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DOI:
10.1097/HJH.0000000000001794

Document Version
Peer reviewed version

Citation for published version (Harvard):
Hirst, A & Marshall, J 2018, 'Endothelium-dependent and cyclooxygenase-dependent cutaneous vasodilatation is blunted in young men with hypertensive parents', *Journal of Hypertension*, vol. 36, no. 11, pp. 2140-2147. https://doi.org/10.1097/HJH.0000000000001794

Endothelium- and COX-dependent cutaneous vasodilation is blunted in young men with hypertensive parents

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Short title: Cutaneous vasodilation in hypertensive offspring

Word count = 5068

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Abstract

Background. Across ethnicities, offspring of parents with essential hypertension (OH) have higher risk of hypertension than offspring of normotensives (ON). Sympathetic hyperactivity and reduced nitric oxide availability have been reported in normotensive OH, but the role of vasodilator cyclooxygenase (COX) products is unclear.

Methods. In 12 OH and 12 ON men (19-24 years old), each group comprising 6 White Europeans (WEs) and 6 South Asians (SAs) with resting ABP <129/89mmHg, reactive hyperaemia and responses evoked by iontophoretic pulses of acetylcholine (ACh) were recorded in forearm skin by laser Doppler fluximetry before and after COX inhibition with aspirin.

Results. Peak reactive hyperaemia) was larger in ON than OH (71.0±7.8 vs 43.4±8.3 perfusion units (PU); $P<0.05$). It was attenuated by COX inhibition in ON (24.8±5.2PU, $P<0.01$), not OH (54.2±7.5PU). Similarly, increases in perfusion evoked by ACh were greater in ON than OH (169.1±20.4 vs 142.1±19.9PU; $P<0.05$) and attenuated by COX inhibition in ON (94.5±13.7; $P<0.05$), not OH (132.6±16.1PU). Considering ethnicities, ACh-evoked dilation, though not reactive hyperaemia was greater in WEs than SAs (176.8±21.7 vs 130.4±15.0; $P<0.01$; 61.0±8.7 vs 51.7±9.2PU). However, within both WEs and SAs, COX inhibition attenuated reactive hyperaemia and ACh-induced dilatation in ON, but not OH.

Conclusions. Reactive hyperaemia and ACh-evoked dilatation in cutaneous circulation are blunted in young, normotensive OH relative to ON men irrespective of WE, or SA ethnicity and are attributable to impaired contribution of COX vasodilator products in OH. These features may provide early markers of endothelial dysfunction that contribute to hypertensive risk in OH men.

Abbreviations. ABP: arterial blood pressure; ACh: acetylcholine, COX: cyclooxygenase; CV: coefficient of variation; CVD: cardiovascular disease; CYP 2C: cytochrome P450 epoxygenase; EDHF: endothelium-dependent hyperpolarising factor; FMD: flow mediated dilatation; NO: nitric oxide; OH: offspring of hypertensives; ON: offspring of normotensives; PU: perfusion units; PG: prostaglandin; PGI_2: prostacyclin; RCF: red cell flux; SA: South Asians; WE: White Europeans

Condensed Abstract. In normotensive young men, aged 19-24 years, who were offspring of hypertensive or normotensive parents (OH, ON respectively), we tested the contribution of cyclooxygenase (COX) products to cutaneous vasodilatation evoked as reactive hyperaemia, or by iontophoresis of acetylcholine (ACh). Equal numbers of OH and ON were White European (WE) and South Asian (SA). COX inhibition attenuated reactive hyperaemia and ACh-evoked vasodilatation in OH only, irrespective of WE, or SA ethnicity. These novel findings indicate that a blunted contribution of vasodilator prostaglandins to cutaneous vasodilatation precedes development of hypertension and may contribute to its pathogenesis

INTRODUCTION

Essential hypertension is a multi-factorial condition with an established familial component that crosses ethnicities, at least amongst those of Black African, White European, Japanese and South Asian descent [1-4]. Given evidence that raised sympathetic nerve activity contributes to the development of hypertension [5], it is not surprising that black and white American teenage girls and boys who were offspring of hypertensive parents (OH), showed greater pressor responses to mental stress than those with normotensive parents (ON) [6]. Further, in young European men (mean age 27 years), mental stress evoked significant increases in muscle sympathetic nerve activity in OH, but not ON [7].

However, there is also evidence that endothelium-dependent dilatation is blunted in young OH. Thus, flow-mediated dilatation (FMD) of the brachial artery, was smaller in young OH, than ON men and women [8]. Further, vasodilator responses evoked in circulation of whole forearm by acetylcholine (ACh) infusion were blunted in normotensive OH men and women, but normalised to those of ON by infusion of L-arginine, the substrate for NO synthesis. By contrast, cyclooxygenase (COX) inhibition had no effect on ACh-induced forearm dilatation in OH, or ON men and women [9]. It was therefore argued that vasodilator prostaglandins (PGs) make *no* contribution to endothelium-dependent vasodilatation in the forearm of OH or ON, but the NO pathway is defective in young OH [10]. In a subsequent, longitudinal study, L-arginine enhanced ACh-induced forearm vasodilatation in patients with essential hypertension who were <30 years old, and at >30 years, COX inhibition *enhanced* ACh dilator responses. Similar changes occurred in normotensives, but at 45-60 and >60 years old respectively [11]. Thus, both ageing and hypertension were accompanied by impairment of the NO pathway followed by up-regulation of a *vasoconstrictor* influence of the COX pathway, but the changes occurred at a younger age during development of hypertension.

Clearly, these studies on whole forearm circulation did not allow assessment of the contributions of *vasodilator* PGs [9]. However, forearm circulation includes skeletal muscle and cutaneous circulations; vasodilator PGs contribute to endothelium-dependent dilatation in the cutaneous circulation of young healthy subjects and their contribution is lost with aging and cardiovascular disease [11-14].

Thus, in the present study, we hypothesised that endothelium-dependent dilatation is impaired in cutaneous circulation of forearm in young adult OH men relative to ON men and that imbalance in the COX pathway contributes to this. We focussed on men because oestrogen increases production of prostacyclin (PGI_2), and decreases production of vasoconstrictor prostanoids in hypertension [15,16]. These effects seemed likely to complicate comparison of findings in young ON and OH women and to justify a separate study on women. Since we intended to recruit subjects from a student population that is multi-ethnic, we decided to include White Europeans (WEs) and South Asians (SAs). We tested our hypotheses by recording changes in forearm cutaneous red cell flux (RCF) evoked during reactive hyperaemia and iontophoresis of ACh, before and after COX inhibition.

METHODS

Experiments were performed on young, healthy male volunteers, aged 19-24 (mean age ± SEM: 20.9±0.28 years), who were students of the University of Birmingham. Inclusion criteria were that they should be free from known cardiovascular or respiratory disorders, non-smokers and that one or both parents should have essential hypertension, or neither parent was known to have hypertension (OH, ON respectively). The study was approved by the University Research Ethics Committee and performed in accordance with the Helsinki Declaration. Each subject gave informed consent.

Each subject attended the laboratory twice. In brief, on the first visit, anthropometric data were collected, they were familiarised with the equipment and completed a questionnaire on ethnicity, family medical history, diet, smoking, alcohol consumption and level of physical activity. Dietary information included assessment of weekly consumption of Vitamin C-containing fruit and vegetable portions as an index of anti-oxidant vitamin C intake, oily fish portions as an index of omega-3-fatty acids and Vitamin D. Physical activity was rated as sedentary or elective exercise rated as low, medium, high scored as time spent/week (see Table 1). All were White European (WE), or South Asian (SA) and normotensive; resting arterial blood pressure: <139/89 mmHg.

For the second visit, the subject attended the laboratory in the morning. For five days prior to this, they were asked to refrain from any dietary supplements containing vitamin C. Additionally, they were asked not to consume any vasoactive drugs, including non-steroidal anti-inflammatories, or alcohol for 24 h and to avoid caffeine, heavy meals and vigorous exercise for at least 4 h. Each subject acclimatised for 20-30 min and arterial blood pressure (ABP) was recorded by sphygmomanometry. Thereafter, ABP was continuously recorded by photoplethysmography via a finger cuff [17,18]. A laser Doppler probe inserted into an

iontophoresis chamber filled with a 1% solution of ACh in distilled, de-ionised sterile water, was fixed to the left forearm [17,18], to continuously monitor forearm skin perfusion, as cutaneous red cell flux (RCF) in perfusion units (PU).

Experimental protocol. Following baseline recordings for 5 min, reactive hyperaemia was recorded following arterial occlusion at 200 mmHg for 3 min, until perfusion returned to baseline again. ACh was then delivered by iontophoresis: 7 pulses of 0.1 mA and an 8th pulse of 0.2 mA for 20s each, separated by 60s intervals. Recordings of RCF and ABP were made continuously until 5 min following the final iontophoretic pulse [17,18].

The subject drank orange squash containing 600mg aspirin. The iontophoresis chamber and probe were then placed on a different site on the same arm, with a similar baseline as the original site: this was done because skin perfusion remained high for 30-60min following ACh iontophoresis. The protocol was repeated 30 min after aspirin, when COX inhibition is maximal [17,18].

Placebo/reproducibility protocol. The protocol was performed on 8 ON subjects who drank orange squash containing no aspirin (placebo) to test for reproducibility of reactive hyperaemia and ACh-evoked responses at different sites on forearm skin.

Data analyses.

For reactive hyperaemia, RCF was measured as change from baseline at peak, 30s, 1 and 2 min after release of occlusion. Responses evoked by ACh were calculated as change from baseline to maximal RCF for each iontophoresis pulse; a compacted mean for each subject was also calculated as the average of these changes [17,18]. Differences between and within ON and OH groups were assessed by two-way ANOVA and two-way repeated measures ANOVA respectively. For reactive hyperaemia, Scheffe′'s *post hoc* test was used to

determine times at which responses differed. Compacted means for ACh responses were compared between and within groups by Student's un-paired and paired t-tests respectively. Anthropometric and baseline data were compared between OH and ON with one-way ANOVA. Comparable analyses were performed between and within the 12 WEs and 12 SAs and for the subgroups of OH and ON within WEs and SAs. Power calculations based on previous and pilot data indicated that a subgroup size of 6 was sufficient to detect a 50% reduction in peak reactive hyperaemia and a 30% reduction in mean ACh responses after aspirin with 80% power in both the WE and SA ONs at $P<0.05$. For the placebo protocol, a coefficient of variation (CV) was calculated on data collected on repetition of the stimuli. $P<0.05$ was considered significant throughout.

RESULTS

There were no significant differences between OH and ON groups for anthropometric, cardiovascular or lifestyle characteristics (Table 1). Baseline RCF values were not significantly different between OH and ON before (13.0±0.7 vs 13.3±0.7PU respectively), or after aspirin (14.7±1.1vs 13.8±0.7PU); P>0.05 in each case.

Reactive hyperaemia

Peak reactive hyperaemia was substantially higher in ON than OH, the disparity being maintained until 30s (Figure 1A cf 1B*).* By contrast, after aspirin, reactive hyperaemia was greater in OH, than ON (Figure 1B). Within ON, reactive hyperaemia was significantly attenuated by aspirin (Figure 1A & C). By contrast, aspirin had no significant effect on peak reactive hyperaemia in OH (Figure 1B); it was attenuated by >50% in 2 individuals, augmented by >30% in 5, and changed by <10% in the remainder (Figure 1D).

ACh-evoked dilatation

Before aspirin, cutaneous vasodilator responses evoked by ACh were blunted in OH relative to ON (Figure 2A cf 2B). By contrast, following aspirin, ACh-evoked responses were blunted in ON relative to OH (Figure 2B). Within groups, ACh-evoked responses were attenuated after aspirin in ON (see Figure 2A & C), the mean response decreasing from 169.1±20.4 to 95.4±13.7 PU (P<0.01). By contrast, aspirin had no significant effect on ACh-evoked responses in OH (Figure 2B & D); they were attenuated by ≥30% from control response in 3 individuals, augmented by 15-40% in 4 and changed by <10% in the remainder.

Comparisons between White Europeans (WEs) *and South Asians* (SAs). None of the baseline characteristics was significantly different between SAs and WEs except diastolic pressure and MABP, which were higher in SAs (Table 2). Peak reactive hyperaemia was not significantly different between SAs and WEs before or after aspirin (Figure 3A & B). Moreover, aspirin

did not cause significant attenuation of peak hyperaemia in WEs or SAs. By contrast, both before and after aspirin, vasodilator responses evoked by ACh were blunted in SAs relative to WEs (Figure 4A & B), but any effect of aspirin did not reach statistical significance in either ethnicity (P=0.12 in WEs; P= 0.14 in SAs).

Considering the effects of aspirin within the ON and OH subgroups of WEs and SAs, reactive hyperaemia was attenuated by aspirin in ONs of both WEs and SAs (Figure 3C&D), but not in OHs of either ethnicity (Figure 3E & F). Similarly, within WEs and SAs, ACh responses were attenuated by aspirin in ON, but not OH (Figure 4 C-F): mean ACh-evoked responses were reduced from 186.0±34.0 to 98.2±22.8PU in ON WEs and from 143.1±18.2 to 120.6±16.8PU in ON SAs (P=0.02, 0.03 respectively).

Placebo/reproducibility protocol. There was no significant difference between two different sites on the forearm of ON subjects for reactive hyperaemia, or for ACh-evoked responses. Peak reactive hyperaemia, was 83.4±7.6 vs 83.1±9.2 PU at sites 1 and 2 respectively with a CV between paired values of 7.6%. Mean ACh-evoked dilatation was 139.9±19.0 vs 149.6±20.6 PU at sites 1 and 2 respectively with a CV of 4.9%.

DISCUSSION

The present study showed that both reactive hyperaemia and dilatation evoked in cutaneous circulation by the endothelium-dependent dilator ACh were blunted in young OH men relative to ON men. Further, COX inhibition with aspirin attenuated reactive hyperaemia and the ACh-evoked responses in ON, but not in OH.

Impaired endothelial-dependent dilatation in cutaneous circulation of OH

That forearm cutaneous vasodilatation evoked by iontophoresis of ACh was blunted in young OH men, is consistent with vasodilatation induced in whole forearm by infusion of ACh being blunted in OH men and women aged 23-24 y [9]; our finding that reactive hyperaemia was blunted is novel. There is a structural limitation on maximal vasodilatation and rarefaction of capillaries in essential hypertension, and it has been suggested these changes begin early in the development of hypertension [19]. However, it is unlikely structural abnormalities explain the present results for in young men (22-33 y) who were ON or OH, but had normal, or high resting ABP, only the hypertensive OH group showed reduced maximal vasodilatation and capillary rarefaction in forearm cutaneous circulation [20]. Thus, blunted endothelium-dependent dilatation in the young OH men is the more probable explanation.

Contribution of COX products to cutaneous vasodilatation. That COX inhibition attenuated ACh-evoked cutaneous dilatation of ON, but not OH men contrasts with the lack of effect of COX inhibition on ACh- evoked dilatation in the whole forearm of mixed male/female groups of 8 ON and 10 OH [9]. The simplest explanations for this disparity are that responses evoked by ACh in skeletal muscle overshadowed those in cutaneous circulation and/or inclusion of women confounded the issue [15,16]. Since COX inhibition also attenuated reactive hyperaemia in the cutaneous circulation of young ON, we deduce that vasodilator PGs, probably prostacyclin (PGI_2) the commonest endothelial PG [21], contribute to both vasodilator responses in cutaneous circulation of young ON, but not OH men.

Our finding that COX inhibition hardly changed peak reactive hyperaemia or ACh-evoked dilatation in 40% of OH, and substantially *augmented* them in ~40% of OH agrees with evidence that IP receptors for PGI_2 become dysfunctional during development of hypertension in young spontaneously hypertensive rats and with ageing, while the TP receptors that respond to thromboxane and mediate vasoconstriction, become responsive to PGI_2 [21]. Accordingly, a TP receptor antagonist augmented ACh-evoked dilatation and FMD in patients with coronary artery disease or atherosclerosis [22,23]. We acknowledge that TP receptor inhibition did not affect reactive hyperaemia in the skin of normotensive men aged 20-30 y [24], but their OH/ON status was not recorded. In view of the present results, a study on TP receptor inhibition on young OH and ON men would seem justified.

The question arises as to why the cutaneous dilator responses that persisted after COX inhibition were substantially larger in OH, than ON men. COX products can *inhibit* NO synthesis in cutaneous circulation [25]. Thus, COX inhibition might have allowed NO to make a larger contribution to ACh-evoked dilatation and reactive hyperaemia in OH, than ON men. This is unlikely given NO makes little contribution to either response in cutaneous circulation [13,26-29] and given NO availability is attenuated during development of hypertension in young OH men [9,10]. Alternatively, COX inhibition may have revealed the dilator influence of endothelium-derived hyperpolarising factor/s (EDHFs), which include a cytochrome P450 epoxygenase (CYP 2C) metabolite [26,30,31]. EDHFs provide a dilator reserve early in endothelial dysfunction [21] and CYP 2C metabolites compensated for impaired NO availability in the forearm of newly diagnosed hypertensives who were OH [31]. Thus, CYP 2C metabolites may help maintain cutaneous vasodilator responses of young OH men at a stage when the dilator influences of PGs are deficient. This remains to be explored.

In previous studies, the effects of COX inhibition on cutaneous circulation of normotensive subjects have been highly variable. COX inhibition attenuated ACh-evoked dilatation in 6 men aged 23-36 y [11], in mixed male/female groups of 12, 23 or 11 subjects of 23-30 y [13,14,32] and reactive hyperaemia in 6 men and women aged 27-40 y [12]. By contrast, COX inhibition had no effect on these responses in mixed groups of 9, or 6 men and women aged 18-30 y [33,34], but *augmented* reactive hyperaemia in 12 men and women aged 19-25 y [25]. Given the small group sizes, the influences of gender and age on the contributions of COX products [13,15-18] and the fact that ON/OH status was not considered, it seems likely these variable outcomes reflected within group variability. Indeed, the present results indicate that in healthy men in young adulthood (19-24 y), of similar life styles and levels of physical activity, ON/OH status is the dominant factor determining the effects of COX inhibition on reactive hyperaemia and ACh-evoked dilatation. The high reproducibility of both responses in ON men who received placebo rather than COX inhibitor is consistent with that proposal.

Effects of ethnicity

We recruited equal numbers of WE and SA in the ON and OH groups, knowing the prevalence of cardiovascular disease (CVD) is higher in SAs and associated with endothelial dysfunction [35]. Accordingly, diastolic pressure was higher in the young SA than WE men consistent with increased peripheral resistance in young SA men. Further, ACh-evoked dilatator responses though not reactive hyperaemia, were blunted in young SA, relative to WE men. Similarly, we recently showed that reactive hyperaemia and vasodilator responses to mental stress were blunted in forearm of young normotensive SA men, relative to WE men [36]. Moreover, FMD and the tonic dilator influence of NO on forearm were also blunted in young normotensive SA, relative to WE men [37]. Indeed, it seems endothelium-dependent dilatation is impaired even in young SA men with no overt CVD.

Nevertheless, a key finding of the present study is that in both young WE *and* SA men, COX inhibition attenuated reactive hyperaemia and ACh-evoked dilatation in cutaneous circulation

of ON, just as in the full group of mixed ethnicity. Given the heritable proportion of ABP is 40-60% in WEs *and* SAs [1,4], it may be the future risk of hypertension is lower in the minority of individual OH in whom COX inhibition attenuates cutaneous dilator responses, but higher in those in whom responses are augmented. Factors such as central obesity, physical inactivity, high life stress and hyper-responsiveness to stressors determine whether individual OH, or ON develop hypertension [1-4,38] and the prevalence of central obesity and diabetes is higher among SAs [35]. Thus, it would not be surprising if the proportion of OH men who actually develop hypertensive and CVD were greater amongst SAs than WEs.

Study Limitations

Like most studies on ON/OH [see 6-8], we did not verify whether the parents of ON had hypertension. Thus, some may have been undiagnosed, or develop hypertension in the future. However, young OH men whose parents have hypertension in early middle-age are at higher risk of hypertension than those whose parents develop hypertension in late middle-age [39]. It is therefore reasonable to assume the OH men were the higher risk group. It is a limitation that we assessed BMI but not body fat distribution. However, in a recent study, BMI, waist: hip ratio and fasting glucose levels were similar in young SA and WE men, but fasting insulin was at the high end of the normal range in SAs, consistent with their depressed endothelium-dependent dilatation [37]. Thus, we have missed early signs of diabetes in some SA men. Importantly, our proposals should be tested in a larger population of young men and women.

Considering our experimental stimuli, release of sensory nerve peptides has been implicated in reactive hyperaemia and vasodilatation evoked by iontophoresis of ACh [26,33]. We used a shorter period of arterial occlusion (3 vs 5min) and delivered ACh with a lower current than those studies. Moreover, we showed that iontophoresis of vehicle with the same protocol as we used for ACh had minimal effects on cutaneous perfusion, while COX inhibition had no effect on responses evoked by iontophoresis of the endothelium-independent dilator sodium-

nitroprusside [17,18]. Thus, whilst we cannot exclude involvement of neuropeptides released directly by ischaemia or current, or by their ability to stimulate synthesis of mediators, such as PGs [26,33], it seems reasonable to assume the responses we report mainly reflect direct effects of ischemia and ACh on endothelium-dependent dilatation.

Conclusions

The present study on normotensive men in the 19-24 y age range, indicates that OH men of WE and SA ethnicity show blunted reactive hyperaemia and ACh-evoked vasodilatation in cutaneous circulation relative to ON men, attributable to a depressed contribution of vasodilator PGs. These early signs of endothelial dysfunction precede development of frank hypertension, but may contribute causally to its pathogenesis [40].

Acknowledgements

The authors thank those who gave their time to take part in this study.

Sources of funding

None to declare

Conflicts of Interest

The authors have none to declare

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	ON	OH	P value
Number of subjects	12	12	
Age (years)	21.1±0.3	20.7±0.4	NS
BMI (Kg/m^2)	24.4±0.9	22.9±0.3	NS
Systolic BP (mmHg)	121.1±3.3	122.3±1.7	NS
Diastolic BP (mmHg)	74.9±2.8	76.8±1.6	NS
MABP (mmHg)	90.3±2.8	91.9±1.4	NS
Alcohol units/wk	2.1±0.7	3.2±1.2	NS
Caffeine beverages/day	0.4±0.2	0.4±0.3	NS
Vit C-containing portions/day	2.8±0.3	3.1±0.4	NS
Oily Fish portions/wk	1.9±0.3	1.2±0.3	NS
Physical activity/wk	3.1±0.2	2.9±0.2	NS

Table 1. Characteristics of ON and OH subjects. Vit C-containing portions; high Vitamin C-containing fruit and vegetable portions. Physical activity rated as sedentary (minimal), elective exercise: low (30min<3 times/week), medium (30min≤3 times/week), high intensity (30min>3 times/week) scored as 0-4 respectively. NS, P>0.05; ON vs OH.

	WE	SA	P value
Number of subjects	12	12	
Age (years)	20.3±0.3	21.6±0.4	NS
BMI (Kg/m^2)	23.2±0.6	24.3±0.5	NS
Systolic BP (mmHg)	119±2.9	124.4±1.9	NS
Diastolic BP (mmHg)	72.9±2.1	78.75±2.0$^{\#}$	<0.05
MABP (mmHg)	88.3±2.2	93.9±1.9$^{\#}$	<0.05
Alcohol units/wk	3.2±0.5	2.3±1.4	NS
Caffeine beverages/day	0.4±0.3	0.4±0.2	NS
Vit-C containing portions/day	2.9±0.3	3.0±0.4	NS
Oily Fish portions/wk	1.7±0.3	1.2±0.3	NS
Physical activity/wk	2.9±0.2	3.2±0.2	NS

Table 2. Characteristics of WE and SA subjects. Abbreviations as in Table 1. NS, $^{\#}$: P>0.05, P<0.05 respectively; SA vs WE.

Figure 1. Reactive hyperemia in OH and ON men before and after aspirin. A, B: control responses in ON and OH respectively, before and after aspirin. C, D peak reactive hyperaemia (RH) in individual ON and OH respectively, before and after aspirin; individual data connected by fine line. Cross-hatched squares indicate mean peak responses ±SEM; before and after aspirin connected by thick line. **, *: $p<0.001$, $P<0.05$ respectively within group before vs after aspirin. $^{\#, \S}$: $p<0.05$ between ON and OH groups before and after aspirin respectively.

Figure 2. Vascular responses evoked by ACh in OH and ON men before and after aspirin. A, B: control responses in ON and OH respectively, before and after aspirin. C, D: mean dilator response to ACh in individual OH and ON respectively, before and after aspirin; individual data connected by fine line. Cross-hatched squares indicate mean ACh responses ±SEM; before and after aspirin connected by thick line. **, *: $P<0.001$, $P<0.05$ respectively before vs after aspirin for mean response to ACh. $^{\# \S}$: $p<0.05$ between ON and OH groups before and after aspirin respectively.

Figure 3. Reactive Hyperaemia in WE and SA men before and after aspirin. A: control responses in WEs and SAs; B: responses in WEs and SAs after aspirin; C,D: responses in ON WEs and SAs before and after aspirin. E, F: responses in OH WEs and SAs before and after aspirin. *: $P<0.05$ within WE, or SA, before vs after aspirin.

Figure 4. Vascular responses evoked by ACh in WE and SA men before and after aspirin. A: control responses in WEs and SAs; B: responses in WEs and SAs after aspirin; C, D: responses in ON WEs and SAs before and after aspirin; E, F: responses in OH WEs and SAs before and after aspirin;. $^{\#}$: $P<0.05$ between WE and SA groups. *: $P<0.05$ within WE, or SA, before vs after aspirin.