Coronary $\beta_2$-adrenoreceptors mediate endothelium-dependent vasoreactivity in humans: novel insights from an in vivo intravascular ultrasound study

Rishi Puri$^1$, Gary Y.H. Liew$^{1,2}$, Stephen J. Nicholls$^3$, Adam J. Nelson$^1$, Darryl P. Leong$^1$, Angelo Carbone$^1$, Barbara Copus$^2$, Dennis T.L. Wong$^1$, John F. Beltrame$^{1,4}$, Stephen G. Worthley$^{1,2}$, and Matthew I. Worthley$^{1,2}$*

1Discipline of Medicine, University of Adelaide, Adelaide, South Australia, Australia; 2Cardiovascular Investigational Unit, Royal Adelaide Hospital, Level 6 Theatre Block, North Terrace, Adelaide, South Australia 5000, Australia; 3Department of Cardiovascular Medicine, Heart and Vascular Institute, Cleveland Clinic, Cleveland, OH, USA; and 4Cardiology Unit, The Queen Elizabeth Hospital, Woodville, South Australia, Australia

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Aims
The interaction between coronary $\beta_2$-adrenoreceptors and segmental plaque burden is complex and poorly understood in humans. We aimed to validate intracoronary (IC) salbutamol as a novel endothelium-dependent vasodilator utilizing intravascular ultrasound (IVUS), and thus assess relationships between coronary $\beta_2$-adrenoreceptors, regional plaque burden and segmental endothelial function.

Methods and results
In 29 patients with near-normal coronary angiograms, IVUS-upon-Doppler Flowire imaging protocols were performed. Protocol 1: incremental IC salbutamol (0.15, 0.30, 0.60 $\mu$g/min) infusions (15 patients, 103 segments); protocol 2: salbutamol (0.30 $\mu$g/min) infusion before and after IC administration of N$\text{G}$-monomethyl-L-arginine (L-NMMA) (10 patients, 82 segments). Vehicle infusions (IC dextrose) were performed in 4 patients (21 segments). Macrovascular response [% change segmental lumen volume ($\triangle$SLV)] and plaque burden [per cent atheroma volume (PAV)] were studied in 5-mm coronary segments. Microvascular response [per cent change in coronary blood flow ($\triangle$CBF)] was calculated following each infusion. Intracoronary salbutamol demonstrated significant dose-response $\triangle$SLV and $\triangle$CBF from baseline, respectively (0.15 $\mu$g/min: 3.5 $\pm$ 1.3%, 28 $\pm$ 14%, $P = 0.04$, $P = \text{NS}$; 0.30 $\mu$g/min: 5.5 $\pm$ 1.4%, 54 $\pm$ 17%, $P = 0.001$, $P < 0.0001$; 0.60 $\mu$g/min: 4.8 $\pm$ 1.6%, 66 $\pm$ 15%, $P = 0.02$, $P < 0.0001$), with $\triangle$SLV responses further exemplified in low vs. high plaque burden groups. Salbutamol vasomotor responses were suppressed by L-NMMA, supporting nitric oxide-dependent mechanisms. Vehicle infusions resulted in no significant $\triangle$SLV or $\triangle$CBF. Multivariate analysis including conventional cardiovascular risk factors, PAV, segmental remodelling and plaque eccentricity indices identified PAV as the only significant predictor of a $\triangle$SLV to IC salbutamol (coefficient $-0.18$, 95% CI $-0.32$ to $-0.04$, $P = 0.015$).

Conclusions
Intracoronary salbutamol is a novel endothelium-dependent epicardial and microvascular coronary vasodilator. Intravascular ultrasound-derived regional plaque burden is a major determinant of segmental coronary endothelial function.

Keywords
salbutamol • $\beta_2$-adrenoreceptors • IVUS • endothelial function • atherosclerosis

* Corresponding author. Tel: +61 8 8222 4000, Fax: +61 8 8222 2454, Email: matthew.worthley@adelaide.edu.au

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Introduction

The in vivo evaluation of the human coronary adrenergic system is complex,1 whereby the significant interspecies and inter-territory variability of adrenoceptor (AR) sub-type expression has resulted in numerous prior conflicting observations.2–5 Specifically, the in vivo functional role of human coronary β2-ARs in eliciting nitric-oxide (NO)-dependent vasoreactivity in varying stages of atherosclerosis remains unclear. Endothelial dysfunction is a systemic process shown to be an independent predictor of major cardiac events.6–8 Conduit vessel endothelial dysfunction has been recently shown to occur in a segmental fashion,9 and thought to play a mechanic role in mediating focal plaque disruption.10 Additionally, the intravascular ultrasound (IVUS)-derived burden of coronary atherosclerosis has been shown to be a strong predictor of future cardiac events.11 However, the fundamental in vivo relationship between segmental coronary endothelial function and regional plaque burden in humans is unknown. Defining this dynamic relationship might provide incremental information regarding plaque stability, progression, and relationship to future coronary events.

The present study was designed to (i) assess the safety and physiological responses of selective coronary β2-AR stimulation [with intracoronary (IC) salbutamol delivery] during IVUS and Doppler Flowire imaging, and (ii) test that the coronary arterial salbutamol response is NO-dependent, (iii) utilize this validated stimulus and IVUS imaging to investigate the fundamental determinants of segmental coronary endothelial function in humans, with a particular emphasis upon this dynamic relationship with corresponding regional plaque burden.

Methods

Study subjects

This study enrolled 29 patients (aged ≥18 years) electively referred to the Royal Adelaide Hospital Cardiac Catheterization Laboratories for the investigation of chest pain. Informed consent was obtained >48 h prior to the procedure and all vasoactive medications were held for 24 h prior to the study. All procedures were performed on a morning list following an overnight fast. Patients with significant valvular heart disease, left ventricular dysfunction, prior percutaneous or surgical coronary revascularization, recent acute coronary syndrome (within 4 weeks), known predilection to coronary artery spasm, calculated creatinine clearance of <60 mL/min, chronic β-blocker therapy or the use of short or long acting β2-agonists within the previous 12 h were excluded. Target vessels with visual angiographic stenoses of >30% were excluded. Fasting blood samples for serum lipids, high sensitivity C-reactive protein, and biochemistry were obtained at angiography. This study was approved by the Royal Adelaide Hospital Human Research Ethics Committee.

Catheterization, imaging, and coronary endothelial function testing protocols

Coronary angiography was performed via a standard 6-French technique. Intravenous heparin (70 IU/kg) was administered for the research protocol. Following intubation of the left (or right) coronary system, a 0.014 in. Doppler guide wire (Flowire; Volcano Therapeutics, Rancho Cordova, CA, USA) was placed into the target vessel within its mid-segment away from major side-branches. This wire was also used to monorail a 2.5F 40-MHz Atlantis SR Pro IVUS catheter (Boston Scientific, Natick, MA, USA) into the study artery. This technique allowed for the dual and simultaneous assessment of macro- and microvascular coronary responses to IC infusions. All IC infusions were administered through a Medrad infusion pump at 2 mL/min via the coronary guiding catheter for a period of 5 min. Intravascular ultrasound was performed in the usual fashion, but without pre-treatment of the target vessel with IC nitroglycerin. After 3 min of IC infusion, the instantaneous average peak velocity (APV) was recorded, followed by the guide wire being repositioned into the distal vessel and IVUS pull-back commenced at 0.5 mm/s. The infusions continued for the remaining 2 min during which IVUS acquisition/pullback was undertaken. All IVUS images were saved and recorded on a DVD for off-line analysis.

Infusion protocols

This study involved two main active infusion protocols summarized in Figure 1. Protocol 1 assessed the safety and efficacy of the imaging methodology with incremental IC salbutamol doses. Concentrations of IC salbutamol previously shown not to significantly affect heart rate and blood pressure were evaluated.12 Following a baseline 5-min period of 5% IC dextrose infusion, 15 patients underwent sequential 5-min infusions of IC salbutamol at doses of 0.15, 0.30 and 0.60 μg/min, respectively, with APV measurements and the initiation of the IVUS pullback performed at the 3-min mark (Figure 1A). The second protocol was designed to assess the endothelium-dependent nature of IC salbutamol upon the epicardial coronary conduit vessel. An additional 10 patients underwent a 5-min IC salbutamol infusion at 0.30 μg/min (selected upon the findings from protocol 1) both before and after NO synthase inhibition with Nω-monomethyl-L-arginine (L-NMMA, Bachem Distribution Services GmbH, Weil am Rhein, Switzerland) given at 20 μmol/min for 5-min13,14 (Figure 1B). All patients received IC nitroglycerin at the completion of the protocol, and cine angiography was performed to confirm the absence of coronary spasm or local dissection.

To determine the variation in segmentary coronary lumen measurements and coronary blood flow (CBF) over time, another four patients underwent IVUS and Doppler Flowire imaging following each of four consecutive IC dextrose (vehicle) infusions.

Data acquisition and analysis

Haemodynamics

Haemodynamic data (aortic pressure) and ECG was digitally recorded and printed at the end of each step of the infusion protocol.

Intravascular ultrasound

All IVUS data were analysed using echoPlaque 3.0.53 (Indec Systems, Santa Clara, CA, USA). From the initial IVUS run, the common most distal and proximal fiducial markers (anatomical side-branches) were chosen to define the region of vessel to be analysed. Cross-sectional images were selected every 30 frames (0.5 mm) apart. Each IVUS run was precisely divided into pre-defined 5-mm segments comprising of 10 cross-sectional frames spaced 0.5-mm apart (Figure 2). Owing to angiographically proven heterogeneity of coronary vasodilator function,15 each segment was evaluated separately. Using MIB software (Indec Medical Systems, Santa Clara, CA, USA), the baseline IVUS run (with numbered frames) was simultaneously played with each subsequent IVUS run in order to precisely frame match anatomical fiducial markers between each run. This technique ensured that the same arterial segments were consistently analysed for each infusion. The leading edges of the lumen and external elastic membrane (EEM)
were traced by manual planimetry. Plaque area was defined as the area occupied between these leading edges. Only cross-sectional images deemed acceptable for complete lumen and EEM analysis were included. Per cent atheroma volume (PAV) was chosen as our determinant of segmental plaque burden. This was calculated as the proportion of the entire vessel cross-sectional area (CSA) of that segment occupied by atherosclerotic plaque:

$$\text{PAV} = \frac{\sum (\text{EEM}_{\text{area}} - \text{Lumen}_{\text{area}})}{\sum \text{EEM}_{\text{area}}} \times 100.$$  

Segmental lumen volumes (SLV) were calculated as the summation of lumen area in each measured image. The SLV for each 5-mm segment was normalized to account for differences in the number of analysable frames within each pre-defined segment:

$$\text{SLV}_{\text{normalized}} = \frac{\sum (\text{Lumen}_{\text{area}})}{\text{number of images in segment}} \times 10.$$  

Percentage changes in SLV ($\triangle \text{SLV}$) were used as the primary analysis of segmental vasomotor response. Low and high plaque burden groups were defined around the mean PAV for respective analysis regarding plaque burden. Segmental remodelling indices (RIs) were determined by calculating the average segmental EEM$_{\text{area}}$ dividing this by a reference EEM$_{\text{area}}$ taken from either a proximal or distal reference point located within 10-mm from the index segment with the least plaque burden. Segmental eccentricity indices (EIs) were determined by calculating the average of all EI's of each analysable frame within a coronary segment (EI = ratio of maximal to minimal plaque thickness). All measurements were performed with the analyst blinded to the specific segment, and the degree of plaque burden and lumen response from preceding infusion sequences.

Coronary blood flow
A Doppler flowwire-derived APV was determined from instantaneous velocity signals from the Doppler wire by an online fast Fourier transform. Coronary blood flow was calculated from the product of IVUS-derived average segmental CSA and Doppler flowwire-derived APV using the equation: $\text{CBF} = \text{CSA} \times \text{APV}$/2. Per cent change in CBF ($\triangle \text{CBF}$) from baseline was assessed for each patient for each run in all infusion protocols.

Statistical analysis
Data are expressed as mean $\pm$ SEM or median (IQR) as appropriate. The effect of salbutamol dose and covariates upon $\triangle \text{SLV}$ was evaluated by mixed effects modelling. To identify univariate predictors of SLV and SLV response to salbutamol, the covariate of interest and salbutamol dose, and the covariate*dose interaction were modelled as fixed effects, with subject identity and arterial segment modelled as random effects to account for repeated measures within arterial segments within patients. For significant covariate*dose interactions, post hoc testing was performed at each dose. Two multivariate models were then constructed. First, any covariate with significant dose-dependent association with SLV on univariate analysis and second, any covariate whose main effect on SLV independent of salbutamol dose exhibited a $P$-value $< 0.2$ was included. Independent predictors of change in SLV were then identified by multivariate mixed-effects modelling using backward elimination. In the second analysis, we determined whether incremental predictive value for change in SLV was added by the stepwise inclusion of covariates found to or previously reported to influence SLV, using the likelihood-ratio test. Intra- and inter-observer variability analysis was performed following planimetry of lumen and plaque areas from 20 randomly selected IVUS frames by two-independent observers and...
by one observer at two separate time points. All statistical tests were two-sided and a $P$-value $<0.05$ considered significant. Statistical analysis was performed with STATA 11 (Stata Corp, College Station, TX, USA).

**Results**

**Clinical, haemodynamic, and observer variability data**

Baseline demographics of the study cohort are shown in Table 1. Two patients experienced transient coronary spasm during instrumentation, prior to IC infusions (one during Flowire manipulation and second with the IVUS catheter), both responding promptly to IC nitroglycerin. These patients were excluded from the research protocol, and data not included in the analysis. In protocol 1, successive doses of IC salbutamol, and in protocol 2, the infusion of L-NMMA each had no significant effect upon baseline blood pressure or heart rate, respectively. For coronary lumen measurements, the intra-observer coefficient of variation was 1.1%, and the inter-observer coefficient of variation was 2.6%. For plaque measurements, the intra-observer coefficient of variation was 1.8%, and the inter-observer coefficient of variation was 3.8%.

**Intravascular ultrasound and coronary blood flow results (protocol 1)**

Analysis from protocol 1 ($n = 103$ segments) suggests a dose-dependent increase in SLV across all segments from baseline ($\Delta$SLV: 0.15 μg/min: 3.5 ± 1.3%, $P = 0.04$; 0.30 μg/min: 5.5 ± 1.4%, $P = 0.001$; 0.60 μg/min: 4.8 ± 1.6%, $P = 0.02$) (Figure 3A). Absolute variations of mean SLV values across all segments (including for stratification of plaque burden) at various time points are shown in Table 2 and largely reflect the corresponding per cent changes in SLV that were observed from baseline. Segmental plaque burden was dichotomized into low (mean PAV 15 ± 0.6%) and high (mean PAV 38 ± 1.5%) plaque burden groups using the mean sample PAV as the cut-off. The vasodilator properties of IC salbutamol were observed at all doses within the low plaque burden group ($\Delta$SLV: 0.15 μg/min: 5.8 ± 1.8%, $P = 0.015$; 0.30 μg/min: 9.1 ± 1.9%, $P < 0.0001$; 0.60 μg/min: 8.8 ± 2.1%, $P = 0.001$) but were no longer significant in the high plaque burden group ($\Delta$SLV:...
There was a progressive and significant △CBF from baseline with escalating doses of IC salbutamol (△CBF: 0.15 μg/min: 28 ± 14%, P = 0.04; 0.30 μg/min: 54 ± 17%, P < 0.0001; 0.60 μg/min: 66 ± 15%, P < 0.0001) (Figure 3B).

Based upon the results of protocol 1, a salbutamol dose was chosen to resemble a submaximal ‘EC50’ dose, in order to provide the optimal chance of differentiating subtle changes in vasomotor reactivity according to varying degrees of plaque burden for the planned L-NMMA infusion in protocol 2. While changes (per cent change from baseline and absolute change) in conduit vessel volumes were similar between the 0.30 and 0.60 μg/min salbutamol doses, microvascular effects were less when comparing the effects of the 0.30 μg/min with the 0.60 μg/min dose of salbutamol from protocol 1, respectively (Figure 3). Hence, for protocol 2, the 0.30 μg/min of salbutamol was chosen (with L-NMMA) to evaluate its NO-dependent vasomotor properties.

### Intravascular ultrasound and coronary blood flow results (protocol 2)

The infusion sequence in protocol 2 was designed to assess the potential endothelium-dependent effects of IC salbutamol within the epicardial coronary vasculature (n = 82 segments). In all segments (irrespective of plaque burden), salbutamol produced a significant increase in △SLV compared with baseline (4.7 ± 1.6%, P = 0.03). Following IC L-NMMA, repeat IC salbutamol infusion no longer caused a significant increase in △SLV (0.03 ± 1.4%, P = 0.78) (Figure 4A), and this response was significantly impaired compared with the vasomotor changes observed from the initial salbutamol infusion (P = 0.03). Absolute variations of mean SLV values (including for stratification of plaque burden) across all segments at various time points are shown in Table 2 and largely reflect the corresponding per cent changes in SLV that were observed from baseline. Conduit segments were further stratified according to low (mean PAV 19 ± 1.3%) and high (mean PAV 41 ± 1.0%) plaque burden groups. In the

![Figure 3](https://example.com/figure3.png)

**Figure 3** Salbutamol dose–responses: macro- and microvasculature (protocol 1). (A) All segments. *P < 0.05 vs. baseline (B) coronary blood flow response to incremental salbutamol dosing. *P < 0.0001 vs. baseline, #P < 0.05 vs. low dose salbutamol.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics</th>
<th>Entire cohort, n = 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>58 ± 3</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>13 (45)</td>
<td></td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>16 (55)</td>
<td></td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>10 (34)</td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>Family history CAD, n (%)</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>15 (52)</td>
<td></td>
</tr>
<tr>
<td>Statin</td>
<td>9 (31)</td>
<td></td>
</tr>
<tr>
<td>ACE-I/ARB</td>
<td>8 (26)</td>
<td></td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>3 (10)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>176 ± 4</td>
<td></td>
</tr>
<tr>
<td>TGL</td>
<td>62 (45.98)</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>50 ± 11</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>112 ± 6</td>
<td></td>
</tr>
<tr>
<td>High sensitivity C-reactive protein, mg/L</td>
<td>2.5 (1.6,4.6)</td>
<td></td>
</tr>
<tr>
<td>Artery Investigated, n (%)</td>
<td>LAD 24 (83)</td>
<td></td>
</tr>
<tr>
<td>LCx</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>RCA</td>
<td>1 (3)</td>
<td></td>
</tr>
</tbody>
</table>

ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CAD, coronary artery disease; HDL, high-density lipoprotein; LAD, left anterior descending artery; LCx, left circumflex artery; LDL, low-density lipoprotein; RCA, right coronary artery; TGL, triglycerides.
low plaque burden group, salbutamol infusion resulted in a significant ΔSLV of 7.0 ± 1.5% from baseline (P < 0.0001). The addition of L-NMMA resulted in a significant blunted salbutamol response, however with incomplete vasodilator abolishment noted (ΔSLV of 3.8 ± 1.9%, P = 0.09 vs. baseline). In the high plaque burden group, salbutamol infusion resulted in an insignificant degree of vasodilatation from baseline (ΔSLV 0.74 ± 2.0%, P = 0.9). The addition of L-NMMA resulted in a net degree of vasoconstriction compared with baseline (ΔSLV –3.8 ± 1.9%, P = 0.09), which was significant lower compared with the initial salbutamol infusion (P < 0.01).

In protocol 2, ΔCBF significantly increased from baseline following the first infusion of salbutamol (ΔCBF 56 ± 22%, P < 0.01). Following IC L-NMMA, repeat IC salbutamol infusion no longer caused a significant increase in ΔCBF (5.5 ± 12, P = 0.9) (Figure 4B), and this response was also significantly impaired compared with the vasomotor changes observed from the initial salbutamol infusion (P < 0.01).

### Intravascular ultrasound and coronary blood flow results (repeated intracoronary vehicle infusions)

In the four patients (21 segments) who underwent four consecutive vehicle (IC dextrose) infusions, there was no significant variation in ΔSLV (Run 2: –2.3 ± 1.9%, P = 0.24; Run 3: 2.7 ± 2.1%, P = 0.20; Run 4: –0.38 ± 2.7%, P = 0.73) and ΔCBF (Run 2: 6.4 ± 1.6%, P = 0.20; Run 3: 7.8 ± 2.1%, P = 0.15; Run 4: 1.4 ± 7.3%, P = 0.85) following each infusion compared with baseline (Run 1). These experiments confirm no impact of IC vehicle infusion or coronary instrumentation on dynamic vascular measurements over time. Mean absolute SLV values at each time point are depicted in Table 2.

### Table 2: Mean absolute segmental lumen volume values at each time point per infusion protocol

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Mean SLV at each time point (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol 1</td>
<td></td>
</tr>
<tr>
<td>All segments</td>
<td>101.4 ± 4.0 103.4 ± 3.9 106.3 ± 4.2** 104.7 ± 4.0</td>
</tr>
<tr>
<td>Low PAV</td>
<td>119.5 ± 5.6 123.7 ± 5.3 127.9 ± 5.4**** 127.2 ± 5.2**</td>
</tr>
<tr>
<td>High PAV</td>
<td>82.2 ± 4.4 81.9 ± 3.9 83.4 ± 4.6 80.9 ± 4.1</td>
</tr>
<tr>
<td>Protocol 2</td>
<td></td>
</tr>
<tr>
<td>All segments</td>
<td>83.4 ± 3.4 87.2 ± 3.3**** 81.6 ± 3.3**** 82.5 ± 3.3****</td>
</tr>
<tr>
<td>Low PAV</td>
<td>88.1 ± 5.2 93.1 ± 5.2* 89.6 ± 4.7 90.3 ± 5.0</td>
</tr>
<tr>
<td>High PAV</td>
<td>78.7 ± 4.3 78.2 ± 4.2 73.6 ± 4.5****** 74.7 ± 4.0</td>
</tr>
<tr>
<td>Control</td>
<td>Run 1 Run 2 Run 3 Run 4</td>
</tr>
<tr>
<td>All segments</td>
<td>79.7 ± 7.2 77.9 ± 7.3 81.7 ± 4.9 80.4 ± 8.1</td>
</tr>
</tbody>
</table>

PAV, per cent atheroma volume; SLV, segmental lumen volume.

*P < 0.02 vs. baseline, **P < 0.01 vs. baseline, ***P < 0.001 vs. baseline, ****P < 0.0001 vs. baseline, *****P = 0.03 vs. salbutamol dose # 1, ******P < 0.01 vs. baseline and salbutamol dose # 1.

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**Figure 4** Salbutamol and L-N⁵-monomethyl-L-arginine responses: macro- and microvasculature (protocol 2). (A) All segments. *P < 0.05 salbutamol vs. baseline, #P < 0.05 salbutamol vs. salbutamol + L-N⁵-monomethyl-L-arginine. (B) Coronary blood flow responses. *P < 0.01 salbutamol vs. baseline, #P < 0.01 salbutamol vs. salbutamol + L-N⁵-monomethyl-L-arginine.
Determinants of segmental coronary endothelial function

Systemic patient factors (age, hypertension, smoking, LDL, HDL, high sensitivity C-reactive protein) and local plaque/vessel factors (segmental PAV, RI, EI) were each evaluated as univariate predictors of segmental endothelial function. Segmental plaque burden (PAV) was found to be the only significant univariate predictor (coefficient $-0.18$, 95% CI: $-0.32$ to $-0.044$, $P = 0.009$) of $\Delta$SLV (Table 3). Age and LDL were forced into a multivariate model with PAV to identify independent predictors of $\Delta$SLV response to salbutamol. Per cent atheroma volume was found to be the sole independent predictor of $\Delta$SLV from baseline ($P = 0.015$).

Likelihood ratio testing was performed to determine the predictive capacity of cardiovascular risk factors (age, hypertension, smoking, LDL, HDL, high sensitivity C-reactive protein—each of which have been previously shown to influence coronary vasoreactivity), and plaque burden (PAV) in determining a change in the salbutamol-endothelial conduit vessel response. As observed in Figure 5, each risk factor imparted a significant, individual effect upon the salbutamol-endothelial conduit vessel response, when assessed in a stepwise manner. For example, age was a significant predictor of the change in the salbutamol-endothelial vasomotor response; however, the combination of age and hypertension was more predictive of the salbutamol-endothelial response than age alone, and so on. Furthermore, plaque burden remained a major predictor of the segmental-endothelial conduit vessel response, above and beyond the cumulative predictive effect of all cardiovascular risk factors summated in the model.

Table 3  Univariate and multivariate predictors of salbutamol-induced SLV

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Coefficient (95% CI)</th>
<th>P-value</th>
<th>Multivariate Coefficient (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI</td>
<td>$-0.021$ ($-0.34$ to $0.29$)</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>$-0.30$ ($-9.1$ to $8.5$)</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>$0.69$ ($-5.7$ to $7.1$)</td>
<td>0.8</td>
<td></td>
<td></td>
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<tr>
<td>High sensitivity C-reactive protein</td>
<td>$-0.17$ ($-0.53$ to $0.19$)</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RI</td>
<td>$-7.0$ ($-19$ to $5.2$)</td>
<td>0.3</td>
<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>$3.7$ ($-2.0$ to $9.4$)</td>
<td>0.21</td>
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<tr>
<td>Age*</td>
<td>$-0.20$ ($-0.43$ to $0.033$)</td>
<td>0.1</td>
<td>$-0.16$ ($-0.38$ to $0.060$)</td>
<td>0.3</td>
</tr>
<tr>
<td>LDL*</td>
<td>$-2.1$ ($-4.8$ to $0.50$)</td>
<td>0.1</td>
<td>$-1.4$ ($-1.9$ to $1.1$)</td>
<td>0.2</td>
</tr>
<tr>
<td>PAV*</td>
<td>$-0.18$ ($-0.32$ to $-0.044$)</td>
<td>0.01</td>
<td>$-0.17$ ($-0.31$ to $-0.03$)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Univariate predictors entered into multivariate model.

EI, eccentricity index; HDL, high-density lipoprotein; RI, remodelling index; LDL, low-density lipoprotein; PAV, per cent atheroma volume.

Figure 5  Cumulative value of cardiovascular risk factors and plaque burden in predicting segmental endothelial function. Each nested model’s ability to predict segmental coronary endothelial function is compared with the adjacent model using the likelihood ratio test.

Discussion

We have successfully described the physiological role of coronary $\beta_2$AR’s in mediating endothelium-dependent vasoreactivity across various stages of atherosclerotic disease within the in vivo human
epicardial coronary circulation. The study achieved its objectives demonstrating that IC salbutamol mediates its vasoactive effects via NO within both the coronary macro- and microvasculature, supporting earlier in vitro,20–22 and human in vivo observations within the peripheral vasculature.23,24 Furthermore, we also show for the first time the incremental predictive capacity of regional plaque burden in determining corresponding segmental coronary endothelial responses, above and beyond that of the cumulative burden of traditional cardiovascular risk factors. These insights provide an explanation of the observed heterogeneity in vasomotor response within an epicardial coronary artery in a majority of our patients, which has been elusive with conventional quantitative coronary angiographic (QCA) assessment.

**β₂-Adrenoceptors, salbutamol, and human coronary arteries**

Although the presence of β₂AR’s upon human coronary vascular endothelial cells has been well described,26 their physiological responses within an intact human coronary arterial system are complex23,24 with such responses being further susceptible to a number of genetic polymorphisms that result in functional alterations of the receptor complex and subsequent vascular response.27 Since the original finding that removal of endothelium reduces βAR-agonist-induced vasorelaxation of canine coronary arteries,21 accumulating evidence has suggested that endothelium-mediated β-adrenergic-induced vasorelaxation is impaired by endothelial removal or inhibition of NO synthesis.22,28–31

Very few studies have investigated the in vivo function of endothelial β₂AR’s in humans. Either direct infusion of salbutamol into the intact human brachial artery or inhaled salbutamol has been shown to possess endothelium-dependent properties within the peripheral vasculature.23,24 The only prior study conducted within intact human coronary arteries was undertaken by Barbato et al., who utilized QCA and Flowire methodology to elegantly show a microvascular endothelium-dependent vasomotor effect of salbutamol, as well as impaired β₂AR responsiveness coupled with enhanced αAR tone in angiographically stenotic conduit segments, which together mediated a constrictive effect of salbutamol in these segments.12 Similar mechanisms were observed in our experiments when subgroup analysis was done according to the degree of plaque burden present. Although it may be possible that the residual degree of salbutamol-mediated vasodilatation observed in the low plaque burden group following L-NMMA may be due to salbutamol-mediated smooth muscle cell effects,32 it is more likely that our findings are a result of the variability in L-NMMA-mediated NOS inhibition in relation to the degree of plaque burden present. It is also likely that low atheroma burden segments require higher doses of L-NMMA for complete NOS inhibition, which may reflect the residual endothelium-dependent vasodilating effects of salbutamol observed. In contrast, higher atheroma burden segments may release much less NO, and thus require lower doses of L-NMMA to completely inhibit the residual amount of NOS present. This was evident with complete abolition of NOS resulting in net coronary vasoconstriction, due to impaired β₂AR responsiveness and enhanced αAR tone, as described by Barbato et al.12

Utilizing IVUS, a more sensitive imaging methodology, our study shows that IC salbutamol (and epicardial β₂AR stimulation) possesses NO-dependent properties within the intact human coronary vasculature, synonymous with acetylcholine-induced muscarinic receptor stimulation mediating NO-dependent vasoreactivity. Moreover, the magnitude of segmental coronary vasomotor reactivity observed within our study is consistent with responses observed in some prior studies evaluating the dose–response effects of IC acetylcholine,33,34 and similar in magnitude to the IC salbutamol responses reported by Barbato et al.12 However from a mechanistic viewpoint, unlike acetylcholine, βAR-agonists are not known to activate the inositol 1,4,5 triphosphate signalling pathway. Therefore, alternative mechanisms are likely to explain our observations. These may include (i) synergism between the actions upon adenylyl cyclase of exogenous βAR-agonists and endothelium-derived prostaglandins,21 (ii) inhibition of cyclic adenosine monophosphate (cAMP) phosphodiesterase by cyclic guanosine monophosphate (produced within vascular smooth muscle cells) in response to basal endothelium-NO release;35 (iii) β₂AR-mediated endothelial cAMP synthesis to directly stimulate NO synthase and subsequent release of endothelial NO,22 and/or (iv) activation of potassium channel-induced endothelial hyperpolarization and subsequent NO-synthase activation via Ca²⁺/calmodulin.36 Despite these postulated mechanisms, the functional significance of β₂AR’s within the human coronary arterial system, however, cannot be understated. Adrenergic stimulation plays an important role in the regulation of coronary vasomotor tone, whereby both endothelial β₁ and β₂ AR’s contribute towards vasodilatory drive, opposing the vasoconstrictive effects of endothelial αAR’s, particularly within atherosclerotic coronary arteries.37,38 A recent characterization of the relative expression of α and βAR-subtypes within human epicardial coronary arteries has uncovered that two-thirds of all such AR’s are of the βAR type, of which 99% are of the β₂AR sub-type.39 Given the recent discovery of the α₁DAR being the predominant αAR responsible for epicardial coronary vasoconstriction,38,39 the interplay between coronary β₂ and α₁DAR function will therefore be important for the selective modulation of coronary vasomotor tone as a potential novel therapeutic strategy.

**Plaque burden and vessel function seen with intravascular ultrasound**

The discrepancy between findings on coronary angiography (or ‘lumenography’) and IVUS in the assessment of plaque burden has been well documented.40 Intravascular ultrasound frequently demonstrates the ubiquitous presence of plaque within angiographically normal coronary arterial segments. Prior attempts to characterize the structure–function relationship between plaque burden and epicardial coronary vasoactivity failed to systematically evaluate volumetric indices of plaque burden, and at best relied upon the off-line matching of QCA-derived lumen diameter responses with plaque topography measured separately with IVUS.9,41–43 The comprehensive method of assessing pan-segmental volumetric endothelial luminal response with IVUS has confirmed the heterogeneous dynamic properties of the human epicardial coronary vasculature, with all but three patients
analysing both segmental vasodilation and vasoconstriction in adjacent segments. Our data support the heterogeneity of segmental lumen reactivity to be intrinsically related to plaque burden, not detected angiographically.

Recent analysis has demonstrated a significant relationship between the baseline extent and progression of disease, as determined by IVUS, with the prospective risk of major adverse cardiac events. These observations were made in stable patients within non-critically diseased vessels. The mean baseline PAV value of 38.6% in these trial patients compared similarly with the mean PAV within segments that were stratified as having high plaque burden in our protocols. Most of the segments with this extent of PAV exhibited blunted vasomotor (or mild vasoconstrictive) responses to IC salbutamol. We also found that PAV provides a significant and incremental prediction of focal coronary endothelial function beyond what the cumulative burden of atherosclerosis-risk factors provide. This strengthens the validity of PAV as an important biomarker of coronary risk. It remains unclear from the large collection of patients in the various atheroma progression–regression trials as to whether plaque burden itself, component risk factors or localized endothelial dysfunction, mediates further disease progression.

Clinical and future implications

Further work is required in this area to evaluate the impact of segmental coronary endothelial function upon atheroma progression, plaque instability, and ultimately clinical outcomes. It is increasingly apparent that plaque burden is an important biomarker of future coronary risk. Factors linking plaque burden to vessel function may therefore be pivotal in determining the likelihood of a clinical event from a given atheromatous coronary arterial segment. This will require future coronary imaging modalities to incorporate not only structural, but also vascular dynamic information when risk-assessing potentially high-risk plaque segments. Furthermore, there exists a unique opportunity to selectively modulate coronary endothelial function as a method to alter the natural progression of coronary atherosclerosis via the coronary endothelial adrenergic system.

Limitations

Additional infusion sequences to the current experimental protocols would allow for further exploration of the underlying mechanisms involved in the salbutamol-mediated human coronary vasomotor response. However, the practicality of doing this are limited by ethical concerns of infusing novel, vasoconstricting substances in vivo within human coronary arteries during a prolonged and highly invasive protocol. Nevertheless, such limitations of our experimental protocols include the inability to entirely exclude a non-specific vasoconstrictive effect of IC L-NMMA. Further infusions to evaluate the effects of concomitant dosing of a selective β2AR antagonist acting as an endothelium-independent vasoconstrictor (i.e. butoxamine) with progressive doses of IC salbutamol could be performed, as has been the case within the ex vivo human coronary arterial setting, to address this issue. To the best of our knowledge, our study is the first to utilize IVUS to evaluate in vivo lumen responses to IC L-NMMA. However, it is known that while L-NMMA blocks the oxidation of l-arginine, it fails to inhibit the formation of superoxide anions from molecular oxygen, and thus may have provided incomplete NOS inhibition in our low atheroma burden group. Therefore, although rarely used during in vivo human experimentation, l-N^6-nitroarginine is considered to be a more ideal inhibitor of NO synthase. However, its safety of administration within the human in vivo IC setting is uncertain. Time constraints also limited the further evaluation of the impact of segmental plaque burden upon assessing endothelium-independent vasomotion following IC nitroglycerin administration, which would have provided incremental mechanistic information to the above findings.

We also cannot reliably exclude a concomitant direct salbutamol-induced smooth muscle vasorelaxation effect as shown by Sun et al., nor can we exclude an upstream flow-mediated effect due to the observed changes in CBF. Although the use of state-of-the-art imaging software enabled a precise degree of real-time frame matching for segmental analysis with multiple IVUS runs, subtle degrees of horizontal bias due to subtle variations in actual catheter pullback speeds between different runs may result in slight variations in repeated measurements over multiple IVUS runs. We, however, feel this has not significantly impacted upon our results. Finally, true mechanistic studies involving the determination of segmental tissue expression of various effector molecules, and the contribution of genetic polymorphisms, are unable to be achieved during human in vivo studies, and further human ex vivo or in vitro studies will need to be conducted to explore the underlying molecular mechanisms of our findings.

Conclusions

We provide important insights into the functional role of coronary β2AR’s in varying degrees of health and segmental atherosclerotic coronary disease, and outline the NO-dependent properties of these receptors within human coronary arteries in vivo. This study has shown that IC salbutamol is both a macro- and microvascular coronary endothelium-dependent vasodilator. These findings within the macrovasculature have been demonstrated using a novel IVUS-based methodology, which has further shown segmental plaque burden to be a strong independent predictor of endothelium-dependent coronary conduit vessel vasodilatation.

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References

15. R. Puri