



Acute effects of salbutamol on systemic vascular function in people with asthma

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ABSTRACT

Background: Asthmatics are at increased cardiovascular disease risk, which has been linked to beta2(β_2)-agonist use. Inhalation of β_2 -agonists increases sympathetic nerve activity (SNA) in healthy individuals, however the systemic impact of salbutamol in asthmatics using β_2 -agonists regularly is unknown.

Objectives: This study compared the systemic vascular responses to a clinical dose of salbutamol (Phase I) and following an acute increase in SNA (Phase II) in asthmatics and controls.

Methods: Fourteen controls and 14 asthmatics were recruited for Phase I. On separate days, flow-mediated dilation (FMD) and peripheral arterial stiffness (pPWV) were evaluated at baseline and following either 400 μ g inhaled salbutamol or a placebo inhaler. For Phase II, heart rate, blood pressure, vascular conductance, pPWV, and central (c)PWV were evaluated in response to a large increase in SNA brought on by cold-water hand immersion (i.e. cold-pressor test) or body-temperature water hand immersion (i.e. control) in 10 controls and 10 asthmatics.

Results: Following salbutamol, asthmatics demonstrated reduced FMD (-3.0% , $p < 0.05$) and increased pPWV ($+0.7$ m/s, $p < 0.05$); however, salbutamol had no effect in controls. The cold-pressor test resulted in similar increases in blood pressure, vascular flow rates and conductance, pPWV, and cPWV in both asthmatics and controls, suggesting similar neurovascular transduction in asthmatics and controls.

Conclusion: Inhaled Salbutamol leads to increased arterial stiffness and reduced FMD in asthmatics. As asthmatics and controls had similar vascular responses to an increase in SNA, these findings suggest asthmatics have heightened sympathetic responses to β_2 -agonists which may contribute to the increased cardiovascular risk in asthma.

1. Introduction

Although asthma is primarily considered a disease of the airways, people with asthma are also at an increased risk of developing cardiovascular (CV) diseases such as coronary heart disease, cerebrovascular disease, and heart failure [1–5]. The risk of CV disease increases with decreased asthma control [2,4,6,7], and flare-up in asthma symptoms causing increased medication usage, emergency department visits, and/or hospitalizations, are a significant risk factor for CV events [4]. However, the reasons for the elevated CV risk in asthma are unknown.

While it is plausible that flare-ups in inflammation during asthma exacerbations [8] contribute to this increased CV risk, reduced asthma

control is also linked to greater use of asthma medication containing beta2(β_2)-agonists [9–11]. The usage of β_2 -agonists has been associated with increased CV risk independent of obstructive lung disease, smoking history, other CV disease risk factors [5,12]. Specifically, acute coronary events (myocardial infarctions and unstable angina) are up to twice as prevalent among those who have filled prescriptions for β_2 -agonists within the three months prior to the CV events [12]. Thus, it has been suggested that asthma medications containing β_2 -agonists may have deleterious extra-pulmonary effects [5,12].

The sympathetic nervous system is an important regulator of cardiac function, and through the stimulation of β -adrenoceptors, an increase in sympathetic activity has both inotropic and chronotropic effects. The

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short-acting β_2 -agonist salbutamol is commonly used as part of standard asthma management [13], and due to the 4:1 ratio of β_1 vs β_2 -adrenoceptors in the heart [14,15], salbutamol is not considered to have a significant impact on cardiac function. However, both our laboratory and others have found that short-acting β_2 -agonists cause acutely increased sympathetic activity in healthy individuals [16–18], which was demonstrated directly as total sympathetic activity recording with neurography [17]. Importantly, when chronically elevated, sympathetic activity is associated with impaired vascular health [19] and increased CV risk [20]. Consistent with this, patients with asthma have been shown to have increased arterial stiffness [21,22] and impaired vascular function [23], which are both predictive of CV events [24,25].

It is known that repeated usage of salbutamol leads to increased airway tolerance, resulting in a lower degree of bronchoprotection during bronchoconstrictive challenges [11], likely due to decreased β_2 -adrenoceptor sensitivity and density within the lungs [11]. Similarly, desensitization of β_2 -adrenoceptor found in the CV system are believed to impair the physiological responses to increased sympathetic activity [26], leading to an inability to maintain adequate cardiac function. Importantly, as the vast majority of research examining CV consequences of salbutamol has been conducted in salbutamol-naïve individuals ([16–18,27–31]), it is unknown if chronic salbutamol use and/or having asthma lead to a different or adverse acute systemic vascular response following salbutamol inhalation. The purpose of this study was to evaluate the acute effects of inhaled salbutamol on systemic endothelial function and arterial stiffness in asthmatics and non-asthmatic controls (Phase I). Subsequently, the potential mechanisms as to why asthmatics respond differently to salbutamol than controls were explored (Phase II). Here, the cold-pressor test (CPT) was used to elicit a large non-specific increase in sympathetic outflow to determine if the systemic vascular response to a given sympathetic outflow is altered between asthmatics and non-asthmatics.

2. Methods

2.1. Ethics, consent and permissions

This study was approved by the University of Alberta Ethics Board, and all subjects were required to sign informed consent prior to participating.

2.2. Research participants and study overview

Fourteen participants with physician-diagnosed asthma currently using salbutamol for asthma management, and 14 age-, body mass index (BMI)-, and sex-matched healthy controls without asthma or prior salbutamol exposure, were recruited for Phase I of the study. All participants were free from known underlying CV disease or respiratory disease other than asthma. Of the 14 individuals with asthma, six were prescribed controller medications containing a combination of inhaled corticosteroids (ICS) and long acting β_2 -agonists (LABA). The participants were instructed to withhold asthma controller medications for a minimum of 48 h and short-acting β_2 -agonists for 8 h prior to each test day. All participants were requested to withhold food, caffeine, and exercise for 8 h, prior to each test. Participants reported to the laboratory in the morning of two separate days, 48 h apart. The participants were instructed to rest for 10 min in the supine position in a dimly lit and temperature-controlled room. Baseline measures (endothelial function and arterial stiffness) were then recorded, followed by either 1) 400 μ g inhaled salbutamol (Ventolin® inhalation aerosol, Glaxo-SmithKline, Mississauga, Canada) or 2) placebo (placebo inhalation aerosol, Glaxo-SmithKline, Mississauga, Canada) administered orally over four inhalations using a space chamber (ProChamber, Respironics, Parsippany, USA), with each inhalation being held for 10-s (Ventolin® HFA Patient Information, Glaxo-SmithKline, Mississauga, Canada). Previous research from our laboratory found that 400 μ g of inhaled

salbutamol was a large enough dose to elicit detectable changes in arterial stiffness and SNA [17]. Unpublished data from this work suggested that 200 μ g of inhaled salbutamol did not affect SNA, and therefore 400 μ g salbutamol was chosen for the current study. Follow-up measures were recorded 15 min after the last inhalation. The order of the two days was randomized, and the participant was blinded to drug administration. As the heart rate response to salbutamol is prominent, it was not possible for the investigators performing the experiments to be blinded to the drug condition. Subsequent vascular analysis was performed with the investigator blinded to the order of drug administration, and due to the automated nature of the vascular data analysis software, the risk of introducing bias due to lack of blinding was low.

The CPT (Phase II) was completed in a group of 10 asthmatics and 10 non-asthmatic controls (four controls and four asthmatics overlapped as participants from Phase I), using the same inclusion and exclusion criteria, and pre-test instructions as for Phase I. Among the participation with asthma in Phase II, three individuals were prescribed ICS + LABA combination medications, three ICS only, and one participant was prescribed a montelukast controller medication. Baseline measures (brachial and carotid flow and conductance, peripheral and central arterial stiffness, heart rate, blood pressure) were then collected in the supine position over a period of 6 min, followed by the participant lowering their left hand into either 1) body-temperature water (BT) or 2) ice water (IW) for an additional 6 min while measurements were recorded. The order of the testing days was randomized. As FMD cannot be recorded continuously, it was not selected as an outcome in Phase II of the study.

For characteristic purposes, on a separate day, all participants underwent standard spirometry testing according to current guidelines [32].

2.3. Measurements - phase I

2.3.1. Hemodynamic evaluation

Brachial blood pressure, heart rate (BioAmp; ADInstruments, Australia) and finger beat-to-beat blood pressure (Finometer Midi, Amsterdam, Netherlands) were recorded, with heart rate and beat-to-beat blood pressure integrated via LabChart 8.0 (PowerLab 16/30; ADInstruments, Australia).

2.3.2. Arterial stiffness

Peripheral pulse wave velocity (pPWV) between the carotid and radial arteries was assessed as previously described [33]. Briefly, a minimum of 15 consecutive beats were recorded simultaneously at each measuring site using applanation tonometry (Micro-Tip Catheter Transducer model SPT-301, Millar Instruments, Inc., Houston, USA) whereby the distance between the sites on the arteries was measured. The time difference (Δ -time) between pulse waves was manually analyzed offline between the foot of the upslope of each pulse wave using LabChart 8.0 (PowerLabs, ADInstruments). PWV was then calculated as $distance/\Delta$ -time (m/s) and used for evaluation of arterial stiffness [34]. An increase in PWV of 1 m/s corresponds to a 14% increase in risk of CV events [25].

2.3.3. Endothelial function

Baseline diameter and blood flow of the brachial artery measured at the upper right arm was established over 1 min using Doppler ultrasonography (8L-RS 4.0–13.0 MHz probe, Vivid q, GE Healthcare, Mississauga, ON). Following 5 min of occlusion of the lower limb distal to the measuring site, the same section of the brachial artery was imaged for an additional 3 min for evaluation of changes in diameter and blood flow (Medical Imaging Applications, LLC, Coralville, IA, USA; EchoPAC PC software, version 110.x.x, GE Healthcare, Horten, Norway). Shear stress following reactive hyperemia (SSRH) was calculated as $8 \times mean\ velocity\ until\ peak\ FMD/(baseline\ diameter/10)$

(dynes/cm²) until peak dilation [35]. Flow-mediated dilation (FMD, % baseline) was assessed as $\Delta\text{diameter}/\text{baseline diameter} \times 100$ and normalized for SSRH [35]. For each 1% reduction in FMD, cardiovascular disease risk is believed to increase up to 9% [24].

2.4. Measurements - phase II

2.4.1. Hemodynamic measurements

Beat-to-beat blood pressure (Finometer Midi), heart rate (one-lead ECG, BioAmp), and brachial and carotid arterial blood flow (Vivid q, GE Healthcare) data were obtained throughout the 6 min of baseline and the following 6 min of either BT or CPT, and recorded in LabChart 8.0 (PowerLab, ADInstruments). Data recorded at baseline and at 2 min of hand submersion (BT and IW) were used for analysis.

2.4.2. Arterial stiffness

In addition to pPWV, central (cPWV) was assessed as the PWV between the carotid – femoral arteries, using the same approach as described above for pPWV. An average of the first minute at baseline, and a 15 s average at 2 min of hand submersion during the BT and IW were used to evaluate pPWV and cPWV response.

2.4.3. Blood flow and vascular conductance

Carotid and brachial arterial blood flow were evaluated by Doppler ultrasound (8L-RS 4.0–13.0 MHz probe, Vivid q, GE Healthcare; and EchoPAC PC software, version 110.x.x, GE Healthcare) and averaged over 60 s at baseline, and a 15 s average at 2 min of hand submersion for both BT and IW. Vascular conductance was calculated as $\text{blood flow}/\text{mean arterial pressure}$ (ml/min/mmHg).

2.5. Statistical analysis

All statistical analyses were performed using SPSS Statistical software v. 24.0.0.0 (IBM Corp™, Armonk, USA) and graphs generated with SigmaPlot (version 13.0, Systat Software, Inc., San Jose, USA). For all inferential analyses, the probability of Type I error was set at 0.05. Baseline characteristics and pulmonary function for controls and asthmatics were compared separately for Phase I and Phase II using Student's t-test and chi-square tests. Vascular responses to salbutamol and placebo for Phase I were compared between groups before and after drug administration using a three-way repeated measure ANOVA. Hemodynamic, brachial, and carotid responses during Phase II to the IW or BT between control and asthma were evaluated at baseline and at 2 min into BT or IW using a three-way repeated measure ANOVA.

3. Results

3.1. Subject characteristics

As outlined in Table 1, the controls and asthmatics were well matched on baseline characteristics, including sex, age, and BMI. Baseline spirometry values did not differ between controls and asthmatics in Phase I. In Phase II, there were no differences in FEV₁ or FVC (% predicted) without bronchodilator between groups; however, the asthmatics in Phase II had a slightly lower FEV₁/FVC-ratio than controls.

3.2. Phase I

3.2.1. Hemodynamic responses to salbutamol

Following salbutamol inhalation, heart rate (HR) increased by 1.1 ± 6.4 and 0.9 ± 5.2 beats per minute compared to baseline in controls and asthmatics, respectively ($p = 0.01$), but no differences were observed between groups and no increase in HR was seen with placebo. Blood pressure remained stable in both groups following both placebo and salbutamol (see Table 2).

3.2.2. Vascular responses to salbutamol

As displayed in Fig. 1, the endothelial function responses following salbutamol compared to placebo were different between asthmatics and controls; while no overall differences were seen in controls with the two drug conditions (placebo: pre 5.5 ± 1.2 , post $6.0 \pm 1.3\%$; salbutamol: pre 7.0 ± 1.2 , post $7.5 \pm 1.2\%$), the asthmatics demonstrated an impaired FMD response following salbutamol compared to placebo (placebo: pre 6.1 ± 1.1 , post $8.4 \pm 1.1\%$; salbutamol: pre 6.1 ± 1.2 to post $3.1 \pm 1.1\%$, $p = 0.042$). Similarly, the responses in arterial stiffness following placebo and salbutamol were different in asthmatics and controls; in asthma, salbutamol resulted in a larger increase in PWV than did placebo (placebo: 8.3 ± 1.0 to 8.5 ± 1.2 m/s; salbutamol: 8.0 ± 0.8 to 8.7 ± 1.1 m/s), but neither placebo nor salbutamol affected PWV in controls (placebo: 7.5 ± 0.8 to 7.5 ± 0.9 m/s; salbutamol: 7.7 ± 0.8 to 7.6 ± 1.1 m/s, $p = 0.027$ for three-way interaction).

3.3. Phase II

3.3.1. Hemodynamic responses to the CPT

During phase II of testing, both HR and mean arterial pressure (MAP) increased over the first 2 min in a similar pattern in both asthmatics and controls, as indicated by statistically significant interaction ($p < 0.005$ for both) between time-point of measurement (baseline vs 2 min) and type of water condition (BT vs IW) (Fig. 2); however, there were no between group differences in neither HR ($p = 0.374$) nor MAP ($p = 0.848$) with the different water conditions.

3.3.2. Brachial, carotid and PWV responses to the CPT

Brachial blood flow remained stable during both BT and IW hand submersion in both groups ($p = 0.618$, Fig. 2) but carotid blood flow significantly increased during the IW condition in a similar manner between asthma and control ($p = 0.006$ for time-point*water interaction, Fig. 2). While brachial conductance was lower during the IW hand submersion than baseline ($p = 0.034$), carotid conductance remained stable; however, for both parameters there were no differences in responses between asthmatics and controls. Peripheral arterial stiffness increased from baseline to the 2 min mark regardless of disease or water condition ($p = 0.026$). No difference in central arterial stiffness was seen between water conditions, time-point of measurement, or disease conditions ($p = 0.557$, Fig. 3).

4. Discussion

This is the first study to demonstrate that the inhaled β_2 -agonist salbutamol adversely impacts systemic vascular function in asthmatics, as demonstrated by a reduction in vascular function and an increase in arterial stiffness. In the second phase of this study, the systemic vascular responses to a large non-specific sympathetic stimulus, delivered via the CPT, were evaluated in asthmatics and controls. As no between-group differences in the vascular responses to the CPT were observed, these results suggest that asthmatics have a similar vascular response to a given sympathetic outflow (i.e. similar neurovascular transduction), and that the increased PWV with salbutamol in these patients is likely explained by a greater sympathetic response to salbutamol.

CV disease in asthma has previously been linked to β_2 -agonist usage [5,12]. However, studies have typically used health administrative data to demonstrate an association between β_2 -agonist use and CV events, making it difficult to separate medication usage from other risk factors associated with an asthma exacerbation. For example, the use of β_2 -agonists has also been associated with increased risk of asthma death [36], and since β_2 -agonist usage usually increases as asthma control decreases [13], the higher asthma mortality is likely due to poor asthma control resulting in higher β_2 -agonist usage, consequently leading to further airway tolerance and reduced asthma control. The results from

Table 1Subject characteristics. Values are expressed as mean $\pm$ standard deviation.

	Phase I		Phase II	
	Control	Asthma	Control	Asthma
Sample size (male/female)	14 (7/7)	14 (7/7)	10 (4/6)	10 (5/5)
Age (years)	22.8 $\pm$ 3.0	23.1 $\pm$ 2.8	23.3 $\pm$ 3.0	25.9 $\pm$ 4.4
Height (m)	1.7 $\pm$ 0.1	1.7 $\pm$ 0.1	1.7 $\pm$ 0.1	1.7 $\pm$ 0.1
Weight (kg)	73.0 $\pm$ 13.0	73.8 $\pm$ 15.1	73.3 $\pm$ 15.7	80.4 $\pm$ 19.4
BMI (kg/m ²)	24.1 $\pm$ 3.0	24.3 $\pm$ 3.1	24.3 $\pm$ 3.7	27.5 $\pm$ 5.3
Allergies (%)	50.0	78.6	20.0	80.0*
FEV ₁ (% predicted)	102.1 $\pm$ 17.1	96.7 $\pm$ 12.8	104.7 $\pm$ 0.8	96.9 $\pm$ 14.2
FVC (% predicted)	102.6 $\pm$ 13.5	105.4 $\pm$ 15.0	106.2 $\pm$ 16.3	108.0 $\pm$ 10.7
FEV ₁ /FVC	83.0 $\pm$ 6.0	77.6 $\pm$ 10.1	82.7 $\pm$ 4.4	75.4 $\pm$ 7.9*
<i>Short-acting β-agonist usage</i>				
Duration (years)	0.0 $\pm$ 0.0	13.1 $\pm$ 6.8	0.0 $\pm$ 0.0	10.2 $\pm$ 5.3
Intensity (puffs/week)	0.0 $\pm$ 0.0	2.8 $\pm$ 2.4	0.0 $\pm$ 0.0	2.1 $\pm$ 1.2

BMI: body mass index; FEV₁: forced expired volume in 1 s; FVC: forced vital capacity. *p < 0.05 between asthma and control.

the current study demonstrate adverse changes in both arterial stiffness and endothelial function acutely following salbutamol administration in asthma, independent of changes in lung function. Based on previous work, the increase in arterial stiffness and impairment in endothelial function seen with salbutamol would be associated with an acute increase in CV risk of between 10 and 25% [24,25]. Importantly, the increased CV risk of salbutamol during an actual asthma exacerbation may be greater, being that the medication would be used during a period of heightened inflammation [8], with potential hypoxemia and hypercapnia.

Numerous studies have examined the vascular responses to both intravenous [27] and inhaled [16–18] β_2 -agonists, and the results typically depend on dosage and method of administration. Common responses to β_2 -agonists include increased HR [17,18], reduced vascular resistance [16, 18], but no changes in BP ([16–18,29]). In a previous study from our laboratory, participants with no previous salbutamol exposure demonstrated a 23% increase in total muscle SNA following salbutamol inhalation without concurrent changes in blood pressure [17]. These results indicate that salbutamol administration acutely increases sympathetic drive independently of baroreflex feedback. Chronically increased sympathetic outflow can affect vascular remodelling and tone [19]; therefore, regular use of salbutamol may contribute to the chronically elevated arterial stiffness [7,22,37] and reduced endothelial function [6] seen in asthmatics.

With repeated β_2 -agonist exposure, the density and sensitivity of the β_2 -adrenoceptor in the airways decrease [11,38]. In heart disease, chronic sympathetic activation leads to a desensitization of the β_2 -adrenoceptor located in the CV system [39] but is it unclear if chronic inhaled β_2 -agonist use leads to desensitization of the β_2 -receptors in the CV system. Thus, it is plausible that modifications in receptor sensitivity

and density alters neurovascular transduction in asthma. To further investigate the mechanism(s) for the altered vascular responses to salbutamol in asthmatics in Phase I of the current study, a non-specific increase in SNA, elicited by the CPT, was used in Phase II to examine whether there was a divergent vascular response between asthmatics and controls. The CPT typically increases muscle SNA 30–35% in health [40] and disease [41,42], with the peak response usually seen within the first 2 min of hand submersion [43]. Contrary to our hypothesis, there were no between-group differences in the vascular responses to the increase in sympathetic output, suggesting asthmatics maintain normal neurovascular transduction to a given sympathetic stimulus. Thus, the increases in arterial stiffness and reduced endothelial function observed following β_2 -agonist inhalation in asthmatics likely stems from differences upstream from the β_2 -adrenoceptor-smooth muscle coupling, such as increased sympathoexcitation in response to salbutamol inhalation.

4.1. Limitations

While the muscle SNA responses to the CPT have been shown to be similar in healthy individuals and in other diseased conditions [41,42], it is unknown if SNA increases in a comparable manner in asthma. Changes in heart rate variability (HRV) correlate well with muscle SNA during stable conditions in healthy people, but not during autonomic challenge tests such as the CPT [40], and thus HRV was not used as a surrogate marker of SNA in the present study. Further research is encouraged to directly measure SNA in asthma both at rest and in response to salbutamol to further understand the autonomic and CV responses to salbutamol in asthma.

As the first study to-date looking at the CV responses to a

Table 2Hemodynamic responses to placebo and salbutamol among controls and asthmatics during Phase I. Values are expressed as mean $\pm$ standard deviation.

	Control Participants				Participants with Asthma			
	Baseline 1	Placebo	Baseline 2	Salbutamol	Baseline 1	Placebo	Baseline 2	Salbutamol
HR (bpm)	60.2 $\pm$ 6.6	56.3 $\pm$ 6.8	61.7 $\pm$ 8.5	62.8 $\pm$ 9.7*	59.5 $\pm$ 8.8	56.4 $\pm$ 10.4	59.4 $\pm$ 8.8	60.3 $\pm$ 9.4*
SBP (mmHg)	106.2 $\pm$ 8.4	104.2 $\pm$ 9.1	106.8 $\pm$ 11.3	109.9 $\pm$ 11.6	108.0 $\pm$ 12.2	108.9 $\pm$ 11.0	106.8 $\pm$ 13.1	108.7 $\pm$ 12.2
DBP (mmHg)	64.6 $\pm$ 8.0	63.3 $\pm$ 6.3	64.8 $\pm$ 6.7	64.5 $\pm$ 6.4	67.0 $\pm$ 9.5	69.3 $\pm$ 6.3	67.0 $\pm$ 10.1	67.7 $\pm$ 7.2
MAP (mmHg)	78.4 $\pm$ 7.6	76.9 $\pm$ 6.6	78.8 $\pm$ 6.8	79.6 $\pm$ 7.0	80.7 $\pm$ 9.8	82.5 $\pm$ 7.3	80.2 $\pm$ 10.7	81.3 $\pm$ 8.7
PP (mmHg)	41.6 $\pm$ 6.5	40.9 $\pm$ 6.7	42.0 $\pm$ 10.9	45.3 $\pm$ 10.4	41.0 $\pm$ 8.0	39.7 $\pm$ 8.0	39.8 $\pm$ 6.9	41.0 $\pm$ 6.4

HR: heart rate; bpm: beats per minute; SBP: systolic blood pressure; mmHg: millimeter mercury; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure. *p < 0.05 for drug*time interaction.

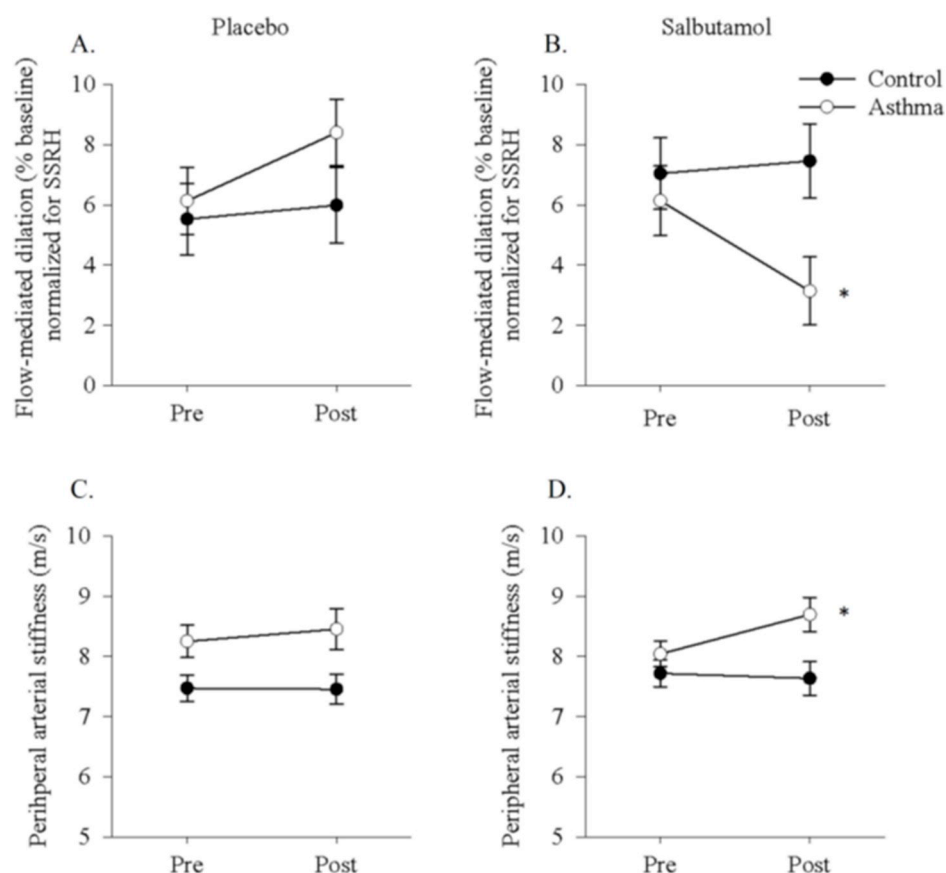


Fig. 1. Vascular responses to placebo and salbutamol in control and asthma. SSRH: shear stress following reactive hyperemia. Graphs display mean $\pm$ SEM. * $p < 0.05$ for interaction between groups and drug conditions.

therapeutic dose of inhaled β_2 -agonists among people with asthma who are chronically exposed to β_2 -agonists, this study would be consistent with epidemiologic data reporting that repeated and/or chronic exposure to salbutamol may be detrimental for CV health [44]. However, while the current study evaluated the acute systemic responses to salbutamol among a clinically representable sample of asthmatics, the medication history was variable. Stewart et al. [10] found that the bronchoprotective effect of salbutamol declines after three doses of 200 μ g taken over two days, and a significant reduction could be seen after only seven doses over four days. Based on this work, the average salbutamol usage of 2.8 and 2.1 doses of 100 μ g/week used in Phase I and Phase II, respectively, was not likely large enough to elicit significant airway tolerance. Furthermore, six of the participants in Phase I, and three of the participants in Phase II were prescribed controller medications containing LABA, a drug also known to cause changes in the sensitivity of the β_2 -adrenoceptors [9]. However, the current study was not designed to evaluate the impact of length and/or intensity of salbutamol or LABA usage, and further research is needed to establish the CV impact of chronic short- and long-acting β_2 -agonists exposure.

Other prescribed medications for asthma, including ICS and montelukasts, may also affect the long-term vascular health among people with asthma; even though all participants were asked to withhold medications prior to each test day, this study did not control for the long-term use of other asthma medications. It is thus possible that history of medication use may have influenced the outcomes in the current study.

Factors that have been associated with asthma previously, such as lower levels of physical activity [45,46] and systemic inflammation [47,48], are known to influence endothelial function and arterial stiffness in health and disease [49–52]. As CV disease development is a

dynamic process occurring over time, it can be speculated that those with long-standing asthma may exhibit lower baseline endothelial function, higher baseline arterial stiffness, and an altered vascular responsiveness to salbutamol as compared to people with newly-onset asthma. Further research is encouraged to assess the relationship between asthma duration and vascular reactivity. Additionally, the inclusion criteria for asthmatics in the current study was physician-diagnosed asthma with a history of salbutamol usage. While the emphasis was on previous salbutamol usage when recruiting asthmatics, it is possible that some of the individuals in the asthma group may not demonstrate confirmed asthma according to current guidelines [13,53].

4.2. Conclusion

Patients with asthma who use salbutamol chronically were found to have adverse systemic vascular responses to inhaled salbutamol, as indicated by reduced vascular function and increased arterial stiffness. The differences in vascular responses to salbutamol between asthmatics and non-asthmatics were not explained by differences in the vascular responses to increased sympathetic output, suggesting the reason asthmatics respond differently to inhaled salbutamol is likely due to greater sympathoexcitation in response to salbutamol, rather than altered neurovascular transduction. The adverse vascular responses to salbutamol among asthmatics may contribute to the increased risk of CV diseases such as coronary heart disease, cerebrovascular disease, and heart failure seen in asthma. These findings would suggest that clinicians should be cautious of significant β_2 -agonist use by their patients.

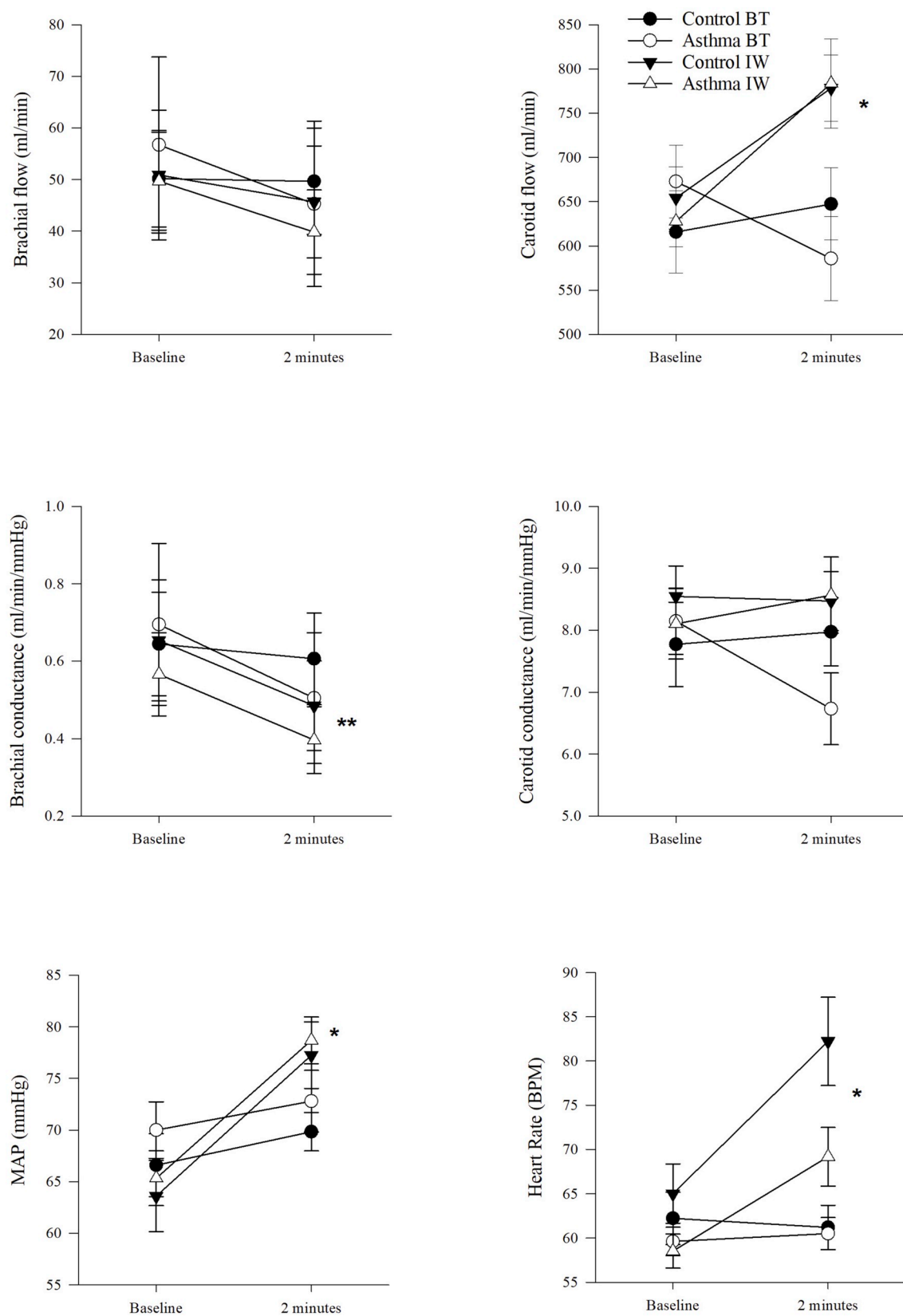


Fig. 2. Hemodynamic responses during body temperature (BT) hand immersion and ice water (IW) among asthmatics and controls. Data displayed as mean $\pm$ SEM. * $p < 0.05$ for time point-water condition interaction, ** $p < 0.05$ for main effect for water condition.

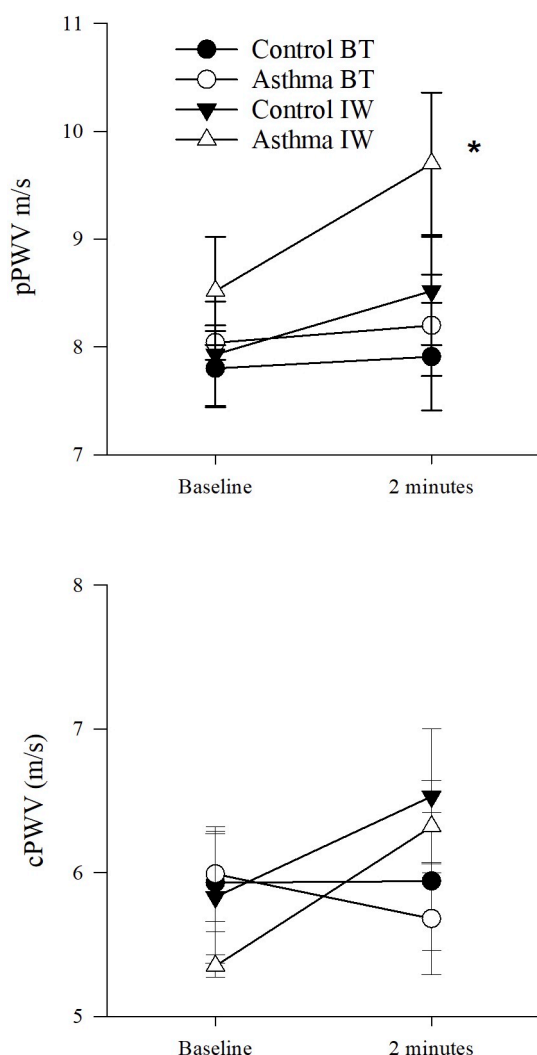


Fig. 3. Responses in arterial stiffness during body temperature (BT) hand immersion and ice water (IW) in asthma and control. Data displayed as mean $\pm$ SEM. pPWV: peripheral pulse wave velocity, cPWV: central pulse wave velocity. * $p < 0.05$ for main time effect.

Acknowledgements

Not applicable.

List of abbreviations

CV	Cardiovascular
β_2	Beta-2
CPT	Cold-pressor test
BMI	Body mass index
BT	Body temperature
IW	Ice water
PWV	pulse wave velocity
FMD	Flow mediated dilation
SSRH	Shear stress following reactive hyperemia
FEV1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
HR	Heart rate
MAP	Mean arterial pressure
SNA	Sympathetic nerve activity
HRV	Heart rate variability

Declarations

Ethics approval and consent to participate: This study was approved by the University of Alberta Ethics Board, and all subjects were required to sign informed consent prior to participating.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare that they have no competing or conflicting interests.

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Authors' contributions

LEM and MKS created the project proposal and research design; LEM, KK, BWB, ARB, DGE, SLH and RBSJ performed the data acquisition and analysis; LEM, KK, BWB, and MKS interpreted the data; LEM drafted the manuscript; all authors edited and approved the final version of the manuscript; all author agree to be accountable for the work completed.

References

- [1] C. Iribarren, I.V. Tolstykh, M.K. Miller, E. Sobel, M.D. Eisner, Adult asthma and risk of coronary heart disease, cerebrovascular disease, and heart failure: a prospective study of 2 matched cohorts, *Am. J. Epidemiol.* 176 (11) (2012) 1014–1024.
- [2] K. Toren, N.B. Lindholm, Do patients with severe asthma run an increased risk from ischaemic heart disease? *Int. J. Epidemiol.* 25 (3) (1996) 617–620.
- [3] S.L. Appleton, R.E. Ruffin, D.H. Wilson, A.W. Taylor, R.J. Adams, Asthma is associated with cardiovascular disease in a representative population sample, *Obes. Res. Clin. Pract.* 2 (2) (2008) 71–142.
- [4] D.W. Bang, C.I. Wi, E.N. Kim, J. Hagan, V. Roger, S. Manemann, et al., Asthma status and risk of incident myocardial infarction: a population-based case-control study, *J. Allergy Clin. Immunol. In Pract.* 4 (5) (2016) 917–923.
- [5] S.L. Appleton, R.E. Ruffin, D.H. Wilson, A.W. Taylor, R.J. Adams, North West Adelaide Cohort Health Study T, Cardiovascular disease risk associated with asthma and respiratory morbidity might be mediated by short-acting beta2-agonists, *J. Allergy Clin. Immunol.* 123 (1) (2009) 124–130 e1.
- [6] P. Yildiz, H. Oflaz, N. Cine, H. Gencallac, N. Erginel-Ünalutuna, A. Yildiz, et al., Endothelial dysfunction in patients with asthma: the role of polymorphisms of ACE and endothelial NOS genes, *J. Asthma* 41 (2) (2004) 159–166.
- [7] Z. Weiler, Y. Zeldin, E. Magen, D. Zamir, M.I. Kidon, Pulmonary function correlates with arterial stiffness in asthmatic patients, *Respir. Med.* 104 (2) (2010) 197–203.
- [8] J.C. Mak, S.P. Ho, A.S. Ho, B.K. Law, A.H. Cheung, J.C. Ho, et al., Sustained elevation of systemic oxidative stress and inflammation in exacerbation and remission of asthma, *ISRN Allergy* 2013 (2013) 561831.
- [9] J. Elers, U. Strandbygaard, L. Pedersen, V. Backer, Daily use of salmeterol causes tolerance to bronchodilation with terbutaline in asthmatic subjects, *Open Respir. Med. J.* 4 (2010) 48–50.
- [10] S.L. Stewart, A.L. Martin, B.E. Davis, D.W. Cockcroft, Salbutamol tolerance to bronchoprotection: course of onset, *Ann. Allergy Asthma Immunol.* 109 (6) (2012) 454–457.
- [11] S.R. Salpeter, T.M. Ormiston, E.E. Salpeter, Meta-analysis: respiratory tolerance to regular beta2-agonist use in patients with asthma, *Ann. Intern. Med.* 140 (10) (2004) 802–813.
- [12] D.H. Au, R.N. Lemaitre, J.R. Curtis, N.L. Smith, B.M. Psaty, The risk of myocardial infarction associated with inhaled beta-adrenoceptor agonists, *Am. J. Respir. Crit. Care Med.* 161 (3 Pt 1) (2000) 827–830.
- [13] E.D. Bateman, S.S. Hurd, P.J. Barnes, J. Bousquet, J.M. Drazen, M. FitzGerald, et al., Global strategy for asthma management and prevention: GINA executive summary, *Eur. Respir. J.* 31 (1) (2008) 143–178.
- [14] O.E. Brodde, S. Schuler, R. Kretsch, M. Brinkmann, H.G. Borst, R. Hetzer, et al.,

- Regional distribution of beta-adrenoceptors in the human heart: coexistence of functional beta 1- and beta 2-adrenoceptors in both atria and ventricles in severe congestive cardiomyopathy, *J. Cardiovasc. Pharmacol.* 8 (6) (1986) 1235–1242.
- [15] M.R. Bristow, R.E. Hersherberger, J.D. Port, W. Minobe, R. Rasmussen, Beta 1- and beta 2-adrenergic receptor-mediated adenylate cyclase stimulation in nonfailing and failing human ventricular myocardium, *Mol. Pharmacol.* 35 (3) (1989) 295–303.
 - [16] L. Cekici, A. Valipour, R. Kohansal, O.C. Burghuber, Short-term effects of inhaled salbutamol on autonomic cardiovascular control in healthy subjects: a placebo-controlled study, *Br. J. Clin. Pharmacol.* 67 (4) (2009) 394–402.
 - [17] H. Edgell, L.E. Moore, C. Chung, B.W. Byers, M.K. Stickland, Short-term cardiovascular and autonomic effects of inhaled salbutamol, *Respir. Physiol. Neurobiol.* 231 (2016) 14–20.
 - [18] E.M. Snyder, E.C. Wong, W.T. Foxx-Lupo, C.M. Wheatley, N.A. Cassuto, A.E. Patanwala, Effects of an inhaled beta2-agonist on cardiovascular function and sympathetic activity in healthy subjects, *Pharmacotherapy* 31 (8) (2011) 748–756.
 - [19] F.A. Dinanno, P.P. Jones, D.R. Seals, H. Tanaka, Age-associated arterial wall thickening is related to elevations in sympathetic activity in healthy humans, *Am. J. Physiol. Heart Circ. Physiol.* 278 (4) (2000) H1205–H1210.
 - [20] H.R. Middlekauff, A.L. Mark, The treatment of heart failure: the role of neurohumoral activation, *Intern. Med.* 37 (2) (1998) 112–122.
 - [21] L.E. Moore, M. Bhutani, S.R. Petersen, M.S. McMurtry, B.W. Byers, V. Tedjasaputra, et al., Physical activity, fitness, and vascular health in patients with asthma, *J. Allergy Clin. Immunol.* 136 (3) (2015) 809–811 e3.
 - [22] W.X. Sun, D. Jin, Y. Li, R.T. Wang, Increased arterial stiffness in stable and severe asthma, *Respir. Med.* 108 (1) (2014) 57–62.
 - [23] P. Yildiz, H. Oflaz, N. Cine, H. Genchallac, N. Erginel-Unaltuna, A. Yildiz, et al., Endothelial dysfunction in patients with asthma: the role of polymorphisms of ACE and endothelial NOS genes, *J. Asthma* 41 (2) (2004) 159–166.
 - [24] D.J. Green, H. Jones, D. Thijssen, N.T. Cable, G. Atkinson, Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? *Hypertension* 57 (3) (2011) 363–369.
 - [25] C. Vlachopoulos, K. Aznaouridis, C. Stefanadis, Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis, *J. Am. Coll. Cardiol.* 55 (13) (2010) 1318–1327.
 - [26] R.J. Lefkowitz, G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization, *J. Biol. Chem.* 273 (30) (1998) 18677–18680.
 - [27] S.P. Beloka, C. Janssen, E. Woff, E. Brassine, G. Deboeck, J. Randria, et al., Effects of beta2-adrenergic stimulation on exercise capacity in normal subjects, *Eur. J. Appl. Physiol.* 111 (9) (2011) 2239–2247.
 - [28] J. Crane, C. Burgess, R. Beasley, Cardiovascular and hypokalaemic effects of inhaled salbutamol, fenoterol, and isoprenaline, *Thorax* 44 (2) (1989) 136–140.
 - [29] A.J. Dagnone, J.L. Parlow, Effects of inhaled albuterol and ipratropium bromide on autonomic control of the cardiovascular system, *Chest* 111 (6) (1997) 1514–1518.
 - [30] A.M. Tahvanainen, A.J. Tikkaoski, M.H. Leskinen, K. Nordhausen, M. Kahonen, T. Koobi, et al., Supine and upright haemodynamic effects of sublingual nitroglycerin and inhaled salbutamol: a double-blind, placebo-controlled, randomized study, *J. Hypertens.* 30 (2) (2012) 297–306.
 - [31] A. Tahvanainen, M. Leskinen, J. Koskela, E. Ilveskoski, J. Alanko, M. Kahonen, et al., Non-invasive measurement of the haemodynamic effects of inhaled salbutamol, intravenous L-arginine and sublingual nitroglycerin, *Br. J. Clin. Pharmacol.* 68 (1) (2009) 23–33.
 - [32] M.R. Miller, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, et al., Standardisation of spirometry, *Eur. Respir. J.* 26 (2) (2005) 319–338.
 - [33] L.E. Moore, B.W. Byers, D.P. Fuhr, E. Wong, M. Bhutani, M.K. Stickland, Cardiovascular benefits from standard pulmonary rehabilitation are related to baseline exercise tolerance levels in chronic obstructive pulmonary disease, *Respir. Med.* 132 (Supplement C) (2017) 56–61.
 - [34] S. Laurent, J. Cockcroft, L. Van Bortel, P. Boutouyrie, C. Giannattasio, D. Hayoz, et al., Expert consensus document on arterial stiffness: methodological issues and clinical applications, *Eur. Heart J.* 27 (21) (2006) 2588–2605.
 - [35] R.A. Harris, S.K. Nishiyama, D.W. Wray, R.S. Richardson, Ultrasound assessment of flow-mediated dilation, *Hypertension* 55 (5) (2010) 1075–1085.
 - [36] W.O. Spitzer, S. Suissa, P. Ernst, R.I. Horwitz, B. Habbick, D. Cockcroft, et al., The use of beta-agonists and the risk of death and near death from asthma, *N. Engl. J. Med.* 326 (8) (1992) 501–506.
 - [37] L.E. Moore, M. Bhutani, S.R. Petersen, M.S. McMurtry, B.W. Byers, V. Tedjasaputra, et al., Physical activity, fitness, and vascular health in patients with asthma, *J. Allergy Clin. Immunol.* 136 (3) (2015) 809–811 e3.
 - [38] W. Liang, S. Austin, Q. Hoang, P.H. Fishman, Resistance of the human beta 1-adrenergic receptor to agonist-mediated down-regulation. Role of the C terminus in determining beta-subtype degradation, *J. Biol. Chem.* 278 (41) (2003) 39773–39781.
 - [39] C. de Lucia, A. Eguchi, W.J. Koch, New insights in cardiac beta-adrenergic signaling during heart failure and aging, *Front. Pharmacol.* 9 (2018) 904.
 - [40] L.D. DeBeck, S.R. Petersen, K.E. Jones, M.K. Stickland, Heart rate variability and muscle sympathetic nerve activity response to acute stress: the effect of breathing, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299 (1) (2010) R80–R91.
 - [41] K. Narkiewicz, C.A. Pesek, P.J. van de Borne, M. Kato, V.K. Somers, Enhanced sympathetic and ventilatory responses to central chemoreflex activation in heart failure, *Circulation* 100 (3) (1999) 262–267.
 - [42] V.A. Imadojemu, Z. Mawji, A. Kunselman, K.S. Gray, C.S. Hogeman, U.A. Leuenberger, Sympathetic chemoreflex responses in obstructive sleep apnea and effects of continuous positive airway pressure therapy, *Chest* 131 (5) (2007) 1406–1413.
 - [43] R.G. Victor, W.N. Leimbach Jr., D.R. Seals, B.G. Wallin, A.L. Mark, Effects of the cold pressor test on muscle sympathetic nerve activity in humans, *Hypertension* 9 (5) (1987) 429–436.
 - [44] S.R. Salpeter, T.M. Ormiston, E.E. Salpeter, Cardiovascular effects of beta-agonists in patients with asthma and COPD: a meta-analysis, *Chest* 125 (6) (2004) 2309–2321.
 - [45] E.S. Ford, G.W. Heath, D.M. Mannino, S.C. Redd, Leisure-time physical activity patterns among US adults with asthma, *Chest* 124 (2) (2003) 432–437.
 - [46] M. Teramoto, S. Moonie, Physical activity participation among adult Nevadans with self-reported asthma, *J. Asthma* 48 (5) (2011) 517–522.
 - [47] Z. Zietkowski, M.M. Tomasiak-Lozowska, R. Skiepkio, B. Mroczko, M. Szmitkowski, A. Bodzenta-Lukaszyk, High-sensitivity C-reactive protein in the exhaled breath condensate and serum in stable and unstable asthma, *Respir. Med.* 103 (3) (2009) 379–385.
 - [48] L.G. Wood, K.J. Baines, J. Fu, H.A. Scott, P.G. Gibson, The neutrophilic inflammatory phenotype is associated with systemic inflammation in asthma, *Chest* 142 (1) (2012) 86–93.
 - [49] A.D. Hingorani, J. Cross, R.K. Kharbanda, M.J. Mullen, K. Bhagat, M. Taylor, et al., Acute systemic inflammation impairs endothelium-dependent dilatation in humans, *Circulation* 102 (9) (2000) 994–999.
 - [50] L. Payvand, A. Dyer, D. McPherson, P. Ades, J. Stein, K. Liu, et al., Physical activity during daily life and brachial artery flow-mediated dilation in peripheral arterial disease, *Vasc. Med.* 14 (3) (2009) 193–201.
 - [51] M.K. Stickland, N. Vogan, S.R. Petersen, E.Y. Wong, M. Bhutani, Physical activity and arterial stiffness in chronic obstructive pulmonary disease, *Respir. Physiol. Neurobiol.* (2013).
 - [52] C. Vlachopoulos, I. Dima, K. Aznaouridis, C. Vasiliadou, N. Ioakeimidis, C. Aggeli, et al., Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals, *Circulation* 112 (14) (2005) 2193–2200.
 - [53] M.D. Loughheed, C. Leniere, F.M. Ducharme, C. Lieskai, S.D. Dell, B.H. Rowe, et al., Canadian Thoracic Society 2012 guideline update: diagnosis and management of asthma in preschoolers, children and adults: executive summary, *Can. Respir. J.* 19 (6) (2012) e81–e88.