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Atazanavir improves cardiometabolic measures but not vascular function in patients with long-standing type 1 diabetes mellitus

Jessica Milian,

Cardiovascular Division, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA

Allison B. Goldfine,

Clinical Research, Joslin Diabetes Center, Boston, MA, USA

Jonah P. Zuflacht,

Cardiovascular Division, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA

Caitlin Parmer, and

Cardiovascular Division, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA

Joshua A. Beckman

Cardiovascular Division, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA

Joshua A. Beckman: jbeckman@partners.org

Abstract

Aims—Vascular disease is the leading cause of morbidity and mortality in type 1 diabetes mellitus (T1DM). We previously demonstrated that patients with T1DM have impaired endothelial function, a forme fruste of atherosclerosis, as a result of increased oxidative stress. Bilirubin has emerged as a potent endogenous antioxidant with higher concentrations associated with lower rates of myocardial infarction and stroke.

Methods—We tested the hypothesis that increasing endogenous bilirubin using atazanavir would improve cardiometabolic risk factors and vascular function in patients with T1DM to determine whether targeting bilirubin may be a novel therapeutic approach to reduce cardiovascular disease risk in this population. In this single-arm, open-label study, we evaluated blood pressure, lipid

Correspondence to: Joshua A. Beckman, jbeckman@partners.org.

Managed by Massimo Federici.

Ethical standard The protocol was approved by the Partners Human Research Committee of Brigham and Women's Hospital and all subjects provided informed consent.

Human and animal rights disclosure All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 [54].

Informed consent disclosure Informed consent was obtained from all patients for being included in the study.

Conflict of interest Dr. Goldfine, Mr. Zuflacht, Ms. Parmer, and Ms. Milian have no conflicts of interest. Dr. Beckman has received honoraria for consulting and support for investigator-initiated research from Bristol Myers Squibb.

profile, and conduit artery function in fifteen subjects (mean age 45 ± 9 years) with T1DM following a 4-day treatment with atazanavir.

Results—As anticipated, atazanavir significantly increased both serum total bilirubin levels ($p < 0.0001$) and plasma total antioxidant capacity ($p < 0.0001$). Reductions in total cholesterol ($p = 0.04$), LDL cholesterol ($p = 0.04$), and mean arterial pressure ($p = 0.04$) were also observed following atazanavir treatment. No changes were seen in either flow-mediated endothelium-dependent ($p = 0.92$) or nitroglycerine-mediated endothelium-independent ($p = 0.68$) vasodilation, measured by high-resolution B-mode ultrasonography at baseline and post-treatment.

Conclusion—Increasing serum bilirubin levels with atazanavir in subjects with T1DM over 4 days favorably reduces LDL and blood pressure but is not associated with improvements in endothelial function of conduit arteries.

Keywords

Type 1 diabetes; Endothelium; Oxidative stress; Bilirubin

Introduction

Type 1 diabetes mellitus (T1DM) confers increased cardiovascular disease risk [1]. The vascular endothelium regulates blood vessel's structure and function by releasing vasodilator and vasoconstriction substances to maintain homeostasis [2]. Endothelium-derived nitric oxide (NO), the most potent vasodilator, protects against atherogenesis through a wide variety of mechanisms, including inhibition of inflammation, prevention of vasoconstriction, inhibition of platelet and leukocyte adhesion, attenuation of vascular smooth muscle cell proliferation, and prevention of lipid peroxidation [3, 4]. Hyperglycemia, inherent to T1DM, induces production of oxygen-derived free radicals, particularly superoxide anion [5], reducing NO bioavailability to cause endothelial dysfunction [6]. Attenuated endothelial function has been linked to increases in cardiovascular events [7, 8] and has been demonstrated in both type 1 and type 2 diabetic populations [9, 10]. Supraphysiological ascorbate infusion [11] and chronic oral [10] antioxidant vitamin therapy restore endothelium-dependent vasodilation in T1DM, but does not reduce cardiovascular disease in patients at high risk of events [12], although not studied specifically in a population with T1DM.

Bilirubin, a product of heme catabolism, has emerged as a potent antioxidant capable of scavenging oxygen-derived free radicals [13]. We have shown that elevated serum bilirubin concentrations are associated with a lower incidence of atherosclerosis [14, 15] and patients with Gilbert's disease, marked by elevated levels of unconjugated bilirubin, have been shown to have a lower incidence of ischemic heart disease [16]. Previously, pharmacologic-mediated increases in serum bilirubin levels improved endothelial function in patients with type 2 diabetes mellitus (T2DM) [14, 17, 18]. Treatment strategies that increase antioxidant capacity by increasing bilirubin may therefore represent a novel approach to alleviate oxidative stress, restore endothelial function, and limit the pro-atherogenic consequences of hyperglycemia [19].

Atazanavir, a protease inhibitor used to treat patients with human immunodeficiency virus (HIV), is known to block UDP glucuronosyltransferase 1A1 (UGT1A1) activity, leading to the accumulation of unconjugated bilirubin [20]. We tested the hypothesis that accumulation of bilirubin via UGT1A1 inhibition would reduce oxidative stress and result in improved endothelial function in patients with T1DM.

Materials and methods

Subject selection

Subjects with T1DM were recruited through online advertising and referrals from the Joslin Diabetes Center in Boston, Massachusetts. Eligible subjects were between 18 and 60 years of age with a long-standing (>20 years) diagnosis of T1DM as defined by recent criteria or evidence of microalbuminuria [21]. All subjects underwent screening medical history, physical examination, and laboratory analyses, including fasting glucose, conjugated and total bilirubin, liver function, creatinine, total and LDL cholesterol, hemoglobin A_{1C}, and urinalysis. All subjects were confirmed HIV negative by antibody screen. Subjects with evidence of unstable cardiovascular disease within 1 year, renal or liver disease, or Gilbert's syndrome were excluded. Active drug abuse, smoking, pregnancy, or use of medications that interfere with atazanavir precluded participation. Any use of proton pump inhibitors was discontinued 2 weeks prior to treatment initiation. The protocol was approved by the Partners Human Research Committee of Brigham and Women's Hospital, and all subjects provided informed consent. This investigation was registered prior to initiation (NCT01421355).

Protocol

In this single-arm, open-label study, subjects received atazanavir 300 mg twice daily for a total of 4 days (Reyataz, Bristol Myers Squibb, Plainsboro, NJ, USA). Conduit artery vascular function was assessed at baseline and on the fourth day of atazanavir treatment to match a study in subjects with T2DM [17]. Fasting blood was collected at both time points for laboratory analysis.

Vascular reactivity testing

Subjects were studied in the morning after overnight fast with rapidly acting insulin held on the morning of vascular study. All vascular studies were performed in a quiet, temperature-controlled, dimly lit room after the subject rested supine for a minimum of 5 min, using an upper-arm sphygmomanometric cuff position, as we have previously performed and according to guidelines [22–25]. High-resolution B-mode ultrasonography using a 7.5 MHz linear array probe (Vivid 7, General Electric) was used to image the brachial artery. Images were obtained using an electrocardiographic R-wave trigger for end diastole. Reactive hyperemia was induced through 5 min of cuff suprasystolic pressure inflation. Flow-mediated, endothelium-dependent vasodilation was assessed at 60–70 s after cuff deflation. We have shown that vasodilation at this time point is endothelium-derived nitric oxide dependent [26, 27]. Vascular function analyses were performed blinded to study visit.

Ten minutes after cuff release, endothelium-independent vasodilation was assessed. The brachial artery was imaged before and 3 min after sublingual administration of 0.4 mg of nitroglycerin (Nitrostat, Parke-David, New York, NY, USA). Brachial artery blood flow velocity was determined via pulsed Doppler velocity–time integral measurement at all time points. Nitroglycerin was not administered if the subject's systolic blood pressure was <100 mmHg or heart rate was <50 beats/min. Analysis was performed using Information Integrity custom-made image acquisition and analysis software (Information Integrity, Stow, Massachusetts).

Laboratory analyses

Blood was collected into Vacutainer tubes (Becton–Dickinson) for chemistry and hematologic analysis at Brigham and Women's Hospital Clinical Laboratories. Plasma samples were stored for subsequent analysis at -80°C from Vacutainer tubes containing K2 EDTA 7.2 mg/4 mL whole blood following centrifugation (1,200g) at 4°C for 10 min. Plasma antioxidant capacity was assessed by ferric-reducing ability of plasma (FRAP) assay at the Clinical and Epidemiologic Research Laboratory of Boston Children's Hospital [28].

The homeostatic model assessment-estimated insulin resistance (HOMA_{IR}) was calculated using the following equation: fasting glucose (mg/dL) $\times$ fasting insulin (mU/mL)/405.

Statistical methods

Descriptive measures are reported as mean $\pm$ standard deviation. Experimental measures are reported as mean $\pm$ standard error. Normality of data was confirmed by the Shapiro–Wilk test. Vascular function parameters were compared using paired t test. Statistical significance was accepted at the 95 % confidence level ($p < 0.05$). All statistical analyses were performed using SPSS Base 22 (IBM; Armonk, NY, USA).

Results

Baseline characteristics

Fifteen subjects with T1DM met eligibility criteria and completed the study. Baseline characteristics of study participants are noted in Table 1. Subjects were 45 ± 9 years of age with an average body mass index of $28.6 \pm 7.8 \text{ kg/m}^2$. The study population included one Hispanic (7 %) and two African-American subjects (13 %). Seven subjects (47 %) were taking angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, and two (13 %) were being treated with statins at the time of enrollment. The mean duration of diabetes was 29.1 ± 10.7 years. Two subjects with <20 years duration of diabetes qualified based on microalbuminuria confirmed by laboratory analysis at screening. Subjects had an average hemoglobin $\text{A}_{1\text{C}}$ value of 8.6 ± 1.3 % at baseline and fasting blood glucose of 178.0 ± 29.4 and 172.4 ± 25.1 mg/dL at baseline and on day 4, respectively. Of the fifteen subjects who completed the study, one subject developed jaundice on day 3 of treatment with atazanavir that resolved within 4 days of medication cessation.

Laboratory analyses

As anticipated, total bilirubin levels were significantly elevated following 4-day treatment with atazanavir (0.50 ± 0.05 mg/dL at baseline and 3.87 ± 0.56 mg/dL post-treatment, $p < 0.0001$). FRAP analysis showed a significant increase in total plasma antioxidant capacity as a result of atazanavir treatment (1.22 ± 0.08 mM at baseline and 1.57 ± 0.10 mM post-treatment, $p < 0.0001$) (Table 2). The sample correlation coefficient for the change in unconjugated bilirubin and FRAP was 0.61, (Pearson $p = 0.016$). Treatment with atazanavir reduced total cholesterol by 4.5 % (192.5 ± 13.3 mg/dL at baseline and 185.5 ± 14.7 mg/dL post-treatment; $p = 0.04$) and LDL cholesterol by 9.9 % (106.8 ± 10.4 mg/dL at baseline and 98.2 ± 11.5 mg/dL post-treatment, $p = 0.04$) (Fig. 1). HDL cholesterol and triglycerides were unchanged following treatment. Neither fasting insulin, fasting glucose, nor HOMA_{IR} were changed significantly with atazanavir treatment. The sample correlation coefficient for the change in unconjugated bilirubin and LDL was -0.55 (Pearson $p = 0.042$).

Hemodynamics and vascular function

Mean arterial pressure was significantly reduced in response to treatment with atazanavir (83.9 ± 2.6 mmHg) compared to baseline (89.1 ± 2.7 mmHg, $p = 0.04$). All other hemodynamic measures remained unchanged following treatment (Table 2). The change in mean arterial pressure correlated neither with the change in bilirubin nor with the change in antioxidant capacity.

Basal brachial artery diameters were unchanged by atazanavir treatment (3.31 ± 0.13 mm at baseline and 3.33 ± 0.14 mm post-treatment, $p = 0.48$) (Table 3). Treatment with atazanavir did not significantly affect the reactive hyperemia stimulus (5.94 ± 0.63 and 5.65 ± 0.58 fold change at baseline and post-treatment, respectively, $p = 0.61$). There was no significant change in flow-mediated, endothelium-dependent vasodilation following treatment with atazanavir (8.08 ± 1.24 % at baseline and 8.01 ± 1.27 % post-treatment; $p = 0.92$) (Fig. 2a, b). Nitroglycerin-mediated, endothelium-independent vasodilation was similarly unchanged following treatment ($p = 0.68$). In exploratory analysis, there was no difference in flow-mediated or nitroglycerin-mediated vasodilation when the subjects with an above-median increase in bilirubin were compared to those with a below-median increase. Similarly, vascular function did not differ when comparing subjects with above- and below-median change in antioxidant capacity.

Discussion

Our study demonstrates that short-term exposure to atazanavir significantly increases serum antioxidant capacity, lowers total and LDL cholesterol, and reduces mean arterial pressure in T1DM. Despite this observed improvement in the vascular environment, both endothelium-dependent vasodilation and endothelium-independent vasodilation remain unchanged after treatment.

Antioxidant capacity and vascular function

Experimental models of diabetes mellitus and non-diabetic animal aortas exposed to high glucose concentrations both demonstrate increased oxidative stress, reduced bioavailability

of endothelium-derived NO, and impaired endothelium-dependent vasodilation [29]. Likewise, endothelium-dependent vasodilation of conduit arteries is attenuated in humans with T1DM [1]. Bilirubin is a potent endogenous scavenger of oxidant species, and its absence increases oxidative stress in human endothelial cells. In Gilbert's syndrome, gene coding regions for UGT1A1 are inactivated, preventing bilirubin excretion and conferring lifelong hyperbilirubinemia. Epidemiologic studies show that patients with Gilbert's syndrome [30] have a reduced incidence of cardiovascular disease. The protease-inhibitor atazanavir blocks UGT1A1 activity, resulting in a state of unconjugated hyperbilirubinemia that mimics the heightened bilirubinemia of Gilbert's syndrome.

Short-term atazanavir exposure significantly increased plasma antioxidant capacity in our subjects with T1DM, comparable to the observed change in a T2DM population [17]. Despite the similar improvement in oxidative stress, conduit artery endothelium-dependent vasodilation in subjects with T1DM was unchanged with atazanavir treatment in this study, contrary to the significant improvement in forearm arteriolar endothelial function reported by Dekker et al. [17] in patients with T2DM. The variations in pathophysiology between T1DM and T2DM or in arterial bed may underlie the differences in our observations. Healthy subjects treated with atazanavir trended to increased endothelium-dependent vasodilation in leg arterioles, whereas no effect was noted in the brachial conduit arteries of well-controlled subjects with HIV [31–34]. Discordance in endothelium-dependent vasodilation between conduit arteries and arteriolar beds has been noted previously [35]. Increasing bilirubin levels may therefore differentially affect endothelial function of the micro- and microvasculature.

Metabolic abnormalities in diabetes

Modification of risk factors for cardiovascular disease, particularly hypertension and dyslipidemia, reduces morbidity and mortality in diabetes mellitus. Current medical therapies are focused on managing blood pressure and normalizing lipid metabolism to reduce adverse cardiovascular events in this population [1]. Superoxide production is increased in humans and animal models of hypercholesterolemia [36, 37], hypertension [38, 39], and diabetes [37]. Several randomized studies have demonstrated a reduction in major cardiovascular complications, including stroke, myocardial infarction, and death, with the use of anti-hypertensive medications [40–42]. Therapy employing hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors, or statins, demonstrates a reduction in coronary heart disease and stroke in patients with diabetes [43, 44] and in patients without additional risk factors [45].

We demonstrate a reduction in both total cholesterol and LDL cholesterol levels in response to atazanavir, consistent with observations in HIV-infected adults receiving atazanavir therapy (46). It has been shown that elevated serum bilirubin is inversely related to total and LDL cholesterol and triglycerides, both in healthy young adults and in diabetic populations [30, 46, 47]. Dekker and colleagues did not report similar metabolic changes in their T2DM cohort. We also observed a decrease in mean arterial pressure [48]. These observations indicate that elevated bilirubin levels may exert therapeutic benefits on cardiovascular health by diminishing diabetic dysmetabolism and improving hemodynamic parameters rather than

through endothelial function. Indeed, Oda and colleagues have shown that increasing total bilirubin levels was associated with lower levels of metabolic syndrome, but, surprisingly, total bilirubin levels were not a risk factor for metabolic syndrome [49]. Thus, the link between bilirubin and cardiometabolic health requires further investigation.

Vascular oxidative stress and endothelial dysfunction predict the risk of cardiovascular disease [50–52]. In our study, a reduction in oxidative stress did not increase the bioavailability of endothelium-derived nitric oxide in conduit arteries of patients with T1DM, but other mechanisms may still promote cardiovascular health. For example, Keshavan et al. [53] demonstrate that bilirubin blocks leukocyte adhesion and migration across the endothelium via inhibition of vascular cell adhesion protein 1 (VCAM-1), an NADPH oxidase- and ROS-dependent cell surface protein. The antioxidant effects of bilirubin may therefore have an important regulatory role in vascular inflammation independent of changes in endothelium-derived nitric oxide bioavailability.

This study has a number of limitations. Our sample size was small but matched enrollment to the study conducted by Dekker and colleagues in a T2DM population. We acknowledge that a larger study (more than tenfold as many subjects based on the effect size) may have detected additional metabolic changes compared to those observed in the current study. Our current study eliminates variability from comparing two different cohorts as each subject served as his own comparator and is consistent with other data in conduit arteries in the literature. In addition, we might have included healthy control subjects to determine whether the metabolic effects were dependent on the presence of diabetes.

In summary, our study is the first to study the effect of enhanced antioxidant capacity through pharmacologically increase in unconjugated bilirubin in patients with longstanding type 1 diabetes mellitus. Based on our results and those in the literature, we note that atazanavir improves endothelium-dependent vasodilation in arterioles but not in conduit arteries, despite significant reductions in oxidative stress. Our study does not exclude a benefit by another mechanism, a benefit that may take longer to occur, or that the dose was optimal, despite the fact that we used the same rationale and procedure as Dekker et al. [17]. The discrepancy in the literature suggests a large trial may be clarifying.

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References

1. Beckman JA, et al. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part II. *Eur Heart J*. 2013; 34(31):2444–2452. [PubMed: 23625211]
2. De Vriese AS, et al. Endothelial dysfunction in diabetes. *Br J Pharmacol*. 2000; 130(5):963–974. [PubMed: 10882379]
3. Stamler JS, et al. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation*. 1994; 89(5):2035–2040. [PubMed: 7514109]

4. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation*. 2006; 113(13):1708–1714. [PubMed: 16585403]
5. Nishikawa T, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. 2000; 404(6779):787–790. [PubMed: 10783895]
6. Beckman JA, et al. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. *Circulation*. 2001; 103(12):1618–1623. [PubMed: 11273987]
7. Gokce N, et al. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation*. 2002; 105(13):1567–1572. [PubMed: 11927524]
8. Heitzer T, et al. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*. 2001; 104(22):2673–2678. [PubMed: 11723017]
9. Johnstone MT, et al. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation*. 1993; 88(6):2510–2516. [PubMed: 8080489]
10. Beckman JA, et al. Oral antioxidant therapy improves endothelial function in Type 1 but not Type 2 diabetes mellitus. *Am J Physiol Heart Circ Physiol*. 2003; 285(6):H2392–H2398. [PubMed: 12881209]
11. Timimi FK, et al. Vitamin C improves endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol*. 1998; 31(3):552–557. [PubMed: 9502634]
12. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002; 360(9326):23–33. [PubMed: 12114037]
13. Stocker R, et al. Bilirubin is an antioxidant of possible physiological importance. *Science*. 1987; 235(4792):1043–1046. [PubMed: 3029864]
14. Perlstein TS, et al. Serum total bilirubin level and prevalent lower-extremity peripheral arterial disease: National Health and Nutrition Examination Survey (NHANES) 1999–2004. *Arterioscler Thromb Vasc Biol*. 2008; 28(1):166–172. [PubMed: 17975120]
15. Perlstein TS, et al. Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999–2004. *Am J Med*. 2008; 121(9):781.e1–788.e1. [PubMed: 18724968]
16. Vitek L, et al. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. *Atherosclerosis*. 2002; 160(2):449–456. [PubMed: 11849670]
17. Dekker D, et al. The bilirubin-increasing drug atazanavir improves endothelial function in patients with type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 2011; 31(2):458–463. [PubMed: 21088253]
18. Ollinger R, et al. Bilirubin: a natural inhibitor of vascular smooth muscle cell proliferation. *Circulation*. 2005; 112(7):1030–1039. [PubMed: 16087796]
19. Forstermann U. Oxidative stress in vascular disease: causes, defense mechanisms and potential therapies. *Nat Clin Pract Cardiovasc Med*. 2008; 5(6):338–349. [PubMed: 18461048]
20. Wood R. Atazanavir: its role in HIV treatment. *Expert Rev Anti Infect Ther*. 2008; 6(6):785–796. [PubMed: 19053892]
21. American Diabetes, A. Standards of medical care in diabetes–2010. *Diabetes Care*. 2010; 33(Suppl 1):S11–S61. [PubMed: 20042772]
22. Beckman JA, et al. Atorvastatin restores endothelial function in normocholesterolemic smokers independent of changes in low-density lipoprotein. *Circ Res*. 2004; 95(2):217–223. [PubMed: 15178637]
23. Beckman JA, et al. Endothelial function varies according to insulin resistance disease type. *Diabetes Care*. 2007; 30(5):1226–1232. [PubMed: 17261751]
24. Nohria A, et al. The effect of salsalate therapy on endothelial function in a broad range of subjects. *J Am Heart Assoc*. 2014; 3(1):e000609. [PubMed: 24390146]
25. Corretti MC, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002; 39(2):257–265. [PubMed: 11788217]

26. Lieberman EH, et al. Flow-induced vasodilation of the human brachial artery is impaired in patients <40 years of age with coronary artery disease. *Am J Cardiol.* 1996; 78(11):1210–1214. [PubMed: 8960576]
27. Owens CD, et al. In vivo human lower extremity saphenous vein bypass grafts manifest flow mediated vasodilation. *J Vasc Surg.* 2009; 50(5):1063–1070. [PubMed: 19679424]
28. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal Biochem.* 1996; 239(1):70–76. [PubMed: 8660627]
29. Paneni F, et al. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *Eur Heart J.* 2013; 34(31):2436–2443. [PubMed: 23641007]
30. Inoguchi T, et al. Relationship between Gilbert syndrome and prevalence of vascular complications in patients with diabetes. *JAMA.* 2007; 298(12):1398–1400. [PubMed: 17895455]
31. Murphy RL, et al. Change to atazanavir/ritonavir treatment improves lipids but not endothelial function in patients on stable antiretroviral therapy. *AIDS.* 2010; 24(6):885–890. [PubMed: 19952712]
32. Flammer AJ, et al. Effect of atazanavir versus other protease inhibitor-containing antiretroviral therapy on endothelial function in HIV-infected persons: randomised controlled trial. *Heart.* 2009; 95(5):385–390. [PubMed: 18653575]
33. Dube MP, et al. No impairment of endothelial function or insulin sensitivity with 4 weeks of the HIV protease inhibitors atazanavir or lopinavir-ritonavir in healthy subjects without HIV infection: a placebo-controlled trial. *Clin Infect Dis.* 2008; 47(4):567–574. [PubMed: 18636958]
34. Hileman C, et al. Relationship between total bilirubin and endothelial function, inflammation and oxidative stress in HIV-infected adults on stable antiretroviral therapy. *HIV Med.* 2012; 13(10):609–616. [PubMed: 22624591]
35. Ingram DG, et al. Chronic nitric oxide synthase inhibition blunts endothelium-dependent function of conduit coronary arteries, not arterioles. *Am J Physiol Heart Circ Physiol.* 2007; 292(6):H2798–H2808. [PubMed: 17259441]
36. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest.* 1993; 91(6):2546–2551. [PubMed: 8390482]
37. Guzik TJ, et al. Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. *Circ Res.* 2000; 86(9):E85–E90. [PubMed: 10807876]
38. Fukui T, et al. p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. *Circ Res.* 1997; 80(1):45–51. [PubMed: 8978321]
39. Mehta JL, et al. Alterations in nitric oxide synthase activity, superoxide anion generation, and platelet aggregation in systemic hypertension, and effects of celiprolol. *Am J Cardiol.* 1994; 74(9):901–905. [PubMed: 7526676]
40. Yusuf S, et al. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The heart outcomes prevention evaluation study investigators. *N Engl J Med.* 2000; 342(3):145–153. [PubMed: 10639539]
41. Curb JD, et al. Effect of diuretic-based antihypertensive treatment on cardiovascular disease risk in older diabetic patients with isolated systolic hypertension. Systolic hypertension in the elderly program cooperative research group. *JAMA.* 1996; 276(23):1886–1892. [PubMed: 8968014]
42. Tuomilehto J, et al. Effects of calcium-channel blockade in older patients with diabetes and systolic hypertension. Systolic hypertension in Europe trial investigators. *N Engl J Med.* 1999; 340(9):677–684. [PubMed: 10053176]
43. Goldberg RB, et al. Cardiovascular events and their reduction with pravastatin in diabetic and glucose-intolerant myocardial infarction survivors with average cholesterol levels: subgroup analyses in the cholesterol and recurrent events (CARE) trial. The care investigators. *Circulation.* 1998; 98(23):2513–2519. [PubMed: 9843456]
44. Cholesterol Treatment Trialists. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. *Lancet.* 2008; 371(9607):117–125. [PubMed: 18191683]
45. Collins R, et al. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet.* 2003; 361(9374):2005–2016. [PubMed: 12814710]

46. Madhavan M, et al. Serum bilirubin distribution and its relation to cardiovascular risk in children and young adults. *Atherosclerosis*. 1997; 131(1):107–113. [PubMed: 9180251]
47. Tapan S, et al. Decreased small dense LDL levels in Gilbert's syndrome. *Clin Biochem*. 2011; 44(4):300–303. [PubMed: 21167147]
48. Kodama S, et al. Meta-analysis of the quantitative relation between pulse pressure and mean arterial pressure and cardiovascular risk in patients with diabetes mellitus. *Am J Cardiol*. 2014; 113(6):1058–1065. [PubMed: 24462067]
49. Oda E, Aizawa Y. Total bilirubin is inversely associated with metabolic syndrome but not a risk factor for metabolic syndrome in Japanese men and women. *Acta Diabetol*. 2013; 50(3):417–422. [PubMed: 23224110]
50. Di Filippo C, et al. Oxidative stress as the leading cause of acute myocardial infarction in diabetics. *Cardiovasc Drug Rev*. 2006; 24(2):77–87. [PubMed: 16961722]
51. Aksoy S, et al. Oxidative stress and severity of coronary artery disease in young smokers with acute myocardial infarction. *Cardiol J*. 2012; 19(4):381–386. [PubMed: 22825899]
52. McMurray J, et al. Evidence of oxidative stress in chronic heart failure in humans. *Eur Heart J*. 1993; 14(11):1493–1498. [PubMed: 8299631]
53. Keshavan P, et al. Unconjugated bilirubin inhibits VCAM-1-mediated transendothelial leukocyte migration. *J Immunol*. 2005; 174(6):3709–3718. [PubMed: 15749910]
54. World Medical Association. I., Declaration of Helsinki. Ethical principles for medical research involving human subjects. *J Indian Med Assoc*. 2009; 107(6):403–405. [PubMed: 19886379]

Fig. 1. Cardiometabolic change with atazanavir treatment. Atazanavir treatment significantly reduced total cholesterol, LDL, and mean arterial pressure (* $p < 0.05$)

Fig. 2.

a Flow-mediated, endothelium-dependent vasodilation of the brachial artery. Flow-mediated, endothelium-dependent vasodilation measured at baseline and 4 days post-treatment with atazanavir remained unchanged ($p = 0.92$). **b** Nitroglycerin-mediated, endothelium-independent vasodilation of the brachial artery. No difference in response to nitroglycerin was noted before and after 4-day treatment with atazanavir ($p = 0.68$)

Table 1Baseline characteristics of study population ($n = 15$)

Characteristics	
Age (years)	45 ± 9
Female (%)	40
Hispanic (%)	7
African-American (%)	13
BMI (kg/m ²)	28.6 ± 7.8
Medications	
ACE/ARB (%)	47
Statin (%)	13
Laboratory values	
Fasting glucose (mg/dL)	178.0 ± 114.0
Hemoglobin A _{1C} (%)	8.6 ± 1.3
Total cholesterol (mg/dL)	192.5 ± 51.6
LDL-c (mg/dL)	106.8 ± 40.5
HDL-c (mg/dL)	61.5 ± 20.5
HOMA _{IR}	22.6 ± 42.6

Values are presented as mean ± standard deviation

BMI body mass index, *ACE* angiotensin-converting enzyme inhibitor, *ARB* angiotensin-receptor blocker, *LDL-c* low-density lipoprotein cholesterol, *HDL-c* high-density lipoprotein cholesterol, *HOMA_{IR}* homeostatic model assessment-estimated insulin resistance

Table 2

Effect of atazanavir treatment on metabolic and hemodynamic measures

	Baseline	Post-treatment	<i>p</i> value
Laboratory values			
Total bilirubin (mg/dL)	0.50 ± 0.05	3.87 ± 0.56	< 0.0001
FRAP (mM)	1.22 ± 0.08	1.57 ± 0.10	< 0.0001
Fasting glucose (mg/dL)	178.0 ± 29.4	172.4 ± 25.1	0.87
Insulin (mU/mL)	57.6 ± 28.1	65.2 ± 30.6	0.08
Total cholesterol (mg/dL)	192.5 ± 13.3	185.5 ± 14.7	0.04
LDL-c (mg/dL)	106.8 ± 10.4	98.2 ± 11.5	0.04
HDL-c (mg/dL)	61.5 ± 5.3	62.2 ± 5.4	0.56
Triglycerides (mg/dL)	127.8 ± 35.2	129.9 ± 37.6	0.84
HOMA _{IR}	22.6 ± 11.4	24.4 ± 12.4	0.22
Hemodynamics			
HR (beats/min)	74.2 ± 3.5	74.9 ± 3.6	0.72
MAP (mmHg)	89.1 ± 2.7	83.9 ± 2.6	0.04

Values are presented as mean ± standard error *FRAP* ferric-reducing ability of plasma, *LDL-c* low-density lipoprotein cholesterol, *HDL-c* high-density lipoprotein cholesterol, *HOMA_{IR}* homeostatic model assessment-estimated insulin resistance, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HR* heart rate, *MAP* mean arterial pressure

Table 3

Effect of atazanavir treatment on vascular function

	Baseline	Post-treatment	<i>p</i> value
Resting diameter (mm)	3.31 ± 0.13	3.33 ± 0.14	0.48
Reactive hyperemic stimulus, fold increase VTI	5.94 ± 0.63	5.65 ± 0.58	0.61
Diameter increase (mm)	0.25 ± 0.03	0.24 ± 0.03	0.79
Endothelium-dependent vasodilation (%)	8.08 ± 1.24	8.01 ± 1.27	0.92
Endothelium-independent vasodilation (%)	15.03 ± 2.12	15.49 ± 2.43	0.68

Values are presented as mean ± standard error *VTI* velocity–time integral