Journal of Geriatric Cardiology (2016) 13: 202–208

Research Article · **Open Access** ·

Gender and tachycardia: independent modulation of platelet reactivity in patients with atrial fibrillation

Nathan EK Procter[1], Jocasta Ball[2], Doan TM Ngo[1], Jeffrey S Isenberg[3], Elaine M Hylek[4], Yuliy Y Chirkov[1], Simon Stewart[2], John D Horowitz[1]

[1]*Basil Hetzel Institute for Translational Research, Queen Elizabeth Hospital, University of Adelaide, Adelaide, Australia*
[2]*National Health and Medical Research Council (NHMRC), Centre of Research Excellence to Reduce Inequality in Heart Disease, Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, Australia*
[3]*Heart, Lung, Blood and Vascular Medicine Institute, Division of Pulmonary, Allergy and Critical Care Medicine Department, University of Pittsburgh, Pittsburgh, Pennsylvania, USA*
[4]*Department of Medicine, Boston University School of Medicine, Boston, Massachusetts, USA*

Abstract

Background Female patients with atrial fibrillation (AF) experience increased risk of thromboembolism compared to males, an observation that is reflected by its inclusion in the CHA_2DS_2VASc score. New onset AF (often associated with tachycardia) also confers upon patients increased thromboembolic risk. The mechanisms underlying this risk are uncertain, but new onset AF is associated with profound impairment of platelet nitric oxide (NO) signalling. Given that cardiovascular responses to catecholamines are gender-dependent, and that the presence of tachycardia in new onset AF may represent a response to catecholaminergic stimulation, we explored the potential impact of gender and tachycardia on platelet aggregation and NO signalling. **Methods** Interactions were sought in 87 AF patients between the extent of adenosine diphosphate (ADP)-induced platelet aggregation, the anti-aggregatory effects of the NO donor, sodium nitroprusside, gender, and admission heart rate. The potential impact of platelet expression of thioredoxin-interacting protein (Txnip) was also evaluated. **Results** Analysis of covariance confirmed the presence of physiological antagonism between platelet ADP and NO responses [$F(1, 74) = 12.212$, $P < 0.01$], while female sex correlated with impaired NO responses independent of platelet aggregability [$F(2, 74) = 8.313$, $P < 0.01$]. Admission heart rate correlated directly with platelet aggregation ($r = 0.235$, $P < 0.05$), and inversely with NO response ($r = –0.331$, $P < 0.01$). Txnip expression varied neither with gender nor with heart rate. **Conclusions** These results indicate that gender and heart rate are independent determinants of platelet function. Prospective studies of the putative benefit of reversal of tachycardia on restoration of normal platelet function are therefore a priority.

J Geriatr Cardiol 2016; 13: 202–208. doi:10.11909/j.issn.1671-5411.2016.03.005

Keywords: Atrial fibrillation; Gender; Heart rate; Nitric oxide; Platelet aggregation

1 Introduction

Clinical factors associated with increased thromboembolic risk in atrial fibrillation (AF) have been identified epidemiologically and formalised into stroke risk algorithm.[1,2] However, the physiological bases underlying these categories of risk are as yet incompletely understood. In particular, while platelet hyperaggregability has been observed in the context of AF,[3,4] its potential role in determining thromboembolic risk remains unclear, studies have so far failed to establish a correlation between platelet reactivity and clinical scores of thromboembolic risk.

Among factors which have been shown to modulate platelet aggregability are the interactions between ambient pro-aggregatory stimuli (such as plasma catecholamine concentrations),[5] and anti-aggregatory autocoids such as nitric oxide (NO). We have previously shown that the inhibitory response of platelets to NO varies widely in patients with AF,[6] and is particularly impaired in 'new onset' AF. Mechanisms to account for this were not fully explored, although theoretically, sources of impaired response to NO might include increased reactive oxygen species (ROS) generation,[7] and/or oxidation/depletion of the haeme component of soluble guanylate cyclase (sGC) (see Chirkov and Horowitz[8] for review).

Correspondence to: John D Horowitz, MD, University of Adelaide, the Queen Elizabeth Hospital, Cardiology Unit, 28 Woodville Rd, Woodville South, SA 5011, Australia. E-mail: john.horowitz@adelaide.edu.au
Received: August 19, 2015 **Revised:** October 26, 2015
Accepted: December 3, 2015 **Published online:** March 27, 2016

Recent onset AF is often associated with relatively rapid ventricular response,[9] symptomatic of acutely increased sympathetic stimulation. In this context, elevated plasma catecholamine concentrations could contribute to platelet hyper-reactivity through direct pro-aggregatory effects,[5] as well as through increased ROS generation,[10–12] and resultant scavenging of NO. Pro-inflammatory effects of catecholamine release measured in myocardium occur predominantly in ageing females,[13] hence there may in theory be gender-specifc aspects to this phenomenon.

Another possible mechanism accounting for the interaction between heart rate and impaired NO response in new onset AF is the stimulation of inflammation. This could, for example, occur via oxidant-stimulated increased expression of the pro-inflammatory protein, thioredoxin-interacting protein (Txnip, see Chong, *et al*.[14] for review), which appears to suppress NO responses.[15]

We have therefore performed further evaluations of the physiological data from Standard *vs*. Atrial Fibrillation spEcific managemenT studY (SAFETY) in order to delineate potential mechanisms underlying the interactions of tachycardia with impaired platelet NO signalling.

2 Methods

2.1 Patient selection

The investigation was conducted as a single centre mechanistic sub-study of the SAFETY, an investigation of non-pharmacological management strategies in patients hospitalized with AF.[16,17] Patients were considered for inclusion if they were admitted to hospital due to AF. Exclusion criteria for SAFETY were age < 45 years, primary diagnosis of valvular heart disease, scheduled catheter ablation of AF, pre-existing NYHA class III–IV heart failure with a documented left ventricular ejection fraction (LVEF) < 45%, alcohol-induced AF and terminal illness requiring palliative care. Patients receiving P_2Y_{12} receptor antagonists were also excluded from the current sub-study because of potential impact of such agents on capacity to measure platelet response to NO. The study was approved by the institutional Ethics of Human Research Committee. Written informed consent was obtained in all cases.

2.2 Clinical data

All patients underwent standardized clinical assessment and routine biochemical investigation. Additional cardiac investigations were resting ECG (which was used for measures of admission heart rate) and transthoracic echocardiography: LVEF was calculated from biplane images using Simpson's method.[18]

2.3 Platelet aggregometry

Platelet aggregometry was performed using whole blood impedance aggregometry as previously described.[19] Briefly, venous blood was collected under vascular stasis from an antecubital vein using a 21G butterfly with a 20 mL syringe into 10 mL tubes containing 1: 10 volume of acid citrate anticoagulant (2 parts 0.1 mol/L citric acid to 3 parts of 0.1 mol/L trisodium citrate). Aggregation was induced with ADP (2.5 μmol/L), and responses were recorded for electrical impedance (Ω) via a computer interface system (Aggrolink, Chrono-log, Havertown, Pennsylvania, USA). The NO donor sodium nitroprusside (SNP, 10 μmol/L) was used to measure platelet response to NO. Inhibition of aggregation by SNP was evaluated as percentage of maximal aggregation in the absence of sodium nitroprusside.

2.4 Platelet thioredoxin-interacting protein determination

Platelet Txnip content was determined as previously reported.[15] Briefly, EDTA-anticoagulated blood was centrifuged to obtain platelet rich plasma, which was smeared onto untreated slides and fixed using 4% (w/v) paraformaldehyde in PBS, then stored at −70°C until assayed. Slides were blocked using 20% (v/v) goat serum in PBS, followed by Txnip detection using rabbit polyclonal anti-human vitamin D3 upregulated protein 1 (VDUP-1) (Invitrogen, USA), 1% (w/v) BSA in PBS and incubating overnight at 2–4°C. Secondary detection was performed using FITC-conjugated swine anti-rabbit polyclonal IgG (Dako, Denmark), as well as primary detection of platelet CD41 using RPE-conjugated mouse monoclonal anti-human CD41 (Dako, Denmark) in PBS. Fluorescence was developed using 'fluorescent mounting medium' (Dako, Denmark) and images acquired at 400× using an Axio Scope.A1 microscope with apotome and AxioVision 4.8 software (Carl Zeiss, Germany). Images were analysed for densitometric fluorescence using AxioVision LE software. The intra-assay coefficient of variation (CV) was 8.5% and the inter-assay CV was 18.6%.

2.5 Statistical methods

Data were analysed by evaluating potential univariate followed by multivariate correlates of (a) platelet response to NO and (b) platelet response to ADP. Clinical factors evaluated were age, sex, LVEF, duration of AF (with "new onset" AF defined on the basis of *de novo* detection), heart rate on admission ECG and CHA_2DS_2VASc score. Analysis of covariance was utilized to evaluate the ADP: NO response relationship in different patient cohorts. Patient characteristics were compared by non-paired *t*-test, Mann-

Whitney *U* test or χ^2 test as appropriate. All data for normally distributed parameters are expressed as mean ± SE unless otherwise stated. Skewed data are expressed as median and interquartile range (IQR). Data were analysed using the IBM SPSS Statistics 20 and GraphPad Prism 6 software packages.

3 Results

The clinical and pharmacological characteristics of the study cohort have been reported previously.[6] Of the total cohort, 85.1% were in AF at the time of initial ECG recording. A breakdown of clinical parameters assessed across gender can be observed in Table 1.

Females displayed lower incidence of known coronary artery disease (CAD), higher LVEF, relatively preserved renal function, and higher CHA_2DS_2VASc scores when compared with males.[2] Pharmacotherapy did not differ significantly across gender, though a trend towards increased statin use in females was noted (Table 2). In particular, there was no significant difference between gender as regards anti-platelet treatment (e.g., aspirin),[20] or with agents that increase platelet NO response, as previously documented with statins and angiotensin-converting enzyme (ACE) inhibitors.[19,21]

We previously reported that platelet aggregation is increased and NO response decreased in female, compared with male AF patients.[6] Currently, we have evaluated the relationship between these parameters in a gender-specific manner analysis of covariance (ANCOVA, Figure 1), in order to determine whether the differing NO response can be explained by physiological antagonism.[22] While physiological antagonism was present, female sex represented an aggregation-independent basis for impaired platelet NO response ($P < 0.01$).

We have also previously reported an inverse correlation between admission heart rate and platelet response to NO ($r = –0.331$, $P < 0.01$),[6] while extent of ADP-induced platelet aggregation was positively correlated ($r = 0.235$, $P < 0.05$). Evaluation by ANCOVA (Figure 2) indicated that the heart

Table 1. Clinical characteristics of the study population, by gender.

	Male (n = 45)	Female (n = 42)	P
Age, yrs	72 (63, 81)	73 (65, 82)	NS
Age ≥ 75 yrs	20 (44.4%)	19 (45.2%)	NS
Comorbidities			
Congestive heart failure	3 (6.7%)	4 (9.5%)	NS
Hypertension	27 (60.0%)	32 (76.2%)	NS
Diabetes mellitus	13 (28.9%)	8 (19.0%)	NS
Prior stroke/TIA	5 (11.1%)	4 (9.5%)	NS
Known coronary artery disease	20 (44.4%)	9 (21.4%)	< 0.05
Clinical presentation			
Admission heart rate, beats/min	85 (72, 134)	96 (70, 136)	NS
LVEF, %	58 (49, 60)	62 (58, 68)	< 0.01
Plasma creatinine, μmol/L	90 (76, 109)	75 (67, 91)	< 0.01
Plasma CRP, mg/L	27.0 (11.0, 46.0)	14.0 (5.4, 45.0)	NS
CHA_2DS_2VASc Score	3 (1, 4)	4 (2, 5)	< 0.01
New onset AF	11 (24.4%)	11 (26.2%)	NS

Data are presented as *n* (%) or median (IQR). AF: atrial fibrillation; CRP: C-reactive protein; IQR: inter quartile range; LVEF: left ventricular ejection fraction; NS: not significant; TIA: transient ischaemic attack.

Table 2. Pharmacotherapy (at time of blood sampling) applied in the study population: variation according to gender.

Antithrombotic therapy	Male (n = 45)	Female (n = 42)	P
Aspirin	13 (28.9%)	16 (38.1%)	NS
Warfarin	24 (53.3%)	26 (61.9%)	NS
Rate and/or rhythm control therapy			
Anti-arrhythmics	11 (24.4%)	14 (33.3%)	NS
β-receptor antagonists	27 (60.0%)	24 (57.1%)	NS
Digoxin	14 (31.1%)	15 (35.7%)	NS
Calcium channel blockers	9 (20.0%)	13 (31.0%)	NS
ACE inhibitors	18 (40.0%)	13 (31.0%)	NS
Angiotensin receptor blockers	8 (17.8%)	14 (33.3%)	0.095
Other medications			
Statins	20 (44.4%)	27 (64.3%)	0.064

ACE: angiotensin-converting enzyme; NS: not significant.

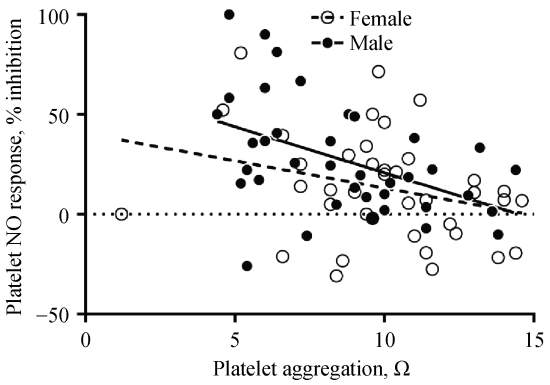

Figure 1. Evaluation of the impact of gender upon the physiological antagonism between pro-aggregatory ADP and anti-aggregatory NO responses in AF patients. ANCOVA demonstrated physiological antagonism: NO response varied inversely with extent of aggregation [$F(1, 74) = 12.212$, $P < 0.01$]. (1) Independent impact of female sex: diminished NO response per unit ADP-induced platelet aggregation [$F(2, 74) = 8.313$, $P < 0.01$]; (2) A non-significant trend [$F(1, 74) = 2.244$, $P = 0.138$] towards diminution of gender differences (as regards platelet NO response) at greater ADP responses. ADP: adenosine diphosphate; AF: atrial fibrillation; ANCOVA: analysis of covariance; NO: nitric oxide.

rate/aggregation relationships did not vary according to duration of AF (similar analysis of the heart rate/NO relationships can be found here).[6]

The potential gender-specificity of the interaction between heart rate and aggregability parameters was also evaluated by ANCOVA (Figure 3). These analyses indicated that for all levels of heart rate, females were hyperaggregable compared to males, and that for all levels of heart rate, females displayed diminished platelet NO response compared to males.

In our previous report,[6] we speculated that plasma 'surges' in catecholamine levels could foster the development of oxidative stress, thus contributing to impaired platelet NO response. Txnip is a proinflammatory protein that interacts reciprocally with NO signalling (see Chong *et al*.[14] for review): we sought to explore whether platelet Txnip expression interacted with new onset AF and/or gender in the context of potential catecholamine-mediated (i.e., tachycardia) oxidative stress (Figure 4). Neither duration of AF nor gender significantly correlated with platelet Txnip expression.

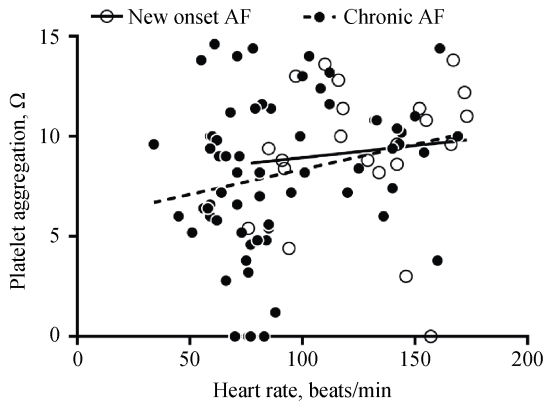

Figure 2. Evaluation of the impact of duration of AF on the interaction between tachycardia and extent of platelet aggregation. ANCOVA confirms that aggregation tends to increase (P = 0.071) with tachycardia. However, the relationship does not vary significantly according to duration of AF [F(1, 83) = 0.192, P = 0.662]. As previously reported,[6] there was a significant (r = 0.235, P < 0.05) relationship overall between heart rate and aggregability. AF: atrial fibrillation; ANCOVA: analysis of covariance.

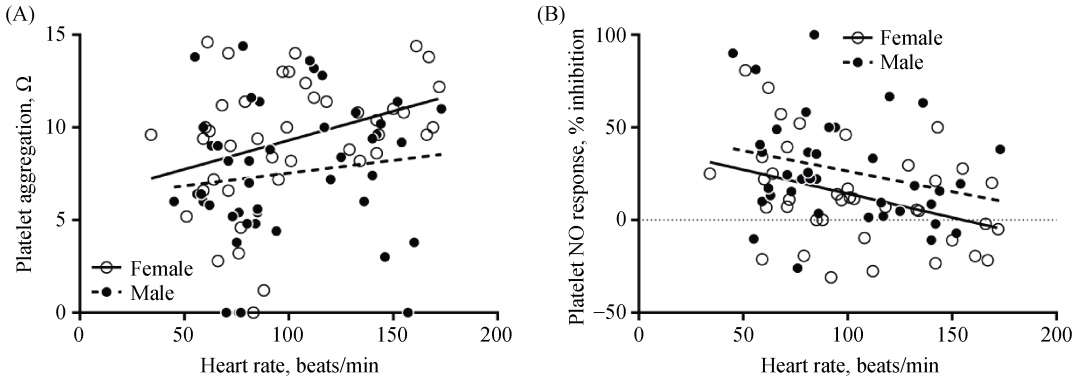

Figure 3. ANCOVA evaluation of the impact of gender on the interaction between tachycardia and extent of platelet aggregation (A) and platelet response to NO (B). While increasing heart rate was associated with both incremental aggregation (P < 0.05) and diminished NO response (P < 0.05), these relationships did not vary significantly according to gender [F(1, 83) = 0.702, P = 0.405; F(1, 73) = 0.049, P = 0.825, respectively]. ANCOVA: analysis of covariance; NO: nitric oxide.

4 Discussion

We have recently demonstrated an association between new onset AF and impaired platelet NO response.[6] The current data demonstrate that the relationship between heart rate and NO response does not vary significantly according to whether AF is of recent onset or chronic. Therefore, the data are strongly consistent with the concept that the tachycardia commonly associated with new onset AF is closely linked with the suppression of platelet NO signaling. One potential mechanism to account for the current observations would be the precipitation of AF in the presence of increased sympathetic discharge,[23] with associated redox stress and NO scavenging.

We evaluated the relationship between heart rate and platelet Txnip content because non-laminar flow may promote expression of this pro-inflammatory protein.[24,25] However, no relationship was found. These data imply that impairment of platelet NO response in the presence of tachycardia is not mediated via increased Txnip expression.

AF in females engenders increased stroke risk when compared to males,[26,27] although the precise mechanisms for this incremental risk remain uncertain. Furthermore, differential stroke incidence in females (compared to males) is independent of anticoagulant therapy.[28,29] The specific thromboembolic risk represented by gender has even been

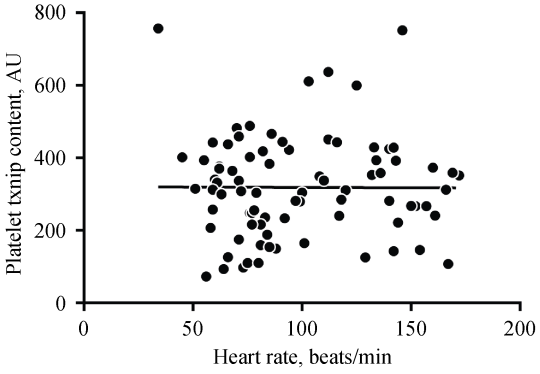

Figure 4. Platelet Txnip content does not vary significantly with heart rate in the entire population evaluation (P = 0.971). ANCOVA (data not shown) revealed no heterogeneity of this relationship according to duration of AF or gender. AF: atrial fibrillation; ANCOVA: analysis of covariance; Txnip: thioredoxin-interacting protein.

incorporated into the CHA_2DS_2VASc stroke risk score, and endorsed by current AHA and ESC guidelines.[2,30,31]

In the current analysis, we postulated that tachycardia might contribute to the impaired NO response, and that differential biochemical response to tachycardia in females might underlie attenuation of NO signaling. Evaluation of heart rate was limited to that on admission ECG: this may not have been completely representative of heart rate around the time of admission, especially in the presence of AF. However, ECG data provided an unbiased if limited sample of patients' heart rates. In any event, the tachycardia: NO response relationship was gender-independent (Figure 3), nor did heart rate vary according to gender (Table 1). The potential explanations for impaired NO responses in females therefore include both physiological antagonism (that is, a consequence of platelet hyperaggregability to ADP),[22] and/or greater susceptibility of ageing females[32–39] to redox stress in response to catecholamine release.[10–12] As demonstrated by the analysis shown in Figure 1, impaired NO responses in females is actually independent of ADP response. In fact, female patients in the current study had a lower prevalence of prior coronary artery disease than males, and would otherwise be expected to exhibit better-preserved NO signaling.[40,41]

There are some limitations to the present study. Of necessity, the sample size of the cohort and the short follow-up period preclude any evaluation and/or conclusions being drawn as regards clinical outcomes. Additionally, determination of heart rate and blood sampling was not always concurrent: the substantial impact of heart rate upon platelet reactivity remained significant despite this. Lastly, ADP was the only agonist used to induce platelet aggregation (as opposed to collagen, for example): this was decided upon on the basis that ADP-induced platelet aggregation is reversible, thus facilitating the evaluation of inhibitory signalling pathways (such as those activated by NO).

In conclusion, while both tachycardia and female gender are associated with impaired NO response in AF patients, they appear to be entirely independent of one another. The potential clinical advantages of reversal of tachycardia in terms of restoration of normal platelet homeostasis have not been adequately explored to date, but should now be evaluated.

Acknowledgements

This investigation was supported by NHMRC Program Grant 519823 and the NHMRC Centre of Research Excellence to Reduce Inequality in Heart Disease. Stewart S, Ball J and Chong CR are supported by the NHMRC. Procter N is supported by the University of Adelaide. Isenberg J is supported by the NIH grants P01 HL103455-01, 1R01HL108954-01, 1R01HL112914-01A1 and 2R01HL089658, and also by the Vascular Medicine Institute, the Institute for Transfusion Medicine and the Hemophilia Center of Western Pennsylvania. Isenberg JS is chair of the Scientific Advisory Boards of Vasculox, Inc. (St. Louis, MO) and radiation Control Technologies, Inc. (RCTI: Garden City, NJ) and holds equity in the same. The other authors report no relationships that could be construed as a conflict of interest.

References

1 Gage BF, Waterman AD, Shannon W, *et al.* Validation of clinical classification schemes for predicting stroke: results from the National Registry of Atrial Fibrillation. *JAMA* 2001; 285: 2864–2870.

2 Lip GY, Nieuwlaat R, Pisters R, *et al.* Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: the Euro Heart Survey on Atrial Fibrillation. *Chest* 2010; 137: 263–272.

3 Ohara K, Inoue H, Nozawa T, *et al.* Accumulation of risk factors enhances the prothrombotic state in atrial fibrillation. *Int J Cardiol* 2008; 126: 316–321.

4 Kamath S, Blann AD, Chin BS, *et al.* A study of platelet activation in atrial fibrillation and the effects of antithrombotic therapy. *Eur Heart J* 2002; 23:1788–1795.

5 Willoughby SR, Chirkova LP, Horowitz JD, *et al.* Multiple agonist induction of aggregation: an approach to examine anti-aggregating effects in vitro. *Platelets* 1996; 7: 329–333.

6 Procter NE, Ball J, Liu S, *et al.* Impaired platelet nitric oxide

response in patients with new onset atrial fibrillation. *Int J Cardiol* 2015; 179: 160–165.
7 Worthley MI, Holmes AS, Willoughby SR, *et al.* The deleterious effects of hyperglycemia on platelet function in diabetic patients with acute coronary syndromes mediation by superoxide production, resolution with intensive insulin administration. *J Am Coll Cardiol* 2007; 49: 304–310.
8 Chirkov YY, Horowitz JD. Impaired tissue responsiveness to organic nitrates and nitric oxide: a new therapeutic frontier? *Pharmacol Ther* 2007; 116: 287–305.
9 Humphries KH, Kerr CR, Connolly SJ, *et al.* New-onset atrial fibrillation: sex differences in presentation, treatment, and outcome. *Circulation* 2001; 103: 2365–2370.
10 Mehta JL, Li D. Epinephrine upregulates superoxide dismutase in human coronary artery endothelial cells. *Free Radic Biol Med* 2001; 30: 148–153.
11 Bleeke T, Zhang H, Madamanchi N, *et al.* Catecholamine-induced vascular wall growth is dependent on generation of reactive oxygen species. *Circ Res* 2004; 94: 37–45.
12 Lu YM, Han F, Shioda N, *et al.* Phenylephrine-induced cardiomyocyte injury is triggered by superoxide generation through uncoupled endothelial nitric-oxide synthase and ameliorated by 3-[2-[4-(3-chloro-2-methylphenyl)-1-piperazinyl] ethyl]-5,6-dimethoxyindazole (DY-9836), a novel calmodulin antagonist. *Mol Pharmacol* 2009; 75: 101–112.
13 Neil CJ, Nguyen TH, Singh K, *et al.* Relation of delayed recovery of myocardial function after takotsubo cardiomyopathy to subsequent quality of life. *Am J Cardiol* 2015; 115: 1085–1089.
14 Chong CR, Chan WP, Nguyen TH, *et al.* Thioredoxin-interacting protein: pathophysiology and emerging pharmacotherapeutics in cardiovascular disease and diabetes. *Cardiovasc Drugs Ther* 2014; 28: 347–360.
15 Sverdlov AL, Chan WP, Procter NE, *et al.* Reciprocal regulation of NO signaling and TXNIP expression in humans: impact of aging and ramipril therapy. *Int J Cardiol* 2013; 168: 4624–4630.
16 Carrington MJ, Ball J, Horowitz JD, *et al.* Navigating the fine line between benefit and risk in chronic atrial fibrillation: rationale and design of the Standard versus Atrial Fibrillation spEcific managemenT studY (SAFETY). *Int J Cardiol* 2013; 166: 359–365.
17 Stewart S, Ball J, Horowitz JD, *et al.* Standard versus atrial fibrillation-specific management strategy (SAFETY) to reduce recurrent admission and prolong survival: pragmatic, multicentre, randomised controlled trial. *Lancet* 2015; 385: 775–784.
18 Lang RM, Bierig M, Devereux RB, *et al.* Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr* 2005; 18: 1440–1463.
19 Willoughby SR, Rajendran S, Chan WP, *et al.* Ramipril sensitizes platelets to nitric oxide: implications for therapy in high-risk patients. *J Am Coll Cardiol* 2012; 60: 887–894.
20 Weiss HJ, Aledort LM, Kochwa S. The effect of salicylates on the hemostatic properties of platelets in man. *J Clin Invest* 1968; 47: 2169–2180.
21 Stepien JM, Prideaux RM, Willoughby SR, *et al.* Pilot study examining the effect of cholesterol lowering on platelet nitric oxide responsiveness and arterial stiffness in subjects with isolated mild hypercholesterolaemia. *Clin Exp Pharmacol Physiol* 2003; 30: 507–512.
22 Henry PJ, Lulich KM, Paterson JW. Experimental testing of Mackay's model for functional antagonism in the isolated costo-uterus of the rat. *Br J Pharmacol* 1985; 86: 131–139.
23 Jayachandran JV, Sih HJ, Winkle W*, et al.* Atrial fibrillation produced by prolonged rapid atrial pacing is associated with heterogeneous changes in atrial sympathetic innervation. *Circulation* 2000; 101: 1185–1191.
24 Yamawaki H, Pan S, Lee RT, *et al.* Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. *J Clin Invest* 2005; 115: 733–738.
25 Wang XQ, Nigro P, World C, *et al.* Thioredoxin interacting protein promotes endothelial cell inflammation in response to disturbed flow by increasing leukocyte adhesion and repressing Kruppel-like factor 2. *Circ Res* 2012; 110: 560–568.
26 Wolf PA, Abbott RD, Kannel WB. Atrial-fibrillation as an independent risk factor for stroke—the Framingham study. *Stroke* 1991; 22: 983–988.
27 Fang MC, Singer DE, Chang Y, *et al.* Gender differences in the risk of ischemic stroke and peripheral embolism in atrial fibrillation: the AnTicoagulation and Risk factors In Atrial fibrillation (ATRIA) study. *Circulation* 2005; 112: 1687–1691.
28 Avgil Tsadok M, Jackevicius CA, Rahme E, *et al.* Sex differences in stroke risk among older patients with recently diagnosed atrial fibrillation. *JAMA* 2012; 307: 1952–1958.
29 Wang TJ, Massaro JM, Levy D*, et al.* A risk score for predicting stroke or death in individuals with new-onset atrial fibrillation in the community: the Framingham Heart Study. *JAMA* 2003; 290: 1049–1056.
30 January CT, Wann LS, Alpert JS, *et al.* 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. *J Am Coll Cardiol* 2014; 64: e1–e76.
31 European Heart Rhythm A, European Association for Cardio-Thoracic S, Camm AJ*, et al.* Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). *Eur Heart J* 2010; 31: 2369–2429.
32 Freedman RR, Sabharwal SC, Desai N. Sex differences in peripheral vascular adrenergic receptors. *Circ Res* 1987; 61: 581–585.
33 Kneale BJ, Chowienczyk PJ, Brett SE, *et al.* Gender differ-

ences in sensitivity to adrenergic agonists of forearm resistance vasculature. *J Am Coll Cardiol* 2000; 36: 1233–1238.
34 Calderone V, Baragatti B, Breschi MC, *et al.* Hormonal influence on the release of endothelial nitric oxide: gender-related dimorphic sensitivity of rat aorta for noradrenaline. *J Pharm Pharmacol* 2002; 54: 523–528.
35 McKee AP, Van Riper DA, Davison CA, *et al.* Gender-dependent modulation of alpha 1-adrenergic responses in rat mesenteric arteries. *Am J Physiol Heart Circ Physiol* 2003; 284: H1737–H1743.
36 Hart EC, Charkoudian N, Wallin BG, *et al.* Sex and ageing differences in resting arterial pressure regulation: the role of the beta-adrenergic receptors. *J Physiol* 2011; 589: 5285–5297.
37 Bohm M, Castellano M, Paul M, *et al.* Cardiac norepinephrine, beta-adrenoceptors, and Gi alpha-proteins in prehypertensive and hypertensive spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 1994; 23: 980-987.
38 Bohm M, Flesch M, Schnabel P. Role of G-proteins in altered beta-adrenergic responsiveness in the failing and hypertrophied myocardium. *Basic Res Cardiol* 1996; 91: 47–51.
39 Jaghoori A, Jakobczak R, Stuklis R, *et al.* Sex-differences in vascular reactivity of internal mammary artery and subcutaneous microvessels. *Heart, Lung & Circulation.* 2012; 21: S8–S9.
40 Chirkov YY, Holmes AS, Chirkova LP, *et al.* Nitrate resistance in platelets from patients with stable angina pectoris. *Circulation* 1999; 100: 129–134.
41 Willoughby SR, Stewart S, Holmes AS, *et al.* Platelet nitric oxide responsiveness: a novel prognostic marker in acute coronary syndromes. *Arterioscler Thromb Vasc Biol* 2005; 25: 2661–2666.