (73%)
Duration of diabetes (years)	8.29 (1.82–20.22)	7.21 (0.22–20.71)
HbA_{1c} (%)	8.30 (7.0–10.90)	8.62 (7.00–10.80)
Glucose (mmol/L)	5.17 (4.78–5.5)	5 (4.72–5.39)
LDL—Cholesterol (mmol/L)	2.49 (2.02–3.03)	2.79 (2.10–3.41)
HDL—Cholesterol (mmol/L)	1.12 (0.95–1.31)	1.18 (1.0–1.44)
Triglycerides (mmol/L)	1.43 (0.83–1.80)	2.17 (1.67–2.40)
Body mass index (kg/m^2)	27.4 (25.2–30.3)	25.6 (22.8–30.2)
Blood pressure (mm Hg)		
Systolic	128 (119–137)	125 (113–136)
Diastolic	79 (73–86)	75 (68–82)
Medications		
ACE-inhibitors or ARB	13 (57%)	17 (65%)
Statins	14 (61%)	10 (38%)
Diabetic therapy		
Insulin only	2 (9%)	3 (11%)
Oral hypoglycemic agent	17 (74%)	22 (85%)
Both insulin and OHA	4 (17%)	1 (4%)

RBX ruboxistaurin, *IQ* interquartile, *HbA1c* hemoglobin A1c, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *ACE* angiotensin converting enzyme

[a]Data are presented for subjects completing visit 3 (ie protocol completers)min 4

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Table 2

Brachial artery data by visit: (a) absolute diameters and (b) absolute change with flow and nitroglycerin

Group	Pre-therapy					Following 6 weeks of treatment				
Diameter (mm) of the brachial artery; mean (±standard deviation (SD))										
Placebo	BL 1	Flow 1 min	Flow 5 min	BL 2	NTG 4 min	BL 1	Flow 1 min	Flow 5 min	BL 2	NTG 4 min
	3.77 (±0.5)	4.14 (±0.5)	4.00 (±0.4)	3.86 (±0.4)	4.53 (±0.5)	3.76 (±0.4)	3.99 (±0.5)	3.92 (±0.4)	3.81 (±0.4)	4.43 (±0.5)
RBX	3.89 (±0.7)	4.29 (±0.6)	4.14 (±0.6)	4.05 (±0.6)	4.62 (±0.7)	3.93 (±0.8)	4.33 (±0.6)	4.22 (±0.7)	4.13 (±0.7)	4.68 (±0.7)
Absolute flow-mediated and nitroglycerin-mediated change (mm); mean (±SD)										
	Flow 1 min	Flow 5 min	NTG 4 min	Flow 1 min	Flow 5 min	NTG 4 min	Difference in 6-week change in FMD between treatments[a]			
							1 min		5 min	
Placebo	0.37 (±0.22)	0.24 (±0.22)	0.63 (±0.27)	0.24 (±0.15)	0.16 (±0.11)	0.58 (±0.25)	0.13 (±0.26) (p=0.08)		0.12 (±0.21) (p<0.02)	
RBX	0.39 (±0.21)	0.24 (±0.20)	0.53 (±0.30)	0.39 (±0.24)	0.28 (±0.26)	0.50 (±0.31)				

RBX ruboxistaurin, *NTG* nitroglycerin, *BL1* first baseline measurement—before cuff inflation, *BL2* second baseline measurement—before nitroglycerin and 20 min after cuff deflation

[a] p-values for RBX drug effect in repeated measures analysis of covariance (ANCOVA)

Table 3

Effect of ruboxistaurin on laboratory parameters

Parameter	Treatment	Baseline	6 weeks	Within group *p*-value	Between group *p*-value
8,12-*iso*-iPF2α-VI (ng/mg creatinine)	Placebo	5.0 (3.2–6.9)[a]	4.9 (3.4–5.9)	0.96	0.91
	RBX 32 mg/day	4.0 (2.6–5.2)	4.1 (2.4–5.6)	0.65	
Urinary albumin excretion rate (μg/mg creatinine)	Placebo	79 (36–168)	81 (32–344)	0.74	0.85
	RBX 32 mg/day	117 (48–285)	91 (62–498)	0.50	
Total cholesterol (mmol/L)	Placebo	4.61 (4.0–5.1)	4.34 (3.9–4.6)	0.15	0.16
	RBX 32 mg/day	4.87 (3.9–5.4)	4.79 (3.8–5.4)	0.45	
HDL cholesterol (mmol/L)	Placebo	1.17 (0.9–1.3)	1.18 (0.9–1.3)	0.57	0.77
	RBX 32 mg/day	1.21 (1.0–1.5)	1.22 (1.0–1.5)	0.16	
LDL cholesterol (mmol/L)	Placebo	2.69 (2.1–3.1)	2.37 (2.0–2.8)	0.001	0.01
	RBX 32 mg/day	2.74 (2.2–3.4)	2.81 (2.3–3.5)	0.92	
Triglycerides (mmol/L)	Placebo	1.53 (1.0–2.1)	1.66 (1.1–2.2)	0.43	0.51
	RBX 32 mg/day	1.60 (1.1–2.1)	1.55 (1.0–2.1)	0.94	

[a]Median (interquartile range)