Effect of ethnicity and sex on vascular responses to environmental stressors: do prostaglandins contribute?

By

Abimbola Olufunmilayo Aiku

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Institute of Cardiovascular Science

College of Medical & Dental Sciences,

The Medical School

Birmingham

B15 2TT

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Abstract

The effect of ethnicity and sex on the cardiovascular responses to environmental stressors and contribution of prostaglandins (PGs) to these responses were investigated in young White European (WE), Black African (BA) and South Asian (SA) men and women. Mental stress elicited responses consistent with the pattern of the alerting response in WEs, BA and SA men including endothelium-dependent forearm vasodilatation, but BA and SA women showed forearm vasoconstriction and BA women showed exaggerated pressor responses. Repetition of mental stress did not induce short, or medium term habituation of alerting responses in any group, but BA women showed sensitization of pressor responses and forearm vasoconstriction indicating vulnerability to mental stress-induced hypertension. On the other hand, BA and SA men, but not women show blunted endothelium-dependent dilatation relative to WEs during reactive hyperaemia and exercise hyperaemia. Vasodilator and vasoconstrictor PGs contributed to the vascular components of the alerting response in BAs and SAs, but not WEs. Further, PGs contributed to reactive hyperaemia and exercise hyperaemia in WE men and women, but in BAs and SAs, PGs played a minimal role in women. `We propose that this endothelial dysfunction makes BA and SA women especially vulnerable to cardiovascular disease caused by mental stress.

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List of abbreviations

WHO	World Health Organisation
CVD	Cardiovascular diseases
ABP	Arterial blood pressure
BAs	Black Africans
SAs	South Asians
WEs	White Europeans
HR	Heart rate
SNS	Sympathetic nervous system
A/DR	Alerting/Defence response
CNS	Central nervous system
DLH	DL-Homocysteic acid
PAG	Periaqueductal grey
PGL	Paragigantocellularis lateralis
RVLM	Rostral ventrolateral medulla
GABA	Gamma-Aminobutyric acid
ORX	Orexin/hypocretin
NTS	Nucleus tractus solitarius
BRS	Baroreceptor sensitivity
MSNA	Muscle sympathetic nerve activity
DBP	Diastolic blood pressure
DCI	Dichlororoisoproppyl noradrenaline
cAMP	cyclic Adenosine monophosphate
PGI2	Prostacyclin
Ach	Acetylcholine
NO	Nitric oxide
NOS	Nitric oxide synthase
eNOS	Endothelial nitric oxide synthase
nNOS	Neuronal nitric oxide synthase
L-NAME	N omega-Nitro-L-arginine methyl ester hydrochloride
SMTC	S-methyl-l-thiocitrulline
L-NMMA	NG-Monomethyl-L-arginine
COX	Cyclooxygenase
Ach	Acetylcholine
FVC	Forearm vascular conductance
NO	Nitric oxide
PGE	Prostaglandin E
PG	Prostaglandin
fMRI	Functional magnetic resonance imaging
FH+	Hypertensive parents
FH-	Normotensive parents
FMD	Flow-meditated dilation
NADPH	Nicotinamide adenine dinucleotide phosphate
ET	Endothelin
BRS	Baroreceptor sensitivity

SNP	Sodium nitroprusside,
LBNP	Lower body negative pressure
CHD	Coronary heart disease
HRV	Heart rate variability
BMI	Body mass index
ABPM	Ambulatory blood pressure monitor
Finapres	Finger blood pressure monitor
VOP	Venous occlusion plethymography
FBF	Forearm blood flow
LDF	Laser doppler fluxmetry
DCRF	Digital cutaneous red cell flux
FCRF	Forearm cutaneous red cell flux
PSS	Perceived stress score
MABP	Mean arterial blood pressure
SEM	Standard error of mean
SIS	Salt intake score
SD	Standard deviation
ANOVA	Analysis of variance
skBF	Skin blood flow
RCF	Red blood cell flux
mC	millicoulombs

List of Publications

Abstracts

AIKU, A. O., MARTIN, U. & MARSHALL, J. M. 2016a. Effect of cyclooxygenase inhibition on reactive hyperaemia and muscle vasodilator responses to mental stress in young Black Africans (BAs) and White Europeans (WEs). Proc Physiol Soc, 37 PCB 340.

AIKU, A. O., MARTIN, U. & MARSHALL, J. M. 2016b. Young Black African (BA) men show blunted endothelial dilator responses and exaggerated pressor responsiveness to environmental stress relative to White European men (WEs): does this predispose BAs to hypertension? Journal of Human Hypertension. , 30, 654. P-29.

AIKU, A. O., ORMSHAW, N. G., JUNEJO, R. T., MARTIN, U. & MARSHALL , J. M. 2016c. Both endothelium-dependent dilatation responses and muscle vasodilator responses to environmental stressors are impaired in young Black Africans and may contribute to their predisposition to develop hypertension. The FASEB Journal, 30, 1206.5-1206.5.

AIKU, A. O., MARTIN, U. & MARSHALL, J. M. 2017a. Do prostaglandins contribute to endothelium-dependent vasodilatation or muscle vasodilator responses to environmental stressors in Young Black African or White European women? Journal Of Human Hypertension, 31, 657. P-25.

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AIKU, A. O. 2018. Absence of sympathetic vasodilation during mental stress with non-dipping circadian blood pressure pattern in young White and Black adults could be an early indicator of hypertension. The FASEB Journal, 32, 847.8-847.8.

AIKU, A. O., MARTIN, U. & MARSHALL, J. M. 2018a. Contribution of cyclooxygenase (COX) products to exercise hyperaemia in young Black Africans (BAs) and White Europeans (WEs): are there gender differences? Proc Physiol Soc 41, 103P. C128.

AIKU, A. O., MARTIN, U. & MARSHALL, J. M. 2018b. Forearm vasoconstriction during mental stress is associated with non-dipping circadian blood pressure pattern in young adult White Europeans (WEs) and Black Africans (BAs): an early indicator of cardiovascular disease? Journal of Human Hypertension, 32, 716. P-43.

AIKU, A. O., MARTIN, U. & MARSHALL, J. M. Effect of cyclooxygenase inhibition on exercise hyperaemia in young Black Africans (BAs) and White

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CHAPTER 1

General Introduction

1. Introduction

1.1 Role of environmental stress in development of cardiovascular disease.

Cardiovascular diseases (CVD) cause majority of deaths globally. Many of the deaths occur in low to middle income countries in the African and South Asian regions (WHO, 2014). This current situation has not always been so, for, in the early 20th century hypertension and cardiovascular complications were rare in Africa. At that time, Africans and Europeans had similar arterial blood pressure (ABP) until middle age, but Europeans then showed an age-related increase in ABP, whereas Africans did not show this effect (Williams, 1941). However, since then, hypertension has become more prevalent in different parts of Africa and about one third of adults in Africa now have hypertension (WHO, 2012). Prevalence of CVD, coronary heart disease (CHD) and risk factors for CVD such as metabolic syndrome and diabetes are higher among South Asians from the Indian subcontinent than White Europeans (Tziomalos *et al.*, 2008). In fact, CVD has an earlier onset and is associated with more severe disease among Black Africans (BAs) and South Asians (SAs) than White Europeans (WEs) living in Europe or America (Cappuccio, 1997; Yusuf *et al.*, 2001).

Several factors contribute to the development of CVD including hypertension such that interaction between genes and the environment facilitate, or inhibit the onset (Hamet, 1995). Notably, there is evidence that rural to urban migration within Africa led to an increase in ABP (Poulter *et al.*, 1984). There is also evidence that migration within South Asia, or internationally has been associated with increased prevalence of CVD (Patel *et al.*, 2006). This has been attributed to lifestyle changes and environmental stress, consistent with the evidence that psychological and environmental stress increases the incidence of CVD (Pickering, 1997). This link has been very evident in

the increased incidence of cardiovascular events following earthquakes and other environmental disasters (Dobson *et al.*, 1991; Kario, 1998; Bazoukis *et al.*, 2018). Similarly, psychosocial stress has been associated with increased CVD (Steptoe & Kivimaki, 2012).

Stress evokes physiological changes mediated by the endocrine and sympathetic nervous systems leading to increased heart rate (HR), increased blood flow to skeletal muscles, reduced blood flow to kidneys, viscera and skin with increased arterial blood pressure (ABP) (Hilton, 1982). Indeed, it has recently been suggested that emotional stress associated with a taxing lifestyle is making such an important contribution to the increasing prevalence of hypertension in sub-Saharan Africa and to associated CVD by disturbing central neural control of blood pressure (Malan & Malan, 2017) that hypertension could be used as a measure of social wellbeing. These associations allow the hypothesis that environmental stress and lifestyle changes may play a role in the increased prevalence of CVD among the SA and BA groups.

On the other hand, there is also evidence that environmental stress can reduce sodium excretion and increase salt retention with subsequent development of increased ABP (Koepke, 1985; Sever & Poulter, 1989). This highlights the fact that although several studies have associated various forms of stress with the development of CVD, it is not clear how stress causes CVD in some individuals, but not others. It has been proposed that individuals who develop hypertension from stress may be those who react to stress with greater sympathetically-driven increases in ABP (Steptoe, 1986). Whether repetition of acute stressors or chronic stress is more likely to cause hypertension has been debated (Sparrenberger *et al.*, 2009). But, there is evidence that repeated exposure

to stress in genetically susceptible individuals, predisposes to development of hypertension (Imumorin *et al.*, 2005). Whether the mechanisms by which acute and chronic stress contribute to the development of hypertension differ, is not clear.

Early researchers, using laboratory experiments to mimic life experiences postulated that there is a link between the acute ABP rises observed when subjects were exposed to situations that stressed them emotionally or physically and the longer-term development of maintained high ABP. In these experiments, the physiological mechanisms underlying the acute responses were explored and links between them and the onset of hypertension were proposed (Shapiro, 1978; Brod, 1982; Folkow, 1991).

In the following sections below, an account is provided of what is known about the responses to environmental stress including the roles ethnicity and gender may play in determining the responses. This chapter focuses on cardiovascular responses evoked by mental stress and the role of endothelium-dependent factors in determining the vascular components is explored. Responses evoked by physical stress in form of reactive hyperaemia following release of arterial occlusion and exercise hyperaemia following muscle contractions are reviewed in Chapters 3 and 6.

1.2 The Alerting/Defence response (A/DR) to environmental stress

In everyday life we experience a considerable amount of stress caused by sudden events, being told stressful news, hearing harsh words, or seeing or imagining things that scare, or annoy us. Such events may occur many times in the course of a single day and from time to time or repeatedly daily over periods of time. Cannon (1915) in his monograph "Bodily changes in pain, hunger, fear and rage" was the first to describe the pattern of response that occurs during what he referred to as "emergency reactions". At

the time of Canon's work, stimulation of the sympathetic nervous system (SNS) was known to increase ABP and HR, cause pupillary dilation, sweating and piloerection, in addition to its influence on the adrenal medulla to release hormonal secretions. Emotions of fear, rage or pain, for example by exposure to a natural enemy such as confrontation of a cat by a barking dog was observed by Canon to cause pupillary dilation, inhibition of digestion, paleness of skin and increased HR piloerection, retraction of ears, hissing, snarling and attack, by striking the dog with claws.

In one of the experiments on frightened cats, Canon (1915) provided evidence for release of a substance referred to as adrenin from the adrenal medulla (now known as adrenaline). He therefore deduced that mass activation of the SNS including the adrenal medulla occur during emotional stress. Even cats with denervated hearts continued to show an increase in HR when confronted by aggressive dog implicating adrenaline in the tachycardia. In addition, Canon observed increased blood glucose level, delayed fatigue of skeletal muscle, and increased limb volume, while the volume of viscera such as kidneys, spleen and intestine decreased. These latter effects were thought to reflect changes in blood flow, but no quantifiable measurements were done. Nevertheless, Canon concluded that the changes that occur during stress ensure adequate blood supply to muscle, which is essential for preservation of the individual. He proposed that both adrenaline and sympathetic nerve activity (SNA) play roles in the response to stress and that the reactions are adaptive, preparing an organism for flight or fight (Cannon, 1915).

Subsequently, a similar pattern of response was elicited by electrical stimulation in parts of the brain; since the animals showed defensive-type behaviour the term "defence reaction" was coined (Hess & Brügger, 1943). In the 1980s, it was noted that the response to aversive stimuli was preceded by this same pattern of cardiovascular

response and it was referred to as the alerting stage of the defence reaction (Hilton, 1982). Currently, "alerting response" or defence reaction are used interchangeably.

1.2.1 The pattern of the alerting response

Following the work of Cannon and other earlier researchers who deduced the pattern of response by observation, quantitative evidence was gained in anaesthetised cats and dogs, that the pattern of the alerting response includes vasodilation in limb muscles, vasoconstriction in splanchnic, renal and cutaneous circulations, increased ABP, increased HR and contractility, increased cardiac output, venoconstriction and increased respiration (Eliasson *et al.*, 1951; Abrahams & Hilton, 1958; Brod *et al.*, 1959; Barcroft *et al.*, 1960; Brod *et al.*, 1976). The alerting response starts soon after application of the stimulus and was short-lasting. In addition, it is graded depending on the intensity of stimuli, and the most readily evoked component is the skeletal muscle vasodilation (Hilton, 1982). In later studies it was shown in conscious animals, that the full response can be elicited as a reflex, but the effective stimuli vary within and between species: in the cat, sight of an aggressive dog, in baboons, the sight of a snake and in dogs, even sudden sound elicited the response (Jordan & Marshall, 1995).

Early on, it was not clear whether the findings in animals could be extrapolated to man. However, a similar pattern of response was evoked in humans by a range of stressful environmental or emotional stimuli and even by electrical stimulation of the brain. Briefly, anxiety accompanying cardiac catheterization elicited increased ABP and increased cardiac output (Stead *et al.*, 1945). Increased ABP was described as a response to stressful life situations (Wolf, 1952), while Hejl (1957) demonstrated an increase in ABP during a stressful mental arithmetic test. Further, the hemodynamic effects of emotional stress evoked by mental arithmetic to the beat of a metronome

investigated, included increased HR, cardiac output, ABP, increased forearm blood flow and reduced forearm vascular resistance indicating forearm vasodilatation, but increased renal vascular resistance and fall in skin temperature indicating renal and cutaneous vasoconstriction (Brod *et al.*, 1959; Brod, 1963). A fall in temperature of exposed intestinal mucosa was also observed during mental stress in subjects with a colostomy indicating splanchnic vasoconstriction (Brod, 1963). Since then, a range of novel, or noxious stimuli such as sudden sound (Zbrozyna & Westwood, 1988; Edwards *et al.*, 1998), the Stroop colour test (Lindqvist *et al.*, 1999; Huang *et al.*, 2010), examination stress and the thought of drinking blood (Blair *et al.*, 1959) were all shown to elicit the alerting pattern of response in humans.

Electrical stimulation of human hypothalamus intraoperatively also elicited transient aggressive behaviour (Bejjani *et al.*, 2002). Further, Schvarcz *et al.* (1972) intraoperatively stimulated the medial posterior hypothalamus and evoked an arousal response with vocalisation, increased ABP, HR and increase in respiratory rate, all of which disappeared within 2 minutes of the stimulus. In addition, lesions in this region of the hypothalamus were also used to successfully treat severely aggressive people (Grossman & Grossman, 1970; Sano *et al.*, 1970).

Taken together, this evidence indicated that in humans, as in other animals, the cardiovascular response to emotional stimuli resembles cardiovascular changes in response to exercise. Brod *et al.* (1959) opined that the response pattern occurred when muscular effort might be necessary for self-preservation (Brod *et al.*, 1959; Brod, 1963). However, it is important to note that Brod (1963) recognised the muscle vasodilation of the alerting response was not associated with muscular effort: it was dissociated from

metabolic changes and considered to be the result of hypothalamic activity and autonomic outflow. Indeed, the evidence gained from the central neural approaches in humans indicated that the hypothalamus plays an important role in the alerting response in humans, as in other animals.

1.2.2 Role of the hypothalamus and brain stem in integrating the alerting response.

Decerebrate animals with hypothalamus spared expressed the alerting response including turning the head towards the stimulus, increased ABP, pupillary dilation, or snarling when an afferent nerve was stimulated; referred to as a pseudo-affective state (Woodworth & Sherrington, 1904). Other workers also observed that dogs or cats who had no cerebral cortex showed a similar pattern of rage as intact animals, but to stimuli that would not normally cause anger, such as touch, elicited rage in these animals (Spiegel *et al.,* 1940). These studies showed that the expression of rage and the sympathetic components depends on subcortical structures in the brain and that the cortex normally exerts an inhibitory effect on the alerting response in the conscious animal. Cannon and Britton (1925) opined that in animals without cerebral cortex, the thalamic region maintains primitive functions and referred to the response shown by decorticated cats – signs of widespread sympathetic activity including raised ABP, erection of hair, profuse sweating and release of adrenaline – as "Sham rage" because it resembles rage seen in intact animals.

Subsequently, Bard delineated the areas of brain stem involved in the response and showed that for the whole pattern to be present and well organised, the caudal hypothalamus needed to be intact (Bard, 1928; Bard, 1958). Further, regions of the diencephalon regions were mapped extending from the tuberal region of hypothamus, just medial, ventral and lateral to the fornix from which mild electrical stimulus evoked,

alerting behaviour, whereas stronger stimuli elicited the "attack reaction" which could turn into a "defence reaction" with vocalisation, piloerection and pupillary dilation and lead to flight. They referred to these behavioural responses as the Defence-Aggression reaction (Abwehrreaktion) (Hess & Brügger, 1943; McDonald, 1951).

Although an increase in muscle blood flow had been reported when brain stem areas were stimulated, and hind limb vasodilation of dogs and cats evoked by stimulation of the sympathetic chain was shown to be due to release of acetylcholine (Burn, 1932; Bulbring & Burn, 1935), no functional connection had yet been made between the sympathetic vasodilator system and the higher parts of the central nervous system (CNS). Following up these studies, a team led by Folkow confirmed that sympathetic vasodilator nerves were exclusively distributed to the hind limb of the cat and that atropine abolished vasodilation elicited by their stimulation, indicating they were cholinergic sympathetic vasodilator nerves. Further, these vasodilator fibers could be activated by electrical stimulation of points extending from the supraoptic region to the mammillary bodies in the hypothalamus of anaesthetized cats (Eliasson *et al.*, 1951).
Importantly, Abrahams and Hilton (1958) showed that stimulation in conscious cats of the hypothalamic areas described by Hess and Brügger (1943) evoked the typical defence reaction. They then showed in anaesthetized cats, that stimulation in these same brain regions produced the full cardiovascular pattern of response (Abrahams *et al.*, 1960), including atropine-sensitive vasodilation in skeletal muscle, vasoconstriction in skin, kidney and intestine, increased ABP, tachycardia plus pupillary dilation, piloerection and increased respiration. Thus, they concluded that the brainstem areas responsible for cholinergic muscle vasodilation are the same as those that integrate the whole alerting response. This group extended the mapping of the defence areas showing

they extend from a narrow strip of undifferentiated grey matter on both sides of midline through the whole length of hypothalamus, tuberal region and mammillary bodies region and caudally into the midbrain tegmentum, in the regions ventral to the superior colliculus, dorsolateral part of central grey matter (Abrahams *et al.*, 1960), Figure 1.1).

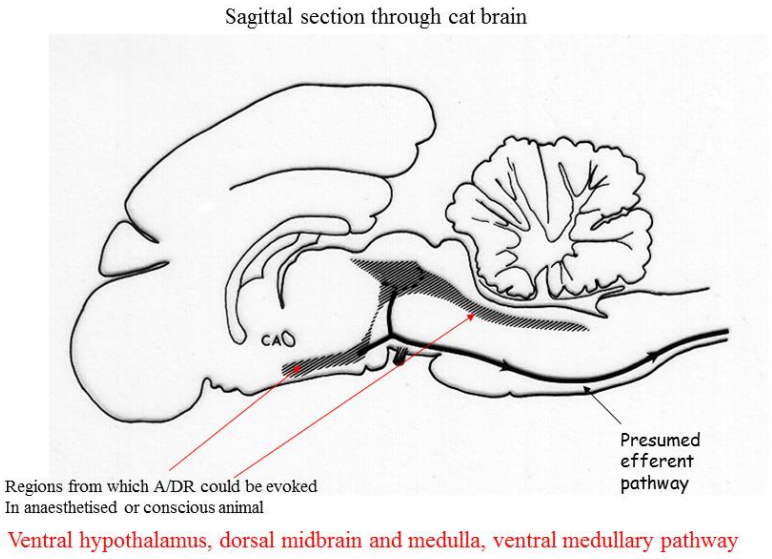


Figure 1.1 Sagittal section of brain stem in cat indicating hypothalamic, midbrain and hindbrain regions associated with integration of the defence areas and ventral pathway; modified from (Abrahams *et al.*, 1960).

In the 1970s, Coote *et al.* (1973) identified a narrow strip within dorsal pontomedullary region (Figure 1.1) starting close to inferior colliculus running into medulla from which the cardiovascular pattern of the alerting response could be elicited in anaesthetised cats and from which the behavioural components of the defence response were evoked in conscious cats. However, the muscle vasodilation elicited from this area was resistant to atropine, but sensitive to guanethidine, which inhibits transmission in adrenergic nerves, suggesting it is mediated by inhibition of sympathetic vasoconstrictor nerve activity to skeletal muscle. Coote *et al.* (1973) proposed that the pontomedullary and hypothalamic-midbrain areas are normally co-activated during the defence reaction and muscle vasodilatation is mediated by inhibition of noradrenergically-mediated vasoconstriction, or activation of sympathetic cholinergic nerves.

Although most of the studies described above were done in cats, similar studies in which the brainstem defence areas were investigated by electrical stimulation under anaesthesia or in the conscious state, were also performed in rabbits (Azevedo *et al.*, 1980), opossum (Azevedo & Veras-Silva, 1987), rats (Yardley & Hilton, 1986), and monkey (Schramm *et al.*, 1971) and the important regions for evoking the full pattern of the alerting response were shown to be analogous to those shown in the cat.

Since electrical stimulation activates both nerve cell bodies and nerve fibres, experiments were performed by using DL-Homocysteic acid (DLH), which only stimulates cell bodies. Such experiments showed the cell bodies that can evoke the full pattern of the defence reaction in rats are in the ventral hypothalamus, and central periaqueductal grey matter of the dorsal midbrain and medulla (Hilton & Redfern, 1986), i.e. in the regions identified by electrical stimulation (Figure 1.2). Similar results were obtained in rabbits by chemical stimulation using microinjections of glutamate ions, which excites cell bodies only, neurones of the defence areas were located in the periaqueductal grey (PAG) of the midbrain, and in the ventromedial nucleus in the hypothalamus (Tan & Dampney, 1983).

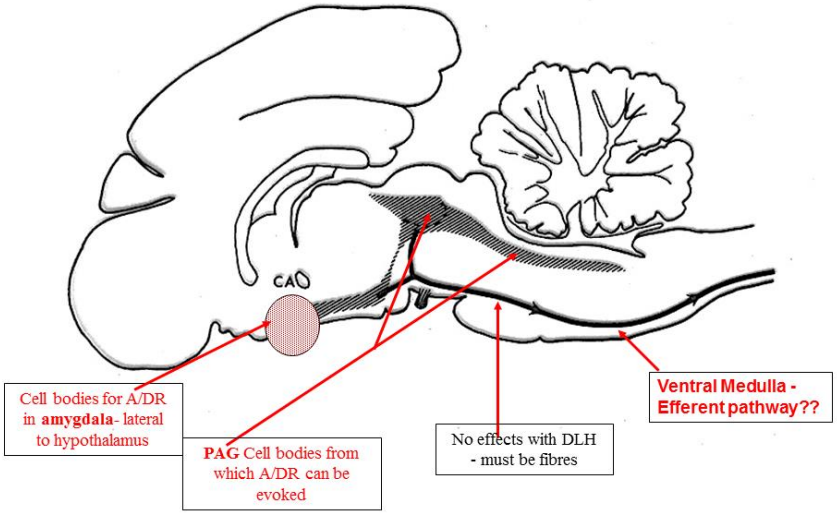


Figure 1.2 Sagittal section of brainstem indicating regions shown cross-hatched from which DLH could evoke the pattern of the alerting response. Area shown in red represents a region of amygdala, lateral to hypothalamus, from which DLH evoked the response.

1.2.3 Efferent pathway for the alerting response.

The ventral efferent pathway from the defence areas of the hypothalamus and mesencephalon were identified to run through the ventral medulla to the spinal cord and thence, to synapse with the preganglonic sympathetic neurones (Lindgren & Uvnas, 1953; Abrahams *et al.*, 1960) (Figure 1.2). However, when attempts were made to follow this pathway through the ventral medulla by stimulating electrically in anaesthetised cats, and recording the full pattern, at a particular level of the ventral medulla only individual components of the alerting response could be elicited (Hilton *et al.*, 1983). This fragmentation occurred in a region originally known as the nucleus paragigantocellularis lateralis, (PGL), but now referred to as the rostral ventrolateral medulla (RVLM), which provides the major tonic excitatory drive to sympathetic neurones in the spinal cord. A lesion applied unilaterally in this region and application of glycine, which inhibits cell bodies to the other side, or bilateral application of glycine not only produced a profound fall in ABP and apnoea, but abolished the alerting response evoked by stimulation in the brainstem defence areas (Hilton *et al.*, 1983).

Thus, it was concluded that these (RVLM) neurones are not only critical in maintaining resting ABP, but in evoking the full pattern of the alerting response (Hilton *et al.*, 1983).

Subsequently, the neurons of RVLM were shown to receive input from the defence areas (Hilton & Smith, 1984; Li & Lovick, 1985) and to act as a relay station for efferents from the deference areas, individual neurones or groups of neurones mediating individual components of the alerting response, including cholinergic muscle vasodilatation (Lovick, 1993) as well as the pressor component of the alerting response (Carrive *et al.*, 1988). Further, neurones within RVLM that are excited from the defence areas were shown to project to the spinal cord intermedolateral region and synapse with preganglionic sympathetic neurones (Amendt *et al.*, 1978). The RVLM generates sympathetic vasoconstrictor tone via glutaminergic synapses and the muscle vasodilator responses, via gamma-Aminobutyric acid (GABA)-ergic synapses (Possas *et al.*, 2001; Cravo *et al.*, 2003).

1.2.4 Role of limbic system in the integration of the alerting response

The amygdala of the limbic system is involved in memory and expression of emotion. Since emotional stress can elicit the alerting responses, the link with the limbic system has been investigated. Gloor (1955) showed that stimulation of the amygdala elicited behavioural effects similar to those elicited by stimulation of brain stem and diencephalic areas and that a projection from the amygdala to these brain stem areas elicited these responses. Electrophysiological studies then demonstrated connections between amygdala and posterior hypothalamus that were associated with the defence-aggression reaction (Magnus & Lammers, 1956). Moreover, Fernandez De Molina and Hunsperger (1959) elicited the alerting response in conscious free moving cats by stimulating parts of amygdala along the stria terminalis at the level of the anterior

commissure. Moreover, stimulation of the amydgala in basolateral regions extending to central nucleus and into the internal capsule elicited arousal, piloerection, pupillary dilation and anticipation (Ursin & Kaada, 1960).

This work was extended by Zbrozyna who showed that the behavioural pattern evoked was also demonstrable in conscious cats by stimulation of the amygdala (Zbrozyna, 1960). However, whereas hypothalamic stimulation immediately elicited the full alerting response and the response disappeared promptly after discontinuation of the stimulation, the response evoked from the amygdala developed gradually and lasted for much longer after the stimulus had ended. This was attributed to an afferent pathway to the amygdala from the hypothalamus which runs through stria terminalis, and a ventral amydalo-fugal pathway connecting the amygdala to the hypothalamic defence areas: the hypothalamic-stria-amygdala-hypothalamic circuit may allow continuing reactivation of the defence regions after the original stimulus (Hilton & Zbrozyna, 1963) (Figure 1.3). Although stimulation in the amygdala evoked behavioural alerting in conscious cats, the cardiovascular components of the defence response could not be demonstrated in cats anesthetised with conventional anaesthetics (Hilton & Zbrozyna, 1963).

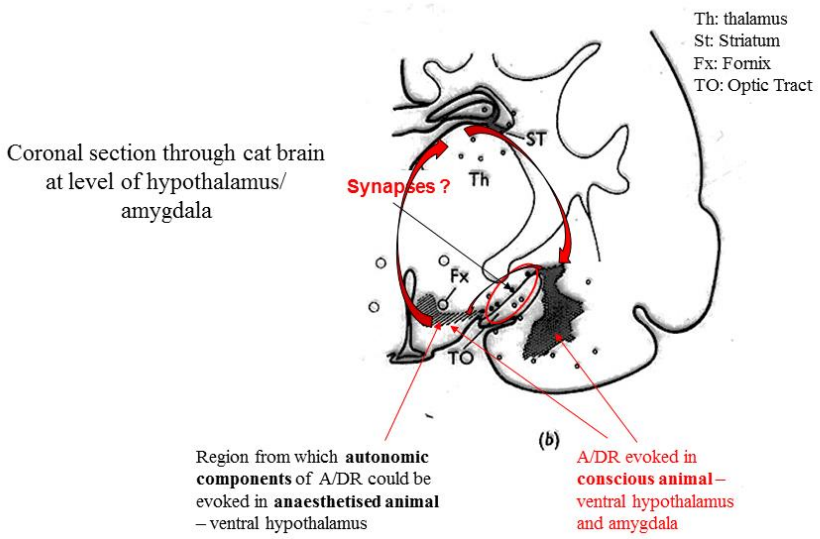


Figure 1.3 Coronal section through forebrain of cat showing defence areas of amygdala and hypothalamus as judged by electrical stimulation in conscious state. Modified from Hilton and Zbrozyna (1963).

In the 1980s, it was discovered that in cats anaesthetised with the steroid anaesthetic Alphaxalone-Alphadalone, the full cardiovascular response, including muscle vasodilatation, could be evoked by stimulation in the basal region of the amygdala (Timms, 1981), inferring that synapses between the amygdala and hypothalamus, are inhibited by conventional anaesthetics. Subsequently, al Maskati and Zbrozyna (1989a) demonstrated by using DLH in rats anaesthetised with alphaxalone-alphadalone that cell bodies of neurons which evoke the full pattern of the alerting response are localised in the basal nucleus of the amygdala (Figure 1.2). It was also demonstrated by Hilton and Marshall (1982) that in cats anaesthetised with alphaxalone-alphadalone, selective carotid chemoreceptor activation elicited the cardiovascular pattern of the alerting response. The muscle vasodilation elicited as a reflex by chemoreceptor stimulation, was generally sensitive to atropine and so was attributable at least in part, to sympathetic cholinergic nerves, but in some cats, it was attenuated by alpha-adrenergic

antagonists consistent with the results of Coote *et al.* (1973) and in others by beta-adrenoreceptor blockade implicating circulating adrenaline, as discussed below.

1.2.5 Role of Orexin neurons in integrating the alerting response.

The defence areas of the perifornical nucleus and dorsomedial hypothalamus contain the wake-promoting neuropeptides called Orexin/hypocretin (ORX) (de Lecea *et al.*, 1998). Because these neuropeptides are highly concentrated in the perifornical hypothalamus their role in the defence reaction has been investigated. Increased HR and respiration similar to that seen during the defence reaction occurred when the Orexin neurones were disinhibited by microinjection of the GABA antagonist, bicuculline methiodide into the perifornical area of rats (Dimicco & Abshire, 1987). Further, injection of GABA agonist (Muscimol) into this same region inhibited behavioural alerting evoked by natural stimuli (Lisa *et al.*, 1989). This suggested that neurons within the perifornical region that inhibit the Orexin neurones are tonically inhibited by neurones that release the excitatory neurotransmitter GABA, such that the defence reaction is not on-going at rest. This may be the cortical inhibitory influence on the defence response that is removed by decerebration. When there is an alerting stimulus, this pathway may be disinhibited or another overriding excitatory input to the defence areas is activated such that the alerting response occurs.

More recently, studies on Orexin-deficient and wild type mice showed that microinjection of the GABA-A receptor antagonist bicuculline into the perifornical region of hypothamalamus and amygdala, evokes the full pattern of alerting including increased ABP, HR, muscle vasodilation and increased respiration providing Orexin is present (Kuwaki, 2011). Further, Orexin-knockout mice showed smaller behavioural alerting, ABP and HR responses to bicuculline than wild type mice. Moreover,

bicuculline microinjected into the basal amygdala of wild type mice produced long-lasting dose-dependent cardiorespiratory excitation, whereas Orexin-knock out mice showed attenuated responses. Therefore, it can be deduced that Orexin neurons play a major role in mediating the defence response at the level of the amygdala and hypothalamus (see Figure 1.4).

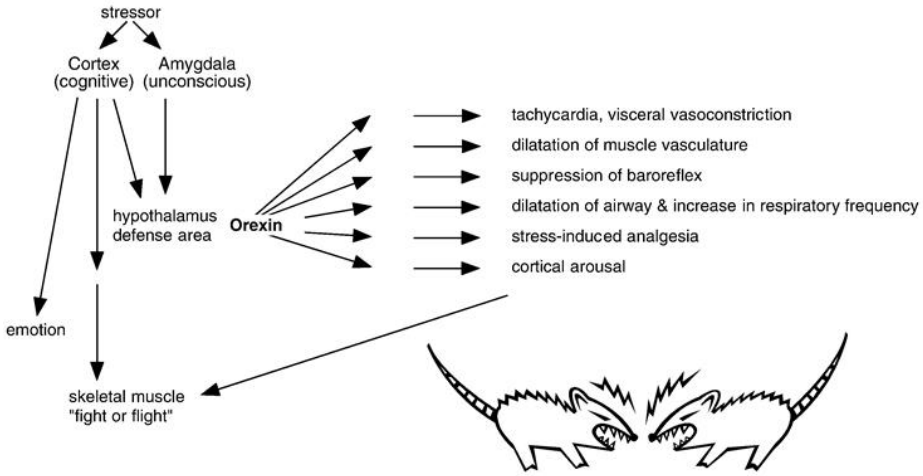


Figure 1.4 Role of orexin in defence response taken from Kuwaki (2011).

1.2.6 Inhibition of baroreceptor reflex during the alerting response

It is known that an increase in ABP stimulates arterial baroreceptors and reflexly attenuates sympathetic outflow to the heart and blood vessels while activating the parasympathetic outflow causing reduced HR. However, during the alerting response there is a simultaneous increase in ABP and HR, which suggests inhibition of the baroreceptor reflex. Coote *et al.* (1979) using anaesthetised cats and an isolated carotid sinus preparation showed that both the bradycardia and decrease in renal sympathetic nerve activity evoked by baroreceptor stimulation were inhibited or abolished during defence area stimulation. Further, Duan *et al.* (1994), using single cell recordings showed that inhibition of the baroreceptor reflex by hypothalamic defence area occurred

at the level of the nucleus tractus solitarius (NTS) within the medulla while Jordan *et al.* (1988) showed this inhibition is mediated by GABA.

Similarly, it was shown in man, by using pulse interval as an index of baroreceptor sensitivity (BRS) during phenylephrine injections that the cardiac BRS is inhibited during the mental stress induced by mental arithmetic, so contributing to the rise in ABP Sleight *et al.* (1978). Further, by using the sequence method to determine BRS, Steptoe and Sawada (1989) demonstrated reduced cardiac BRS during mental stress. Moreover, slope of the relationship between diastolic blood pressure (DBP) and muscle sympathetic nerve activity (MSNA) indicated that the vascular sympathetic component of the baroreflex is also blunted during mental stress. Interestingly, attenuation of the sympathetic BRS was evident during the initial 2 minutes, but not throughout a 5 minute period of mental stress evoked by the Stroop test (Durocher *et al.*, 2011). There is also evidence that the sympathetic baroreceptor reflex is re-set during mental stress such that the threshold is increased (Julien, 2009). Taken together these changes in the baroreflex during mental stress provide a basis for the role of blunted baroreceptor function and resetting in the aetiology of mental stress-induced hypertension.

1.3 Forearm vasodilation during the alerting response evoked by mental stress

1.3.1 Role of sympathetic nervous system (SNS)

As indicated above (section 1.2) evidence that sympathetic cholinergic dilator fibres are involved in the muscle vasodilatation of the altering response was provided in the 1960s. Whether such fibres are present in primates was doubted. Bulbring and Burn (1936), found but no pharmacological evidence for such nerves in rabbits or monkeys. Further, Bolme and Fuxe (1970) showed by using histochemistry that sympathetic

cholinergic nerves supply the intramuscular arterioles, but not the larger arteries of skeletal muscle in cats and dog, but found no histochemical evidence or pharmacological evidence in primates: atropine-sensitive muscle vasodilation occurring after adrenergic blockade of vasoconstrictor nerve activity in cats, dogs, fox, sheep and goats, but not in rat, badger, opossum, rat, hare or 5 different species of primates. Thus, it was concluded that mechanisms other than activation of sympathetic cholinergic fibres must play a key role in the muscle vasodilatation of the alerting response in these animals (Bolme *et al.*, 1970).

Turning to functional evidence in mediating muscle vasodilatation in humans, Barcroft *et al.* (1944), noted that during fainting, a response to emotional stimuli, forearm blood flow increased while ABP fell, implying forearm vasodilatation. On finding that when the arm was nerve-blocked, the forearm vasodilator response was smaller than in the intact forearm during fainting, they deduced the vasodilatation reflected an active dilator process mediated by sympathetic vasodilator nerves as found in cats. Accordingly, Barcroft and Edholm (1945) found that forearm vasodilation did not occur during fainting in surgically sympathectomised forearms. However, when Blair *et al.* (1959) conducted experiments on subjects who had been treated for Meniere's disease by unilateral removal of the stellate ganglion there was little forearm vasodilatation during mental stress in the chronically sympathectomised forearm. Further, Barcroft *et al.* (1960) showed that forearm vasodilation elicited by mental stress was not attenuated in chronically sympathectomised limbs, but it was attenuated in adrenalectomised subjects suggesting a role for adrenaline from adrenal gland. On the other hand, acute stellate ganglion block reduced the forearm vasodilation in 4 out of 5 subjects, suggesting a role for sympathetic nerve activity. Further, intravenous atropine attenuated the muscle

vasodilation in all subjects while local atropine attenuated vasodilation in 10 out 12 subjects (Barcroft *et al.*, 1960). Taken together these results are equivocal, but suggested that both sympathetic nerve fibres and adrenaline may mediate the muscle vasodilator response to mental stress in humans and implicate cholinergic fibres, or at least, a cholinergic mechanism. This evidence was summarised by Greenfield (1962).

Variability in the extent to which sympathetic nerve fibres contribute to the forearm vasodilator response has been reported since the 1950s. Thus, Blair *et al.* (1959) reported the expected forearm vasodilation of mental stress was absent in 9 out of 11 experiments on sympathectomised arms but in the remaining experiments, the increase in forearm blood flow was greater in the sympathectomised than intact limb. These results suggested dissociation between MSNA and forearm vasodilation in some subjects. Similarly, Barcroft *et al.* (1960) demonstrated a reduction in the forearm vasodilation to mental stress in 4 subjects after stellate ganglion blockade, but in one subject, the vasodilation increased and in all subjects, atropine attenuated the forearm vasodilation.

In an attempt to confirm the presence of sympathetic vasodilator fibres in humans, Abboud and Eckstein (1966) used cold as a stimulus to induce vasoconstriction, blocked the actions of vasoconstrictor substances released from sympathetic nerves by using a combination of the two sympatholytic agents (phentolamine and guanethidine), and followed this by inhibition of cholinergic receptors with atropine. They demonstrated that atropine-sensitive muscle vasodilation still occurred in response to mental stress following adrenergic blockade. This result is similar to that obtained in cats by Folkow *et al.* (1948) and so, Abboud and Eckstein (1966) suggested that both vasoconstrictor

and vasodilator sympathetic fibres innervate limb muscles in man. However, Bolme and Fuxe (1970), who used histochemical methods to stain for acetylcholinesterase on biopsy samples of muscle and found no evidence for sympathetic cholinergic nerves in man or monkeys, although they could demonstrate them in cats and dogs.

More recently, the issue has been investigated by recording MSNA by microneurography in the radial or peroneal nerves. The vasoconstrictor nerves were identified by tonic activity at rest with baroreceptor-induced rhythmicity. In one of the earliest studies on normotensive subjects, Delius *et al.* (1972) reported a *decrease* in the bursts recorded in forearm MSNA in association with the forearm vasodilation and increase in ABP induced by mental stress. In a subsequent study, Wallin and Sundlof (1982) reported cessation of leg MSNA during emotional fainting indicating the muscle vasodilatation reflects withdrawal of sympathetic vasoconstrictor tone; there was no evidence of fibres that showed increased activity that might have reflected activation of dilator fibres. On the other hand, Anderson *et al.* (1987b) reported that during emotional stress, there was an *increase* in leg MSNA, but a *no change* in forearm MSNA. However, *decrease* in leg MSNA during mental stress accompanied increased ABP and plasma adrenaline in young normotensive subjects (Matsukawa *et al.*, 1991). Further, Halliwill *et al.* (1997) demonstrated a *decrease* in forearm MSNA during mental stress that was accompanied by forearm vasodilatation, increased ABP and HR. They found no evidence of fibres whose activity increased during forearm vasodilatation and concluded the vasodilation was at least partly due to withdrawal of vasoconstrictor tone, not to activation of sympathetic dilator fibres.

Whether the sympathetic fibres are required at all for forearm vasodilatation to occur in response to emotional stress has also been questioned. Contrary to the evidence mentioned above, that subjects who had chronic sympathectomy, acute stellate ganglion block, or deep nerve block generally showed attenuated forearm vasodilatation during emotional stress (Blair *et al.*, 1959; Barcroft *et al.*, 1960)**.** More recently, Lindqvist *et al.* (1996a) demonstrated that axillary blockade of sympathetic fibres did not alter the forearm blood flow change during mental stress and concluded that forearm vasodilatation can occur in the absence of nervous control. Similarly, Halliwill *et al.* (1997) showed that the forearm dilatation evoked by the Stroop test was associated with sympathetic withdrawal, but persisted after acute sympathetic blockade, showing that sympathetic withdrawal alone could not explain it**.** In fact, in some subjects, the forearm vasodilatation was unchanged after alpha-adrenoreceptor blockade, but was sensitive to beta-adrenergic receptor blockade. Thus, they proposed that the mechanisms of mental stress-induced forearm vasodilation include circulating adrenaline acting on beta adrenergic receptors and sympathetic withdrawal, but not sympathetic vasodilator nerves (Halliwill *et al.*, 1997)**.**

In summary, in humans, it seems the muscle vasodilation that occurs during the alerting response is passive, mediated by withdrawal of MSNA in some subjects. However, whilst withdrawal of MSNA does not occur in all subjects, there is no microneurographic evidence of active vasodilation mediated by sympathetic dilator fibres. Indeed, the available evidence indicates that *non*-neural mechanisms play an important part in the muscle vasodilatation of the alerting response.

1.3.2 Role of adrenaline

As indicated above (section 1.2), in the early 1900s, it was demonstrated that venous effluent from cats subjected to emotional stress applied to intestinal strips caused smooth muscle relaxation, an action was attributed to adrenin (now referred to as adrenaline) (Cannon & De La Paz, 1911) and adrenaline was implicated in the tachycardia (Cannon & Britton, 1925). As noted in section 1.3.1, although cats have cholinergic sympathetic vasodilator fibres, Hilton and Marshall (1982) demonstrated that a later component of the muscle vasodilatation evoked by stimulation in the brain stem defence areas, or as a reflex by peripheral chemoreceptor stimulation was attenuated in some animals by beta-adrenergic blockade implicating adrenaline. Further, in rats that do not possess cholinergic vasodilator fibres, defence area stimulation elicited an initial phase of dilatation that was attenuated by alpha-adrenoreceptor blockade, and a later phase that was reduced by beta-adrenoreceptor blockade, or abolished by bilateral adrenalectomy, implicating adrenaline (Yardley & Hilton, 1987).

In man, the increased muscle blood flow to mental arithmetic similarly occurs in 2 phases; an immediate moderate increase abolished by sympathectomy and a delayed but marked increase abolished by adrenalectomy. Thus, it was proposed that the mental stress-induced vasodilation involved both sympathetic nerves and circulating adrenaline (Wilkins & Eichna, 1941; Glover *et al.*, 1962a). Further, Barcroft *et al.* (1960) demonstrated a residual vasodilation to mental stress after blocking vasodilation with atropine that was attenuated in adrenalectomised patients. Accordingly, Lindqvist *et al.* (1996b) showed that adrenaline is released during mental stress and when they

compared the forearm vasodilatation evoked by mental stress with that induced by exogenous adrenaline, they concluded that circulating adrenaline is responsible for 9-30% of the forearm response to mental stress (Lindqvist *et al.*, 1996b). Others have confirmed that mental stress increases plasma adrenaline levels in humans (Lindqvist *et al.*, 1996a; Lindqvist *et al.*, 1996b; Murakami *et al.*, 1996; Reims *et al.*, 2004).

Pharmacological evidence for the role of beta-adrenoceptors in the muscle vasodilation induced by mental stress has gradually accumulated by using beta-adrenergic receptor antagonists (Glover *et al.* (1962b); Freyschuss *et al.* (1988); Halliwill *et al.* (1997). Further, Lindqvist *et al.* (1997) demonstrated ~20% attenuation of the forearm vasodilatation when propranolol was given 4 minutes after mental stress commenced. Thus, the evidence indicates an adrenaline-mediated, beta adrenergic mechanism contributes to muscle vasodilator response to mental stress, but cannot fully explain it.

Stimulation of β-adrenergic receptors by adrenaline increases cyclic Adenosine monophosphate (cAMP) synthesis within vascular smooth muscle and thence causes smooth muscle relaxation and vasodilatation (Wallukat, 2002). Adrenaline also stimulates beta-adrenergic receptors on the endothelium and increases nitric oxide synthase (NOS) activity leading to synthesis of NO. Indeed, the NOS inhibitor, NG-Monomethyl-L-arginine (L-NMMA) inhibited the forearm vasodilator response to beta 2 selective agonist (Salbutamol) (Dawes *et al.*, 1997). Thus the finding that NOS inhibition attenuated mental stress-induced forearm vasodilatation may be explained at least in part by inhibition of an adrenaline-mediated, beta-adrenoceptor component (Cardillo *et al.*, 1997a). The contribution of NO to mental stress-induced muscle vasodilatation is discussed further below (section 1.3.5).

On the other hand, another study showed that NOS inhibition had no effect on forearm vasodilation induced by the beta-adrenoreceptor agonist Isoproterenol (Limberg *et al.*, 2016), but the non-selective cyclooxygenase (COX) inhibitor, Kerolac) accentuated it and co-infusion of NOS inhibitor and COX inhibitor abolished the vasodilation. These findings suggested that COX products blunt beta-adrenoceptor mediated vasodilation and that the COX pathway suppresses NOS activity (Limberg *et al.*, 2016). They also raise the question of whether or not adrenaline released during mental stress causes the release of vasodilator prostacyclin (PGI_2) from skeletal vascular endothelium, and therefore, whether the COX pathway contributes to the beta adrenoceptor component of stress-induced muscle vasodilation.

1.3.3 Role of acetylcholine (ACh)

As indicated above, many studies have documented a role for acetylcholine (ACh) in mental stress evoked muscle vasodilation in human subjects by using atropine, a muscarinic receptor blocker (Blair *et al.* (1959); (Barcroft *et al.*, 1960; Halliwill *et al.*, 1997). However the source of ACh during mental stress is debatable, for as indicated in section 1.3.1, the role of cholinergic sympathetic nerve fibres in humans, has been questioned due to lack of histochemical and neurophysiological evidence that such fibres exist (Bolme & Fuxe, 1967; Wallin & Sundlof, 1982).

If ACh is not released from sympathetic fibres, then the obvious questions are where is it released from and how does it act? With respect to the last question, Dietz *et al.* (1994) demonstrated that pre-treatment of one arm with atropine attenuated mental stress-induced vasodilation in that arm by about 50%. Further, when atropine and L-NMMA were co-administered there was about 60% attenuation of vasodilation, suggesting that the action of ACh in mental stress is mediated by nitric oxide (NO).

This accords with the finding mentioned above (Cardillo *et al.*, 1997a), that local nitric oxide synthase (NOS) inhibition greatly attenuated the forearm vasodilator response to mental stress.

Regarding the source of ACh in muscle during mental stress, there is evidence that cultured endothelial cells release ACh in response to increased flow (Milner *et al.*, 1990). More recently, ACh was shown to be released from the endothelium of intact mesenteric arteries when shear stress was increased by increasing perfusion rate. The flow-activated release of ACh is non-vesicular and occurs via cation-transporters; on release, ACh evoked vasodilatation by stimulating the synthesis and release of NO (Wilson et al 2016a). This evidence agreed with the earlier finding of Martin *et al.* (1996) who demonstrated that the efflux from perfused endothelium-intact canine coronary rings relaxed a pre-contracted femoral artery section when the flow rate was increased: this relaxation was blocked by the NOS inhibitor L-NMMA and attenuated by atropine and or acetylcholinesterase which rapidly degrades ACh (Martin *et al.*, 1996; Wilson *et al.*, 2016).

The mechanism just described seems likely to contribute to the muscle vasodilatation of mental stress and would explain the evidence that ACh contributes to the dilatation and the evidence that the ACh- component is NO-dependent (Dietz *et al.*, 1994), without the need to implicate sympathetic cholinergic nerves. Nevertheless, since the combination of L-NMMA and atropine attenuated the forearm vasodilatation to mental stress more than atropine alone, NO may also contribute to the dilatation in a manner that is independent of ACh. This issue is discussed in the next section.

1.3.4 Role of nitric oxide (NO).

The vasodilatation evoked in the fore and hind limb of cats following stimulation of the hypothalamic defence area was blunted by the NOS inhibitor, N omega-Nitro-L-arginine methyl ester hydrochloride (L-NAME). Moreover, co-administration of L-NAME and atropine almost abolished the vasodilation. The attenuating effect was found greater in the forelimb than hind limb (Komine *et al.*, 2003). Similar findings were made in rats: hind limb vasodilation evoked by hypothalamic stimulation was attenuated by beta-adrenoceptor blockade or bilateral adrenalectomy and the remaining vasodilation was abolished by L-NAME (Ferreira-Neto *et al.*, 2005).

Regarding the stimulus for release of NO during mental stress, Pike *et al.* (2009) reported a striking correlation between HR and forearm vascular conductance (FVC) following mental stress and suggested the relationship is likely to be mediated mechanical stimulation on the forearm vasculature. Taken together with the results of Martin et al., (1996) and Wilson et al., (2016) it seems that mechanical stimulation via shear stress and release of NO is likely to be one of the key factors that contribute to the forearm vasodilatation during mental stress.

There has also been consideration of whether NO could be released from nerve fibres during the alerting response. There is evidence of release of NO from nitridergic nerves causing vasodilation in canine cerebral blood vessels (Toda & Okamura, 1991). Moreover, markers of NOS were found in sympathetic vasodilator fibres that supply hind limb muscles of rats. In addition, in conscious rats, the muscle vasodilatation of the defence response was abolished by bretylium, which prevents release of catecholamines from sympathetic terminals plus L-NAME, implicating release of NO from nitridergic nerves in a species with no sympathetic cholinergic nerves (Davisson *et al.*, 1994).

Although nitridergic nerves have not been demonstrated human skeletal muscles, recent studies, Seddon *et al.* (2008) showed that the selective neuronal NOS (nNOS) blocker, S-methyl-l-thiocitrulline (SMTC) reduced basal flow in forearm and the vasodilation evoked by mental stress. The question then arises as to whether the nNOS involved in mental stress is located in endothelium, nerve fibres or skeletal muscle. In animals, while endothelial NOS (eNOS) was found throughout the vasculature, nNOS was present in capillary endothelium and on skeletal muscle sarcolemma (Segal *et al.*, 1999). Thus, there is no reason to suppose that nNOS is not present in capillary endothelium in humans and certainly, in humans, there is evidence of nNOS in skeletal muscle sarcolemma: children who lack dystrophin, the membrane protein to which nNOS binds, display blunting of the ability to attenuate exercise-induced vasoconstriction in skeletal muscle (Brenman *et al.*, 1995; Chang *et al.*, 1996; Sander *et al.*, 2000). Therefore, the source of nNOS generated NO at rest and during mental stress could be from skeletal muscle and/or endothelium, (Seddon *et al.*, 2008).

As indicated above, in addition to shear stress-mediated release of NO by ACh, adrenaline acting via beta adrenoceptors could also release NO (Dawes *et al.*, 1997), while prostaglandins (PGs) may also play a role in NO mediated vasodilatation (Limberg *et al.*, 2016) as discussed in the following section.

1.3.5 Potential role of prostaglandins (PGs)

Mental stress activates the sympathetic nerve supply to renal, splanchnic and cutaneous circulations causing vasoconstriction and, in some human subjects, increases MSNA (see Section 1.3.1). Sympathetic stimulation was shown to release PGs in rabbit mesenteric artery (Pipili & Poyser, 1981). Further, in isolated rabbit heart, the beta-

adrenoreceptor agonist isoproterenol stimulated release of PGs (Weis & Malik, 1985). There is also evidence of the formation and release of PGI_2 in isolated animal heart, kidney and cultured coronary endothelial cells by beta adrenoreceptor stimulation (Malik, 1988; Ruan *et al.,* 1997). In addition, as indicated above (section 1.2.3), COX inhibition accentuated beta agonist-mediated forearm vasodilation and combination of NOS and COX inhibition abolished the vasodilation, implying that both NO and PGs play a role in beta-adrenoceptor mediated vasodilation (Limberg *et al.,* 2016). As discussed above, the alerting response involves an increase in sympathetic activity and alpha-adrenoreceptor mediated vasoconstriction in kidneys, skin and splanchnic circulation, an increase or decrease in MSNA and beta-adrenoreceptor mediated vasodilation in skeletal muscle. Thus, PGs may modulate the effects of changes in alpha-mediated vasoconstrictor tone and/or beta adrenoceptor-mediated vasodilation.

Considering first whether PGs may modulate the alpha-adrenoreceptor mediated vasoconstriction of the alerting response, there is published evidence consistent with this possibility. In humans, immersion of one foot in cold water (the cold pressor test), which activates sympathetic fibres to produce alpha-mediated vasoconstriction in the forearm and leg, caused release of PGI_2 and PGE as well as noradrenaline and adrenaline into venous blood (Neri Serneri *et al.,* 1990). When the cold immersion was repeated 5 times, the vasoconstrictor response gradually increased, while the efflux of PGs gradually decreased and noradrenaline efflux did not change. By contrast, after COX inhibition the vasoconstrictor responses were enhanced and did not change on repetition (Neri Serneri *et al.,* 1983). They speculated that PGI_2 acts post-junctionally to attenuate alpha-mediated sympathetic vasoconstriction, while PGE_2 acts pre-junctionally to inhibit the release of noradrenaline.

Whether PGs might modulate the vasoconstrictor and dilator components of the alerting response is of particular interest because during immersion of one foot in cold water vasodilation occurred in the contralateral calf and forearm of 6 out of 14 subjects, but 4 subjects showed vasoconstriction in both limbs. On repetition of the stimulus, the vasodilator responses diminished in most subjects and the vasoconstrictor responses diminished in forearm but not calf (Zbrozyna & Westwood, 1990). The authors concluded these changes represented central neural habituation of the alerting response and did not consider peripheral modulation of the vascular responses in limb muscle, or indeed, of vasoconstriction in skin or visceral circulations. There is evidence that sympathetic withdrawal contributes to cold-induced vasodilation (Flouris & Cheung, 2009). Given the evidence for release of PGs during the cold pressor response, there could also be PG release during alerting response.

Whether PGs play a role in the muscle vasodilatation of the alerting response has not been tested either. This is of interest because as indicated above adrenaline has been implicated in the forearm vasodilatation and PGs have been implicated in adrenaline-induced forearm vasodilatation (Limberg *et al.*, 2016). Further, both ACh and NO have been implicated in the forearm vasodilatation and PGs have implicated in ACh- and NO-induced vasodilatation in human forearm (Kamper *et al.*, 2002). Thus, ACh-induced vasodilation was attenuated by COX inhibition whereas, combined COX and NOS inhibition did not further attenuate the vasodilation, indicating that PGs are partly responsible for ACh-induced vasodilation and that PGs and NO act inter-dependently, rather than independently of one another (see Figure 1.5).

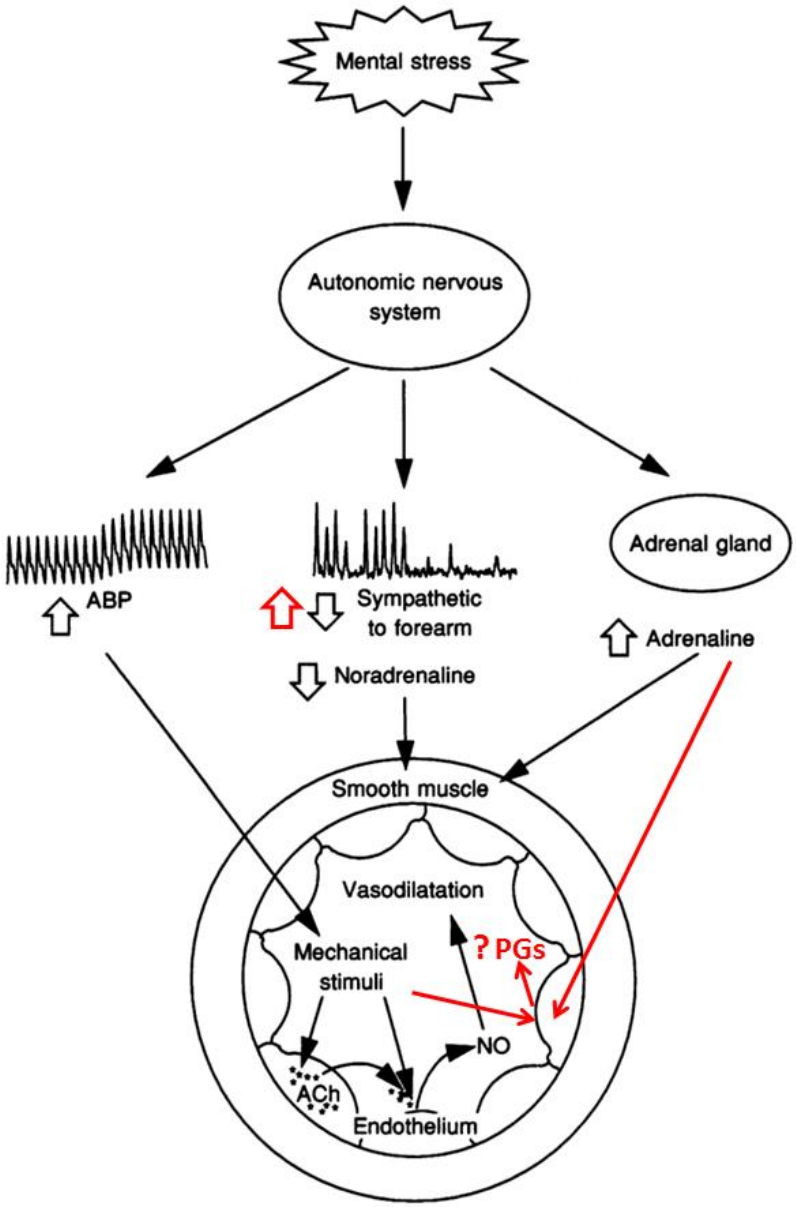


Figure 1.5: The proposed mechanisms of forearm vasodilation evoked by mental stress – adapted from Halliwill *et al.* (1997). It is known that mental stress elicits forearm vasodilatation through increased ABP, increased adrenaline secretion from the adrenal gland with or without sympathetic withdrawal or diminished noradrenaline release. Increases in systemic ABP and forearm flow may stimulate NO from endothelium. We proposed that PGs may be released from the endothelium.

1.4 Modification to alerting response evoked by mental stress

The alerting response can be modulated such that the whole response or individual components of the response wane or persist, or are even accentuated on repetition. (Hilton, 1982). Such modulation of the alerting response occurs within the central nervous system, but exactly how, has not been determined. These issues are briefly reviewed below.

Repeated electrical stimulation of the hypothalamic defence area evoked a stereotyped alerting response even when repeated daily, over days or weeks (Abrahams *et al.*, 1964). However, on repeated exposure to stressful stimuli, the alerting response changes such that in many individuals, repetition leads to gradual reduction in one or more components of the alerting response, or more unusually, one of more components increases with repetition. The former phenomenon is referred to as habituation and the latter, as sensitization (see Schmid *et al.* (2015). For example, in cats, sound or touch elicited the full pattern of alerting response, but, the muscle vasodilatation waned with repetition in some individuals, or even reversed to vasoconstriction. The tachycardic response persisted longer, but waned and reversed to bradycardia, while the pressor response persisted much longer suggesting persistence of vasoconstrictor components of the response (Martin *et al.*, 1976). Similarly in dogs, habituation of the alerting response occurred on repetition of a sound stimulus or repeated confrontations of a cat with the dog, and in some animals, hind limb vasodilatation reversed to vasoconstriction which persisted, or renal vasoconstriction persisted on repetition (Abrahams *et al.*, 1964; Martin *et al.*, 1976; Seal & Zbrozyna, 1978). Similarly, in baboons, prolonged renal vasoconstriction occurred in response to the sight of a snake and in some animals,

repetition of the stimulus led to habituation of renal vasoconstriction, but in others renal vasoconstriction persisted or sensitized (Zbrozyna, 1976).

Comparable patterns of habituation and sensitization have been reported in humans. Repeated immersion of one hand in cold water caused habituation of ABP and HR responses (Glaser *et al.*, 1959), as did repetition of a pure sound, the rate of habituation being faster in men than women and in non-anxious, than anxious subjects (McGuiness, 1973). Further, immersion of one foot in cold water elicited contralateral calf vasodilation in most subjects and vasoconstriction in the remainder, while pure sound elicited calf vasodilation in all subjects. Habituation of both the calf vasodilatation and vasoconstriction occurred during a single session in most individuals, but in some, the vasoconstriction, took up to 4 days to habituate (Zbrozyna & Krebbel, 1985; Zbrozyna & Westwood, 1988; Zbrozyna & Westwood, 1990). In a study on women, (Edwards *et al.*, 1998) the forearm vasodilation to sound did not habituate within a single session, but did habituate when the stimulus was repeated on 3 alternate days. Further, habituation of calf vasodilatation to pure sound or cold immersion occurred during 6 repeated sessions on 6 days, but in some who showed vasodilation during the first session, vasoconstriction occurred on the 6th day (Zbrozyna & Westwood, 1988).

Considering the pathways that might mediate habituation and sensitization, there is evidence of reciprocal connections between the prefrontal cortex and subcortical structures in regions that include the defence areas such as the hypothalamus, the amygdala, the midbrain periaqueductal grey, and pons (Cummings, 1993; Buchanan *et al.*, 1994). The orbitofrontal cortex has been especially implicated in autonomic function and emotional behaviour, electroencephalography studies indicating that

during mental stress, activation of the prefrontal and temporal cortex occurs together with the activation of the autonomic nervous system (Wang *et al.*, 2016).

Functionally, on the basis of lesion and ablation of the frontal cortex in primates and humans, frontal leucotomy and lobotomy was used extensively in psychiatric patients in Europe and the USA to treat aggressive behaviour and extreme anxiety (Robison *et al.*, 2012). Stimulation of the ipsilateral lateral prefrontal cortex in cats *inhibited* defensive/aggressive behaviour evoked from the hypothalamus, whereas stimulation of contralateral cortex did not (Siegel *et al.*, 1975). Further, stimulation at sites within the lateral or anterior sigmoid gyri and anterior part of orbital gyrus in the prefrontal cortex had no effect on cardiovascular baselines, but either facilitated the cardiovascular components of the defence response elicited from amygdala, or inhibited the response pattern elicited from the hypothalamus. It was therefore proposed that sites within the prefrontal cortex facilitated or inhibited the defence response by acting at synapses in the defence pathway between the amygdala and hypothalamus (Timms, 1977).

On the other hand, electrical or chemical (DLH) stimulation of the medial prefrontal cortex of rats for a few seconds before and during stimulation of the hypothalamic or amygdaloid defence areas attenuated or abolished the cardiovascular and behavioural components of the alerting response evoked from both these regions (al Maskati & Zbrozyna, 1989b). Thus, they concluded the pathway from the cortex they had revealed inhibited the defence response at a site more caudal than the hypothalamus, probably at the synapses in the region of RVLM where the ventral pathway synapses with neurones that mediate individual components of the alerting response (section 1.2.3).

Interestingly, Glaser and Griffin (1962) had already demonstrated that bilateral lesions of the frontal cortex in rats inhibited habituation of tachycardia evoked by repeated immersion of the tail in cold water, while electrical stimulation of the prefrontal cortex accelerated habituation. Similarly, monkeys with orbitofrontal cortex lesions showed impaired habituation (Butter, 1964). Thus, it seems the inhibitory areas identified by Timms (1977) and/or al Maskati and Zbrozyna (1989b), may be involved in setting up the process of habituation of the cardiovascular pattern of the alerting response that occurs on repetition of alerting stimuli. Interaction at the level of the amygdala-hypothalamic pathway would be expected to affect all components of the alerting response (see Timms (1977), whereas interaction at the level of RVLM could explain how individual components of the alerting response may be differentially modulated. The facilitatory area of the prefrontal cortex identified by Timms (1977) and which was presumably the target of the frontal lobotomy/leucotomy neurosurgery, may be involved in heightening anxiety and/or the phenomenon of sensitization.

1.5 Cardiovascular responses evoked by mental stress and hypertension

There is evidence that exaggerated responsiveness to the cold pressor test was reproducible after many years and preceded the development of hypertension (Barnett *et al.*, 1963). Likewise, exaggerated blood pressure in response to mental stress preceded development of coronary atherosclerosis by 7 years (Jennings *et al.*, 2004). Further, in a large study on > 4100 young people aged 18-30 years and followed up 14 years later, exaggerated pressor responses to mental stress as young adults, predicted development of hypertension (Matthews *et al.*, 2004). Moreover, a meta-analysis of almost 40 longitudinal studies showed that heightened pressor responsiveness to laboratory stressors and poor recovery from stress was predictive of hypertension and increased

carotid intima-media thickness (Chida & Steptoe, 2010). This evidence agrees with proposal that those who develop hypertension are more likely to be individuals in whom the alerting response fails to habituate when exposed repeatedly to stressors than those who show habituation (Folkow, 1991). Indeed, in view of the discussion above, this proposal can be elaborated to suggest that those who show sensitization of pressor responses on repetition of stress stimuli and/or show primary vasoconstriction in forearm and/or calf in response to mental stress are at greater risk of developing hypertension (Stock *et al.*, 1979).

Taken together these ideas raise obvious questions on the factors that determine how individuals respond to mental stress. The factors that seem most likely to be involved are genetic factors, endothelial dilator function/dysfunction and the level of stress experienced in everyday life. These issues are discussed below.

1.51 Familial hypertension and development of hypertension.

Young people who have one or more hypertensive parents (FH+) have a 3-6-fold greater risk of developing hypertension than those with normotensive parents (FH-) (Lloyd-Jones *et al.*, 2004). Thus, studies of these individuals as young adults are of particular interest in understanding how hypertension develops and how the pattern of cardiovascular response to mental stress changes over time. For example, teenage FH+ exhibited greater ABP increases in response to mental stress compared to FH- teenagers and the increased ABP and HR was sustained after cessation of the stressor (Falkner *et al.*, 1979). Likewise, 14 year-old FH+ showed greater ABP, HR and forearm vasodilator responses (Anderson *et al.*, 1987a), larger increases in MSNA and release of the endothelium-derived vasoconstrictor, endothelin, than FH- (Noll *et al.*, 1996). Similarly, young FH+ (aged 18-40 years) had raised resting ABP that was still within the

normotensive range, but greater increases in MSNA in response to mental stress than FH- (Fonkoue *et al.*, 2016). Moreover, young FH+ in their 20s with borderline hypertension showed higher ABP, HR, MSNA and plasma catecholamines at rest and greater increases in these variables than age-matched normotensive FH- in response to mental arithmetic and cold pressor test (Matsukawa *et al.*, 1991).

Further, in studies on individuals whose familial status was not considered, teenagers with borderline hypertension showed larger and longer-lasting increases in ABP and HR, but smaller forearm vasodilatation during mental stress than normotensive adolescents (Santangelo *et al.*, 1989). Moreover, smaller forearm vasodilatation and greater increases in ABP and peroneal MSNA in response to stress were reported in prehypertensive men in their 20s (Schwartz *et al.*, 2011). Similarly, prehypertensive men and women (aged 34 years) showed smaller forearm vasodilatation, but greater increases in HR and ABP than normotensives during mental stress (Medeiros *et al.*, 2011). On the other hand, young hypertensive men (aged 19-30 years) showed exaggerated pressor responses and either greater calf vasodilatation, or vasoconstriction to cold water immersion than normotensives, but no habituation of pressor, or vascular responses when the stimulus was repeated on each of six days, whereas the normotensives habituated (Zbrozyna & Krebbel, 1985).

Thus, the evidence suggests that in young FH+ adults who are normotensive, the full pattern of the cardiovascular responses to acute mental stress, including the increase in leg MSNA and muscle vasodilatation is exaggerated and more prolonged than in FH-. However, in early hypertensives, whether or not they are FH+, the forearm vasodilatation to acute mental stress is generally blunted, the pressor components are exaggerated and habituation of their alerting response on repetition is impaired, so

increasing their likelihood of experiencing repeated episodes of hypertension in everyday life. In agreement with this proposal, 18-22 year old FH+ who showed exaggerated pressor responses to mental stress showed the highest risk of developing hypertension over a 10 year follow-up period, but even amongst FH-, a higher daily life stress score was associated with higher risk of hypertension (Light *et al.*, 1999). Thus, it seems that stress reactivity in early adulthood is a meaningful predictor of future hypertension when considered in combination with FH+/FH- status and life stress exposure.

1.5.2 Role of endothelial response to mental stress in development of hypertension.

As indicated in sections 1.3.2-1.3.5, there is evidence the endothelium plays an important part in the muscle vasodilator component of the alerting response by generating NO and PGs in response to shear stress and adrenaline. Endothelial dysfunction is associated with blunted endothelium-dependent dilatation and eventually with endothelium-dependent vasoconstriction/vascular spasm by release of vasoconstrictor substances (Widlansky *et al.*, 2003). Importantly, there is evidence firstly that mental stress can evoke endothelial dysfunction, and secondly, that endothelial dysfunction develops very early during development of hypertension as well as being a characteristic feature of established arterial hypertension (Vanhoutte, 1989; Widlansky *et al.*, 2003; Brunner *et al.*, 2005).

Considering first the evidence that mental stress evokes endothelial dysfunction. Mental stress induced in healthy subjects (aged 40-60 years) by requiring them to defend themselves against a false accusation of shoplifting caused depression of flow-meditated dilation (FMD) of the brachial artery, an endothelium-dependent dilatation,

which lasted at least 1.5 hours, recovering in ~4 hours (Ghiadoni *et al.*, 2000). Further, in young normotensive adults (20-25 years), mental arithmetic blunted methacholine-induced forearm vasodilation; Methacholine is a muscarinic receptor agonist like ACh and is similarly endothelium-dependent (Sarabi & Lind, 2001).

The mechanism for this phenomenon may involve the stress-induced rise in ABP, for acute hypertension induced in young adults by intravenous adrenaline or noradrenaline, blunted endothelium dependent vasodilation induced by methacholine and endothelium-independent vasodilation induced by sodium nitroprusside (Millgard & Lind, 1998). Similarly, acute increases in ABP for 1-5 minutes attenuated large artery endothelial function for about 2.5 hours in dogs (Lamping & Dole, 1987) and short periods of elevated ABP due to weight lifting impaired FMD in humans (Jurva *et al.*, 2006).

Alternatively, since acute increases in perfusion pressure in isolated mouse carotid arteries impaired ACh-induced dilatation while increasing superoxide production and nicotinamide adenine dinucleotide phosphate (NADPH) activity, these findings are consistent with the notion that hypertension attenuates endothelial dilatation by inducing oxidative stress and reducing NO availability (Dharmashankar & Widlansky, 2010). On the other hand, mental stress induced by the Stroop test caused impairment of FMD that was prevented by an endothelin (ET)-1A receptor antagonist (Spieker *et al.*, 2002), while the impaired FMD and blunted baroreflex sensitivity induced by emotional stress was associated with increased plasma cortisol and inhibited by pre-treatment with an inhibitor of cortisol production (Broadley *et al.*, 2005). Cortisol increases ET production by endothelial cells and ET antagonises the synthesis and action of NO (Kanse *et al.*, 1991; McEniery & Wilkinson, 2002). Moreover, acute administration of

cortisol impaired the cardiac component of the baroreflex induced by mental stress while accentuating the calf vasoconstriction and attenuating the forearm vasodilatation (Adlan *et al.*, 2018). Thus, raised cortisol during mental stress may also impair endothelium-dependent dilatation, and contributes to impaired regulation of ABP during mental stress, in a manner that would predispose to hypertension.

Turning to the evidence that endothelial dysfunction begins very early during, development of hypertension: FMD was blunted in symptom-free children with family history of hypercholesterolaemia, and adults (18-57 years) who were smokers, groups at high risk of hypertension and atherosclerosis (Celermajer *et al.*, 1992) and was impaired in children with white coat hypertension (Jurko *et al.*, 2018). Further, ACh-induced forearm vasodilatation was impaired relative to age-matched normotensives in non-treated, early hypertensives and in FH+ (aged 18-35 years) and was associated with a blunted effect of NOS inhibition. Moreover, infusion of L-arginine enhanced ACh- and isoproterenol-induced forearm vasodilatation (Taddei *et al.*, 1996; Schlaich *et al.*, 2004). Thus, it is a reasonable hypothesis that both the ACh- and adrenaline-mediated components of the forearm vasodilator response to mental stress are impaired by reduced NO availability in those at risk of hypertension: this has not yet been tested.

Hypertensive patients show blunted forearm vasodilator response to mental stress relative to normotensives and NOS inhibition attenuated vasodilator response to mental stress in normotensives only, suggesting loss of the NO contribution in the hypertensives (Cardillo *et al.*, 1998a). Moreover, Khan *et al.* (2015) demonstrated that selective nNOS inhibition blunted the forearm vasodilator response to mental stress in normotensives, but not in hypertensives, indicating that the contribution of nNOS is blunted in hypertensives. In addition, alpha adrenoreceptor blockade augmented the

forearm vasodilator response to mental stress in hypertensive subjects only. This indicated the impaired NO-mediated dilatation to mental stress in hypertension may reflect exaggerated sympathetically mediated vasoconstriction (Khan *et al.*, 2015).

Taken together, the evidence discussed in this section, suggests that repeated exposure of young individuals to environmental or mental stress would itself lead to endothelial dysfunction and blunting of the forearm vasodilator response to acute mental stress. Those with familial risk of CVD would be most at risk and the outward manifestation would be blunted forearm vasodilatation, or even vasoconstriction to acute mental stress reflecting impaired contribution of NO and/or vasodilator PGs.

1.6 Ethnic differences in cardiovascular responses evoked by mental stress

1.6.1 Differences between White Europeans and Blacks Africans

In the majority of studies on humans discussed above no mention was made of their ethnicity. But, there is evidence that BAs have increased prevalence of hypertension relative to WEs and evidence that their cardiovascular responses to mental stress are abnormal relative to WEs, at least in early middle age. This is reviewed below.

BAs have higher resting ABP than WEs (Akinkugbe *et al.*, 1977; Liu *et al.*, 1989; Profant & Dimsdale, 1999) and the prevalence of hypertension is higher in BAs than in WEs (NCD Risk Factor Collaboration, 2017). Exaggerated vasoconstriction or impaired vasodilatation, leads to increased peripheral resistance and ABP. Therefore, it is not surprising that both have been reported in BAs relative to WEs.

Thus, forearm vasodilator responses induced by intra-arterial infusion of beta-adrenoceptor agonist (Isoproterenol), NO donor (Sodium nitroprusside, SNP) and the muscarinic receptor agonist (Methacholine) were blunted in BAs (aged 30±2.2years)

relative to WEs (aged 28±3.2 years) (Stein *et al.*, 1997). Similarly, forearm vasodilator responses to isoproterenol were blunted in BAs relative to WEs (aged 31 years) (Lang *et al.*, 1995). BAs in their 30s to 40s also showed blunted FMD and blunted forearm vasodilation to NO donor (Nitroglycerin) relative to WEs (Campia *et al.*, 2002b). These results suggest that even in young adult BAs, endothelium-dependent dilatation is blunted, at least partly as a consequence of impaired cGMP signalling, the 2nd messenger for NO. In middle-aged BAs (aged 41-45years), dilator responses to isoproterenol, SNP and ACh were blunted relative to WEs (aged 50-54 years) and dilatation induced by isoproterenol was attenuated in BAs relative to WEs after NOS inhibition (Cardillo *et al.*, 1999). These results suggest that both the cGMP pathway and the cAMP pathway that mediates the action of beta-adrenoceptor stimulation, are impaired, at least in middle aged BAs (Cardillo *et al.*, 1999).

Regarding vasoconstrictor responses, BAs (aged 30 years) showed greater forearm vasoconstriction to infusion of the alpha-adrenoceptor agonist phenylephrine than age-matched WEs. Moreover, forearm vasoconstrictor responses evoked by the cold pressor test were much greater in BAs than WEs even though noradrenaline spillover, an index of sympathetic nerve activity was similar in the two ethnicities (Stein *et al.*, 2000). Thus, the authors argued the exaggerated vasoconstrictor responses to the cold pressor test in the BAs mainly reflected exaggerated responsiveness to noradrenaline (Stein *et al.*, 2000).

Others reported that resting MSNA was similar in BAs and WEs (aged 24 years), but BAs showed greater increases in MSNA and ABP during the cold pressor test than WEs (Calhoun *et al.*, 1993). Moreover, young WE and BA men and women showed similar increases in MSNA in response to lower body negative pressure (LBNP), but the

evoked forearm vasoconstrictor responses were greater in BAs, suggesting enhanced transduction in BAs (Ray & Monahan, 2002). Further, even under resting conditions, young BA and WE men (aged 20-23 years) had similar levels of MSNA, but BAs showed substantially larger increases in leg vascular resistance, in total peripheral resistance and ABP in response to spontaneous increases in MSNA bursts than WEs (Vranish *et al.*, 2018).

Taken together, these findings indicate that BAs show exaggerated vasoconstriction relative to WEs, both as a consequence of increased responsiveness to noradrenaline and greater responsiveness to a given level of MSNA. Given that MSNA is increased during mental stress at least in some individuals (section 1.3.1) and given endothelium-dependent and beta-adrenoceptor mediated dilatation is blunted in BAs relative to WEs (see above), it would be expected the forearm vasodilator response to mental stress would be impaired and the pressor (vasoconstrictor components) augmented in BAs. To date, relatively few studies have investigated this issue.

In middle-aged men and women (aged 40-51 years) forearm vasodilatation evoked by mental arithmetic was blunted in BAs relative to WEs and the vasodilatation was attenuated by NOS inhibition in the WEs only, suggesting the NO contribution was blunted in BAs (Cardillo *et al.*, 1998b). Further, middle aged BA men and women showed larger increases in ABP and HR, and greater inhibition of the baroreflex than WEs during a cold pressor test, (Reimann *et al.*, 2012). In younger individuals, BA men and women (aged 24-28 years) and BA teenage boys showed greater increases in ABP in response to cold pressor tests, than WEs (Treiber *et al.*, 1990; Calhoun *et al.*, 1993) and the evoked increases in MSNA were greater in BAs (Calhoun *et al.*, 1993). Similarly, in response to video games of graded stress with financial incentives, BA

children and teenagers (aged 6-18 years), showed greater increases in ABP and HR than age matched WEs (Murphy *et al.*, 1986; Murphy *et al.*, 1988).

By contrast, mental arithmetic evoked *smaller* increases in ABP and HR in young BA men (aged 18-22 years) (Anderson *et al.*, 1988; Falkner & Kushner, 1989; Fonkoue *et al.*, 2018) and in middle aged BA men (Morell *et al.*, 1988) relative to age matched WEs. Moreover, Anderson *et al.* (1988) reported the forearm vasodilator responses to mental arithmetic were similar in BA and WE men, while Fonkoue *et al.* (2018) demonstrated the increases in MSNA were *smaller* in BAs than WEs, and young BA men showed smaller increases in ABP than WEs during an aversive reaction time task (Fredrikson, 1986). None of these studies indicated whether the BA or WE individuals were FH+ or FH-. But, whilst young BA and WE men showed similar increases in ABP and HR to mental arithmetic (Johnson *et al.*, 1992), or 3 different stressors (mental arithmetic, Stroop test, mirror tracing; (Terrell & Manuck, 1996) in all of these studies, the pressor responses were greater in FH+ than FH-.

It is very difficult to make any firm proposals about the reasons for the major differences between pressor responses evoked by mental stress in WEs and BAs in the studies discussed above because most recorded only ABP and HR, rather than the full alerting response including changes in forearm vasculature and a circulation such as skin that would be expected to show vasoconstriction (section 1.2.1). The variety of different stressors used in different studies adds to the difficulty in comparing the findings, but the additional problem is that it is not clear whether the stimuli were novel to the subjects and therefore whether the pattern of response habituated, or sensitised on repetition. Further, very little has been done to compare the contributions of NO and PGs to the individual components of the alerting response in WEs and BAs. All of these

issues are relevant to the question of whether exaggerated responsiveness to mental stress in young adult BAs might predispose them to develop hypertension.

1.6.2 Differences between White Europeans and South Asians

The prevalence of cardiovascular diseases is higher among South Asians than WEs (Boon *et al.*, 2015). A previous systematic review showed that in UK, there was heterogeneity between SAs who were originally from different countries within the Indian subcontinent, although many studies reported higher prevalence of hypertension in SAs than WEs (Agyemang & Bhopal, 2002). However, a recent meta-analysis from the same authors demonstrated that SA adults in the UK have lower ABP than WEs, but SA children have higher ABP than WE children (Battu *et al.*, 2018). This apparent change in epidemiological profile could be associated with the effect of a western lifestyle, and/or an increased risk of metabolic disorder in Asian children living in affluent western countries, for the majority of SA children are 2^{nd} or 3^{rd} generation migrants to the UK, whereas older SA adults are 1^{st} or 2^{nd} generation immigrants. Irrespective, it suggests the future prevalence of hypertension amongst SA adults will be much higher. In this regard, it should be noted that the prevalence of hypertension among Indians living in South Asia progressively increased from 1.2 to 4% in the 1950s, to 5% in the 1960s, doubling to 12 to 15% in the 1990s and exceeding 30% by 2008 alongside an increase in affluence amongst some sectors of the population (Gupta, 2004; Gupta *et al.*, 2018).

Although the issue of the prevalence of hypertension in SAs is in flux, SAs certainly have higher prevalence of insulin resistance and visceral adiposity and show greater predisposition to developing Type 2 Diabetes mellitus than WEs (McKeigue *et al.*, 1991). Further, SAs have higher risk of CHD than WEs and other Asian groups such as

Chinese Asians (Enas *et al.*, 1996; Lee *et al.*, 2001). The higher prevalence of diabetes and metabolic syndrome is correlated with the higher risk of CHD in SAs living in the UK, the mortality from CHD is higher among adult SAs than other ethnic groups (Balarajan, 1991) and SA women have higher risk than SA men (Balarajan, 1991). Moreover, SA children in the UK as young as 8-11yrs already show a predisposition to insulin resistance as evidenced by higher insulin, although their indices of adiposity were similar to those of WE children (Whincup *et al.*, 2002).

Given the established evidence of endothelial dysfunction in diabetes and the high prevalence of diabetes in SAs, it is not surprising that many studies in which cardiovascular responses have been compared in SAs and WEs have focussed on endothelial function. Relatively few have considered sympathetically-mediated responses, or the cardiovascular components of the alerting response in relation to the increased prevalence of CVD in SAs. The evidence available is reviewed below.

Considering endothelial function, a study done in the USA, showed FMD was attenuated in a mixed gender group of non-diabetic, normotensive SAs (aged 20-60 years) relative to matched WEs. The SAs also showed higher plasma insulin and lower glucose uptake than WEs (Raji *et al.*, 2004). Similarly, FMD was blunted in healthy 20-40 year old SAs relative to WEs, in spite of similar body adiposity and resting ABP. Further, ACh-induced forearm vasodilation was blunted in SAs, NOS inhibition had less effect on the dilatation than in WEs and they showed fewer circulating endothelial progenitor cells at rest (Murphy *et al.*, 2007) and following exercise (Murphy *et al.*, 2007; Cubbon *et al.*, 2010). Similarly, young SA men in their 20s showed blunted reactive hyperaemia in forearm relative to young WE men (Ormshaw *et al.*, 2018): reactive hyperaemia is the increase in blood flow that occurs following the release of

vascular occlusion and is a standard test of endothelial function at microvascular level (Anderson *et al.*, 2011), Moreover, cutaneous ACh-induced vasodilation, but not reactive hyperaemia was blunted in young SA men relative to WE men (Hirst & Marshall, 2018), and maximal cutaneous vasodilator response to heat as well as that evoked during reactive hyperaemia were blunted in young SAs relative to WEs (Petrofsky *et al.*, 2012).

Taken together, these findings indicate that young, healthy normotensive SAs have blunted endothelium-dependent vasodilation in conduit arteries, forearm and cutaneous circulation that may be attributed to reduced NO availability; they also have diminished ability to replace damaged endothelium with progenitor endothelial cells relative to WEs. These points are especially relevant, given impaired peripheral endothelial function correlates well coronary endothelial function preceding overt atherosclerosis, and are highly predictive for CHD and myocardial ischaemia (Kuvin *et al.*, 2001).

Interestingly, among SAs, as amongst WEs, FH+ have increased risk of developing hypertension (Ranasinghe *et al.*, 2015) and, in young SA FH+ (aged 11-18 years) FMD was impaired relative to FH- SA, but brachial artery vasodilator responses to NO donor (Glyceryltrinitrate) were similar (Bharani *et al.*, 2011). These results indicate that young SA FH+s are at particularly high risk of endothelial dysfunction relative to SA FH-s. This agrees with the findings of Hirst and Marshall (2018) who performed a study on groups of young FH- and FH+ WE and SA men. Both ACh-evoked dilatation and reactive hyperaemia evoked in cutaneous circulation were attenuated by COX inhibition in FH-, but not FH+, suggesting that blunting of endothelium-dependent dilatation in both SA and WE FH+ young men is attributable to reduced contribution of PGs.

Turning to sympathetic activity, there is evidence that raised sympathetic activity is predictive of CHD (Alderman *et al.*, 1990), which as mentioned above, is of higher prevalence in SAs than WEs. Accordingly, SA men and women (aged 35-75 years) reported greater, chronic psychological stress than matched WE men and women, as well as higher prevalence of CHD (Williams *et al.*, 2009). Moreover, hostility, an index of anxiety/anger towards life stressors was positively correlated with CHD in both SAs and WEs, but associated with impaired autonomic regulation of heart rate variability (HRV) in SA men, in a direction that suggested increased sympathetic relative to vagal balance (Williams *et al.*, 2011). This finding agreed with the outcome of a study on SAs (aged 37 years) which showed a progressive reduction in vagal and increase in sympathetic determination of HRV and an increase in resting HR in prehypertensive and hypertensive SAs relative to normotensive SAs (Pal *et al.*, 2011). There have been few studies on vascular sympathetic activity in SAs, but a recent study on young WE and SA men and women in Australia (aged 22 years) showed that resting MSNA was similar in the two ethnicities, but showed a stronger relationship between plasma lipid species that are associated with obesity in SAs than WEs even though the SAs had lower BMI than WEs (Eikelis *et al.*, 2017). The authors proposed this association may herald greater end-organ damage and cardiometabolic disease in SAs.

Regarding cardiovascular responses to environmental stressors in SAs, few studies have been performed. A study on young SA men and women (aged 18-30 years) living in the US showed smaller increases in total peripheral resistance and in ABP in response to mental arithmetic, than American WEs (Stoney *et al.*, 2002). Another study showed that SA men and women living in Singapore showed smaller pressor responses to mental arithmetic than Chinese or Malay Asians groups (Kaur & Bishop, 2013). Further, SAs

in the US showed smaller ABP and HR responses to a range of different mental stressors than American WEs (Shen *et al.*, 2004). None of these studies recorded changes in regional vascular resistance that contributed to the changes in total peripheral resistance. Thus, it is impossible to deduce whether or not the vasoconstrictor and vasodilator components of the alerting response differed between WEs and SAs.

The most comprehensive study to date was performed by Ormshaw *et al.* (2018) who recorded ABP, HR and forearm blood flow and vascular resistance responses evoked by repetition of 5 pure sounds at intervals of 5-10 minutes in young healthy WE and SA men (aged 18-24 years). Considered as mean responses across each ethnic group, there were no differences between WEs and SAs for the ABP and HR responses, although WE men showed maintained forearm vasodilation, and SA men showed initial forearm vasodilation followed by vasoconstriction. These responses were consistent across the 5 sounds, indicating no obvious habituation or sensitisation in either ethnicity. However, when responses within individuals were considered and they were separated into subgroups according to whether they showed forearm dilatation or constriction in response to the first sound, a much higher proportion of SAs than WEs showed forearm constriction to the first stimulus (2:1) and whatever the direction of response to the first stimulus this persisted for the 5 stimuli (Ormshaw *et al.*, 2018). Given that forearm vasoconstriction was associated with larger increases in ABP, (Ormshaw *et al.*, 2018) argued that more young SA men than WE men would be at greater risk of repeated pressor responses to stressors in everyday life and the associated risk of future hypertension. However, there are limitations to this study for no attempt was made to test whether the lower preponderance of forearm vasodilatation to mental stress in SAs reflected smaller contribution of endothelium-dependent NO or PGs, or greater

increases in MSNA in SAs, and correspondingly, no attempt was made to follow blood flow changes in a circulation such as skin, which would be expected to show vasoconstriction in response to stress. It should also be noted that their proposals applied only to young SA and WE men.

1.6.3 Gender-related differences in cardiovascular responses to mental stress.

The majority of studies discussed above were performed on men; some were performed in mixed groups of men and women, but very few tested whether there were differences between the genders. Further, several of the studies were performed on subjects of wide age range, therefore spanning the menopause for women. These are important points for ABP is higher in men than women before menopausal; this sex difference begins during adolescence and continues during the premenopausal period. However, post-menopause, women have similar or higher ABP than men due to loss of cardioprotective effect of oestrogen (Reckelhoff, 2001). Further, these gender-related differences are present within both WE and SA populations, and in both ethnicities, the prevalence of hypertension is greater in men than women (de Munter *et al.*, 2011). On the other hand, there is evidence that the prevalence of CHD is higher in SA women than men, that women develop CHD at an earlier age than men and that the mortality rate from CHD is higher in SA women than in women from other ethnicities, both in the Indian subcontinent and in those who have migrated to other countries (Gooneratne, 2013). This gender-related disparity has been associated with conventional risk factors such as higher rates of central obesity, diabetes and lower physical activity, but the pronounced increase in CHD amongst women in urbanised areas raises the possibility that socioeconomic stress also contributes (Gooneratne, 2013). Since the prevalence of CHD in SA women begins to increase at 40-45 years of age, pre-, or peri-menopausally,

it seems likely that markers of future CVD may be present in much younger SA women. This issue has not been investigated.

The picture amongst BAs is also not straightforward. Among American BAs aged 14-18 years, 41% were already pre-hypertensive and males showed higher ABP and greater prevalence of prehypertension than females (Covelli *et al.*, 2012). However, a study across 4 African countries reported higher prevalence of hypertension among adult BA men than women in some regions and greater prevalence among women in other regions, while in the remainder, there were no sex-related differences (Gómez-Olivé *et al.*, 2017). There was also no sex difference in the prevalence of hypertension between adult BA men and women in Ghana (Cappuccio & Miller, 2016). On the contrary, in the US, BA women showed higher prevalence of hypertension even premenopausally at the age of 40 years, than BA men and WE women and men (Geronimus *et al.*, 2007). Moreover, in the US between 1988-1994 and 1999-2002, there was a progressive increase in prevalence of hypertension in all ethnic groups, but between 1999 and 2002, BA men showed higher prevalence of hypertension than WE men (39% vs 27%), while BA women showed higher prevalence than both WE women (44% vs 30%) *and* BA men (44% vs 39%) (Hertz *et al.*, 2005). A review of worldwide prevalence of hypertension for the period 1980-2003 also showed that BA women had a higher prevalence than BA men (36% vs 31%) (Kearney *et al.*, 2004). Of particular relevance in the present context, the higher prevalence of hypertension in BA women in the US was associated with psychological stress, attributable at least in part, to racism and the stress inherent in being economic and care providers (Geronimus *et al.*, 2007). Indeed, the findings of these studies raise the possibility that BA women living in

westernised or urban environments may experience early aging of the endothelium, or earlier loss, or even absence of the cardioprotective effect of oestrogen.

It is known that oestrogen stimulates formation of NO by genomic effects, upregulating expression of eNOS, and by non-genomic effects, increasing NOS activation (Florian *et al.*, 2004; Nevzati *et al.*, 2015; Usselman *et al.*, 2016). Accordingly, women have been shown to have greater basal NO bioavailability than men (Kneale *et al.*, 1997). In addition, oestrogen stimulates formation of PGs by increasing expression of COX and PG synthase (Sobrino *et al.*, 2010). Further, oestrogen has dilator effects on vascular smooth muscle, by inhibiting entry of calcium (Crews & Khalil, 1999) and increasing hyperpolarization by actions on potassium channels in (Wellman *et al.*, 1996). Consistent with these effects, endothelium-dependent dilatation FMD was augmented during the high oestrogen phase of the menstrual cycle in women (Hashimoto *et al.*, 1995). Further, FMD declines earlier with age in men than premenopausal women, but after menopause this effect is lost (Celermajer *et al.*, 1994). Indeed, oestrogen supplementation increased FMD and plasma NO metabolites in post-menopausal women, but not age-matched men (Kawano *et al.*, 1997).

The forearm vasodilator response to the beta 2-adrenoreceptor agonist (Albuterol), was *greater* during the high oestrogen phase of the menstrual cycle in young women relative to men, but was similarly attenuated by NOS inhibition in both genders (Kneale *et al.*, 2000). Further, graded doses of noradrenaline induced blunted forearm vasoconstriction in women relative to men, and the beta-receptor antagonist propranolol enhanced these vasoconstrictor responses in women but not men (Kneale *et al.*, 2000). Similarly, beta-adrenoreceptor blockade increased noradrenaline-induced forearm vasoconstriction in pre-menopausal women, but not in age-matched men, or post-menopausal women (Hart

et al., 2011). Taken together these results indicate the blunting of noradrenaline-induced vasoconstriction in women is oestrogen-dependent and mediated by beta 2 adrenergic receptors acting in an NO-independent manner, probably via receptors on vascular smooth muscle.

In some contrast, Limberg *et al.* (2016) reported no difference between young men and women in forearm vasodilator responses to a non-selective beta adrenoreceptor agonist, Isoproterenol. Moreover, these responses were not affected by NOS inhibition but were augmented by COX inhibition and this effect was lost when NOS and COX inhibition were combined (Limberg *et al.*, 2016). They concluded that β-adrenergic mediated vasodilation is similar in men and women, but in both sexes, beta 2 adrenoreceptor-induced stimulation of COX restrains NO synthesis and release.

Seen against this background it might be expected that women have less sympathetic vasoconstrictor tone contributing to ABP at rest and that the alpha-adenoreceptor mediated vasoconstrictor components of the response to mental stress would be blunted, whereas the limb vasodilator component would be augmented by enhanced beta-adrenoreceptor mediated and endothelium-dependent signalling. In agreement with these ideas, resting SBP and plasma catecholamine concentrations were lower in women than men (Christou *et al.*, 2005). Using mental arithmetic as a mental stress, the evoked changes in MSNA, ABP, HR and forearm vasodilatation were similar in men and women (aged 20-42 years) (Jones *et al.*, 1996), but, women showed greater calf vasodilatation (Yang *et al.*, 2013). Similarly during visual orientation tasks forearm and calf vasodilator responses were greater in women, more men than women showing calf vasoconstriction than women (Butt *et al.*, 1999), although the magnitude of the vascular responses evoked by the Stroop test were similar in men and women (Butt *et al.*, 1999).

More detailed analyses of the pressor and HR responses to mental stress suggest further gender-related differences. Thus, changes in MSNA, perceived stress and HR in response to mental arithmetic did not differ between men and women in their 20s, but men showed greater increases in ABP (Carter & Ray, 2009). Similarly, in a meta-analysis, young women showed higher resting HR and greater HR response to stressors than young men, but the men showed higher resting ABP and greater increases in ABP and plasma catecholamines (Stoney *et al.,* 1987). Others showed greater ABP changes in men and greater HR changes in women in response to stress (Forsman & Lindblad, 1983; Dimsdale *et al.*, 1990; McAdoo *et al.*, 1990; Allen *et al.*, 1993), which has led to men being tagged as "vascular reactors and women being tagged cardiac reactors" (Allen *et al.,* 1993). Consistent with this proposal, alpha adrenergic blockade had a smaller blunting effect on the ABP change evoked by mental stress in women than men (Sudhir *et al.*, 1997).

However, these studies are by no means conclusive for none of them indicated whether the male or female subjects were FH+ or FH-. This is important because young normotensive FH+ men show greater increases in ABP during mental stress than FH- men (Jorgensen & Houston, 1981), but there has been no study on FH+/FH- women. Further, none of them indicated the ethnicity of the subjects. Given the studies were mostly performed in the US and ethnicity was not mentioned, it seems likely most were performed on WEs, but whether or not this is the case, there has been no study to date comparing the pattern of cardiovascular responses evoked by mental stress in men and women of different ethnicities.

1.7 General aims and hypotheses

In view of the discussion above, the general aim of the project was to compare the cardiovascular responses evoked by environmental stress between WEs and BAs and between WEs and SAs with particular focus on the forearm vasodilator component of the response and including in each ethnicity, comparison between men and women, as well as to determine the role of PGs in these responses. Since there is evidence shown above that the vasodilator response is endothelium dependent, reactive hyperaemia was also used as a test stimulus, since this is a standard test of microvascular dilator function.

Hypotheses of the experimental studies were as follows

- Chapter 3: BAs and SAs will each show initial blunted muscle vasodilatation but exaggerated pressor responses to sound (alerting) stimulus on first exposure to sound relative to WEs. However, with repetition of 5 sounds, forearm vasodilator responses will habituate in BAs and SAs, but pressor responses may sensitize. Further, reactive hyperaemia will be smaller in BAs and in SAs than WEs,
- Chapter 4: Over three alternate days of repetition of sounds, pressor responses to repeated sound will fail to habituate in BAs.
- Chapter 5: Forearm vasodilatation to mental stress and reactive hyperaemia will be attenuated by cyclooxygenase inhibition in each ethnicity, but BAs and SAs who have NO impairment will show a greater contribution of PGs relative to WEs.

  Using these outcomes as foundation, 3 more studies were performed

(i) To investigate differences in forearm vasodilator responses evoked by rhythmic forearm contractions between BAs and WEs, and between SAs and WEs and the contribution of PGs (Chapter 6).

(ii) Compare ambulatory recordings of ABP and HR between WEs and BAs and to consider the relationship between nocturnal dipping of blood pressure with responses to environmental stress (Chapter 7).

(iii) Compare cutaneous reactive hyperaemia and acetylcholine mediated vasodilation between WEs and BAs and to investigate the role contribution of PGs (Chapter 8).

The background to these studies is provided in the relevant chapters.

CHAPTER 2

General Methods And Materials

2.1 Study participants

The participants were mainly young men and women aged 18-26 years. All participants were non-smoking, had body mass index (BMI) of 18-29.9kg/m^2 and were recreationally active. None had a history of smoking, current pregnancy or history of hypertension or cardiovascular disorder. The participants were recruited mainly from the University of Birmingham via word of mouth, advertisement in College newsletters, University-wide research advertisement site, Society meetings, distribution of flyers at fairs and in public spaces on the University of Birmingham campus.

Participants were recruited from three ethnic groups; White Europeans (WEs), Black Africans (BAs) and South Asians (SAs). Ethnicity was self-reported and was defined as advised by the Office for National Statistics (https: // www. ons .gov .uk /methodology/classificationsandstandards/measuringequality/ethnicgroupnationalidentityandreligion). Participants were considered to be eligible if both parents were of the same ethnic origin, non-smoking, not obese and free from any cardiovascular or respiratory disease.

Criteria for exclusion from participation include: presence of any known cardiovascular or respiratory diseases, pregnancy, mixed racial ancestry and positive history of smoking. All participants were requested to abstain from consuming any non-steroidal anti-inflammatory drugs (e.g. Aspirin, Neurofen, etc) and alcohol as well as to refrain from vigorous exercise for at least 24 hours prior to each visit. They were asked not to consume any .caffeinated drinks (tea, coffee, Red Bull, etc) or heavy meals for at least 12 hours prior to each visit. The female participants were requested to attend experimental sessions within first 7 days of starting menstruation, when the female

hormones were low in order to minimize the effects of raised oestrogen and progesterone which are known to affect vascular function (Srinivasa & Marshall, 2011). The time experiments started was controlled for each participant who took part in experiments repeated on separate days.

All studies were approved by the University of Birmingham's Ethics Committee (see Appendix 1). Each participant was given a study information sheet and the procedures were verbally explained. All those who took part gave informed consent, as confirmed by signature on a consent form (Appendix 2); they were also made aware of their right to withdraw from the study at any time. Each participant completed a general questionnaire which contained questions on medical history, parental medical history, intake of caffeinated drinks, fruits intake and exercise (Appendix 3). In addition, they completed the Cohen's perceived stress scale which is a well-established method for assessing perceived stress in everyday life and has a good internal reliability (Cohen *et al.*, 1983) and a purpose-designed salt intake questionnaire (see Appendices 4 and 5).

2.2 General experimental condition

Experiments were performed in a quiet room. The room was arranged with the equipment out of view of the subject in order to minimize distractions and anticipatory stress. The subject reclined in semi- recumbent position on a couch with both arms supported at heart level with the aid of foam pads as shown in Figure 2.1.

2.3 Measurements

2.3.1 Anthropometric measurements

Each subject's anthropometric measurement was taken. Briefly, height was taken with a stadiometer and recorded in meters (m). Weight was taken with light clothing on and

recorded in kilograms (kg). Waist circumference was taken at level of the umbilicus in centimeters (cm). Forearm circumference was taken at the widest part of the forearm below the elbow in centimeters (cm).

2.3.2 Arterial Blood pressure (ABP)

Arterial blood pressure was measured using 3 devices; automated blood pressure monitor, automatically calibrating beat to beat finger blood pressure monitor (Finapres) and ambulatory blood pressure monitor (ABPM). These different methods were used at different times during the study, as indicated below.

Baseline ABP recordings

Upon arrival at the laboratory, the subject rested semi-reclined for a period of rest of 10-15 minutes (min) following which 3 ABP readings were taken at 1-2 min interval using an automated blood pressure monitor (OMRON M4, Omron Healthcare, Ltd, UK). The OMRON blood pressure monitor uses the oscillometric method of blood pressure (BP) measurement. The automated blood pressure monitor consists of an inflatable cuff, the manometer, and a mechanism for inflation that is operated electrically.

In order to record BP, an appropriately sized cuff was wrapped around the upper arm at the level of the heart. Cuff inflation was triggered manually by pressing a button. The monitor then inflates to ~20 mmHg above systolic blood pressure (SBP) and deflation of the cuff is automatically triggered at rate of 4 mmHg per second (s). When the pressure falls to just below the SBP, turbulent blood flow through the narrow lumen of the brachial artery creates vibrations, which increase in amplitude during the pulse pressure and more or less cease when pressure fell below diastolic blood pressure (DBP). The point of maximal oscillation corresponds to MABP. Using algorithms, the value of mean ABP (MABP) would be used to determine the systolic and diastolic

pressures (SBP, DBP). These vibrations were sensed and converted into digital readings. The frequency of the pulses in the vibrations is used to calculate the heart rate (HR). BP measured in the laboratory was categorized as normotension (BP < 120/80 mmHg), prehypertension (BP = 120-139/80-89 mmHg), and hypertension (BP ≥ 140/90 mmHg) (Collier & Landram, 2012; Gupta *et al.*, 2013; Odunaiya *et al.*, 2015).

Ambulatory blood pressure monitoring

In Chapter 7, an ambulatory blood pressure monitoring device (Model 90217A-1, Spacelabs Healthcare Ltd, Hertford, UK) was used to record BP over a period of 24 hours while the subject conducted their normal daily activities. The ambulatory blood pressure monitor (ABPM) uses the oscillometric method of BP measurement. The ABPM consists of a battery-operated monitoring device, a washable inflatable cuff and a removable bladder. The device was placed in a pouch, which was fastened around the waist with aid of a belt, or placed in a pocket. The device was generally connected to an inflatable cuff wrapped around the non-dominant arm. However, before fixing the ABPM on the subject, blood pressure was recorded in both arms in sitting position following 10-15 minutes of rest. This was to determine if there was inter-arm BP difference. If there was an inter-arm difference in SBP exceeding 20 mmHg, the cuff was wrapped on the arm with higher BP. Before attaching to the subject, the monitor was initialized on a computer with Spacelabs Software with the subject's details. The monitor was set to record blood pressure at 30 minutes interval during day-time and once per hour during night-time. Day-time was set as 7am till 11pm, while night-time was from 11pm until 7am the next morning. The subject was instructed to remain stationary or be seated and not to talk during each measurement with arms maintained at heart level. Each subject was given a diary to record their activities at the time of

measurements as well as sleep and wake times. At the end of a 24 hour period, the recordings were uploaded onto the software on the computer. The output provided day-time (12 hour), night-time (12 hour) and 24-hour averages with standard deviation (SD) for SBP, DBP, pulse pressure (PP), MABP with HR as well as percentage nocturnal BP dipping (see original record of ABPM data summary page, Figure 2.2). Based on the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) guideline, hypertension was categorized day-time BP of >135/85 mmHg (Williams et al., 2018).

Beat to beat ABP measurement

During experimental studies in the laboratory, beat to beat ABP was continuously recorded throughout each experiment by using a Finapres monitor (Ohmeda Model 2300, Englewood, USA): Finapres is an acronym for FINger Arterial PRESsure. The device consists of an inflatable finger cuff with photo electric (infra-red) plethysmograph, a servo-pressure controlling unit located in a small black wrist unit and a monitoring unit. The cuff and wrist unit are connected to the main unit which contains the air pump and computer. The wrist unit is fixed by Velcro strap to the back of the hand and connected to the finger cuff by air hose tubing and the cable connector. The wrist unit contains an air pressure control valve, a pressure transducer, fast servo-controlled pressuring system and electronics for the infrared photoplethysmograph in the finger cuff. The Finapres uses infrared light transmission to measure arterial pressure in the finger based on the volume clamp technique that was described by Penaz (Boehmer, 1987; Wesseling, 1996; Imholz *et al.*, 1998).

To record ABP, a finger cuff of appropriate size was wrapped around the middle phalanx of the middle finger with the hand maintained at heart level on layers of foam.

The output of the Finapres was displayed on the monitor as SBP, DBP, pulse rate (PR) and mean ABP. The Finapres was connected to the Powerlab 8SP (AD instruments), and the output was displayed digitally as waveform using Labchart version 7 (AD instruments).

2.3.3 Heart rate (HR)

Heart rate (HR) in beats per minute (bpm) was computed from the Finapres ABP recording using the cyclic measurement option and rate determination function on Lab chart Version 7 (AD instruments). HR was also determined by electrocardiography (ECG) using ECG-Bio amp (AD instruments), just in case the HR determination from the ABP recording failed. To this end, bipolar Lead II, which records the difference in potential between the right arm and left leg, with the left leg serving as the positive pole was used. Generally, three electrodes were attached to the right wrist, right leg and the left leg. However, during the forearm exercise experiments (Chapter 6), the electrodes were attached to the right shoulder, the right lower trunk and left lower trunk. The ECG waveform was digitally recorded on the computer through PowerLab 8-SP and HR determined with the Labchart version 7 software (AD instrument).

2.3.4 Total forearm blood flow (FBF)

To determine the FBF, venous occlusion plethymography (VOP) technique was used (Whitney, 1953; Greenfield *et al.*, 1963). This technique aims to arrest venous return from the arm, by using a cuff inflated to supravenous pressure but subdiastolic pressure of 50 mmHg for a few seconds without interrupting arterial inflow. When venous outflow from the arm is occluded and arterial inflow into the arm continues, there is an increase in the forearm volume due to accumulation of blood mainly in the venous vessels. The initial rate of swelling during venous occlusion corresponds to the apparent

rate of arterial inflow (Greenfield *et al.*, 1963). Although this methodology was first described almost a century ago, it is still recognized as being the most reliable method for accurately recording absolute blood flow in the forearm (Joyner *et al.*, 2001; Wilkinson & Webb, 2001; Junejo *et al.*, 2019). The VOP measurements have been shown to be highly reproducible and more reproducible than Doppler ultrasound (Pallares *et al.*, 1994; Thijssen *et al.*, 2005). Further, there is substantial evidence reviewed by Wilkinson and others (Joyner *et al.*, 2001; Wilkinson & Webb, 2001) and recently (Junejo *et al.*, 2019) showing that venous occlusion plethysmography is highly reliable for recording absolute blood flow at rest and in response to various stimuli in different groups of individuals.

An electrically calibrating plethysmograph device (EC6 Plethysmograph, Hokanson Inc., USA) was used to measure FBF. An appropriately sized indium-gallium filled silastic tube strain-gauge was placed onto the widest part of the forearm just below the elbow. The left forearm was used during mental stress experiments (Chapter 3, 4 and 5 using protocol 1), while the dominant forearm was used in protocol 2 during the rhythmic hand grip experiments. The limb of interest was placed on foam pad, positioned to avoid the silastic tube coming into contact with the foam pad and maintained at heart level. A small sphygmomanometer cuff (6.5 cm wide) was wrapped around the wrist so that it could be manually inflated to a supra-arterial pressure of approximately 200 mmHg for 1 minute before measurement of FBF. This ensured exclusion of the hand circulation from any measurements obtained (Higashi, 2015). A large cuff (10.5 cm), which was connected to the rapid cuff inflator, was wrapped around the upper arm of the same limb.

In each experiment, prior to any measurement of FBF, calibration of the plethysmograph was performed at rest. Calibration was achieved by deflecting the small lever on the plethysmograph to achieve a deflection of 1%. The deflection resulted in stretching of the silastic tubing of the strain gauge and changed the resistance of the strain gauge to produce an upward calibration deflection on the digital output, which is equivalent to 1% change in forearm volume: this was recorded in volts. As shown in Figure 2.4, three calibration deflections were obtained, the average calibration value (c) was extracted from digital output using the Lab chart software.

For each measurement of FBF, the wrist cuff was inflated to ~200 mmHg and ~1 min later the upper arm cuff was inflated to ~50 mmHg, using the rapid inflation system (AG101 Cuff Inflator Air Source and E20 Rapid Cuff Inflator Hokanson Inc., USA) (Wythe *et al.*, 2015; Junejo *et al.*, 2019). The upper arm cuff was kept inflated for 7s to prevent forearm venous outflow and then deflated for 8s; at this time the upper arm cuff could be re-inflated again to allow another measurement of FBF. The times at which FBF was measured in different studies is indicated below.

Once the upper arm cuff was inflated, the change in forearm volume following inflow of arterial blood stretched the silastic tubing and the change in resistance was recorded by the plethysmograph. The output was digitally displayed through the Powerlab 8 SP-AD instrument as an upward slope on the computer. The portion of the slope over the first 2 heart beats corresponded to the initial slope ("b") of the increase in the forearm circumference after inflation of the arm cuff (Junejo *et al.*, 2019). The value "b" was extracted with the Lab Chart software using the "average slope" function (see Figure

2.3) and was used in determining the rate of inflow of blood into the forearm (FBF) as ml/100 ml of tissue/min, according to the formula:

FBF (ml/100 ml of tissue/min) = (2b/ c) x 60.

Where b = gradient of the initial slope of the plethymogram (mV/min).

c = value of 1% calibration deflection (mV), as described above.

2.3.4.1 Baseline blood flow measurement

FBF was recorded at baseline by using VOP over 15s cycles, the wrist cuff having been inflated to 200 mmHg 1 min before. Each cycle consisted of inflation of the upper cuff for 7s and deflation for 8s. Thus, 4 cycles of recordings were taken within 1 min (Wilkinson & Webb, 2001). These were averaged to give a single mean value for baseline FBF (see Figure 2.5 for an original tracing).

2.3.4.2 Reactive hyperaemia

To induce reactive hyperaemia, the upper arm cuff was inflated to 200 mmHg and kept inflated for a period of 2 min. Then, 10s before the end of the 2 min period, the wrist cuff was inflated to 200 mmHg, to exclude hand circulation. At the end of 2 min of arterial occlusion, the upper arm cuff was deflated and as rapidly as possible (within 0-5s) and was re-inflated to 50 mmHg to perform VOP and record FBF. FBF was measured using 15s cycles for the first 1min and over 30 s cycles for the 2nd min post occlusion, such that recordings were done at 0-5s (peak), 15s, 30s, 45s, 60s, 90s and 120s. Following this, both upper arm and wrist cuffs were deflated (see Figure 2.5 for original tracing).

2.3.4.3 Exercise hyperaemia

To induce exercise hyperaemia, rhythmic handgrip exercise was performed over a period of 2 min using hand dynamometer (Lafayette 70718, Lafayette Instruments Company, UK), with 1s contraction and 1s relaxation to the beat of a metronome. The dynamometer was connected to a visual display and also to the Powerlab for digital display of the voltage.

To record post exercise FBF, 10s before the end of the 2 min period, the wrist cuff was inflated to 200 mmHg, to exclude hand circulation and at the end of 2 min, the upper cuff was inflated to 50 mmHg to record FBF within 0-5 s after cessation of exercise, for the peak FBF. Other recordings of FBF were then made at 30s, 60s, 90s and 120s, and subsequently at each minute until 7 min post exercise (Junejo, 2017) (see Figure 2.6 for original tracing).

2.3.4.4 FBF during alerting response to mental stress

To evoke alerting response, sound stimulus was applied through headphones using the MAICO MA40 audiometer. 5 pure sounds of 100 dB, 2 kHz was applied for 30s each through headphones at randomised intervals between 5 and 10 min to avoid conditioning the subject (Edwards *et al.*, 1998). Repeated sounds of these characteristics have been used in previous studies of the cardiovascular response to mental stress in various groups of human subjects and in particular, have been used to investigate the phenomena of habituation and sensitisation (Zbrozyna & Westwood, 1988; Edwards *et al.*, 1998; Ormshaw *et al.*, 2018). During the sound stimuli, FBF recordings were made at 5s, 15s, and 30s. To do this, the wrist cuff was inflated to 200 mmHg immediately the 1st sound followed by inflation of the upper cuff to 50 mmHg at 5s to allow the first measurement of FBF, followed by deflation. Two further measurements of FBF were

made at 15s, and at 30s. At 37s both cuffs were deflated. The same process was repeated for the other 4 sounds (see Figure 2.7 for an original tracing). Following each sound, the subject was asked to rate how stressful they found the sound on a scale of 0-10. A maximum score of 10 represented the most stressful sound and a minimum score of 1 was used for the least stressful sound. This method of measuring discomfort during the sound has been used previously in a similar protocol (Edwards et al., 1998).

2.3.5 Digital and Forearm Cutaneous Red Cell Flux

Digital and forearm cutaneous red cell flux was measured by using Laser Doppler fluxmetry (LDF). LDF is a noninvasive method that enables continuous, noninvasive assessment of perfusion in the microcirculation of the skin, uninfluenced by blood flow in the underlying skeletal muscle (Saumet *et al.*, 1988).

In the present study, during mental stress experiments, a dual channel Laser Doppler equipment (VMS-LDF2, Moor Instruments Ltd, UK) and 2 laser probes were used to measure cutaneous red cell flux. One laser probe was attached to the theca eminence by using a double sided adhesive disc, to record digital cutaneous red cell flux (DCRF) and another laser probe was attached in the same manner to the volar aspect of the forearm, to measure forearm cutaneous red cell flux (FCRF).

The technique relies on low power light of a single frequency (monochromatic) being conducted through the probes to the tissue. The light is scattered within the tissue, some being absorbed and only a small fraction is scattered back from moving red blood cells. The light undergoes a change in wavelength when it hits the moving blood cells. This is referred to as the Doppler shift. The change in wavelength is directly related to the number and velocity of the moving red cells. The Doppler shifts results in a broadening of the optical spectrum of the reflected light. The reflected light is transmitted through

optical fibers guides to yield a signal that is a product of the number of moving red cells and the average velocity of movement of the cells (Sarnik *et al.*, 2007). The signal is then converted into electrical output expressed in millivolts.

To obtain consistent recordings, the probes must be calibrated. During calibration, the tip of the probe was inserted into a motility fluid according to manufacturer's instructions. With the one or both probes attached to the skin, the output/s were recorded digitally using the PowerLab 8SP (AD instruments), in volts. This was converted to perfusion units (PU), using the calibration that 1 Volt is equivalent to 200 PU by using Labchart version 7 (AD instrument).

2.4 Drug administration.

In some of studies Chapters 5, 6 and 8, 600mg of Aspirin (Aspirin dispersible tablets, Boots Pharmaceuticals, UK) was given dissolved in orange flavoured squash drink. This dose achieves cyclooxygenase inhibition for 30 to 90 minutes post-consumption (Heavey *et al.*, 1985). Orange squash drink was used as placebo. This same dose of aspirin has been used in previous studies investigating the role of prostaglandins in vascular responses (Easter & Marshall, 2005; Win & Marshall, 2005) and was also recently shown to attenuate the substantial increase in release of PGI2 and PGE2 evoked during forearm contractions at 60% MVC (Junejo, 2017). Further, there is evidence of effectiveness of aspirin in attenuating blood flow during reactive hyperaemia and exercise (Carlsson & Wennmalm, 1983; Cowley *et al.*, 1984).

2.5 Cohen Stress Score

The subject's perception of stress in the period before the experiment was assessed using the Cohen's perceived stress score (PSS, see appendix 4). The PSS measures the

degree to which an individual perceived life as stressful (Cohen *et al.*, 1983). Cohen's stress score is a validated and widely used stress scoring method. The 10 item PSS containing 6 negative and 4 positive items was used. The scale provides questions answered on a likert scale (0 = Never; 1 = almost never; 2 = sometimes; 3 = fairly often; 4 = very often). In the 10 item scale, questions 4, 5, 7, and 8 are the positively stated items and scores were obtained by reversing (e.g., 0 = 4, 1 = 3, 2 = 2, 3 = 1, and 4 = 0) during calculation of the scores selected by the subject. All the scores were then summed across all 10 items. The maximum obtainable score was 40 and the minimum obtainable score was 16.

2.6 Salt intake scoring

A salt intake questionnaire (Appendix 5) was designed to determine how often foods with an established high salt content were consumed (Charlton *et al.*, 2008). The responses were recorded on a likert scale: always (daily) = 5; often (4-6 days) = 4; sometimes (2-3 days) = 3, rarely (≤ 1 day) = 2, never =1). Salt intake score (SIS) was calculated by summing the answers. There were 16 questions, the lowest score obtainable was 16 and the highest score was 80.

2.7 Data Acquisition

A desktop computer connected to Power Lab (AD Instruments, USA) and provided with Labchart software (Version 7) was used for data acquisition. The operating system on the computer was Windows 7, Microsoft. The biofeedback unit was an AD Instruments Powerlab 8SP unit. The output of the Finapres, Laser Doppler monitor, ECG-BioAmp, and Plethysmograph recorded via PowerLab 8SP were translated into recordings on the computer monitor by using Lab Chart 7 software (AD Instruments, USA).

At appropriate time points during each protocol (see Methods section of Chapters 3-6), values of MABP (mmHg), HR (bpm), DCRCF (PU), FCRCF (PU) and the corresponding slopes for measuring FBF were extracted from the Lab Chart tracings offline. Occasionally, due to automated calibration of the Finapres monitor, it was impossible to obtain MABP and HR from the Finapres plethysmogram tracings. At those time points ECG tracings were used to determine HR, while MABP was extracted from nearest adjacent recording. The data obtained were transferred from the data pad of Labchart into Microsoft Excel spread sheets (version 2010).

The SBP, DBP and HR obtained from the automated blood pressure monitor while the subject was resting at the very beginning of the session, were used as the baseline values of ABP.

2.8 Data Extraction

Body mass index was calculated using the formula, BMI = weight (kg)/height $(m)^2$ (Keys *et al.*, 1972). Mean arterial blood pressure was calculated using the formula MABP = DBP + 1/3(SBP – DBP) (Meaney *et al.*, 2000; DeMers & Wachs, 2019). The SBP and DBP were obtained from the automated blood pressure monitor. The MABP obtained is referred to as baseline "mean $ABP^{1"}$ in the results section of Chapters 3-8. The MABP values obtained from the Finapres recording was calculated using the LabChart software and is referred to as "mean $ABP^{2"}$: These values were used when considering the effect of experimental interventions on MABP during the various protocols presented in the results sections of Chapters 3-8.

Peak reactive and exercise hyperemia were determined from the first FBF measurement taken within 5s of cuff release or cessation of exercise respectively. Other FBF values were taken at the times points indicated above. Each FBF value was divided by the

corresponding MABP value in order to calculate forearm vascular conductance (FVC). Digital cutaneous and forearm cutaneous vascular conductance (DCVC and FCVC) were calculated by dividing the relevant red cell flux (DCRF, FCRF) measured in perfusion units by the corresponding MABP.

All data were expressed as mean ± standard error of mean (SEM) for each physiological variable (FBF, FVC, MABP, HR, DCRF, DCVC, FRCF, and FCVC) as well as PSS SIS and perception of sound stress score. The mean baseline value for each of the variables was obtained as an average of the 3 or 4 baseline values. Changes from baseline values (ΔFVC, ΔMABP, ΔHR, ΔDCVC, ΔFCVC) were determined by subtracting the baseline value from values obtained during reactive hyperemia, sound stimuli and following handgrip exercise.

2.9 Statistical analysis

The baseline characteristics of subjects were presented in frequency tables and data were compared using independent sample Student's T test. 2-way or 3-way mixed factorial, one-way or 2-way repeated measures, 2-way or univariate analysis of variance (ANOVA) were done with Bonferroni post hoc tests when appropriate. Where appropriate, paired and un-paired Student's t-test, Fishers exact test or Chi Squared test were done. The details are provided in the relevant Chapters. In all cases, the level of statistical significance was set at $p < 0.05$.

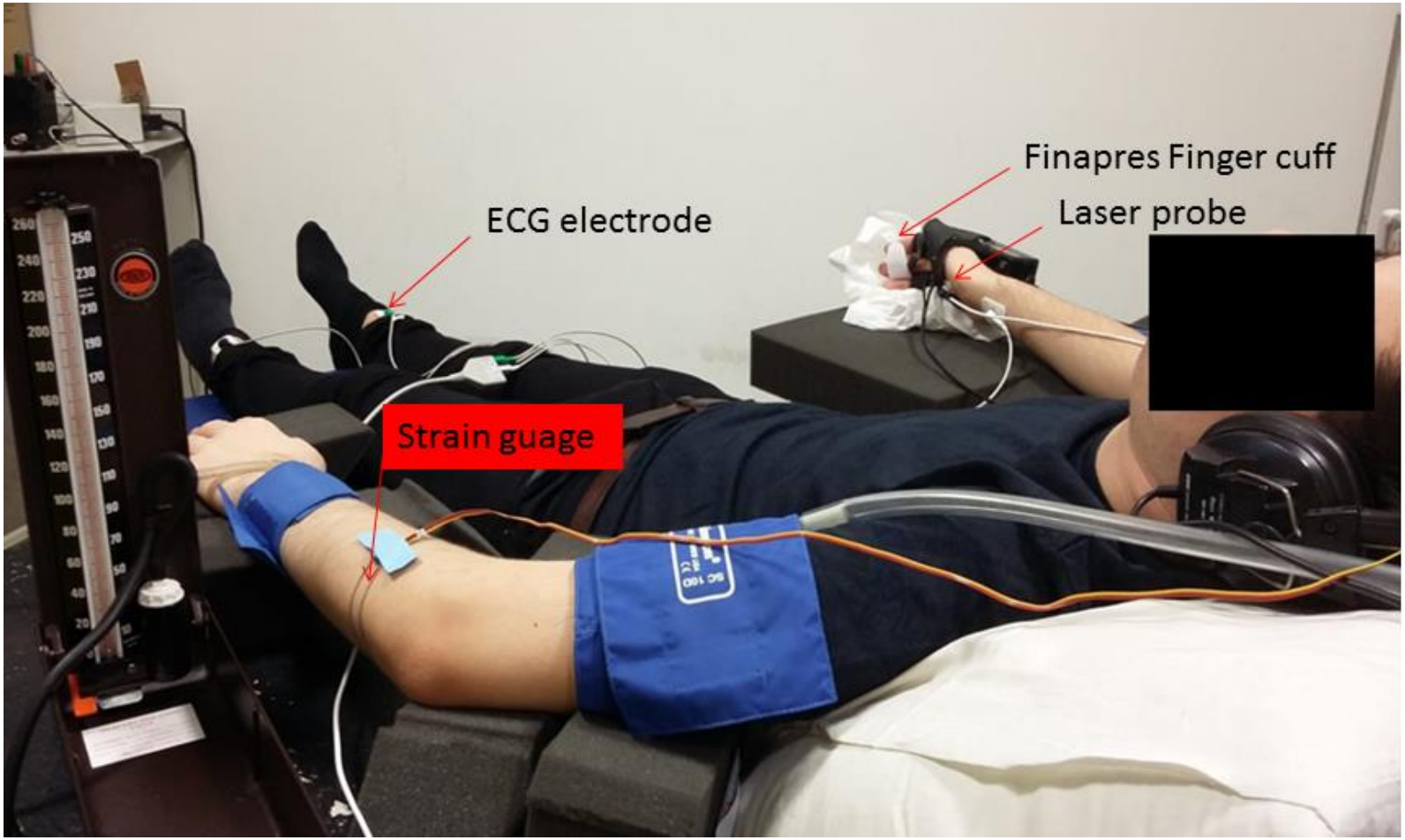
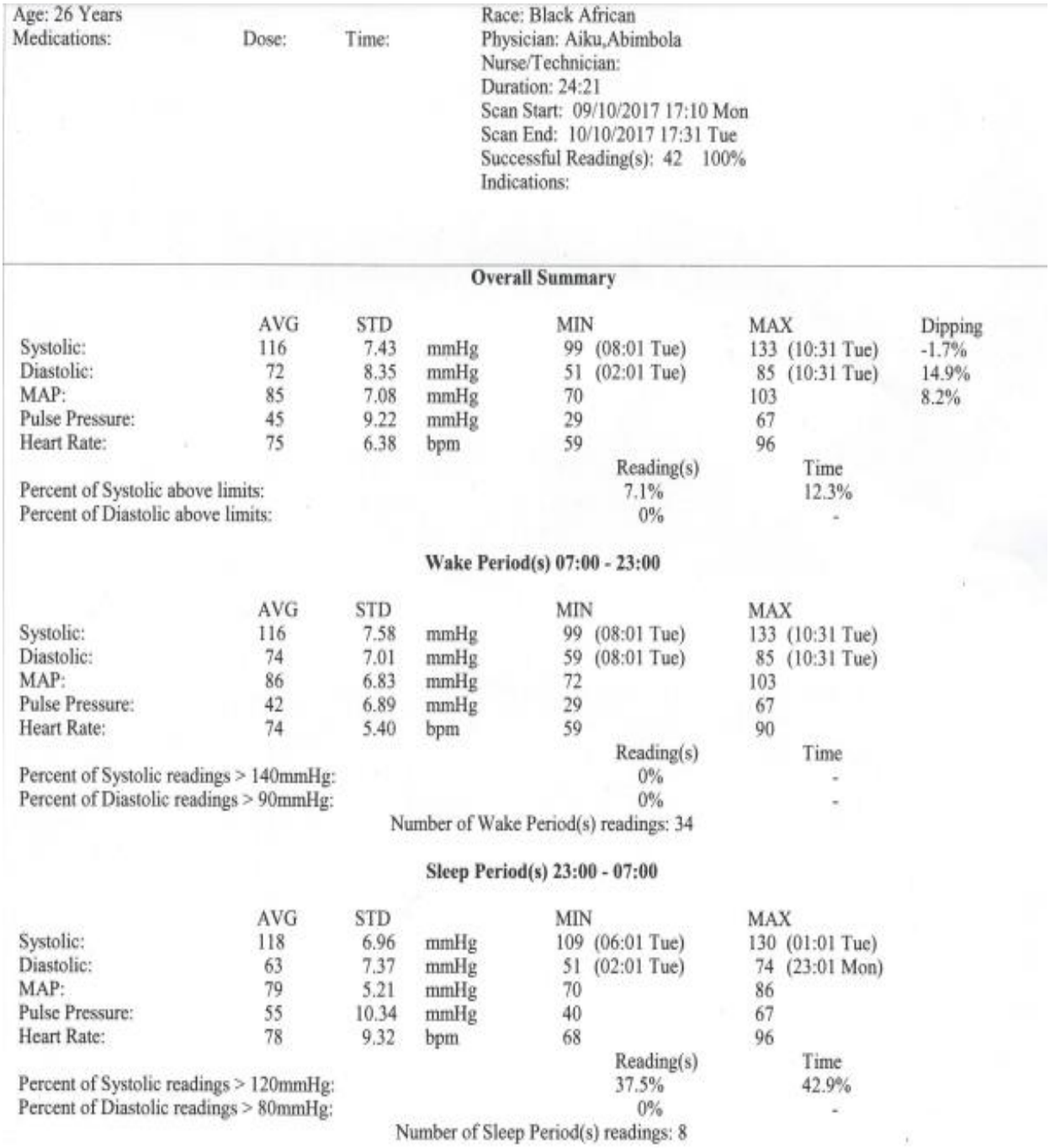


Figure 2.1: Subject lying on the couch with upper and lower cuffs wrapped, ECG electrodes, Laser Doppler probes, strain gauge attached and wearing head phones during mental stress experiments.



Age: 26 Years
Medications: Dose: Time:

Race: Black African
Physician: Aiku,Abimbola
Nurse/Technician:
Duration: 24:21
Scan Start: 09/10/2017 17:10 Mon
Scan End: 10/10/2017 17:31 Tue
Successful Reading(s): 42 100%
Indications:

Overall Summary

	AVG	STD		MIN	MAX	Dipping
Systolic:	116	7.43	mmHg	99 (08:01 Tue)	133 (10:31 Tue)	-1.7%
Diastolic:	72	8.35	mmHg	51 (02:01 Tue)	85 (10:31 Tue)	14.9%
MAP:	85	7.08	mmHg	70	103	8.2%
Pulse Pressure:	45	9.22	mmHg	29	67	
Heart Rate:	75	6.38	bpm	59	96	

	Reading(s)	Time
Percent of Systolic above limits:	7.1%	12.3%
Percent of Diastolic above limits:	0%	-

Wake Period(s) 07:00 - 23:00

	AVG	STD		MIN	MAX
Systolic:	116	7.58	mmHg	99 (08:01 Tue)	133 (10:31 Tue)
Diastolic:	74	7.01	mmHg	59 (08:01 Tue)	85 (10:31 Tue)
MAP:	86	6.83	mmHg	72	103
Pulse Pressure:	42	6.89	mmHg	29	67
Heart Rate:	74	5.40	bpm	59	90

	Reading(s)	Time
Percent of Systolic readings > 140mmHg:	0%	-
Percent of Diastolic readings > 90mmHg:	0%	-

Number of Wake Period(s) readings: 34

Sleep Period(s) 23:00 - 07:00

	AVG	STD		MIN	MAX
Systolic:	118	6.96	mmHg	109 (06:01 Tue)	130 (01:01 Tue)
Diastolic:	63	7.37	mmHg	51 (02:01 Tue)	74 (23:01 Mon)
MAP:	79	5.21	mmHg	70	86
Pulse Pressure:	55	10.34	mmHg	40	67
Heart Rate:	78	9.32	bpm	68	96

	Reading(s)	Time
Percent of Systolic readings > 120mmHg:	37.5%	42.9%
Percent of Diastolic readings > 80mmHg:	0%	-

Number of Sleep Period(s) readings: 8

Figure 2.2: Original ambulatory blood pressure measurement summary page.

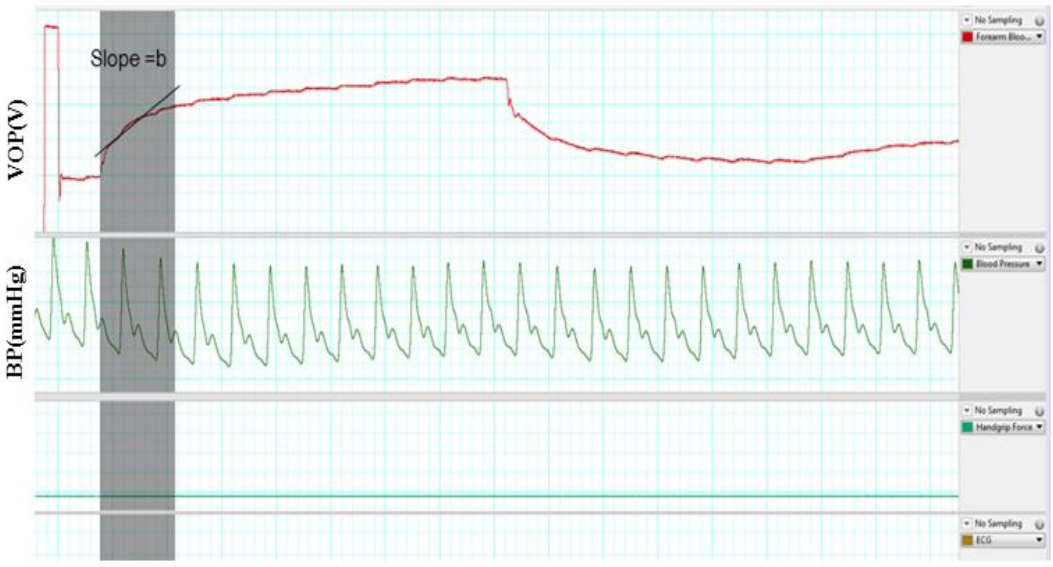


Figure 2.3: Original trace showing plethsmogram of a segment used for extraction of slope value (b).

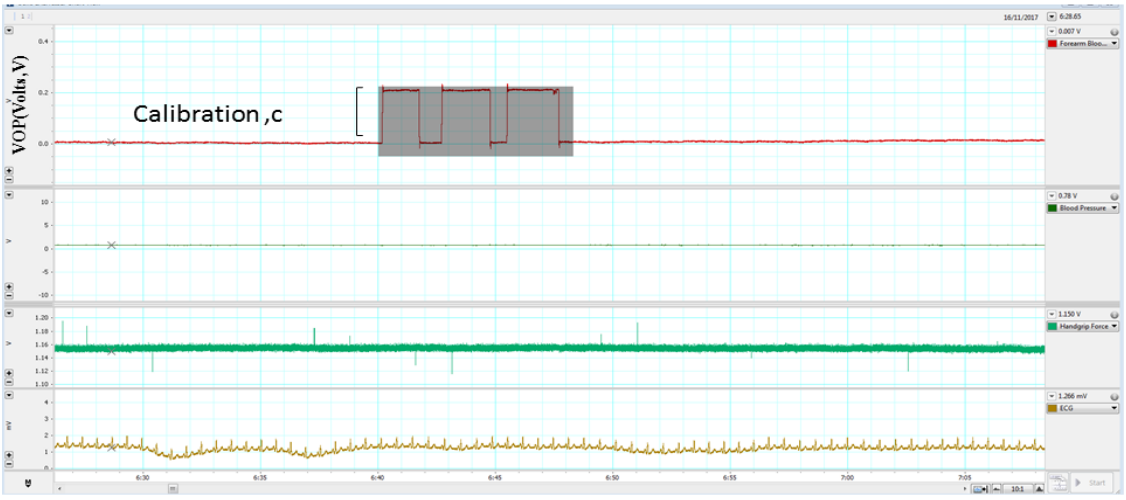


Figure 2.4: Original tracing of plethysmogram showing segment used for extraction of calibration value (c).

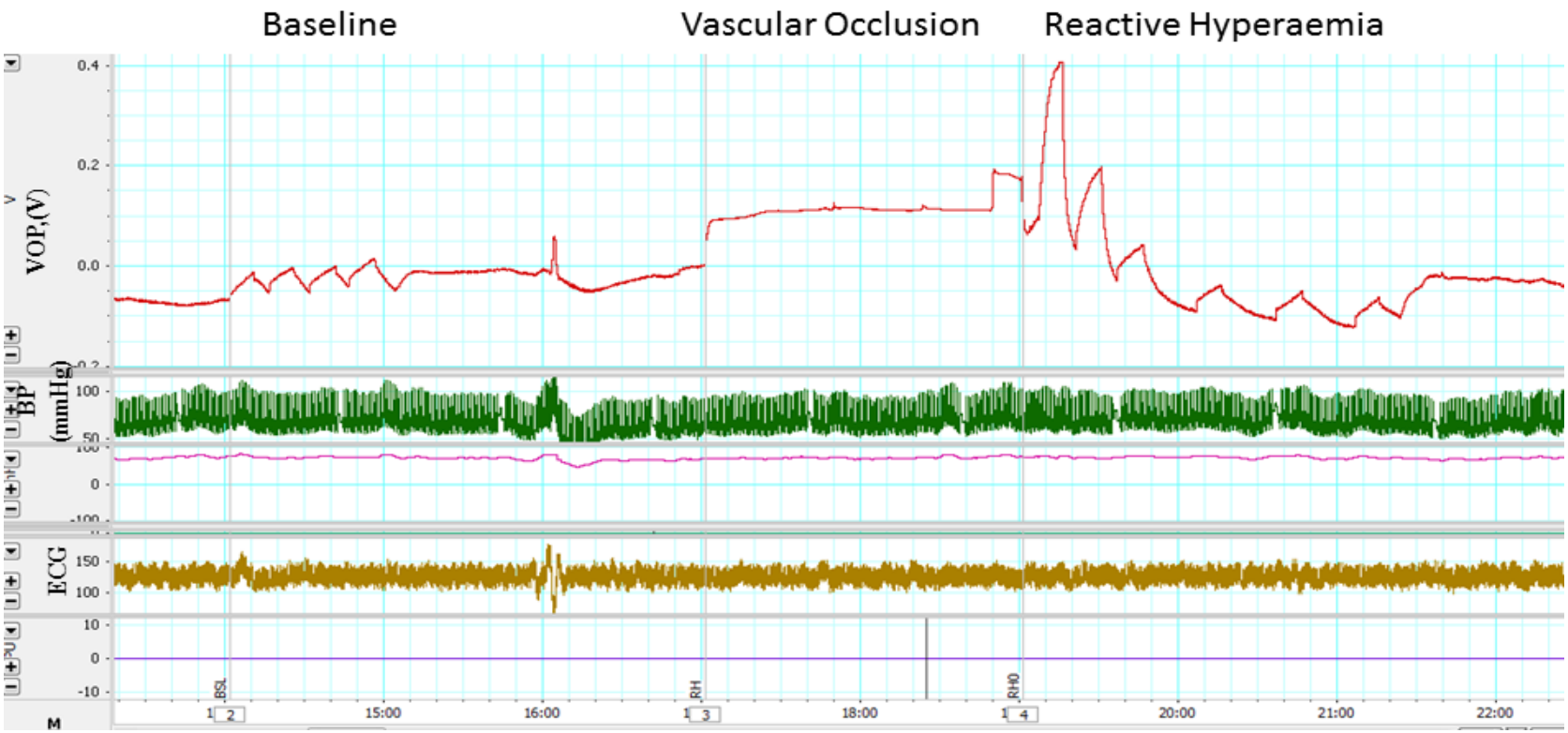


Figure 2.5: Original tracing illustrating cardiovascular responses evoked during baseline and reactive hyperaemia.

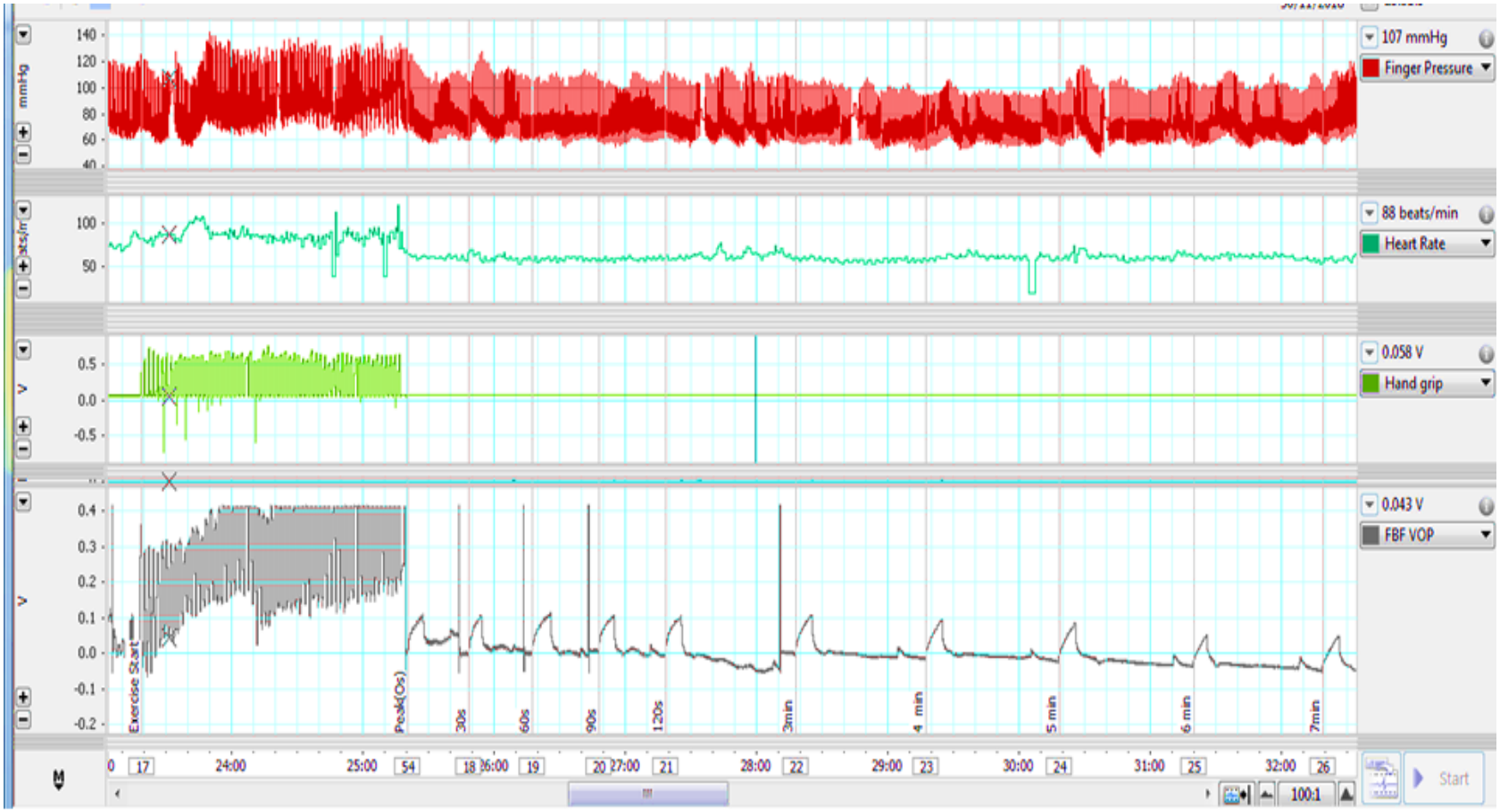


Figure 2.6: Original tracing illustrating cardiovascular responses evoked during and after rhythmic handgrip exercise.

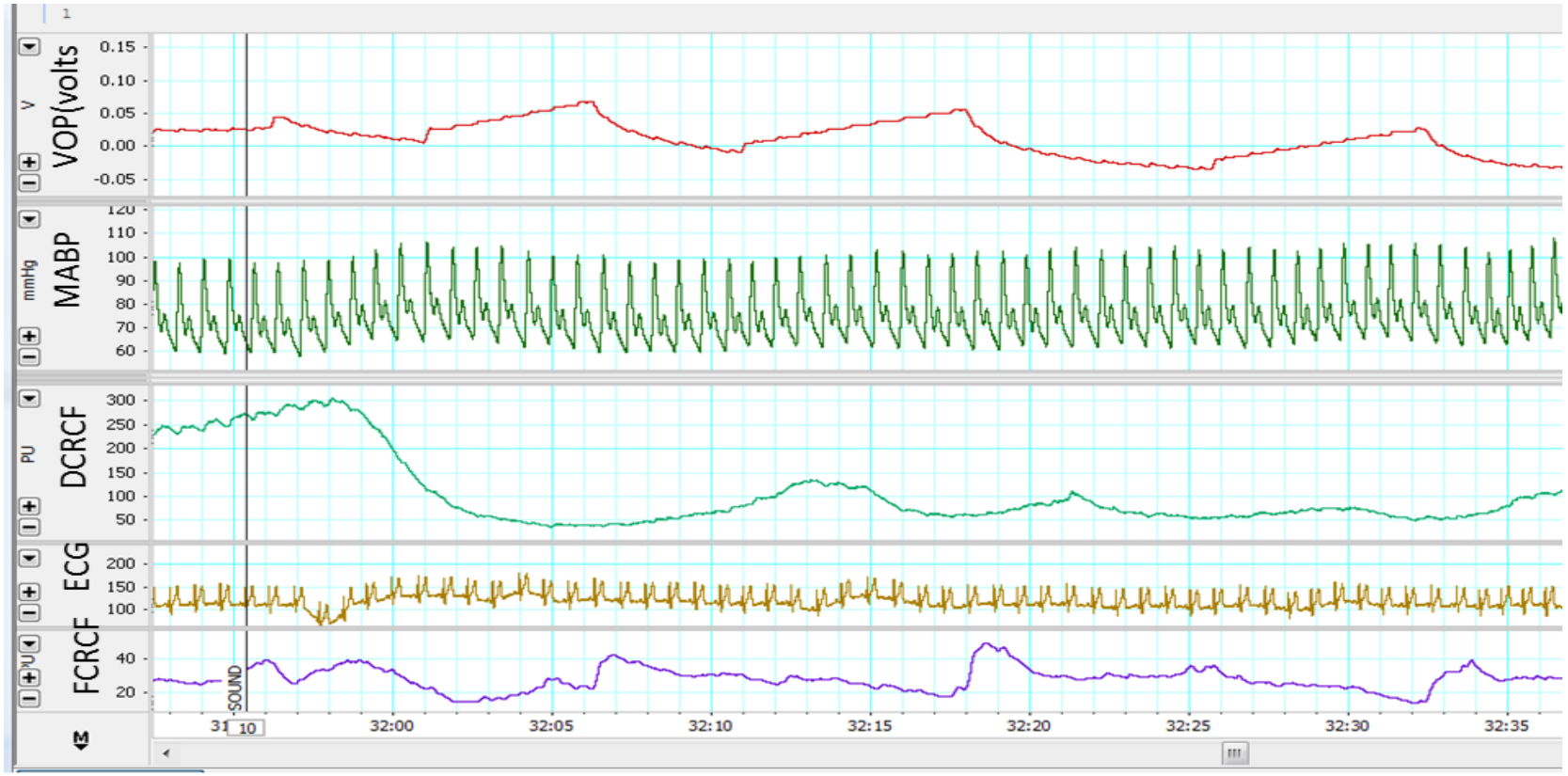


Figure 2.7: Original tracing illustrating cardiovascular responses evoked during sound (mental stress).

CHAPTER 3

Role Of Ethnicity And Sex In Cardiovascular Responses Evoked By Repeated Acute Mental Stress In White Europeans, Black Africans And South Asians

3.1 Introduction

It is well established that the prevalence of cardiovascular diseases (CVD) is higher in Black Africans (BAs) and South Asians (SAs) than in White Europeans (WEs) and in BAs at least, the prevalence of hypertension is greater than in WEs (section 1). It is well known that mental stress elicits the characteristic pattern of the alerting response, which is controlled by the central nervous system via autonomic nerve supply to heart and blood vessels, as well as by vasodilator substances released from the endothelium as discussed in section 1.2.

A major contributory factor to the development of hypertension is increased peripheral resistance due to raised vasoconstrictor tone associated with raised sympathetic nerve activity and endothelial dysfunction (Ushakov *et al.*, 2016). There is evidence that even acute mental stress induces endothelial dysfunction; this could be worsened by repeated episodes of stress as part of daily living and therefore, could contribute future development of hypertension (Ushakov *et al.*, 2016). There is also evidence that the pressor responses to mental stress can induce structural remodelling in the blood vessels leading to increased total peripheral resistance and arterial blood pressure (ABP) and that individuals with hypertensive parents (FH+) are more vulnerable to such changes (Folkow, 1982). Moreover, exaggerated pressor responses to, and slow recovery from, laboratory stressors predicts future incidence of hypertension and increased thickness of the carotid artery wall (Chida & Steptoe, 2010).

The latter point is of interest because even though the characteristic of the alerting response is muscle vasodilatation with vasoconstriction elsewhere (responders), some individuals show forearm or calf vasoconstriction and/or an increase in MSNA (non-responders; responders (Section 1.3.1). Whether an individual is a responder or a non-

responder, the direction of the response is consistent between different stress stimuli and is consistent to a given stimulus when the stimulus is repeated a few times (Fonkoue & Carter, 2015). Further, in most individuals when the stimulus is repeated many times within an experimental session or in several sessions repeated over days or weeks, some or all the components of the alerting response gradually change, a reduction or an increase in magnitude being known as habituation or sensitization respectively (Zbrozyna & Westwood, 1988; Edwards *et al.*, 1998). It has been proposed that habituation of the forearm vasodilation and persistence or sensitization of the forearm vasoconstriction, in those individuals who show it and persistence of pressor component of the alerting response may lead to hypertension (Hilton, 1982).

Consistent with this hypothesis, it was shown that teenage and young adult FH+, who are at higher risk of hypertension than FH-, showed larger increases in ABP, HR and MSNA and greater muscle vasodilatation in response to mental stress than FH-, (Section 1.5.1; Anderson et al, 1987; Noll et al, 1996; Fonkoue et al, 2016). Further, in a study of young labile hypertensives and normotensives, within each group, immersion of one foot in cold water evoked muscle vasodilatation in some, but muscle vasoconstriction in others, but the vasodilator response was exaggerated in the hypertensives relative to the normotensives in response to the first stimulus. Moreover, in the labile hypertensives, the muscle vasodilatation showed only transient habituation within the first session or over several sessions and the pressor responses were bigger and persisted on repetition (Zbrozyna & Westwood, 1988). Taken together, these results indicate that the alerting response is altered early in the development of hypertension and the muscle vasodilatation is particularly labile.

Considering what is already known of the WE, SA and BA ethnic groups in relation to the stress theory of hypertension outlined above, endothelium-dependent dilatation is impaired in BAs and SAs relative to WEs as discussed in section 1.6 and impaired NO availability has been implicated in BAs in the late 20s-30 years age range (Stein *et al.*, 1997). Further, that in mixed gender groups of middle-aged American BAs, mental stress-induced forearm vasodilatation is attenuated relative to that seen in age matched WEs and this was attributed to impaired NO availability in BAs (Cardillo *et al.*, 1998b, a). Further, it has recently been reported that a higher proportion of young SA men than WE men consistently show forearm vasoconstriction rather than vasodilatation in response to a repeated sound stress stimulus (Ormshaw *et al.*, 2018).

The response evoked by mental stressors in young BAs, when early markers of cardiovascular disease may already be present, has not been investigated and there has been no study of whether BAs habituate or sensitise on repetition of the mental stressor. There has been no investigation of a vascular bed that characteristically shows vasoconstriction during an alerting response in either SAs or BAs and there have been no comparison of how mental stressors affect men and women in the 3 ethnic groups. The latter issue is of particular interest given recent evidence that SA and BA women may be at greater risk of CVD than men and may be particularly vulnerable to stressors in everyday life (section 1.6).

3.1.2 Aims

The aims of the present study are to test reactive hyperaemia (an endothelium-dependent response) and the pattern of cardiovascular response evoked by 5 repetitions

of a sound stimulus, a recognised alerting stimulus (Zbrozyna & Westwood, 1988; Edwards *et al.*, 1998) in young men and women of the 3 ethnic groups.

We aimed to determine

1. Whether peak and or total reactive hyperaemia differs between WEs and BAs, or between WEs and SAs and whether there are sex differences within each ethnic group.
2. Whether BAs or SAs differ from WEs in the pattern of response evoked by the first alerting stimulus.
3. Whether there are sex differences within each ethnic group in the responses evoked by repeated alerting stimuli.
4. Whether any component of the alerting response habituates or sensitizes and whether ethnicity affects the magnitude of habituation or sensitization.

3.1.3 Hypotheses

1. Reactive hyperaemia will be smaller in BAs and in SAs relative to WEs, and the men in each ethnic group will show smaller responses than women.
2. BAs will show an initial blunted forearm vasodilatation and exaggerated pressor response to the 1st sound. Further, on repetition, the vasodilator response will habituate while the pressor components will remain high compared with WEs.

Fewer SA men and women than WEs will show muscle vasodilator responses to the 1st sound, the remainder showing vasoconstriction and any muscle vasodilatation will be depressed relative to WEs. The forearm dilator responses will habituate on repetition of sound and pressor responses will sensitize.

3.2 Methods

3.2.1 Protocol 1

3.2.1.1 Study participants

The experiments were performed on 70 young adults aged 18 to 26 years; 22 WEs (13 men and 9 women), 28 BAs (16 men and 12 women) and 20 SAs (9 men and 11 women).

3.2.1.2 Procedure

The methodology was described in detail in Chapter 2. Briefly, the subject reclined semi-inclined on the couch while the recording equipment was attached. Following a rest period of 10-15 min, resting brachial ABP was recorded from the non-dominant arm using an automated blood pressure monitor. Each Subject was given an orange squash drink that contained Aspirin or plain orange squash 30 min before the experiment started. The effects of aspirin are described in in Chapter 5.

Thereafter, continuous beat to beat ABP was recorded using the Finapres from the middle finger of the right hand and maintained at heart level. Heart rate (HR) was computed from the ABP recording or from the R-R intervals of an ECG. Cutaneous red cell flux (CRF) was recorded continuously from the thenar eminence (DCRCF) and from the volar aspect of the forearm between the wrist and the elbow joints (FCRCF) using laser Doppler probes. Forearm blood flow (FBF) was recorded from the left arm using venous occlusion plethysmography with Indium-Gallium strain gauge.

Baseline blood flow was recorded as described in Chapter 2, see section 2.3.4.1. Reactive hyperaemia was recorded as described in Chapter 2, see section 2.3.4.2. Blood flow was recorded during sound stimuli as described in Chapter 2, see section 2.3.4.4. The subjects were asked to score the stressfulness of each sound as described in section 2.3.4.4.

The protocol is shown below (Figure 3. 1).

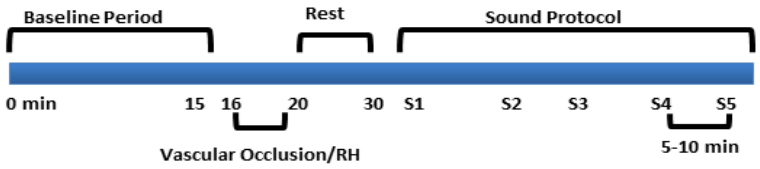


Figure 3.1 . Protocol 1. Baseline period of 15 min followed by 2 min period of arterial occlusion of one arm with reactive hyperaemia (RH) recorded for 2 minutes, followed at 30 min by 5 sound stimuli (S1-S5) applied at 5-10 min intervals for 30 s each. FBF was recorded following release of arterial occlusion and at 5, 15, 30 s into each sound.

3.2.3 Data analysis

All variables are expressed as mean ± standard error of mean (SEM) unless indicated otherwise. Anthropometric measurements and recorded resting (baseline) measurements and other characteristics of the participants were summarized and presented in Tables 3.1-3.3. Three to 4 baseline measurements of FBF, HR, MABP, DCRCF, FCRCF were taken. FVC DCVC, FCVC were calculated using FBF/MABP, DCRCF/MABP and FCRCF/MABP respectively, using ABP values extracted over the same time period as FBF was measured.

Measurements of FBF, HR, MABP, DCRCF, FCRCF done during reactive hyperaemia at peak (0s), 15s, 30s, 45s, 60s, 90s and 120s and in response to each sound at 5s, 15s and 30s were recorded as absolute values. FVC was recorded as absolute values during reactive hyperaemia at intervals following release of occlusion and at intervals during each sound. Change from baseline values were computed by subtracting the corresponding baseline value from the timed absolute values and labelled as ΔFBF, ΔFVC, ΔMABP, ΔHR, ΔDCRCF, ΔFCRCF, ΔDCVC and ΔFCVC**.** Group comparisons of anthropometric and baseline values were done using independent Student's T tests. Comparisons between ethnicities for the proportion of individuals who were pre-

hypertensive (PH+, resting SBP >120mmHg) and FH+ vs FH- were made using Fisher's exact test.

Three-way mixed ANOVA was used to determine the effects of two independent variables (ethnicity and sex as between subjects factors) on ΔFVC during reactive hyperaemia measured over the 7 time points (time as the within subject factor). Bonferroni post hoc test were done as appropriate. In addition, 2-way ANOVA was done to determine the effects of ethnicity and sex on the peak of reactive hyperaemia.

Three-way mixed ANOVA was used to determine the effects of ethnicity and sex as between subjects factors on cardiovascular responses to 1^{st} sound measured over the 3 time points (5, 15 and 30s) with time as the within subject factor. Further, when there were significant interactions, 2-way repeated measures (mixed) ANOVA was used to compare groups of WEs vs BAs or WEs vs SAs.

Changes in FVC and changes in MABP were plotted in scattergraphs for each ethnic group and correlation analysis was done to determine whether those who showed less also show heightened BP response. Correlation analysis was done and coefficients with two tailed p values presented.

The consistency of changes evoked in each variable at 15s into the 5 sounds within individuals was tested by intraclass correlation (ICC) analysis; the coefficient of consistency (Chronbach's alpha (α)) value is reported with two tailed p values.

The effect of repetition of the sound stimulus (S1-S5, 5 time points) within one session was tested within each ethnic group in groups consisting of men and women as well as groups of only men or women using one-way repeated measure ANOVA: these analyses provided assessment of habituation or sensitization of the responses within the

session for each group of subjects, as whole ethnic groups or in gender specific ethnic groups as appropriate.

Three-way mixed ANOVA was used to determine effect of ethnicity and sex (between subjects factors) on repeated sounds measured over the 5 time points at 15s into each sound (time as the within subjects factor). Two way repeated measures ANOVA was done to determine differences between BAs and WEs; SAs and WEs or between men and women in each ethnic group when appropriate. Bonferroni post hoc test were performed as appropriate. In all cases, $p<0.05$ was taken as significant.

Individuals were divided into "vasodilators, VDs" and "vasoconstrictors, VCs" according to the direction of change in FVC at 15s into S1, when the evoked change in cutaneous vasoconstriction and ABP generally reach their maximum (Edwards *et al.*, 1998; Ormshaw *et al.*, 2018). The proportion of vasodilators and vasoconstrictors within groups were compared by Fisher's exact test. Scattergraph of ΔFVC and ΔMABP at 15s during S1-S5 was plotted. Factorial ANOVAs were used to determine effect of ethnicity and vasodilator status (as the between subjects factors) on the responses to repeated sounds measured over 3 time points during S1 and over the 5 time points at 15s into each sound (time as the within subjects factor) as well as on reactive hyperaemia measured over 7 time points. Bonferroni post hoc test were performed as appropriate. In all cases, $p<0.05$ was taken as significant.

3.3 Results

3.3.1 Baseline characteristics

3.3.1.1 Baseline characteristics in whole ethnic groups

There were no differences between ethnic groups for anthropometric characteristics (Table 3.1). In addition, salt intake as assessed by dietary questionnaire was similar in each ethnic group. There were also no differences between ethnicities in their perceived stress score (PSS) assessment of daily stress. However, a higher proportion of BAs than WEs were FH+ (30.4% vs 4.55%, $p<0.05$). Further, from resting measurements of ABP made with sphygmomanometer at the beginning of the first experimental session, DBP was higher in BAs than WEs ($p=0.046$) and SBP tended to be higher in BAs ($p=0.06$). In addition, a higher proportion of BAs than WEs were pre-hypertensive (21.4% vs 0%, $p<0.05$). There were no differences between WEs and SAs for baseline values except that a higher proportion of SAs were pre-hypertensive (15% vs 0%, $p=0.01$) and/or were FH+ (30% vs 4.55%, $p=0.04$; Table 3.1). The values of cardiovascular variables measured before the protocol started and from which changes in each variable were calculated for reactive hyperaemia and responses to sound are shown in Table 3.2, FCRCF was lower in BAs relative to WEs ($p=0.02$).

3.3.1.2 Sex differences in baseline characteristics within each ethnic group

There were no differences in anthropometric, salt intake, PSS or family history of hypertension between males and females in each ethnic group. However, female BAs were slightly older than male BAs. Moreover, men had higher SBP relative to the women (WEs: $p=0.004$; BAs: $p=0.009$; SAs: $p=0.03$, see Table 3.2). In addition, men had significantly higher forearm circumference (FAC) relative to the respective women

($p<0.05$). WE men also had higher waist circumference relative to WE women ($p=0.003$, see Table 3.2).

3.3.1.3 Cardiovascular baselines in sex-dependent groups within ethnicity.

There were no significant differences between WE and BA men, or WE and SA men except for a higher prevalence of prehypertension in BA men (37.5% vs 0%, $p=0.02$) and in SA men (22% vs 0%, $p<0.05$). In addition, there were more FH+ in BA men (18.8%) and SA men (33.3%) than WE men (0%; $p<0.05$ in each case). BA women had lower FCRCF than WE women ($p=0.02$; Table 3.2). In addition, DBP and MABP tended to be higher in BA women than WEs ($p=0.07$, $p=0.09$ respectively). There were no significant differences between WE and SA women (Table 3.2).

3.3.2 Reactive hyperaemia (RH)

3.3.2.1 Reactive hyperaemia in whole ethnic groups

Forearm blood flow increased from 4.22 ± 0.27 at baseline to 38.07 ± 3.07 ml/100 ml tissue/min at peak in WEs, from 4.23 ± 0.37 to 34.49 ± 2.15 ml/100 ml tissue/min in BAs and from 4.61 ± 0.31 to 34.95 ± 2.04 ml/100ml tissue/min in SA ($p<0.001$ in each case). There were no changes in ABP (values not reported). Therefore, FVC values also increased in each ethnicity (Figure 3.2), from +0.05 ± 0.004 at baseline to +0.47 ± 0.038 CU at peak in WEs, from +0.05 ± 0.005 to + 0.41 ± 0.028 CU in BAs and from +0.06 ± 0.00 to +0.42 ± 0.03 CU in SAs ($p<0.001$ in each case). The increases in FBF and FVC were short lived, and declined to baseline level by 30s after release of occlusion in all 3 ethnic groups.

Whole group of BAs showed blunted reactive hyperaemia relative to WEs ($F(1,48)=5.08$, $p=0.03$, partial $\eta^2=0.96$), but reactive hyperaemia was similar in WEs and SAs ($F(1,40)=1.51$, $p=0.23$, partial $\eta^2=0.04$; see Figure 3.2).

There was significant 3-way interaction between time, sex and ethnicity ($F(3,88)=2.85$, $p=0.046$, partial $\eta^2= 0.82$) and a significant two-way interaction between time and sex on ΔFVC during reactive hyperaemia measured over 7 time points ($F(3,88)=10.78$, $p<0.001$, partial $\eta^2=0.14$). The interactions of time and ethnicity or ethnicity and sex were not significant ($p>0.05$). There was a significant simple main effect of sex at peak and 15s of reactive hyperaemia ($F(1,64)=13.13$, $p=0.001$, partial $\eta^2=0.17$ and $F(1,64)=6.50$, $p=0.01$, partial $\eta^2= 0.09$) but not at other time points ($p>0.05$). Bonferroni corrections were made with comparisons within significant simple main effect. Reactive hyperaemia was greater at peak and at 15s in men relative to women ($F(3,88)=13.13$, $p=0.001$, partial $\eta^2=0.17$ and ($F(3,88)=6.50$, $p=0.013$, partial $\eta^2=0.09$ respectively). Relative to WE men, WE women showed blunted peak reactive hyperaemia ($p<0.001$). At 15s during reactive hyperaemia, SA women showed blunted reactive hyperaemia relative to SA men ($p=0.03$, Figure 3.3).

Considering the responses at only peak of reactive hyperaemia, there was significant 3-way interaction between ethnicity and sex on peak reactive hyperaemia ($F(2,64)=3.23$, $p=0.046$, partial $\eta^2=0.09$). There was significant 2-way interaction between time and sex ($F(2,64)=10.78$, $p<0.001$, partial $\eta^2=0.14$) but not between time and ethnicity, or between ethnicity and sex ($p>0.05$). Simple main effect of sex on peak of reactive hyperaemia was significant for WEs ($F(1,64)=14.66$, $p<0.001$, partial $\eta^2 =0.19$) but not for BAs or SAs ($F(1,64)=2.55$, $p<0.62$, partial $\eta^2=0.004$ and ($F(1,64)=3.17$, $p<0.08$, partial $\eta^2 =0.05$, see Figure 3.3). Bonferroni corrections were made with comparisons within the significant simple main effect. The peak reactive hyperaemia was greater in WE men than WE women ($F(1,64)=14.66$, $p=0.001$, partial $\eta^2 =0.19$), see Figure 3.3). There was a significant simple main effect of ethnicity on the peak reactive hyperaemia

between men ($F(2,64)=3.43$, $p<0.04$, partial $\eta^2=0.10$) but not between women ($F(2, 64)=0.63$, $p<0.54$, partial $\eta^2=0.02$). Bonferroni corrections were made with comparisons within simple main effect. The peak reactive hyperaemia was greater in WE men than BA men ($F(2,64)=3.43$, $p=0.03$, partial $\eta^2=0.10$) but not SA men ($p>0.05$), (Figure 3.4).

3.3.2.2 Sex-related differences in reactive hyperaemia

Peak and total reactive hyperaemia were blunted in WE women relative to WE men ($p<0.05$). SA women showed blunted at near peak but not total reactive hyperaemia relative to SA men). By contrast, BA women did not show attenuated peak or total reactive hyperaemia relative to BA men (Figure 3.3).

3.3.2.3 Comparison of reactive hyperaemia between ethnicities for same sex groups

BA men had attenuated peak reactive hyperaemia relative to WEs ($p<0.05$). However, SA men showed similar reactive hyperaemia to WE men ($p>0.05$, Figure 3.4). There were no significant differences in reactive hyperaemia between WE and BA women or SA women ($p>0.05$, Figure 3.4).

3.3.3 Cardiovascular response evoked by the first sound stimulus (S1)

3.3.3.1 Responses evoked by S1 in whole ethnic groups

When men and women were combined within each ethnicity, the pattern of response evoked by S1 in all WEs, all BAs and all SAs groups was similar, except for changes in FVC. Thus, at 5, 15 and 30s of S1 there was an increase in ABP and a decrease in DCVC and FCVC indicating forearm and digital cutaneous vasoconstriction. Any changes in HR were small and variable in all 3 ethnicities (Figure 3.5).

At 5s into S1, ΔFVC increased WEs and SAs but decreased in BAs. At 15s into the sound, ΔFVC increased in WEs and all BAs but decreased in SAs indicating net vasodilatation in the whole circulation of the forearm, but ΔFVC decreased in SAs indicating forearm vasoconstriction (p=0.02). At 30s, when the stimulus ended, ΔFVC decreased WEs and BAs and SAs. The greatest vasodilatory response was observed at 15s into the sound as reported by Edwards *et al.* (1998) (Figure 3.5). At 5s, 15s and 30s, MABP increased in WEs, BAs and SAs. The greatest pressor response occurring at 5s into the sound, but the decrease in FCVC and DCVC, reflecting cutaneous vasoconstriction occurred at 5 or 15s as observed previously by Edwards et al. (1998). WEs showed modest tachycardia at 5s, and 15s but bradycardia at 30s. BAs and SAs showed bradycardia. In the three ethnic groups, there was forearm and digital cutaneous vasoconstriction. There were no significant differences in ΔMABP, ΔHR, ΔDCVC, ΔFCVC between whole groups of WEs and BAs or SAs (p>0.05, see Figure 3.5).

There was no significant 3-way interaction between time, sex and ethnicity on ΔFVC during S1 (p>0.05). There was no significant interaction between time and sex or between ethnicity and sex (p>0.05). There was a significant interaction between effect of time and ethnicity on ΔFVC during S1 ($F(4,128)=4.992$, $p=0.001$, partial $\eta^2=0.14$). Simple main effect of ethnicity on ΔFVC during S1 was significant at 5s and 15s but not at 30s ($F(1,64)=3.69$, $p=0.03$, partial $\eta^2=0.10$; $F(1,64)=3.43$, $p=0.04$, partial $\eta^2=0.04$ and ($F(1,64)=1.16$, $p=0.32$, partial $\eta^2=0.04$). Bonferroni corrections were made with comparisons within the simple main effect. ΔFVC at 15s was greater in WEs relative to SAs (p=0.04) but not relative to BAs (p>0.05), see Figure 3.5. In addition, BA men showed greater ΔFVC relative to BA women (p=0.01). There were no differences between WE and SA men and women (p>0.05, Figure 3.6).

There was significant 3-way interaction between time, ethnicity and sex for ΔMABP during S1 ($F(4,124)=2.69$, $p=0.03$, partial $\eta^2=0.08$) and significant 2-way interaction between ethnicity and sex ($F(2,62)=6.35$, $p=0.003$, partial $\eta^2=0.17$). Simple main effect of ethnicity was significant among women but not men, BA women showed greater pressor response relative to WE women ($F(2.64)=3.60$, $p=0.03$ partial $\eta^2=0.17$, Figure 3.7). Simple main effect of sex on ΔMABP during S1 was significant for BAs ($F(1,62)=9.53$, $p=0.03$, partial $\eta^2=0.13$). Bonferroni corrections were made with comparisons within the simple main effect. BA women showed greater pressor response relative to BA men ($p=0.003$). There were no significant differences between WE or SA men and women (Figure 3.6). There was no significant 3-way or 2-way interaction between time, ethnicity and sex for ΔHR or ΔDCVC or ΔFCVC ($p>0.05$ in each case).

3.3.3.2 Sex-related differences in responses to S1

WE men and women showed similar forearm skeletal vasodilation, pressor response, digital and cutaneous vasoconstriction. WE women showed net tachycardia relative to WE men. BA men showed forearm skeletal vasodilation whereas the BA women showed forearm skeletal vasoconstriction and greater pressor response. There were no sex differences in heart rate digital and cutaneous vasoconstriction responses in BAs. There were no sex differences in the responses to S1 in SAs (Figure 3.6).

3.3.3.3 Comparison of responses to S1 between ethnicities for same sex groups

BA men showed similar forearm vasodilation, heart rate changes, digital and cutaneous vasoconstriction but significantly smaller pressor responses relative to WE men ($p=0.01$). BA women showed net forearm vasoconstriction while WE women showed forearm vasodilation ($p=0.01$). BA women showed significantly smaller FVC relative to

WEs (p=0.03). BA women showed greater increases in MABP (p=0.03) and bradycardia relative to WE women (p=0.03, see Figure 3.7).

There were no significant differences between SA and WE men in any component of the responses to S1. However, SA women showed vasoconstriction rather than vasodilation at 15s during S1 relative to WE women (p=0.02) as well as smaller forearm cutaneous vasoconstriction relative to WE women (p=0.04). There were no other differences in the responses between the women (see Figure 3.8).

3.3.3.4 Correlation of ΔFVC and ΔMABP at 15s during S1

When considered as a group consisting of all WEs, BAs and SAs, there was significant negative correlation between ΔFVC and ΔMABP (Pearson's correlation coefficient, (r) = -0.47, $p<0.001$). When considered in whole ethnic groups, there was significant negative correlation between ΔFVC and ΔMABP in WEs and BAs (r= -0.45, p=0.03, and r= -0.57, p=0.002) but not in SAs (r= -0.22, p=0.36, see Figure 3.9).

3.3.4 Cardiovascular responses evoked by repeated sound stimuli (S1-S5)

3.3.4.1 Responses evoked by S1-S5 in whole ethnic groups

Habituation or sensitization of responses to S1-S5 in whole ethnic groups

Considering responses at 15s into each sound (the time point selected by Edwards et al, 1998), the changes evoked in each variable were highly consistent within each individual in each ethnic group, see Table 3.7 which shows Cronbach's α for the outcome of intracclass coefficient (ICC) analyses. The exception to this consistency was the direction of HR change, which was variable across S1-S5 in individual SAs.

Table 3.8 shows the main effects of repetition of sound on alerting responses for the assessment of habituation or sensitization within a session for each ethnic group.

Within the WE and BA groups, MABP increased progressively during S1-S5 at 15s into each sound, indicating sensitization of the pressor response ($F(2,48)=3.81$, $p=0.02$, partial $\eta^2=0.16$; $F(2,48)=3.58$, $p=0.03$. partial $\eta^2=0.12$ respectively). For other variables, there was no obvious habituation or sensitisation across S1-S5 in WEs, BAs or SAs (Figure 3.10).

Ethnic differences in responses to S1-S5

Comparing whole ethnic groups at 15s into each sound (S1-S5), BAs showed attenuated forearm skeletal vasodilation and bradycardia relative to WEs ($p<0.05$, Figure 3.10). Similarly, SAs showed blunted forearm skeletal vasodilation relative to WEs across S1-S5; the mean change suggesting forearm vasoconstriction in SAs (Figure 3.10).

3.3.4.2 Sex-related differences in responses to S1-S5

Habituation or sensitization of responses in sex-dependent groups within ethnicity

Table 3.9 shows the main effects of repetition of sound on alerting responses for the assessment of habituation or sensitization within a session for men and women within each ethnic group. BA women but not BA men showed progressive increase in MABP during S1-S5 indicating sensitization of the pressor response ($p=0.04$). For other variables, there was no obvious habituation or sensitisation across S1-S5 in men or women in WE, BA or SA ethnic groups (Figure 3.11).

Ethnic differences in responses to S1-S5 in sex-dependent groups within ethnicity

Sex differences within each ethnic group were displayed in Figure 3.11. Across S1-S5, WE men and women showed forearm vasodilation, pressor responses and forearm and digital cutaneous vasoconstriction. By contrast, BA women showed greater pressor responses than BA men across S1-S5 ($p=0.001$, Figure 3.11 middle). There was an

apparent trend for BA women to show mean forearm vasoconstriction across S1-S5 whereas BA men showed forearm vasodilation: any difference between men and women did not reach statistical significance ($p>0.05$).

Similarly, there was a trend for SA women to show forearm vasoconstriction and for SA men to show forearm vasodilation but any difference was not statistically significant. Both SA men and women showed pressor responses, tachycardia and forearm and digital vasoconstriction.

3.3.4.3 Comparison of responses to S1-S5 between ethnicities for same sex groups

Considering men, shown in Figure 3.12, WE men showed greater pressor responses relative to BA men ($p=0.05$) but other than this BA and SA men showed similar responses in individual variables as WE men.

Considering women only, shown in Figure 3.13, WEs showed forearm vasodilation while BAs and SAs showed consistent forearm vasoconstriction over S1-S5. There were no other differences between WE and BA women or between WE and SA women.

3.3.5 Perception of sound

As shown on Table 3.10, BAs tended to have higher perception of S1 relative to WEs ($p=0.07$). During S5, BAs had significantly higher perception of sound ($p=0.01$). There was no difference between WEs and SAs or between men and women in any of the 3 ethnic groups (Figure 3.14).

3.3.6 Responses evoked in vasodilators (VDs) and vasoconstrictors (VCs)

As indicated in Methods (3.2.3), some subjects in each ethnic group showed forearm vasoconstriction at 15s during the first sound stimulus while others showed vasodilation, as also reported by Edwards *et al.* (1998) and Ormshaw *et al.* (2018). This

prompted categorisation into two groups (vasodilators, VDs or vasoconstrictors, VCs) based on the forearm vascular response to S1 (Ormshaw *et al.*, 2018).

3.3.6.1 Baseline

As shown on Table 3.3, the baseline characteristics were similar between VDs and VCs within WE and SA groups. In the BA group, VDs showed higher FAC (p=0.02), higher SBP (p=0.02) and higher MABP (p=0.01) and higher proportion of pre-hypertensives (p=0.02) relative to VCs.

As shown on Table 3.4, the proportion of VDs or VCs was not different between whole group of WEs and BAs or between WEs and SAs (p=0.39, p=0.12). BA females had a higher proportion of VCs relative to BA males (p=0.01) and WE females (p=0.03). There were no differences between WEs and SAs (Table 3.5). There were no sex differences in proportions of VDs and VCs within the WE or SA groups (Table 3.6).

3.3.6.2 Reactive hyperaemia

There was a significant 3-way interaction between time, ethnicity and vasodilator status on ΔFVC ($F(3,86)=4.62$, $p=0.01$, partial $\eta^2=0.12$) and a significant 2-way interaction between ethnicity and vasodilator status ($F(2,64)=4.09$, $p=0.02$, partial $\eta^2=0.11$). Simple main effect of vasodilator status on ΔFVC was significant in SAs ($F(1,64)=7.46$, $p=0.01$, partial $\eta^2=0.10$). Bonferroni corrections were made with comparisons within simple main effect. There was no significant difference in reactive hyperaemia between WE and BA vasodilators and vasoconstrictors. However, SA vasoconstrictors showed blunted reactive hyperaemia relative to SA VDs ($p<0.05$, Figure 3.15).

3.3.6.3 Responses evoked by sound 1 (S1)

Reflecting the fact that individuals were grouped according to whether they showed a forearm vasodilator or vasoconstrictor response to S1, in each of the WE, BA and SA

groups, FVC responses were significantly different between the VDs and the VCs in each ethnic group ($p<0.005$ in each case).

In the 3 ethnic groups, VCs showed greater MABP relative to VDs ($p<0.05$ respectively). In addition, in the WE group, VDs showed tachycardia whereas VCs showed bradycardia ($p<0.05$, Figure 3.16). Figure 3.17 shows scatterplot of ΔFVC and ΔMABP in VDs and VCs within each ethnic group.

3.3.6.4 Responses evoked by repeated sound (S1-S5)

WE VDs showed consistent increases in FVC during S1-S5. Vasoconstriction was not consistent in WE VCs who reverted to vasodilation. Changes in MABP, HR, DCVC and FCVC were similar between the WE VDs and VCs. In BA and SA groups, VDs showed consistent increases in FVC and vasoconstrictors showed consistent decreases in FVC over S1-S5. In the BA and SA VCs, there was progressive increase in MABP over S1-S5 ($p<0.005$, 0.05 respectively). There were no differences in changes in HR or DCVC between VDs and VCs in BA and SA groups. However, BA VDs showed forearm cutaneous vasodilation while BA VCs showed consistent cutaneous vasoconstriction during S1-S5 ($p=0.05$), (Figure 3.18).

3.4 Discussion

3.4.1 Baseline characteristics

The higher resting ABP, higher proportion of pre-hypertensives and higher parental history of hypertension in young BAs in their 20s are consistent with previous reports (Ejike *et al.*, 2010). In the UK, the mean systolic blood pressure of BAs is higher than that of WEs (Chaturvedi *et al.*, 1993); the change becomes apparent by the age of 16 years and was more pronounced in boys than girls (Harding et al., 2010). The present finding that there were no significant differences between all WEs and SAs in resting ABP, or the proportions of those with pre-hypertension is also consistent with the findings of population studies for although some studies have reported higher prevalence of hypertension amongst WEs compared with SAs living in the UK (Primatesta *et al.*, 2000; Lane *et al.*, 2002; Eastwood *et al.*, 2015), these reports have not been consistent due to heterogeneity among the sub-sets of SAs (Battu *et al.*, 2018). Nevertheless, population data does indicate that South Asians have younger age of onset of CVD and a higher cardiovascular event rate relative to WEs (Khattar *et al.*, 2000; Bellary *et al.*, 2010). The higher history of hypertension suggests that the genetic component to hypertension is higher in the BAs and SAs than in WEs.

In each of the 3 ethnic groups, the men had higher SBP relative to the respective women groups. This is consistent with previous reports that men are more predisposed to developing hypertension (de Munter *et al.*, 2011; Shen *et al.*, 2017). There was a trend for MABP and DBP to be higher in BA than WE females and accordingly, for a higher proportion of BA females to have hypertensive parents, but these differences did not reach statistical significance. These trends are consistent with evidence that BA women

develop hypertension earlier than BA men and WE men or women (Geronimus *et al.*, 2007).

Considering other cardiovascular baselines, the only one that was different between ethnicities was forearm cutaneous red cell flux (FRCF), an index of skin perfusion, which was lower in BAs than WEs, this being attributable to attenuated FRCF in female but not male BAs relative to their WE counterparts. A recent study reported attenuated skin vascular conductance in American BAs relative to WE young men and women (Patik *et al.*, 2018). Moreover, resting skin blood flow (skBF) was reported to be reduced in hypertensives relative to normotensives (Carberry *et al.*, 1992). Thus, the tendency for BA women to have higher DBP, MABP and to have significantly lower skBF may all be indications of future hypertension.

3.4.2 Reactive hyperaemia

Blunted total and peak reactive hyperaemia in BAs relative to WEs is consistent with previous studies showing the impairment of NO oxide mediated FMD in BAs (Campia *et al.*, 2002b). Moreover, BA men but women showed blunted reactive hyperaemia relative to sex groups of WE. This suggests that the blunted reactive hyperaemia seen in whole BA group was due to attenuation in BA men (Stein *et al.*, 1997; Campia *et al.*, 2002b). However, the finding that SA men did not show blunted reactive hyperaemia conflicts with the report of Ormshaw *et al.* (2018). Nevertheless, in this present study, the SAs who showed forearm vasoconstriction in response to mental stress showed blunted reactive hyperaemia, relative to those who showed forearm vasodilation to mental stress. This outcome is similar to the finding of Ormshaw *et al.* (2018) who showed that young SA men not only showed attenuated reactive hyperaemia relative to WEs but a higher proportion of SA than WE men showed a forearm vasoconstrictor

response to mental stress. Taken together, these findings suggest that impaired endothelial function is not a universal phenomenon among SA and that, SAs who have no endothelial dysfunction as indicated by a non-blunted reactive hyperaemia relative to WEs do show characteristic forearm vasodilator responses to mental stress. On the other hand, the forearm vasoconstrictor response to mental stress could be a marker of coexistent endothelial dysfunction and a predictor of future CVD in the young SAs.

That WE and SA women showed blunted peak and total reactive hyperaemia relative to men is consistent with previous report on young individuals whose ethnicity was not mentioned (Hodges *et al.*, 2010). The fact that BA women did not show blunted reactive hyperaemia relative to BA men taken together with the finding that there was no difference in reactive hyperaemia between WE and BA women, raises the possibility that the lack of sex difference in BAs was mainly due to blunted reactive hyperaemia in BA men. In line with this idea, FMD was particularly blunted in BA men relative to WE men and comparable to FMD in BA women (Campia *et al.*, 2002b).

The peak of reactive hyperaemia is mediated by vasodilatory prostaglandins, myogenic relaxation as well as to opening of K^+ channels and endothelial derived hyperpolarizing factor (EDHF) (Kilbom & Wennmalm, 1976; Carlsson *et al.*, 1987; Crecelius *et al.*, 2013). By contrast, Nitric oxide (NO) does not contribute to the of peak reactive hyperaemia but may contribute modestly to maintaining reactive hyperaemia (Tagawa *et al.*, 1994; Nugent *et al.*, 1999). Thus, although, impairment of NO mediated vasodilation has been documented in BAs (Stein *et al.*, 1997), impairment of other vasodilator substances are more likely to contribute the blunted peak of RH in BA and possibly WE and SA women. The role of PGs in reactive hyperaemia was investigated and will be discussed in Chapter 5.

3.4.3 Responses to first mental stress

Consistent with previous reports, WEs and BAs showed responses to the first sound stimulus consistent with alerting response consisting of forearm vasodilation, increased blood pressure, forearm and cutaneous vasodilation (Hilton, 1982). The finding that, SAs showed forearm vasoconstriction while other components of the response were similar to the WEs is consistent with previous report on SA men (Ormshaw *et al.*, 2018). However, further analyses showed that within WEs, both men and women showed mean vasodilation to S1 whereas in BAs, the mean forearm vasodilatation in the full group disguised the fact that forearm vasodilatation occurred in BA men, but forearm vasoconstriction occurred in BA women. On the other hand, within SAs, mean forearm vasoconstriction to S1 occurred in women as well as men.

These findings underscore the need for sex specific studies in order to clearly understand the specific mechanisms involved in stress-related cardiovascular reactivity. As far as we are aware, the present study is the first to show both gender- and ethnicity-related differences in forearm vascular to mental stress.

The forearm vasodilator response to mental stress is mediated by sympathetic withdrawal, circulating adrenaline as well as other endothelium-dependent vasodilators such as NO (Section 1.3). Therefore, forearm vasoconstriction in BA women and SA women, suggests exaggerated sympathetic vasoconstrictor tone and/or altered endothelial response to circulating adrenaline, impairment of NO or impairment of release of other endothelial vasodilators such as PGs. In addition, to endothelial dysfunction, exaggerated sympathetic vasoconstrictor tone could contribute in BA women also who showed exaggerated pressor responses. There is evidence that NO mediates forearm vasodilation during mental stress (Cardillo *et al.*, 1997b; Dietz *et al.*,

1997). Indeed, the attenuated forearm vasodilation seen in BA women in the present study is consistent with the findings of blunted forearm vasodilator responses to mental stress due to impairment of NO in middle aged BAs relative to WEs (Cardillo *et al.*, 1998b). Recently, it was shown that NO generated by neuronal nitric oxide synthase (nNOS) mediates the forearm vasodilation during mental stress and nNO impairment was demonstrated in hypertensives (Khan *et al.*, 2015). Therefore in the young BAs women in particular, there may be nNO impairment which predisposes them to development of hypertension as they age. Similarly, it could be that nNO impairment contributes to the impairment of forearm vasodilator responses to mental stress in SAs: this could be tested. However, the involvement of other mediators other mediators such as PGs may also contribute to the vasodilation during mental stress in BAs and SAs, we therefore examined the role of PGs during mental stress and this will be discussed in Chapter 5.

Turning to the other components of the response to mental stress, we did not demonstrate exaggerated pressor responses or exaggerated cutaneous vasoconstriction to S1 in whole groups of BAs, or SAs compared with WEs as we hypothesised. Indeed, WE men showed greater pressor responses than BA men but BA women showed greater pressor responses than both BA men and WE women. The pressor response to mental stress may occur as a result of increase in cardiac output and or increase in peripheral resistance. During the alerting response, the skeletal vasodilation modulates the effect renal, splanchnic and cutaneous vasoconstriction on blood pressure, such that greater magnitude of vasodilation attenuates the pressor response (Brod *et al.*, 1976). The finding, contrary to our hypothesis, that BA men showed smaller pressor responses than WE men suggests that their forearm vasodilator responses were sufficient to blunt the

effect of renal vasoconstriction in the kidneys and elsewhere. According to the stress hyperreactivity theory of hypertension, the present findings suggest that the pathogenesis of hypertension is unlikely to be related to stress in BA men but may be related to stress in WE men.

Interestingly, greater pressor responses in response to mental stress in WEs relative to BAs have been reported by others when using stress induced by mental arithmetic (Fredrikson, 1986; Anderson *et al.*, 1988; Morell *et al.*, 1988; Falkner & Kushner, 1989; Fonkoue *et al.*, 2018), whereas cold water immersions elicited greater pressor responses in BAs (Treiber *et al.*, 1990; Somova, 1992; Calhoun *et al.*, 1993; Reimann *et al.*, 2012). The reasons for the disparities are not clear.

In WE men, cutaneous vasoconstriction, tachycardia as well as increased blood pressure observed during mental stress suggest that the increase in blood pressure could be due to sympathetic cardiac activity increasing cardiac output through changes in heart rate. However, in BAs, the increase in blood pressure in the presence of bradycardia suggests that vascular changes mediated the pressor response rather than cardiac changes.

In the BA women, forearm vasoconstriction suggested dysfunctional endothelial response during mental stress. Vasoconstriction in skeletal vascular bed contributes about 20-25% of total peripheral resistance. Moreover, there was bradycardia as well as vasoconstriction, suggesting that cardiac output was not significantly increased. It is probable that in BA women, endothelial dysfunction as well as heightened sympathetic vasoconstrictor tone in viscera contributed to the pressor response.

Unlike BAs, SA women showed forearm vasoconstriction but the pressor responses that were not greater than WE women, this is consistent with lower pressor responses to

stress that have been documented in SAs (Stoney *et al.*, 2002; Shen *et al.*, 2004). The findings of this present study suggest that SA may show smaller renal vasoconstriction.

The pattern of response in BA women is similar to the response to confrontation observed in a cat, consisting of skeletal vasoconstriction, visceral vasoconstriction as well as bradycardia (Adams *et al.*, 1969). This was suggested to be a second type of response to an alerting stimulus, however, has been debated and considered to have been conditioned response (Hilton, 1982). The pattern of response in BA men is similar to the pattern of skeletal vasodilation, as well as renal vasoconstriction and bradycardia observed in rabbits following stimulation of defence areas (Azevedo *et al.*, 1980).

The negative correlation of MAP and FVC implies that decreases in FVC were associated with increases in MABP, consistent with the observation in BA women who showed greater pressor responses with net forearm vasoconstriction. It is therefore vasoconstrictors in any ethnic group that would be predisposed to greater pressor responses during mental stress than vasodilators.

3.4.4 Effect of repetition of mental stress

Habituation or sensitization of alerting response during repetition of mental stress

Consistent with previous findings in WEs who were predominantly women by Edwards *et al.* (1998), there was no habituation of the forearm vasodilator or vasoconstrictor responses or cutaneous vasoconstrictor responses within a session of repeated sound stimuli in the full group of WEs or WE men and WE women of the present study. This is contrary to findings of Zbrozyna and Westwood (1988), who showed that 9 repetitions of pure sound at 90 or 100 dB and 1 kHz in WE men, caused habituation of forearm vasodilator responses within one session. However, Edwards *et al.* (1998) used 5 repetitions of pure sound at 90 dB and 2 kHz, and the present study

used 5 repetitions of pure sound at 100 dB and 2 kHz. Thus the lack of habituation could have been due to the fewer number of repetitions in the present study and that of Edwards *et al.* (1998). The number of exposures required to cause habituation has been shown to vary in animal experiments. In some dogs, repeated confrontation with a cat did not cause habituation of the alerting response but it did in other dogs (Seal & Zbrozyna, 1978). Further, in sessions of 10 daily confrontations, some animals required 2-7 daily exposures before habituation occurred while others required over 300 confrontations done over 47 days (Martin *et al.*, 1976). It is therefore probable that longer repetitions of sound stimuli would readily induce habituation in young WEs.

The new findings of the present study was that the full groups of BAs and SAs also showed no habituation or sensitization of the forearm vasodilator responses to 5 repetitions of the sound seen in BA men, or of the net forearm vasoconstriction in BA women and SA men and women, nor of their cutaneous vasoconstrictor responses. This contrasts with the hypothesis that BAs and SAs would show habituation of the vasodilator responses.

Ethnic and gender differences in responses to repeated mental stress

In the whole groups of WEs and BAs, there was sensitization of the pressor responses. The higher pressor response in WE men relative to WE women is consistent with previous report where men showed higher blood pressure and heart rate responses to mental stress (Martin *et al.*, 2008). It has recently been reported that BA women show higher prevalence of hypertension that is stress related (Hertz *et al.*, 2005; Geronimus *et al.*, 2007). Failure of habituation of the pressor responses and vasoconstriction in the forearm may explain the greater predisposition of BA women to stress induced hypertension.

Forearm vasodilation in SA men contrasts the report by Ormshaw *et al.* (2018) who demonstrated forearm skeletal vasoconstriction in SA men showed while WEs showed vasodilation. Moreover, both WEs and SAs showed bradycardia as well as reduced MABP. When individual responses were considered, however 5 of the 15 SAs did not show forearm vasoconstriction. The SA vasodilators in that study did not differ from WE vasodilators as we have also demonstrated in this present study.

The consistent impairment of forearm vasodilation during mental stress in the BA and SA women who did not show attenuation of reactive hyperaemia suggests that repeated mental stress would consistently elicit endothelial dysfunction in these women, thereby predisposing them to CVD while WE women remain protected. These women did not show blunted reactive hyperaemia, suggesting that stress induced endothelial dysfunction precedes dysfunctional response to ischaemia. Mental stress induced vasodilation involves NO from nNOS while reactive hyperaemia involves PGs and NO from eNOS. Therefore, it is possible to have impaired mental stress vasodilator response without concurrent blunting of reactive hyperaemia as we have observed in these women. There is evidence for impaired responsiveness to beta adrenergic agonist, Isoproterenol in SAs (Kapoor *et al.*, 1996) as well as in BA men (Stein *et al.*, 1997). Further, there is increased alpha adrenergic vasoconstriction in BA men (Stein *et al.*, 2000). These may explain the blunted vasodilation observed in BAs.

In this study, lower pressor reactivity to mental stress was observed in both SA men and women is consistent with findings of Stoney *et al.* (2002). The lower pressor response in the presence of forearm and digital cutaneous vasoconstriction as well as forearm vasoconstriction in SA women would suggest that peripheral resistance did not rise sufficiently to alter blood pressure in spite of lack of skeletal vasodilation. The

major defect in SAs being impaired endothelial function without heightened sympathetic vasoconstrictor activity.

3.4.5 Association between vasoconstrictor response during mental stress and exaggerated blood pressure changes

Vasoconstriction in the skeletal vascular beds contributed to increased total peripheral resistance and augment pressor response. In each ethnic group, there were vasoconstrictors, however the finding of a higher proportion of vasoconstrictors among BA women, provides the link between stress and higher incidence of hypertension observed by Geronimus *et al.* (2007).

Vasoconstrictors showed exaggerated pressor responses, a response which predicts development of hypertension in later years (Matthews *et al.*, 2003; Chida & Steptoe, 2010). In these studies only the pressor responses were measured, we have now provided evidence of negative correlation between forearm vascular conductance and MABP. Further, we provide additional finding of underlying forearm vasoconstriction in those who showed exaggerated pressor responses. The finding of this study suggests that the vasoconstrictor response to mental stress is an early indicator of cardiovascular disorder that precedes overt endothelial dysfunction and would serve as an early indicator of cardiovascular disorder. Our findings also suggest that in BAs and SAs but not WEs, the female gender is a risk factor for mental stress induced CVD.

3.4.6 Conclusion

In line with the hypotheses, mental stress elicited different patterns of response in the 3 ethnic groups and between genders within ethnicity, BA and SA women more commonly showing forearm vasoconstriction. According to the general hypothesis of

this thesis (P 81), in all the ethnic groups, subset of individuals who show forearm vasoconstriction to mental stress would be expected to be more susceptible to stress-induced CVD. This was further tested in Chapter 4 for BAs who were exposed to repeated sounds on each of 3 alternate days. In addition, the results of the present chapter showed that reactive hyperaemia is blunted in WE women relative to WE men and in SA women relative to SA men but not in BA women relative to BA men. Further, BA men but not SA men showed blunted reactive hyperaemia relative to WE men.

3.4.7 Limitations

Mental stress was studied using pure sound that lasted for 30s, this prevented venous effluent collection as the time was short. We did not assay for release of vasoactive mediators during mental stress which could have shown whether or not vasoconstrictors have impairment of specific vasodilatory mediators or release more of vasoconstrictive mediators such as endothelin. MSNA was not assessed due to lack of facilities, we would have been able to correlate vasodilator responses with the MSNA and in addition determine whether vascular baroreflex was altered or not. The grouping of vasodilators and vasoconstrictors was done on absolute change in vascular conductance using zero as the cut-off point. In the future, percentage change in vascular conductance and or resistance will be used to validate these findings.

Table 3.1 Baseline values of anthropometric values and cardiovascular variables in all WEs, BAs and SAs, men and women being grouped together

Baseline variables	White Europeans (n=22)	Black Africans (n= 28)	South Asians n=20
Age (years)	21.00±0.51	21.54±0.46	20.67±0.52
Men, n (%)	13 (59.1)	16 (57.1)	9 (45)
Women, n (%)	9 (40.9)	12 (42.9)	11 (55)
BMI (kg/m^2)	22.91±0.49	23.26±0.69	22.68±0.80
WC (cm)	78.65±2.17	77.77±1.36	75.81±3.28
FAC (cm)	24.57±0.40	24.79±0.48	23.80±0.60
SBP[1] (mmHg)	104.55±1.90	109.28±2.47	102.90±3.22
DBP[1] (mmHg)	63.68±1.26	67.53±1.34*	63.30±1.51
HR[1] (bpm)	66.64±2.03	67.76±1.50	66.47±3.16
Mean ABP[1] (mmHg)	77.30±1.29	81.45±1.63	73.90±3.42
Perceived stress score (PSS)	15.57±1.10	17.79±1.43	16.76±1.45
Salt intake score (SIS)	42.85±2.08	44.17±1.86	44.06±2.52
	Cardiovascular variables		
Mean ABP[2] (mmHg)	79.05±2.12	84.58±2.86	77.14±4.56
HR[2] (bpm)	65.56±1.97	66.93±1.69	62.47±2.45
FBF(ml/100ml /min)	4.29±0.30	4.23±0.37	4.61±0.31
FVC (CU)	0.06±0.00	0.05±0.01	0.06±0.00
DCRCF (PU)	160.97±28.77	154.23±29.01	130.24±23.21
DCVC (CU)	2.08±0.40	1.99±0.39	2.01±0.40
FCRCF (PU)	21.03±2.89	14.04±1.32*	21.43±5.23
FCVC (CU)	0.27±0.04	0.20±0.03	0.27±0.06
PH+ n (%)	0 (0%)	6 (21.4%)*	3 (15%)
FH+ n (%)	1/22 (4.55%)	7/23 (30.4%)*	6/20 (30%)§

Values are shown as mean ± SEM, except for sex, prehypertension (PH+) and parental hypertension (FH+) which are shown as number (n) and % of total. Values of ABP[1] in upper part were recorded by sphygmomanometer, those in lower part (ABP[2)] by Finapres.*$p<0.05$: WEs vs BAs, § $p<0.05$: WEs vs SAs.

Table 3.2 Baseline values of anthropometric and cardiovascular variables in WE, BA and SA men and women.

	Men			Women		
	WE (n=13)	**BA (n=16)**	**SA (n=9)**	**WE(n=9)**	**BA(n=12)**	**SA(n=11)**
Age (years	22.31±0.79	20.69±0.66	21.00±0.80	20.56±0.53	22.64±0.64*	20.40±0.70
BMI (kg/m^2)	23.52±0.68	23.26±0.69	22.51±0.82	22.03±0.61	22.21±0.74	22.82±1.32
WC (cm)	84.00±2.87	78.77±2.34	81.88±3.42	72.11±1.58	74.15±2.26	70.95±4.83
FAC (cm)	25.58±0.50	26.09±0.50	25.56±0.67	23.22±0.33	22.70±0.47	22.36±0.69
SBP[1] (mmHg)	109.54±1.43	114.69±3.52	110.44±4.11	97.33±2.77	102.08±2.11	96.73±4.04
DBP[1] (mmHg)	64.92±1.67	67.94±1.92	63.00±1.78	61.89±1.88	66.98±1.86	63.55±2.40
HR[1] (bpm)	68.31±2.22	66.53±2.13	63.44±5.74	64.22±3.80	69.42±2.05	69.20±3.15
Mean ABP[1] (mmHg)	79.79±1.28	83.52±2.43	84.26±4.24	73.70±2.10	78.68±1.82	65.42±3.57
PSS	16.08±1.50	16.21±1.44	17.50±2.60	14.89±1.67	20.67±2.75	16.11±1.59
SIS	45.33±1.86	45.29±2.21	41.63±4.14	39.13±4.24	42.60±3.29	46.00±3.15
	Cardiovascular variables					
MABP[2] (mmHg)	79.33±2.74	89.65±3.34*	85.15±3.98	78.65±3.54	77.82±4.39	70.24±7.13
HR[2] (bpm)	66.63±2.51	65.59±2.22	59.08±3.74	64.02±3.27	68.71±2.62	65.25±3.13
FBF(ml/100ml/min)	4.64±0.38	4.40±0.51	4.90±0.38	3.78±0.45	4.00±0.55	4.37±0.47
FVC (CU)	0.06±0.00	0.05±0.01	0.06±0.00	0.05±0.01	0.05±0.01	0.05±0.01
DCRCF (PU)	203.56±49.56	190.55±36.83	149.30±38.69	123.12±28.92	101.77±43.32	111.18±26.77
DCVC (CU)	2.75±0.72	2.32±0.45	1.94±0.54	1.48±0.31	1.51±0.69	2.08±0.62
FCRCF(PU)	19.07±4.89	15.07±1.96	15.07±1.65	22.77±3.47	12.55±1.56*	27.00±9.53
FCVC (CU)	0.26±0.08	0.18±0.03	0.19±0.03	0.29±0.04	0.23±0.07	0.34±0.11
PH+ n (%)	0 (0%)	6(37.5%)*	2 (22.2%)	0 (0%)	0 (0%)	1(9.1%)
FH+, n (%)	0(0%)	3(18.8%)	3 (33.3%)	1(11.1%)	4(33.3%)	3 (27.3%)

Values are shown as mean ± SEM, except for sex, pre-hypertension (PH+) and parental hypertension (FH+) which are shown as number (n) and % of total. Values of ABP[1] in upper part were recorded by sphygmomanometer, those in lower part (ABP[2]) by Finapres. *p<0.05: WE vs BAs, § p<0.05: WEs vs SAs.

Table 3.3 Baseline values of anthropometric values and cardiovascular baselines in WEs, BAs and SAs vasodilators (VDs) and vasoconstrictors (VCs).

	White Europeans		Black Africans		South Asians	
	VD (n=14)	**VC (n=8)**	**VD n=14**	**VC (n=14)**	**VD (n=7)**	**VC (n=13)**
Age(yrs)	19.75±0.31	22.25±1.11	21.43±0.74	21.67±0.73	20.83±1.11	20.58±0.58
BMI(kg/m^2)	22.89±0.50	22.94±1.08	23.61±0.69	21.88±0.69	23.39±1.15	22.30±1.07
WC (cm)	79.62±3.04	76.86±2.73	79.50±2.74	73.91±1.75	79.42±4.09	74.00±4.49
FAC (cm)	24.68±0.51	24.36±0.70	26.04±0.59*	23.33±0.54	24.71±0.42	23.31±0.87
SBP[1] (mmHg)	104.64±2.83	104.38±1.93	116.57±3.44*	102.00±2.35	104.71±4.92	101.92±4.31
DBP[1] (mmHg)	62.71±1.68	65.38±1.83	70.00±1.70	65.06±1.89	66.43±3.13	61.62±1.49
HR[1] (bpm)	66.43±2.76	67.00±3.04	68.18±2.41	67.36±1.89	66.14±6.96	66.67±3.24
MeanABP[1] (mmHg)	76.69±1.84	78.38±1.56	85.52±2.22*	77.37±1.90	73.19±4.59	73.90±3.42
PSS	16.54±1.51	14.00±1.45	16.55±1.76	19.25±2.22	13.33±2.04	18.64±1.74
SIS	43.85±2.34	41.00±4.24	44.50±1.88	43.83±3.29	43.17±3.20	44.50±3.51
	Cardiovascular Variables					
Mean ABP[2] (mmHg)	78.01±2.93	80.87±2.90	88.14±3.97	81.02±4.02	79.93±3.25	75.82±6.31
HR[2](bpm)	65.76±2.76	65.22±2.67	66.32±2.53	67.54±2.32	57.49±2.99	64.13±3.16
FBF (ml/100ml/min)	4.06±0.37	4.69±0.49	4.66±0.54	3.80±0.49	4.37±0.52	4.74±0.39
FVC (CU)	0.05±0.00	0.06±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.06±0.01
DCRCF (PU)	157.92±35.57	166.57±53.47	148.89±38.22	159.57±45.47	77.16±28.18	151.47±28.40
DCVC (CU)	2.10±0.51	2.03±0.69	1.86±0.49	2.12±0.62	1.98±1.10	2.03±0.40
FCRCF (PU)	23.06±4.01	17.30±3.53	14.04±2.41	14.05±1.23	33.80±14.66	15.25±1.79
FCVC (CU)	0.31±0.06	0.21±0.04	0.17±0.03	0.23±0.06	0.41±0.17	0.20±0.03
PH+, n (%)	0(0%)	0(0%)	6/14(42.86%)	0(0%)	1 (14.3%)	0(0%)
FH+ n (%)	1(7%)	0(0%)	4/14(28.57%)	3/14(21.43%)	0(0%)	3 (21%)

Values are shown as mean ± SEM, except for sex, pre-hypertension (PH+) and parental hypertension (FH+) which are shown as number (n) and % of total. Values of ABP[1] in upper part were recorded by sphygmomanometer, those in lower part (ABP[2]) by Finapres *$p<0.05$: VDs vs VCs.

Table 3.4 Proportions of vasodilators and vasoconstrictors in whole ethnic groups of WEs, BAs and SAs

	All WE n (%)	**All BA** n (%)	**All SA n (%)**
VDs	14(63.6)	14 (50)	7(35)
VCs	8 (36.4)	14(50)	13(65)
Total	22	28	20

Values are shown as number (n) and percentage (%) of total.

Table 3.5 Proportions of vasodilators and vasoconstrictors in WE, BA SA men and women.

	Men n (%)			**Women n (%)**		
	WEs	**BAs**	**SAs**	WEs	BAs	SAs
VDs	8 (61.5)	12(75)	3(33.3)	6(66.7)	2(16.7)	4(36.4)
VCs	5 (38.50)	4(25)	6(66.7)	3 (33.3)	10(83.3)*	7(63.6)
Total	13	16	9	9	12	11

Values are shown as number (n) and percentage (%) of total. *p=0.03: WE women vs BA women.

Table 3.6 Proportions of vasodilators and vasoconstrictors in men and women within each ethnic group.

	WEs n (%)		**BAs n (%)**		**SAs n (%)**	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
VDs	8 (61.5)	6(66.7)	12(75)	2(16.7)	3(33.3)	4(36.4)
VCs	5 (38.50)	3 (33.3)	4(25)	10(83.3)*	6(66.7)	7(63.6)
Total	13	9	16	12	9	11

Values are shown as number (n) and percentage (%) of total. *p=0.01: BA men vs BA women.

Table 3.7 Measures of reproducibility of results showing Cronbach's α Coefficient (p) during S1-S5 in WEs, BAs and SAs.

	White Europeans		Black Africans		South Asians	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
ΔFVC (CU)	0.929 (p=0.000)	0.865 (p=0.000)	0.956 (p=0.000)	0.989 (p=0.000)	0.692 (p=0.008)	0.872 (p=0.000)
ΔMAP (mmHg)	0.989 (p=0.000)	0.844 (p=0.000)	0.833 (p=0.000)	0.940 (p=0.000)	0.939 (p=0.000)	0.951 (p=0.000)
ΔHR (bpm)	0.944 (p=0.000)	0.795 (p=0.000)	0.732 (p=0.000)	0.775 (p=0.000)	0.434 (p=1.21)	0.501 (p=0.059)
ΔDCVC (CU)	0.961 (p=0.000)	0.948 (p=0.000)	0.963 (p=0.000)	0.986 (p=0.000)	0.933 (p=0.000)	0.925 (p=0.000)
ΔFCVC (CU)	0.968 (p=0.000)	0.201 (p=0.282)*	0.982 (p=0.000)	0.111 (p=0.351)*	0.896 (p=0.000)	0.930 (p=0.000)

Values shown are Cronbach's α coefficient and p values.

Table 3.8 Test of habituation or sensitization of responses during S1-S5 in whole ethnic groups.

	Ethnicity	**F(degrees of freedom)**	**P**	**Partial Eta Squared(η^2)**	**Post hoc**
ΔFVC	WEs	F(2,45)=0.506	0.731	0.024	
	BAs	F(2,45)=0.571	0.684	0.021	
	SAs	F(2,45)=1.105	0.347	0.055	
Δ MABP	WEs	F(2,48)=3.814	0.022*	0.160	S4 vs S5, p=0.004
	BAs	F(2,48)=3.582	0.029*	0.117	S2 vs S3 p=0.012
	SAs	F(2,48)=2.025	0.115	0.096	
Δ HR	WEs	F(4,84)=0.146	0.964	0.007	
	BAs	F(4,84)=1.699	0.684	0.059	
	SAs	F(4,84)=0.432	0.785	0.022	
Δ DCVC	WEs	F(2,39)=1.574	0.192	0.090	
	BAs	F(3,60)=0.580	0.624	0.027	
	SAs	F(3,38)=1.058	0.380	0.070	
ΔFCVC	WEs	F(2,33)=0.875	0.436	0.051	
	BAs	F(2,38)=2.801	0.076	0.123	
	SAs	F(2,34)=0.747	0.498	0.047	

Table 3.9 Test of habituation or sensitization of responses during S1-S5 in sex dependent groups within each ethnic group.

	Ethnicity	Sex	F (degrees of freedom)	p value	Partial Eta Squared (η^2)
ΔFVC	WEs				
		Men	F(2,22)=0.433	0.640	0.035
		Women	F(4,32)=1.187	0.335	0.129
	BAs				
		Men	F(3,38)=0.799	0.483	0.051
		Women	F(4,44)=0.997	0.420	0.083
	SAs				
		Men	F(4,32)=0.461	0.764	0.054
		Women	F(4,40)=2.127	0.095	0.175
Δ MABP	WEs				
		Men	F(2,21)=1.632	0.220	0.120
		Women	F(4,28)=2.323	0.081	0.249
	BAs				
		Men	F(2,29)=1.650	0.221	0.099
		Women	F(3,28)=3.355	0.039*	0.234
	SAs				
		Men	F(3,22)=1.526	0.218	0.160
		Women	F(3,27)=0.711	0.581	0.066
Δ HR	WEs				
		Men	F(2,22)=0.433	0.640	0.035
		Women	F(4,32)=1.187	0.335	0.129
	BAs				
		Men	F(3,38)=0.799	0.531	0.051
		Women	F(4,44)=0.997	0.410	0.083
	SAs				
		Men	F(4,32)=0.461	0.764	0.054
		Women	F(4,40)=2.127	0.123	0.175
Δ DCVC	WEs				
		Men	F(2,13)=2.356	0.136	0.252
		Women	F(2,16)=0.146	0.867	0.018
	BAs				
		Men	F(2,28)=0.368	0.830	0.030
		Women	F(1,9)=0.554	0.489	0.065
	SAs				
		Men	F(4,24)=0.275	0.891	0.044
		Women	F(2,13)=2.698	0.106	0.278
ΔFCVC	WEs				
		Men	F(1,8)=0.810	0.411	0.104
		Women	F(2,13)=1.553	0.246	0.163
	BAs				
		Men	F(2,20)=2.908	0.084	0.209
		Women	F(1,11)=0.474	0.559	0.056
	SAs				
		Men	F(4,28)=1.301	0.294	0.157
		Women	F(2,12)=1.977	0.182	0.220

Table 3.10 Perception of sound scores during S1-S5 in WEs, BAs and SAs.

	All WE	All BA	All SA	p value (WEs vs BAs)	p value (WEs vs SAs)
S1	5.64±0.52	6.82±0.40	5.55±0.46	0.07	0.90
S2	5.55±0.48	6.93±0.35	5.83±0.44		
S3	5.55±0.49	6.85±0.37	5.85±0.40		
S4	5.41±0.47	6.91±0.37	5.75±0.38		
S5	5.32±0.46	7.02±0.38*	5.70±0.44	0.01	0.55
		Men			
	WE	**BA**	**SA**	**p value**	**p value**
S1	5.54±0.56	7.06±0.52	5.00±0.50	0.06	0.51
S2	5.38±0.55	7.06±0.44	5.06±0.75		
S3	5.54±0.58	6.88±0.46	5.00±0.60		
S4	5.46±0.61	6.66±0.49	4.67±0.55		
S5	5.38±0.59	7.03±0.53	4.78±0.52	0.05	0.48
		Women			
	WE	**BA**	**SA**	**p value**	**p value**
S1	5.78±1.01	6.50±0.62	6.00±0.73	0.53	0.85
S2	5.78±0.89	6.75±0.59	6.45±0.47		
S3	5.56±0.90	6.82±0.64	6.55±0.45		
S4	5.33±0.78	7.27±0.57	6.64±0.34		
S5	5.22±0.78	7.00±0.58	6.45±0.59	0.08	0.20

Values are mean ± SEM. *****$p<0.05$: WEs vs BAs.

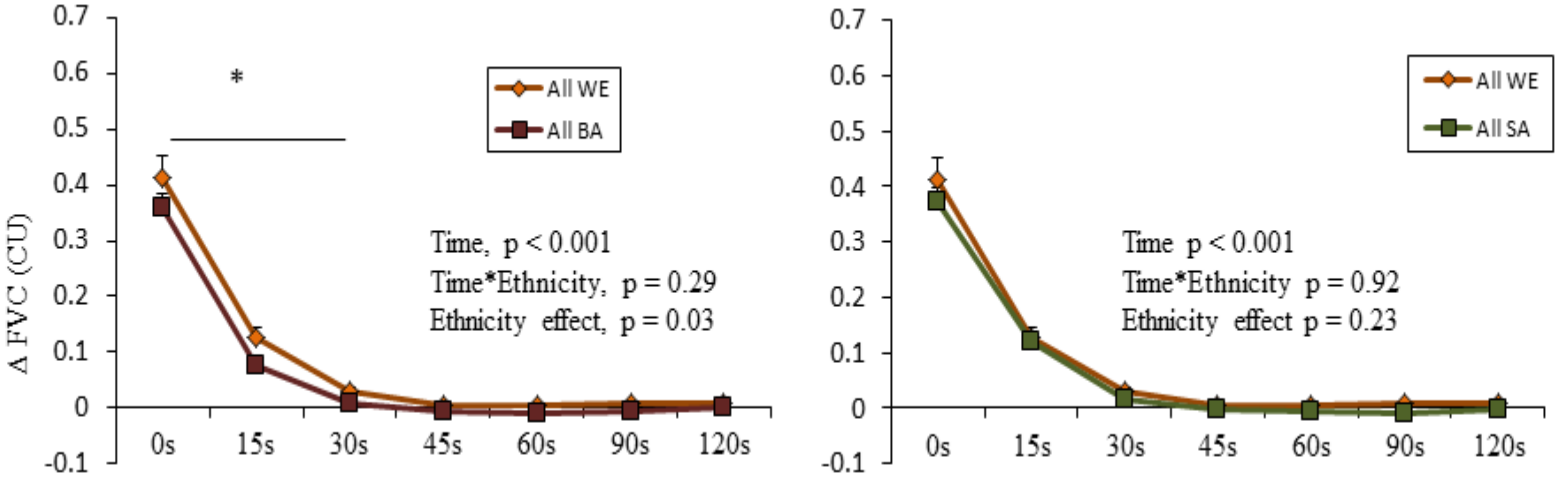


Figure 3.2: Change from baseline values of forearm vascular conductance (FVC) during reactive hyperaemia in whole ethnic groups of WEs, BAs and SAs. Values are mean ± SEM. *p <0.05: main effect of ethnicity in WEs vs BAs or WEs vs SAs with 2-way mixed ANOVA.

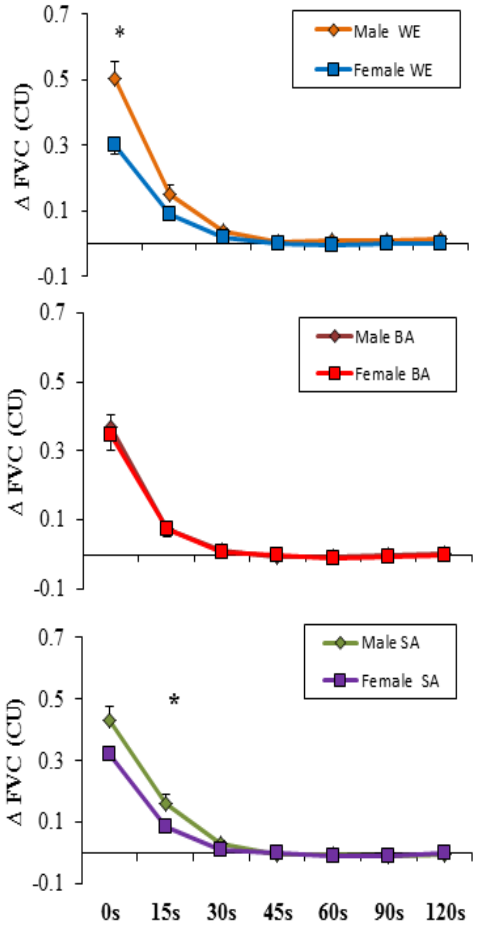


Figure 3.3: Change from baseline values of forearm vascular conductance (FVC) during reactive hyperaemia in WEs, BAs and SAs. Values are mean ± SEM. *p <0.05: males vs female, 3-way mixed ANOVA with Bonferroni post hoc test.

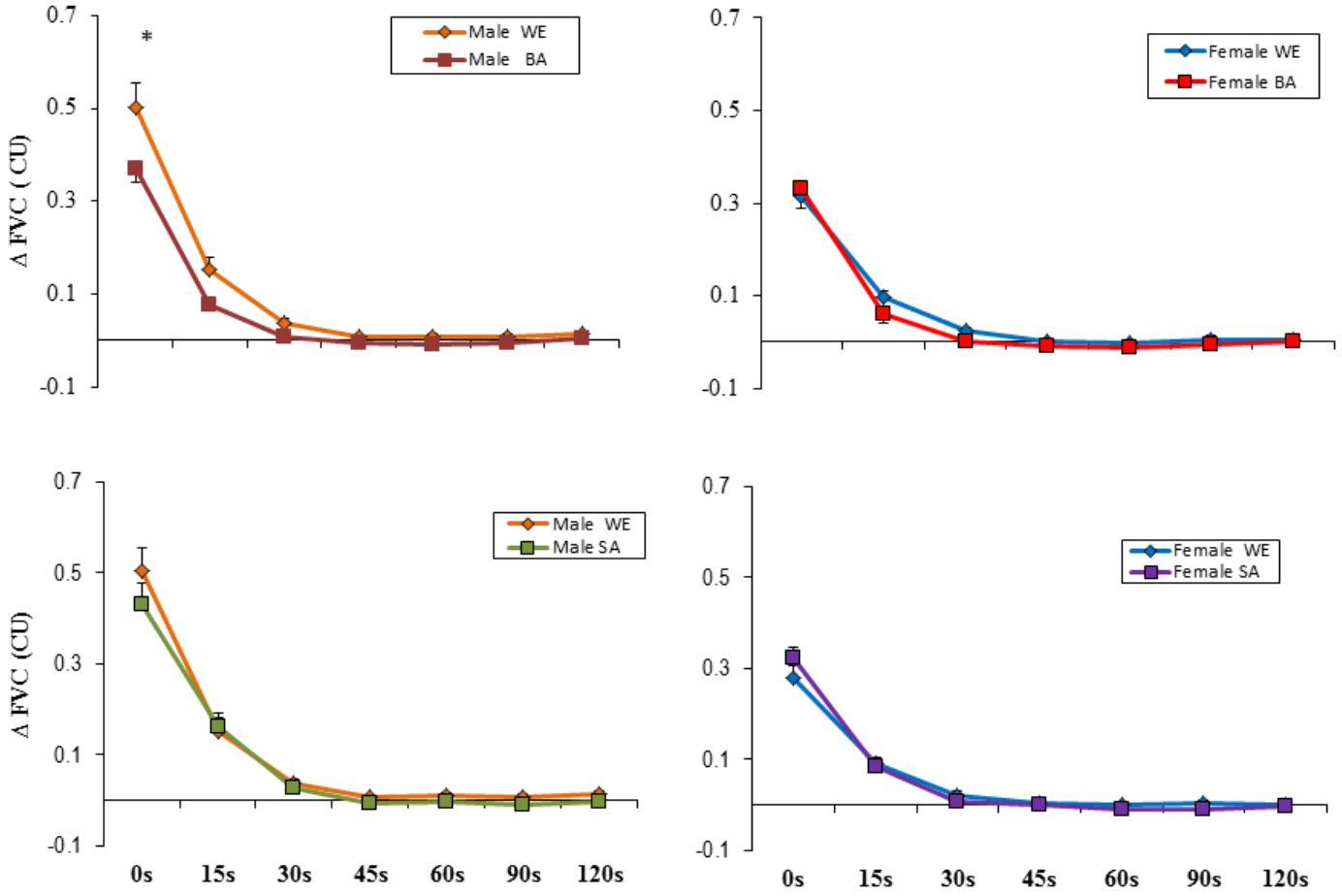


Figure 3.4: Change from baseline values of forearm vascular conductance (FVC) during reactive hyperaemia in male and female WEs, BAs and SAs. Values are mean ± SEM. *p <0. 05: WE vs BA men, 3-way mixed ANOVA with Bonferroni post hoc test.

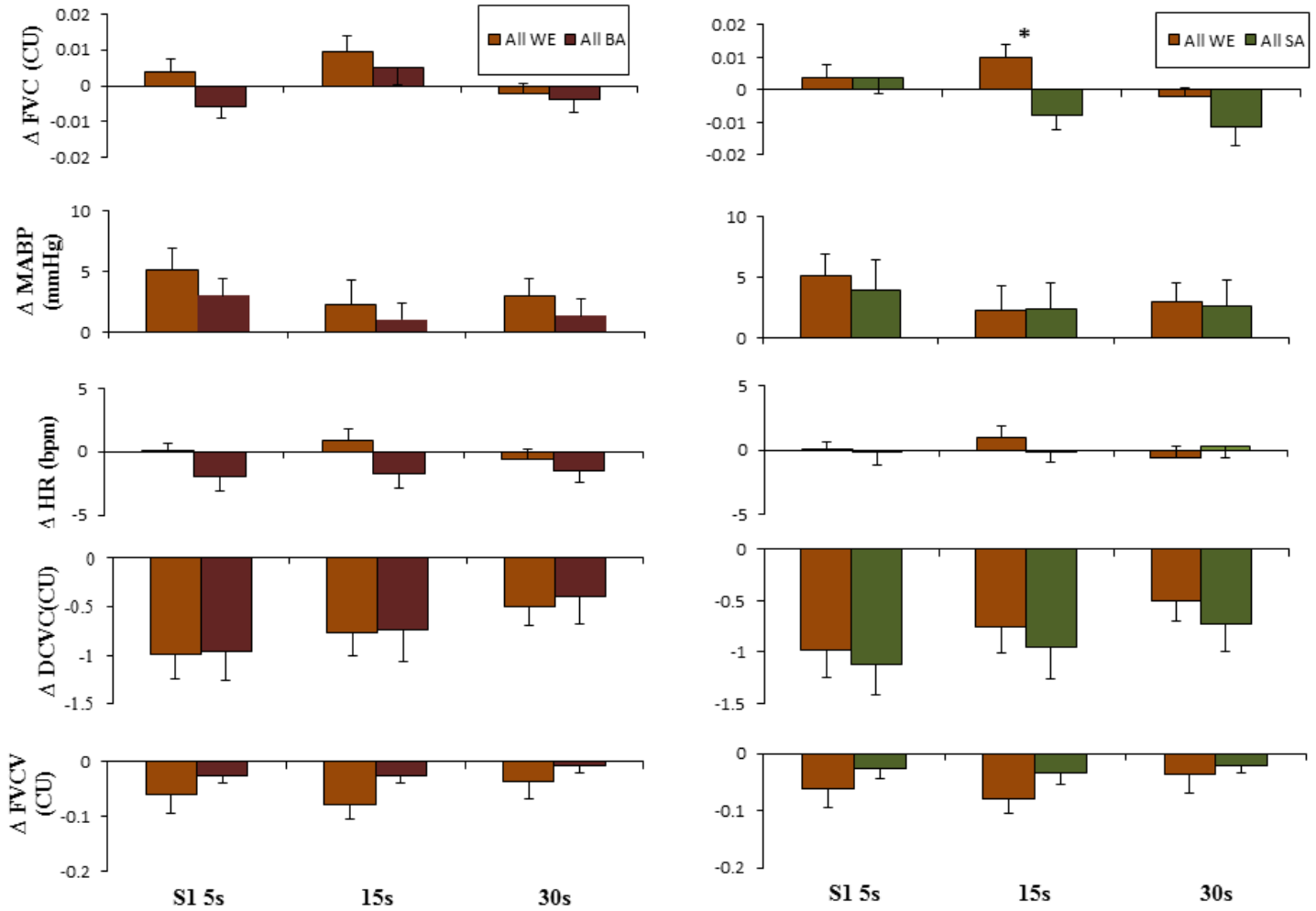


Figure 3.5: Change from baseline values of forearm vascular conductance (FVC), mean ABP (MABP), heart rate (HR), digital vascular conductance (DCVC) and forearm vascular conductance (FCVC) during sound 1 in whole group of WEs, BAs and SAs. Values are mean ± SEM. *p<0.05: WEs vs SAs, 3-way mixed ANOVA with Bonferroni post hoc test.

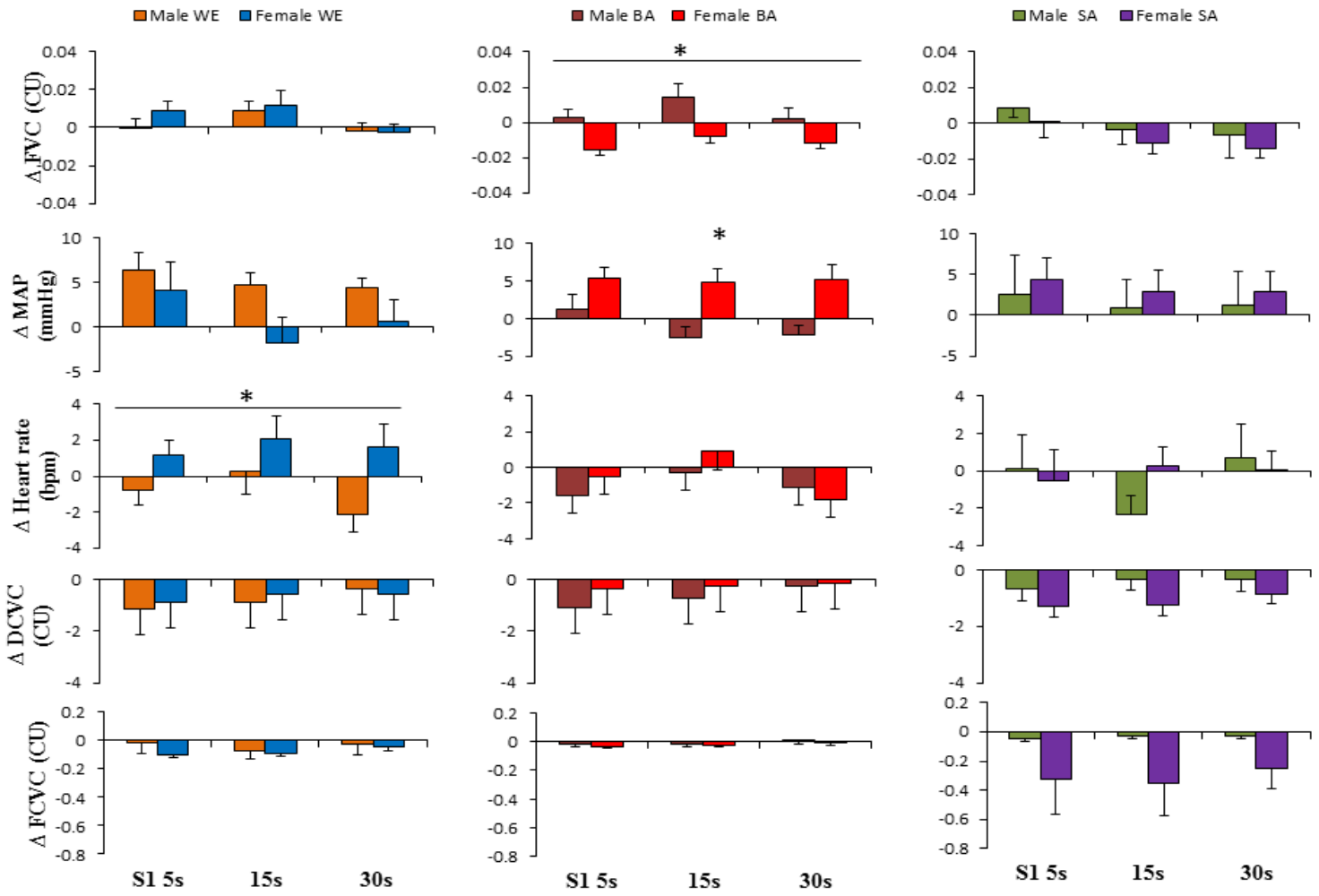


Figure 3.6: Change from baseline values of FVC, MAP, HR DCVC and FCVC during sound 1 in WE, BA and SA males and females. Values are mean ± SEM. *p <0.05: males vs females with 3-way mixed ANOVA with Bonferroni post hoc test.

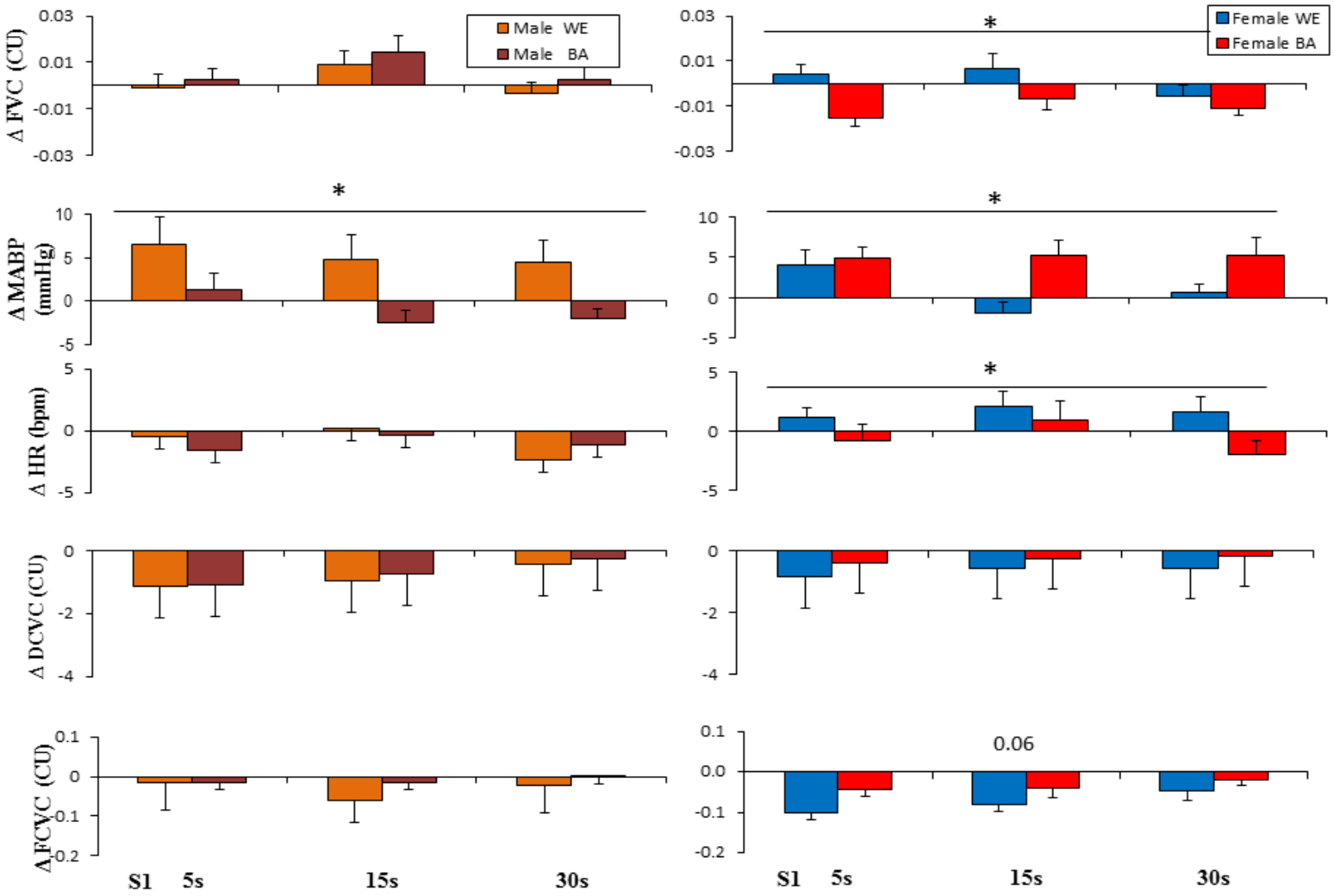


Figure 3.7: Change from baseline values of FVC, MAP, HR DCVC and FCVC during sound 1 in males (WEs vs BAs) and females (WEs vs BAs). Values are mean ± SEM. *p <0.05, main effects with 2-way mixed ANOVA in gender groups of WEs vs BAs.

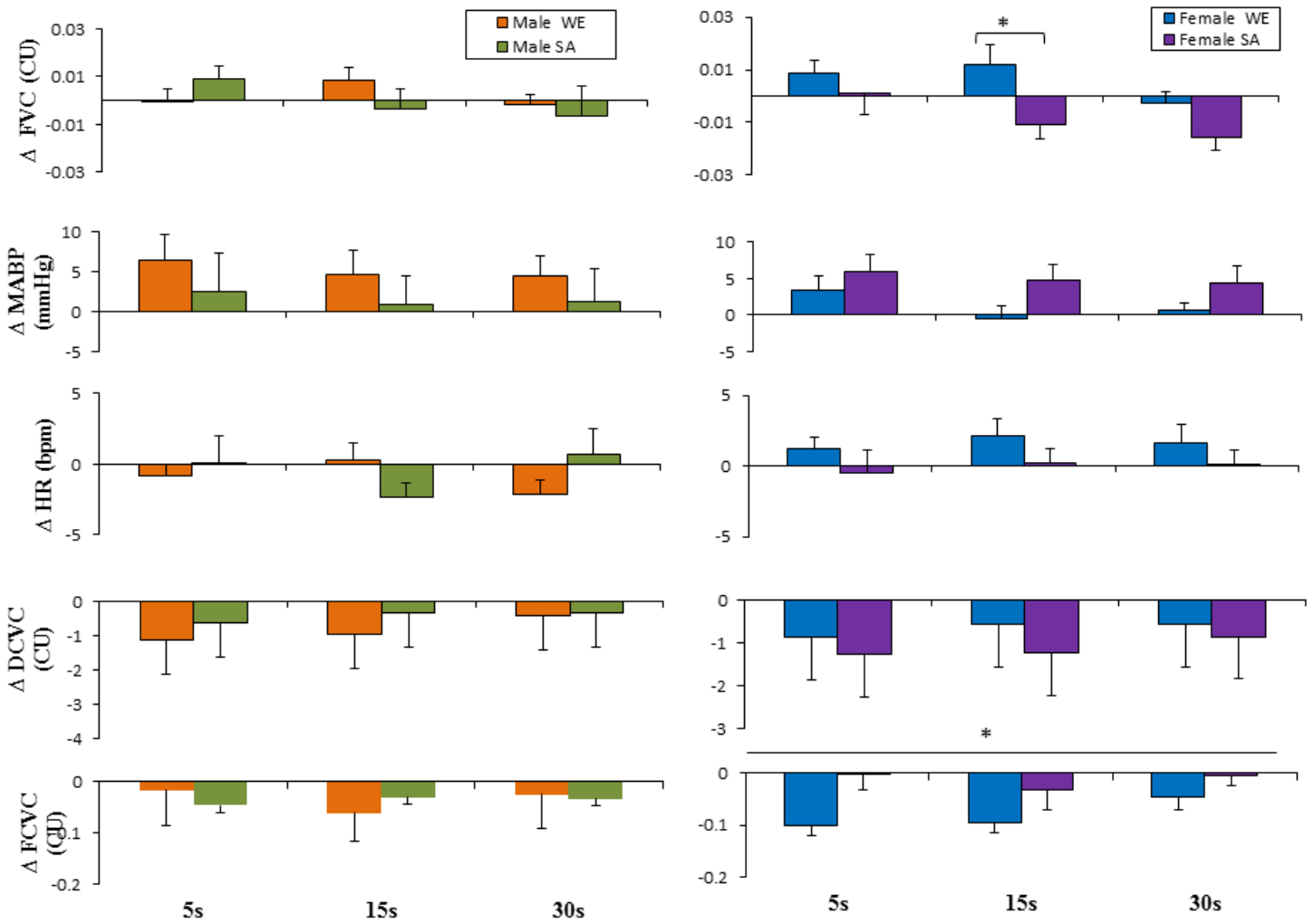


Figure 3.8: Change from baseline values of FVC, MAP, HR DCVC and FCVC during sound 1 in males (WEs vs SAs) and females (WEs vs SAs). Values are mean ± SEM. $^{§}p < 0.05$: main effects with 2-way mixed ANOVA in gender groups of WEs vs SAs

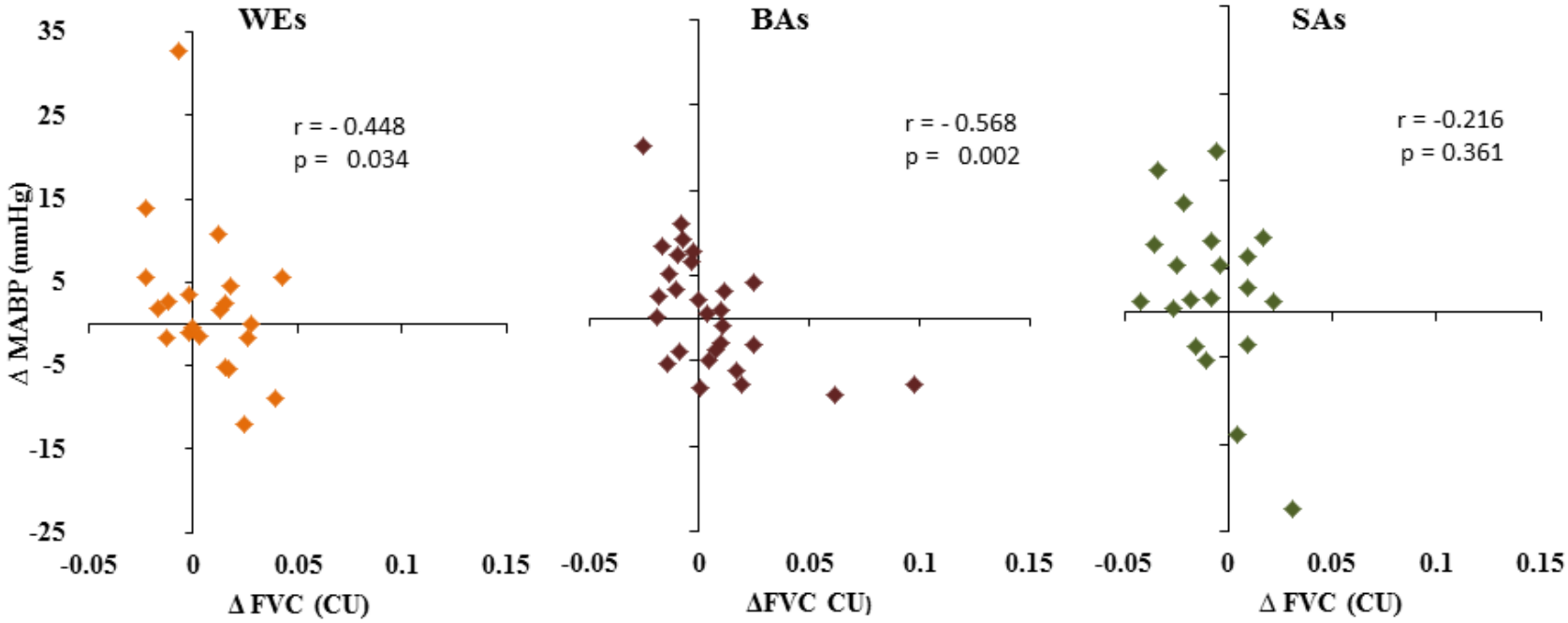


Figure 3.9: Scatterplot of relationship between change in forearm vascular conductance (Δ FVC) and change in mean arterial pressure (ΔMAP) in each ethnic group. Pearson's correlation coefficient, r=-0.448 (WEs), -0.568 (BAs) and -0.216 (SAs).

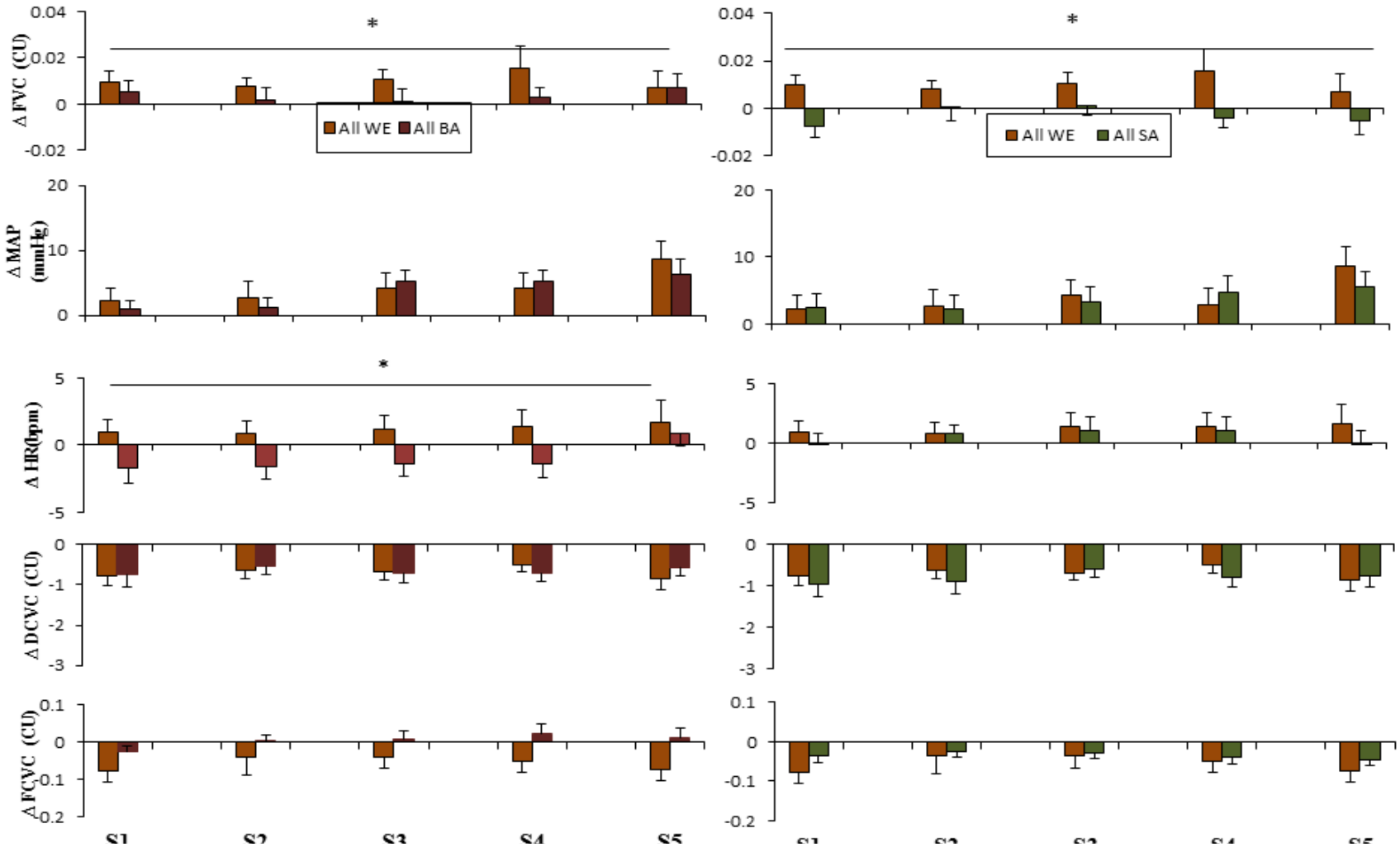


Figure 3.10: Change from baseline values of FVC, MAP, HR DCVC and FCVC during sound 1-5 at 15s into each sound in whole groups of WEs, BAs and SAs. Values are mean ± SEM. * $p<0.05$: main effect of ethnicity with 2-way mixed ANOVA.

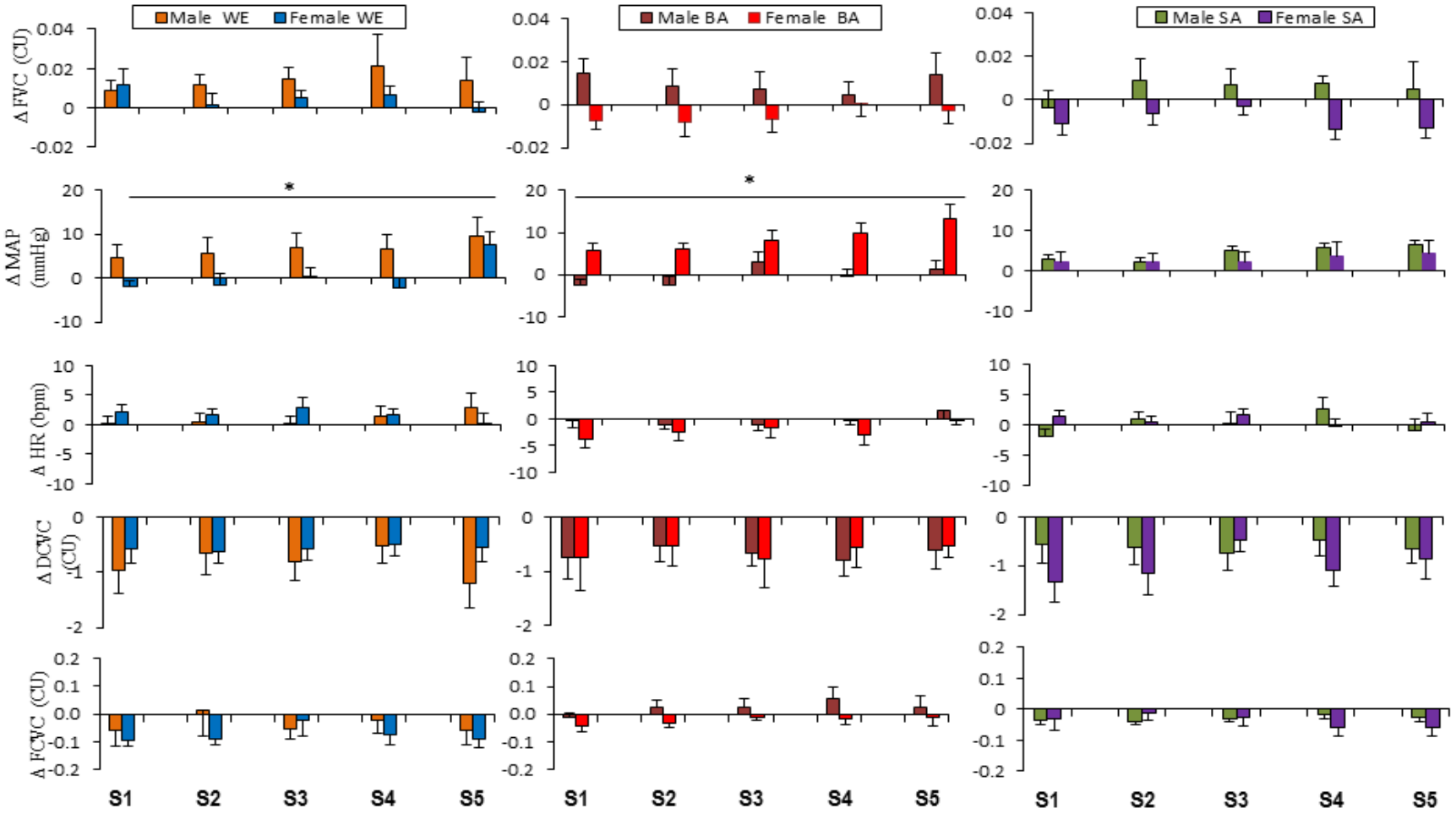


Figure 3.11: Change from baseline values of FVC, MAP, HR, DCVC and FCVC during sound 1-5 at 15s into each sound in WEs, BAs and SAs. Values are mean ± SEM. * $p<0.05$: main effect with 2-way mixed ANOVAs.

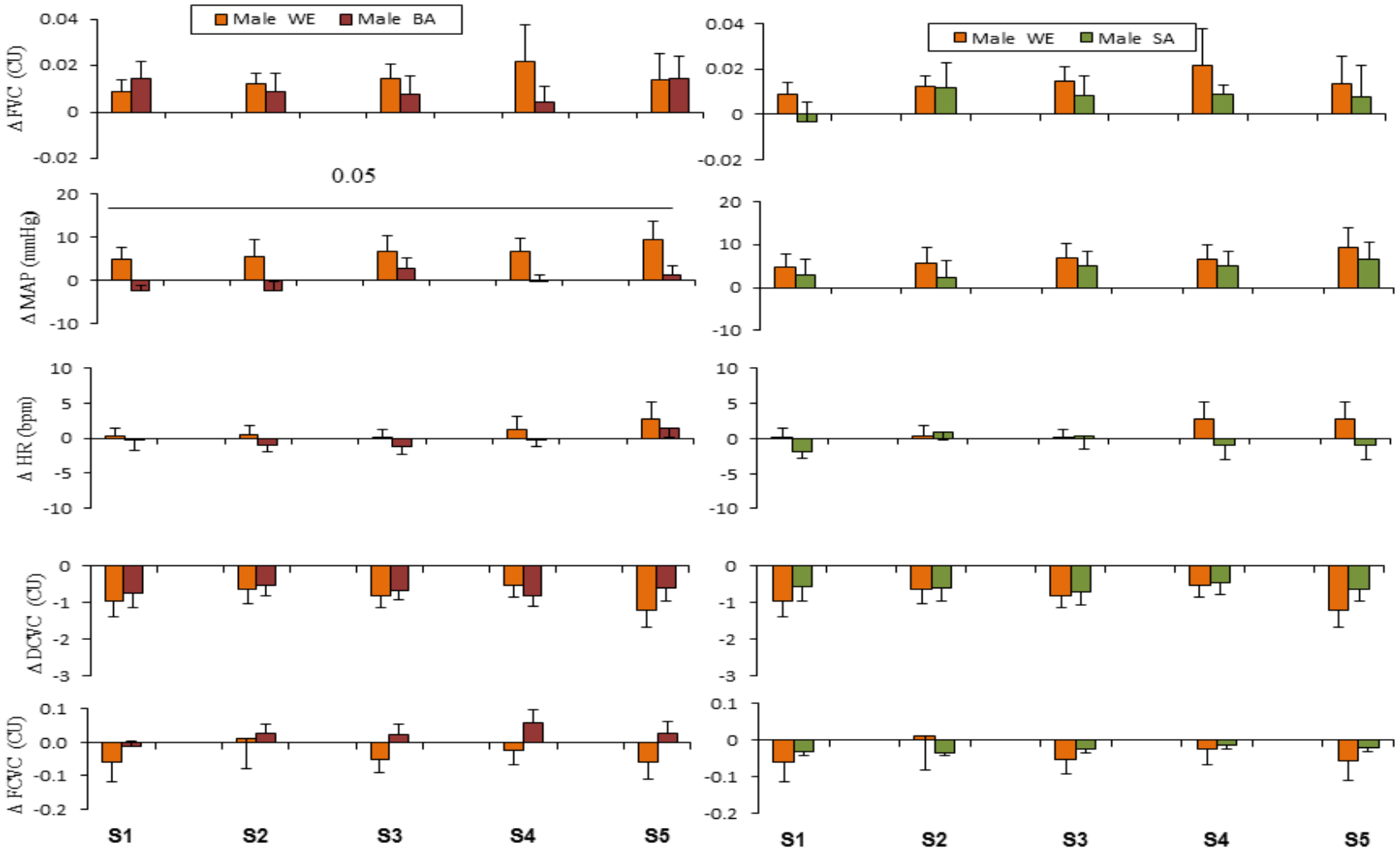


Figure 3.12: Change from baseline values of FVC, MAP, HR, DCVC and FCVC during sound 1-5 at 15s into each sound in WE, BA and SA males. Values are mean ± SEM. $p<0.05$: main effects with 2-way mixed ANOVAs.

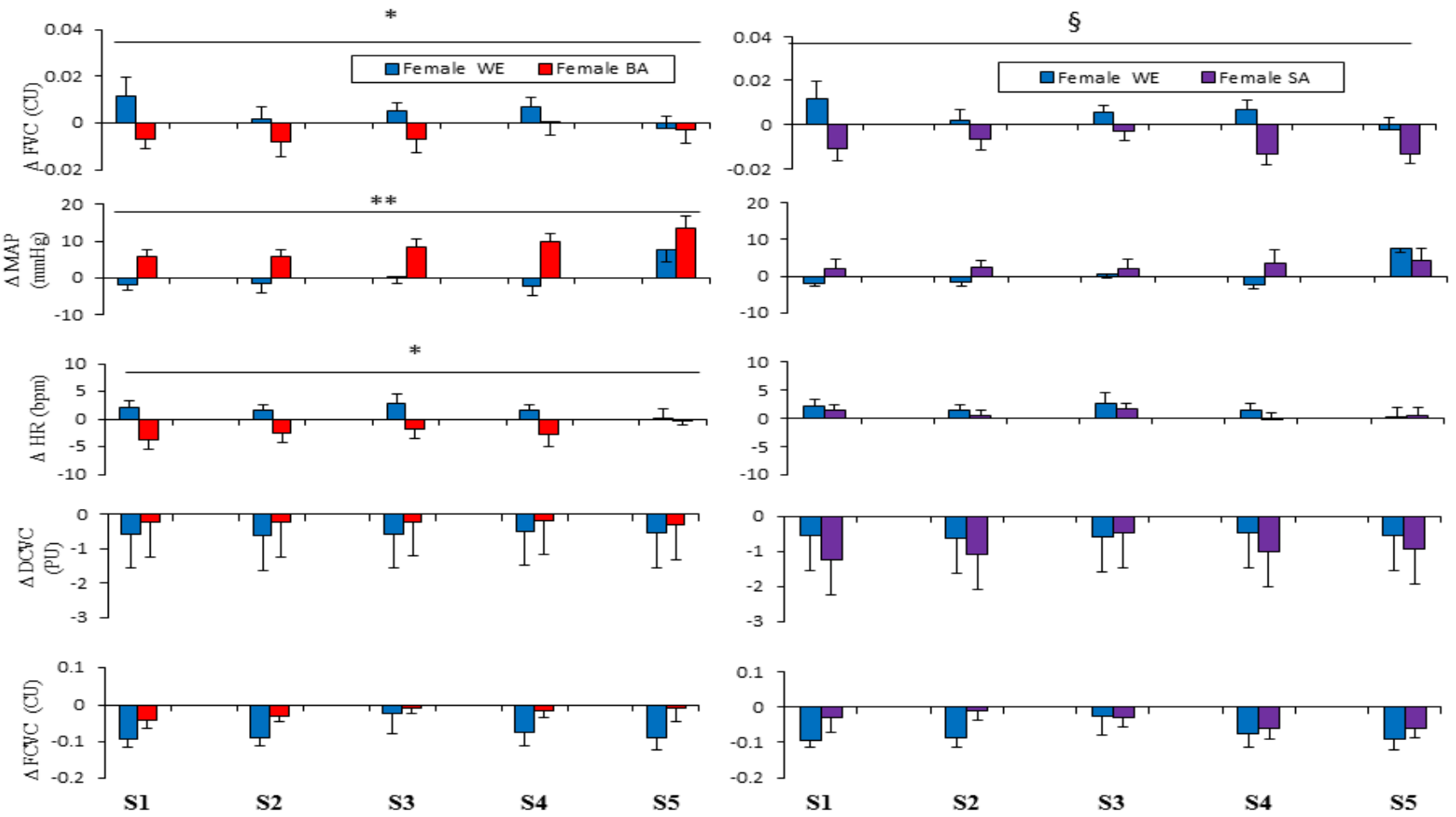


Figure 3.13: Change from baseline values of FVC, MAP, HR, DCVC and FCVC during sound 1-5 at 15s into each sound in WE, BA and SA females. Values are mean ± SEM. * p<0.05, **p<0.005 WEs vs BAs: §p<0.05: WE vs SAs. main effects with 2-way mixed ANOVAs.

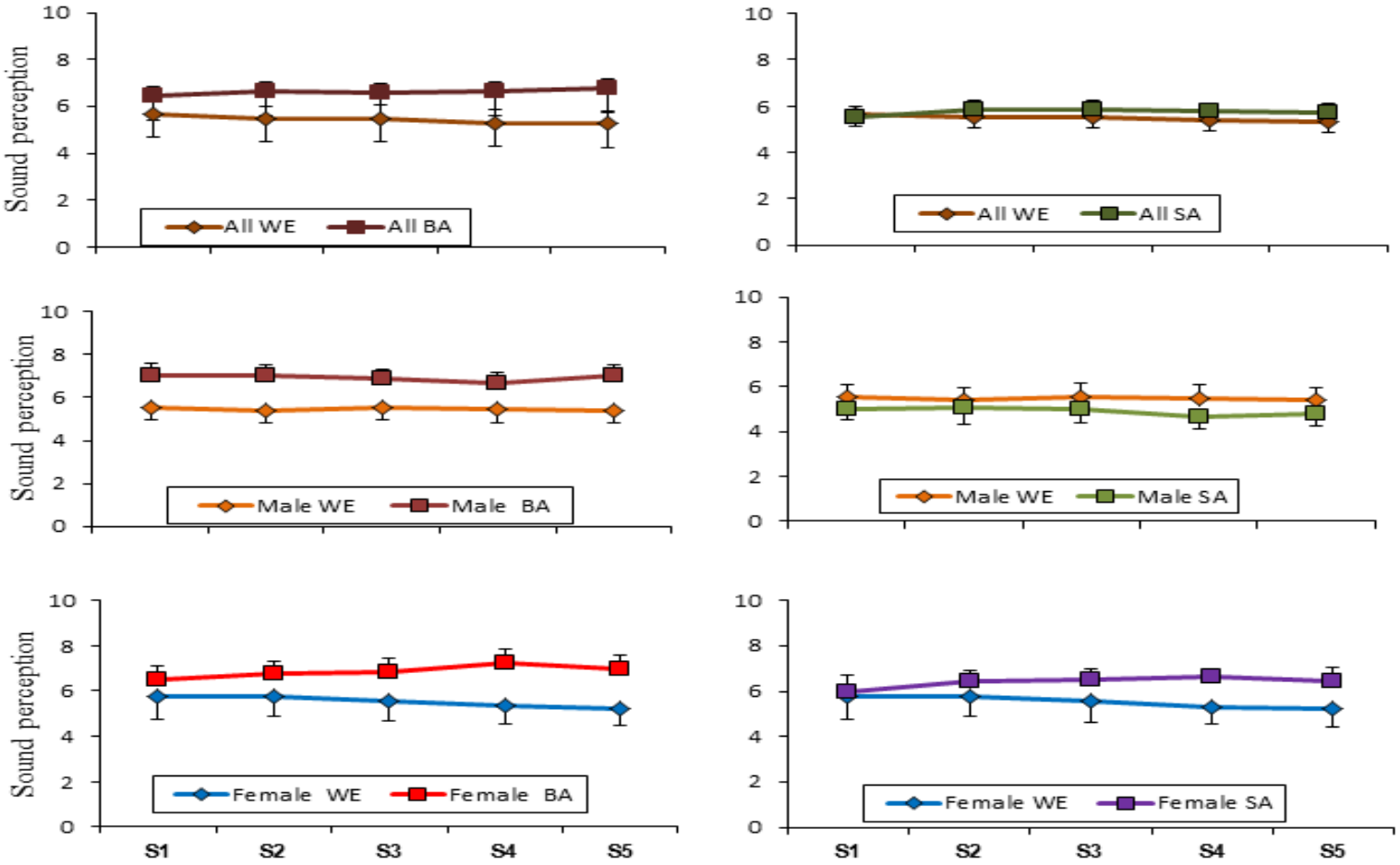


Figure 3.14: Perception of stressfulness of sound score during sound 1-5 at 15s into each sound in WE, BA and SA. Values are mean ± SEM.

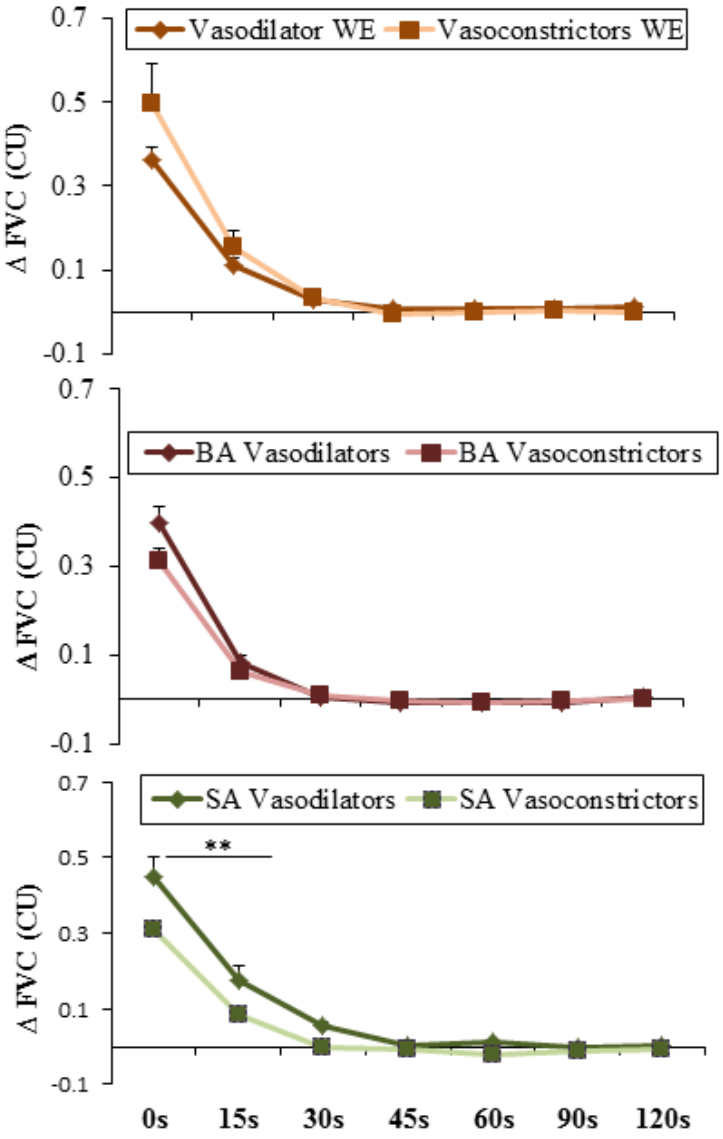


Figure 3.15: Change from baseline values of FVC during reactive hyperaemia in WE, BA and SA vasodilators (VDs) and vasoconstrictors (VCs). Values are mean ± SEM. **p<0.005: VDs vs VCs, 3-way mixed ANOVA with Bonferroni posthoc test.

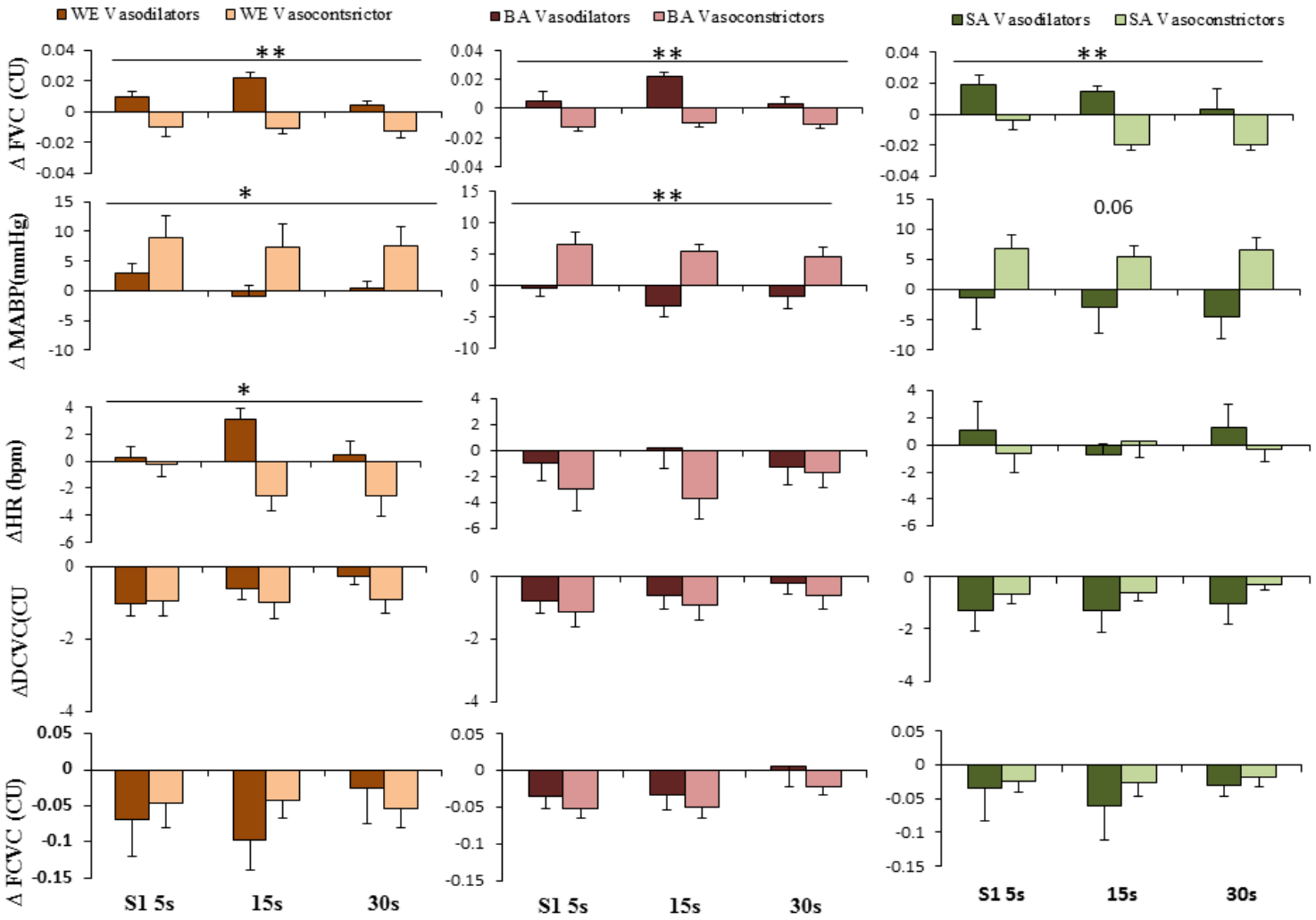


Figure 3.16: Change from baseline values of FVC, MAP, HR, DCVC and FCVC during sound 1 in WE, BA and SA vasodilators (VDs) and vasoconstrictors (VCs). Values are mean ± SEM. * p<0.05: VDs vs VCs, main effects with 2-way mixed ANOVA.

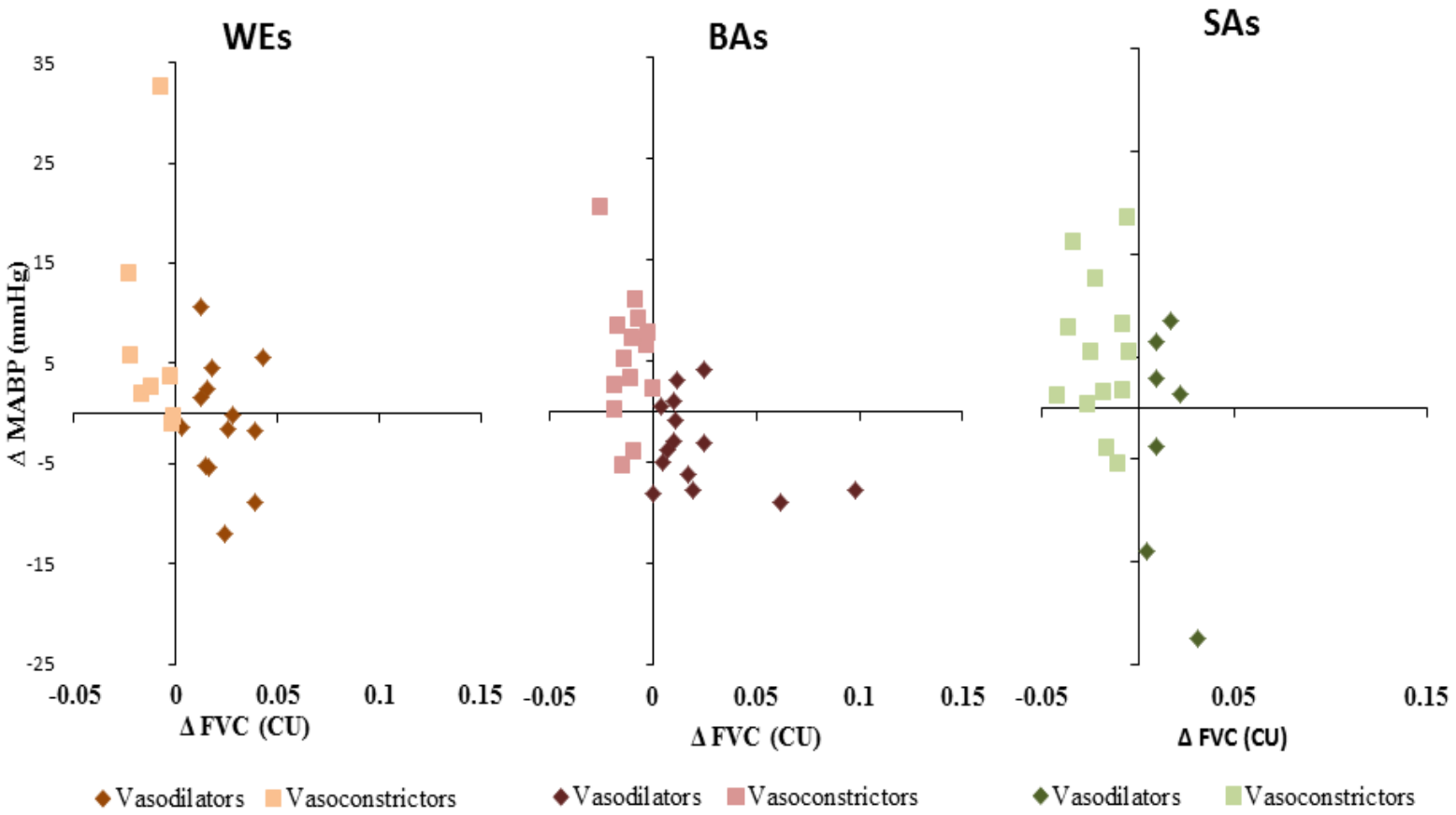


Figure 3.17: Scatterplot of change from baseline values of FVC and MAP at 15s during sound 1 in WE, BA and SA vasodilators (VDs) and vasoconstrictors (VCs).

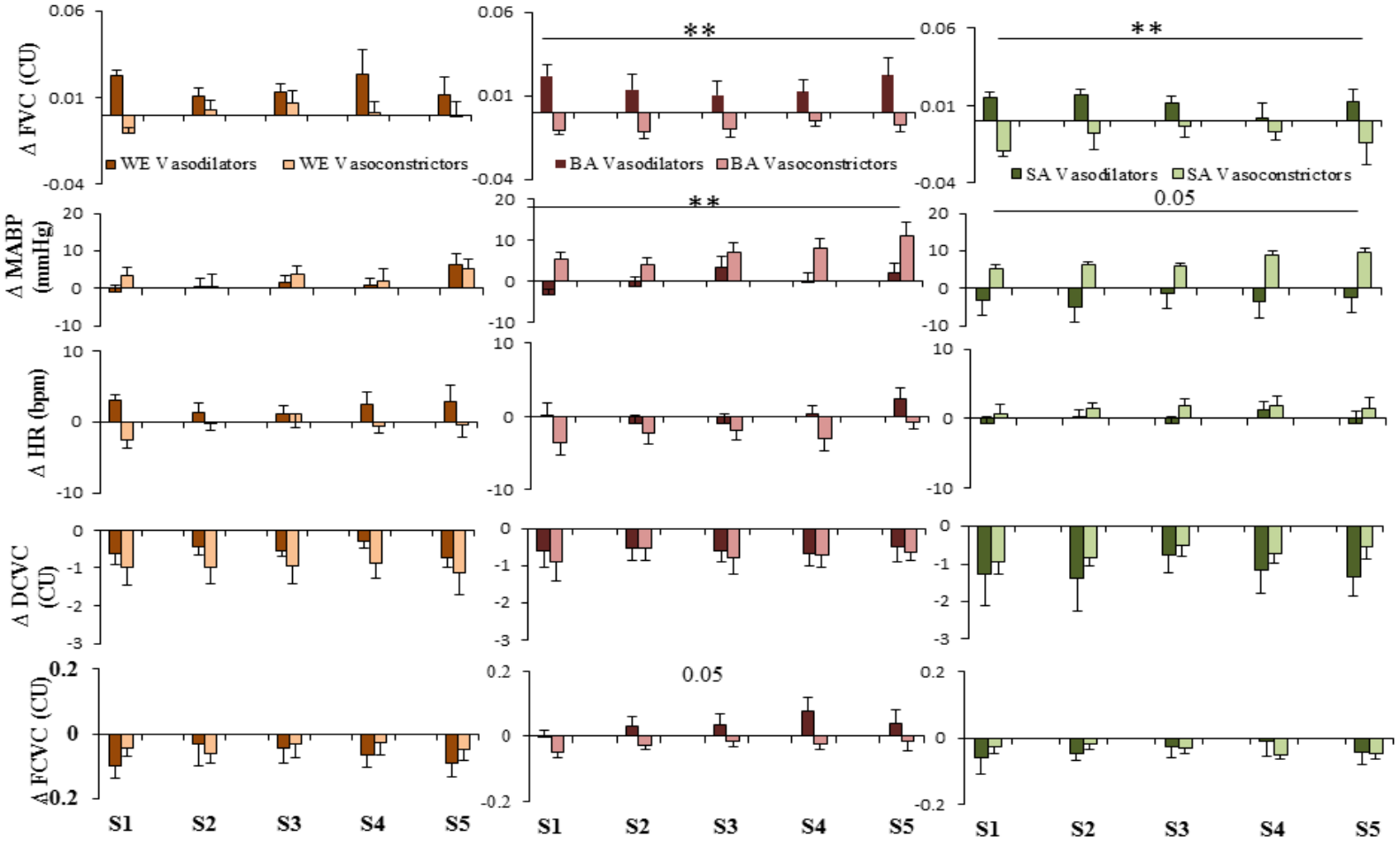


Figure 3.18: Change from baseline values of FVC, MAP, HR, DCVC and FCVC during sound 1-5 at 15s into each sound in WE, BA and SA vasodilators (VDs) and vasoconstrictors (VCs). Values are mean ± SEM. * $p<0.05$, **$p<0.005$: main effect of vasodilator status with 2-way mixed ANOVA,

CHAPTER 4

Changes In Cardiovascular Responses Evoked By Repeated Mental Stress On 3 Alternate Days

4.1 Introduction

Although novel, stressful stimuli evoke the pattern of the alerting response, which characteristically includes a pressor response, tachycardia, vasoconstriction in cutaneous, renal and splanchnic circulations, and vasodilatation in skeletal muscle, some individuals show vasoconstriction in skeletal muscle and/or an increase in MSNA (Chapter 1, 1.2). The pattern of response evoked in any given individual has been shown to be consistent when the same stimulus is repeated a few times and when different stimuli are applied (Fonkoue & Carter, 2015). Nevertheless, when the same stimulus is repeated many times within the same day, and on different days, short-, and medium-term habituation, or sensitisation occur such that responses become smaller or larger respectively (Hilton, 1982). The muscle vasodilatation is particularly vulnerable to these processes and can habituate completely, or even reverse to vasoconstriction, leaving the pressor components (Hilton, 1982). These findings are of particular interest in the context of hypertension because it is recognised that exaggerated pressor responses to laboratory stressors are predictive of future hypertension (Chida & Steptoe, 2010).

Studies performed in humans on habituation and sensitisations were generally performed on WE men and women. For example, repetition of 9 sound stimuli, or immersions of one foot in cold water within 1 experimental session evoked the pattern of the alerting response in WE men and women with muscle vasodilatation in most, but vasoconstriction in a few and these responses were generally consistent on repetition. However, when the protocol was repeated on 6 daily sessions, most individuals showed medium-term habituation (Zbrozyna & Westwood, 1988). Similarly, in a group of WE women, repetition of sound stimuli within a session evoked the alerting response with

forearm vasodilatation that showed no habituation, but when the sounds were repeated on 3 alternate days, all components of the response showed medium-term habituation (Edwards *et al.*, 1998). More recently, Ormshaw *et al.* (2018) showed in young normotensive SA and WE men that the pattern of response evoked by repetition of sounds showed no short-term habituation within session in either ethnicity; whether medium-term habituation occurs was not tested.

By contrast, in a study on young normotensive and labile hypertensive men, repeated immersion of one foot in cold water on each of 6 days evoked muscle vasodilatation in some, vasoconstriction in others, but whereas muscle vasodilatation showed medium-term habituation in the normotensives, muscle vasodilatation was initially larger in the hypertensives and any habituation was transient; the muscle vasodilatation generally recovered. Moreover, muscle vasoconstriction was more common in the hypertensives and showed no sign of short- or medium-term habituation, while the pressor responses persisted (Zbrozyna & Krebbel, 1985). In other words, young labile hypertensives behaved in a manner expected to predict, or exacerbate hypertension (Chida & Steptoe, 2010).

In the study described in Chapter 3, BA women showed larger increases in ABP in response to repeated sound stimuli within a single session than WE women and showed forearm vasoconstriction rather than vasodilatation, which was consistent on repetition On the other hand, BA men had higher resting ABP than WE men, but showed smaller increases in ABP in response to sound than WE men, and whether forearm vasodilation or vasoconstriction occurred in individual BA or WE men, the response was consistent within session in both groups. Thus, in the context of the stress hyperreactivity theory of hypertension (Chida & Steptoe, 2010) and given BAs have a higher prevalence of

hypertension, while BA women develop the disorder earlier than BA men (Geronimus *et al.*, 2007) it was important to test whether young BA women or men show medium-term habituation, or sensitisation of the cardiovascular responses evoked by mental stressors.

4.1.2. Aim of study

The aim of the present study was to test responses evoked in young BA men and women by 5 repetitions of a sound stimulus on each of 3 alternate days.

We aimed to determine

1. Whether the cardiovascular responses to repeated sound stimulus are altered over 3 days in BAs.
2. Whether there is a sex differences within BAs in the responses evoked by repeated sound stimuli on 3 alternate days.
3. Whether any component of the alerting response habituates or sensitizes over the 3 alternate days and whether the magnitude of habituation or sensitization is different from that previously reported in WEs.

4.1.3 Hypotheses

1. BA women will show initial exaggerated pressor responses during the first day of repeated sound stimuli and the pressor responses will sensitize during the 3 days of repetition of sound.
2. BA men will show small pressor responses to repeated sound during the first day of repeated sound, but the pressor responses will sensitize during the 3 days of repetition of 5 sets of sound.

3. BA men will show habituation of forearm vasodilator responses to repeated sound during the 3 days of repetition of sound, while BA women will show habituation of forearm vasodilatation and persistence of vasoconstrictor responses during the 3 days.

4.2 Methods

4.2.1 Study participants

The experiments were performed on Black African (BA) young adults aged 18 to 26 years; 7 men and 7 women.

4.2.2 Procedure

As described in Chapters 2 and 3, resting ABP was measured with a sphygmomanometer at the beginning of the experimental session while the subject reclined on the laboratory couch. Thereafter, as described in Chapter 3, ABP was continuously recorded via a finger cuff with the hand held at heart level, DCRCF was recorded from the thenar eminence and FRCF was recorded from the volar aspect of the forearm. FBF was recorded at intervals by venous occlusion plethysmography. Vascular conductance values (DCVC, FCVC and FVC) were calculated by dividing the relevant RCF or FBF value by ABP, as described in Chapters 2 and 3. The protocol was exactly as described in full in Chapter 3: in brief, recordings were made before and during reactive hyperaemia and at 5, 15 and 30s during each of 5 sound stimuli (100 Db, 2 kHz for 30s each) at intervals of 5-10 min (see Figure 4.1). The protocol was repeated on 2 alternate days, 3 times in total. The protocol is shown below (Figure 4. 1).

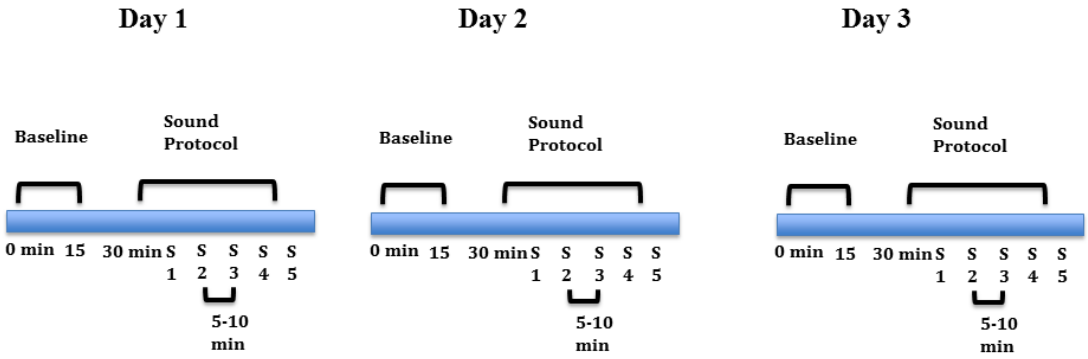


Figure 4.1: Protocol used on each of 3 alternate days. Baseline period of 15 minutes followed at 30 mins by 5 sets of sound (S1-S5) applied at 5-10 min intervals for 30s each. FBF was recorded at 5, 15s and 30s into each sound.

4.2.3 Data analysis

All data are presented as mean ± standard error of mean (SEM) except where stated otherwise. Comparisons between men and women for anthropometric measurements and cardiovascular baselines were done using independent Student's T tests.

Responses to sound were recorded as absolute values and are presented as change from the appropriate baseline values as ΔFBF, ΔFVC, ΔMABP, ΔHR, ΔDCRCF, ΔFCRCF, ΔDCVC and ΔFCVC. Only responses elicited by sound stimuli at 15s were used in this chapter. The effect of repetition of sound was tested within men and women groups on each experimental day by using one way repeated measures ANOVA in order to assess habituation or sensitization within each of the 3 daily sessions.

A 3-way mixed ANOVA with 2 within subject factors (days (3) and sound stimuli (5) and one between factor (sex), was used to assess group differences in habituation or sensitization due to exposure to 3 alternate days of repeated sound stimuli. Bonferroni post hoc test was done as appropriate. In presence of significant 3 way interactions, the data was split by gender and analysed using a 2 way repeated measures ANOVA. In all cases, $p < 0.05$ was taken as significant.

4.3 Results

4.3.1 Baseline characteristics

Women tended to be older than males (21.14±1.06 vs 23.71±0.87 years, p =0.08). Men had higher waist circumference, forearm circumference than women. Systolic blood pressure (SBP) was also higher in men than women BAs (115.71±3.33 vs 100.64±4.54 mmHg, p=0.02) and mean ABP (MABP) tended to be higher in men (84.86±2.64 vs 76.86±3.30 mmHg, p=0.08). There were no differences in other variables (Table 4. 1). There were no differences between men and women in the cardiovascular variables taken at baseline during the experiments on day 1, 2 and 3 (Table 4.2).

4.3.2. Changes evoked by repetition of sound stimuli

There was significant 3-way interaction between days, sound stimuli and sex on ΔFVC during S1-S5 ($F(8,96)=2.23$, $p=0.03$, partial $\eta^2=0.16$). There were no significant 2-way interactions: between days and sex ($F(2,24)=0.57$, $p=0.57$, partial $\eta^2=0.05$), between sound and sex ($F(4,48)=0.41$, $p=0.80$, partial $\eta^2=0.03$), or between days and sound stimuli ($F(8,96)=1.64$, $p=0.12$, partial $\eta^2=0.12$). There were no significant effects of days or sound ($p>0.05$).

There was significant 3-way interaction between days, sound stimuli and sex on ΔMABP during S1-S5 ($F(8,96)=2.25$, $p=0.03$, partial $\eta^2=0.18$) and between days and sound stimuli ($F(8,96)=2.57$, $p=0.01$, partial $\eta^2=0.18$). There was no significant 2-way interaction between days and gender or between sound and sex ($p>0.05$). There was a significant interaction between day and sound for men ($F(4,48)=4.11$, $p=0.001$, partial $\eta^2=0.41$) but not for women ($p>0.05$). Bonferroni post hoc analysis revealed effect of sound but not days was significant for the men ($F(2,24)=3.55$, $p=0.02$, partial $\eta^2=0.37$)

and ($F(2,24)=0.13$, $p=0.88$, partial $\eta^2=0.02$). There was no significant 3-way interaction between day, sound and sex for ΔHR ($p>0.05$). There was significant 2 way interaction between days and sex ($F(2,24)=4.94$, $p=0.02$, partial $\eta^2=0.29$), There was no significant interaction between sound stimuli and sex, or between days and sound stimuli ($p>0.05$). Bonferroni post hoc analysis revealed effect of days in women ($F(2,12)=8,39$, $p=0.01$, partial $\eta^2=0.58$) with decrease in HR on day 3 relative to day 1 ($p=0.04$).

There was no significant 3-way interaction between days, sound and sex or any significant 2-way interaction for ΔDCVC or ΔFCVC ($p>0.05$). There were no significant main effects ($p>0.05$).

Further, when considered in sex dependent groups with 2-way repeated measures ANOVA, there was no significant interaction between days and sounds in men or women for ΔFVC. The main effects were not significant for ΔFVC over the 3 days ($p>0.05$).

2-way repeated measures ANOVA showed significant interaction between days and sounds for ΔMABP in men but not women ($F(8,48)=4.12$, $p=0.01$, partial $\eta^2=0.41$): ($F(8, 48)=0.87$, $p=0.55$, partial $\eta^2=0.13$). There was significant main effect of sound in men ($F(4,24)=3.55$, $p=0.02$, partial $\eta^2=0.37$) but not in women ($p>0.5$). The effect of days was not significant in men or women ($p>0.05$). Bonferroni post hoc analysis revealed significant effect on day 2 in men ($p=0.01$, partial $\eta^2=0.98$: S1 vs S2, $p=0.041$, S2 vs S5, $p=0.014$) and in women on day 3 ($p=0.02$, partial $\eta2 =0.96$, S1 vs S3, $p=0.003$, S1 vs S4, $p=0.04$).

2-way repeated measures ANOVA showed no significant interaction between days and sounds for ΔHR in men and women ($p>0.05$). There was significant main effect of days

in women ($F(2,12)=8.39$, $p=0.02$, partial $\eta^2=0.58$) but not in men ($p>0.05$). The effect of sound was not significant in men or women ($p>0.05$). Bonferroni post hoc analysis revealed significant difference between day 1 and day 3 in women ($p=0.02$).

4.3.3 Changes evoked by repetition of sound in men.

On the first day, as shown on Figure 4.2, the first sound elicited responses consistent with the alerting response consisting of increased MABP and HR, forearm vasodilation, but digital and forearm cutaneous vasoconstriction. In 5 (71%) subjects there was forearm vasodilation while 2 (29%) showed forearm vasoconstriction. The evoked increase in ABP was augmented during S1-S5, indicating sensitization (see Fig 4.2, Table 4.3; $p=0.03$), but all other responses were consistent during the 5 repetitions.

On day 2, S1-S5 elicited forearm vasoconstriction (Figure 4.1), rather than forearm vasodilatation and any HR responses were small, but otherwise the changes in each variable were similar to those of day 1; the evoked increase in ABP was augmented across S1-S5 ($p<0.0001$, Table 4.3).

On day 3, forearm vasodilator responses to S1-S5 and tachycardia showed a tendency to recover, and again, the evoked increases in MABP were augmented during S1-S5 ($p=0.02$, Table 4.3). The forearm cutaneous vasoconstriction observed on days 1 and 2 reverted to vasodilation on day 3 (Figure 4.2).

When the responses for each variable to S1-S5 were averaged to give a mean response for each day (Figure 4.4), it can be seen that the net forearm vasodilation, on day 1, habituated on day 2 but recovered by day 3; there was no difference across days 1-3 ($p=0.19$). Further, the pressor responses did not change over the 3 days ($p=0.16$). There was a trend for bradycardia on day 2, but the differences in HR across day1-3 were not

significant (p=0.70). Changes FCVC and DCVC across the 3 days were also not significant. Thus, mean responses on day 3 were very similar to day 1 (Figure 4.4).

4.3.4 Changes evoked by repetition of sound in women.

On day 1, S1 elicited an increase in MABP, with vasoconstriction in digital and forearm skin, but there was a trend for a decrease in FVC, indicating forearm vasoconstriction, this occurring in 3/7 (43%) women and there was bradycardia. There were no changes in any of these responses across S1-S5 (Figure 4.3, Table 4.3).

On day 2, the responses evoked in all variables were similar to those on day 1 except that there was more obvious forearm vasoconstriction. Again these responses did not change over S1-S5 (Figure 4.3 and Table 4.3).

On day 3, there was a small forearm vasodilation during S1, which reverted to forearm vasoconstriction during S2-S4, forearm vasodilation occurring during S5; these differences were statistically significant (p=0.03, Table 4.3). Concomitantly, S1 elicited a small decrease in MABP but there was a progressive increased in MABP from S1-S5 (p=0.004, Table 4.3) and a pronounced bradycardia which was not significantly different between S1-S5 (p=0.30, see Figure 4.3 and Table 4.3). There were also pronounced digital and forearm cutaneous vasoconstrictor responses which were maintained across S1-S5. However, digital cutaneous vasoconstriction tended to sensitize as it increased from S1-S5 (p>0.05, see Figure 4.3).

As shown in Figure 4.4, when the mean responses on each day are considered, there was no difference in ΔFVC across days 1-3 (p=0.74), although forearm vasoconstriction occurred on days 2 and 3. Similarly mean ΔMABP, ΔDCVC and ΔFCVC were not

different across days 1-3 (p=0.98, 0.45, 0.88 respectively). By contrast, over the 3 days, there was a significant increase in the evoked bradycardia (p=0.006; Figure 4.4).

Thus, in BA women, the mean responses evoked by repeated sounds on day 3 comprised pronounced forearm vasoconstriction and cutaneous vasoconstriction together with a pronounced increase in MABP and bradycardia.

4.4 Discussion

The major findings of the present study were that when exposed to 5 sound stimuli on each of 3 alternate days, young men and women showed pressor responses with forearm and cutaneous vasoconstriction, which persisted until day 3 with no habituation. In addition, men showed forearm vasodilation and tachycardia, which reversed to forearm vasoconstriction and bradycardia on day 2 but recovered to forearm vasodilation and tachycardia on day 3, whereas women showed forearm vasoconstriction and bradycardia, which became more pronounced from day 1-3. These findings are important because lack of habituation, especially of the pressor components of the responses to environmental stressors but also the muscle vasodilatation, has been associated with development of hypertension (Hilton, 1982; Zbrozyna, 1982). To the best of our knowledge, this is the first study to examine whether or not BAs show habituation to repeated mental stress over several days as previous studies were done in WEs.

Before considering these findings in more detail, it should be noted that BA women had lower BMI, waist and forearm circumference than men as might be expected of young adults in their early 20s. In addition, as reported in Chapter 3, BA women had lower SBP but comparable HR and DBP to BA men, with a trend for MABP also to be lower in BA women. Hart *et al.* (2009b) showed that relative to men, young women had lower BPs (SBP, DBP and MABP), smaller stroke volume and cardiac output while HR was similar thus the reduced cardiac output reflected a lower stroke volume in women rather than lower HR. Hart *et al.* (2009b) who performed their study in the US did not indicate the ethnicity of their subjects, which may indicate they were WEs. In this present study, the similarity in DBP but not SBP between BA men and women, suggests elevated

peripheral resistance in BA women while cardiac output may be lower, unlike the situation in WE women who showed lower DBP and SBP relative to the men. Therefore, it would appear that the balance of factors that contributes to resting ABP differs between BAs and WEs.

4.4.1 Responses to repeated sound on day 1

As in Chapter 3 in BA men, the first sound elicited a pattern of response consistent with the full pattern alerting response as reported in WE men and women, including forearm vasodilatation, cutaneous vasoconstriction, increase in MABP and HR (Brod *et al.*, 1959; Edwards *et al.*, 1998; Ormshaw *et al.*, 2018). The tachycardia was more obvious in the present study than in Chapter 3, where the mean HR response to the first sound in BA men was bradycardia. However, this probably reflects the fact that 3/7 (29%) of the BA men of the present study showed forearm vasodilatation rather than forearm vasoconstriction. For, in Chapter 3, when BAs and WEs were divided into subgroups depending on whether they showed forearm vasodilatation or constriction and irrespective of whether they were male or women, the vasoconstrictors showed consistent bradycardia whereas vasodilators showed tachycardia.

In accord with this pattern, the BA women of the present study showed forearm vasoconstriction and bradycardia in response to the first sound, together with pressor responses and cutaneous vasoconstriction as in Chapter 3. Not surprisingly, as in Chapter 3, the pattern of response evoked by S1 on day 1 persisted during S2-S4 in both BA men and BA women, with no sign of habituation; in fact, the pressor response progressively increased in BA men suggesting sensitisation.

4.4.2 Responses to repeated sound over days 1-3

In accord with the hypotheses for the present study, forearm vasodilator responses to sound showed habituation in BA men on day 2, revealing forearm vasoconstriction. However the dilator responses recovered on day 3. Moreover, the pressor responses continued to increase on repetition of the sound in BA men on days 2 and 3, even though the cutaneous vasoconstrictor responses showed no obvious change. This suggests that in BA men, the pressor responses probably reflected renal and visceral vasoconstrictor components of the alerting response increasing total peripheral resistance, for any increase in cardiac output was unlikely to have made a big contribution, given the HR changes on day 2 were small and variable.

In previous studies on young normotensive WE men and women, forearm or calf vasodilator responses evoked by repeated sound stimuli or immersion of one foot in cold water habituated during the 1st or 2nd experimental session and did not return even when the stimuli were repeated in six successive sessions (Zbrozyna & Krebbel, 1985; Zbrozyna & Westwood, 1988; Edwards *et al.*, 1998). However, the forearm vasoconstrictor responses, which occurred in some individuals during these same stimuli persisted along with the pressor responses. Moreover, in young WEs with labile hypertension, calf vasodilator responses to repeated immersion of one foot in cold water showed only transient habituation on day 1 or 2 and then fully recovered, accompanied throughout by strong pressor responses (Zbrozyna & Krebbel, 1985). Thus, the changes that occurred in young, normotensive BA men on repetition of sound over 3 days were far more similar to those reported in labile hypertensive than in normotensive WE men.

As far as BA women are concerned the present findings are consistent with the hypothesis that they would show persistent forearm vasoconstriction and pressor

responses to sound over the 3 days, although the forearm vasoconstrictor responses showed some variability on day 3, while the pressor responses showed sensitization. Given skeletal muscle vasculature represents ~20% of total peripheral resistance, it seems likely that the pressor responses in women reflected skeletal muscle, as well as cutaneous, and visceral vasoconstriction. These findings are very different from those reported in WE women for their forearm vasodilator, cutaneous vasoconstriction and pressor responses to repeated sounds all habituated over 3 days such that they were virtually abolished (Edwards *et al.*, 1998). Thus, the implication of the present findings is that young normotensive BA women are less likely to show habituation and more likely to show increased persistent pressor responses to stressful stimuli than BA men as well as WE women.

As discussed in Chapter 3, simultaneous bradycardia and pressor responses during the response to alerting, stressful stimuli would be considered an alteration of what is classically described as the alerting response (Hilton, 1982). Indeed, during the alerting response, the baroreceptor reflex is inhibited such that ABP and HR increase at the same time. Evidence for this was provided when electrical stimulation of the defence area in cats, which elicited the full alerting response was combined with application of raised pressure to the carotid sinus: both the bradycardia and increase in renal sympathetic nerve activity were inhibited (Coote *et al.*, 1979). Further depression of the cardiac component of the baroreceptor reflex by mental stress was also demonstrated in humans (Steptoe & Sawada, 1989).

As discussed in Chapter 3, the fact that in BAs, and some WEs, particularly those who showed forearm vasoconstriction rather than dilatation, there was no evidence of inhibition of the cardiac and vascular components of the baroreceptor reflex implies that

in these individuals, defence area activation did not inhibit the baroreceptor reflex at the earliest stage, at nucleus tractus solitarius (Jordan *et al.*, 1988). Rather the present finding that the vasoconstrictor components of the alerting response appeared to sensitise on each of the 3 days, or at least showed a maintained magnitude on repetition, alongside sensitization of the pressor response and bradycardia (see Figures 4.1 and 4.2), especially in BA women, suggests that it was inhibition of the cardiac component of the baroreceptor reflex that was altered. It has been argued that the cardiac component of the alerting response is particularly vulnerable to inhibition during the alerting response because defence area activation increases central respiratory drive, which exerts an inhibitory influence on the vagal component of the baroreceptor reflex (see Julien 2009; Coote et al, 1979). It may therefore be that this influence on the baroreceptor reflex is dysfunctional in BAs, especially in BA women. In future studies, the effect of mental stress on the cardiac and vascular components of the baroreceptor reflex should be directly compared in young BAs and WEs.

Turning then to the issues of habituation and sensitization, although these processes have been recognised to affect evoked responses on repetition of a given stimulus since the time of the ancient Greeks, the mechanisms underlying them are still not understood (Thompson, 2009). A widely held view is that habituation and sensitization occur simultaneously and interact, and that the rate at which sensitization occurs depends on the state of arousal when the stimulus is an alerting stimulus. This is known as the Dual Process Theory (Thompson, 2009). According to this theory, habituation occurs when the level of arousal gradually decreases, whereas sensitization occurs when the level of arousal increases. Recovery from habituation generally occurs when the stimulus that has been repeated is replaced by a stronger or different stimulus. Whether or not

habituation occurs from the re-set response depends again on the dual processes of habituation, sensitization and arousal state (Thompson, 2009). This may explain the lack of habituation or sensitization in BAs in the present study. In addition, there may be alterations in the neural processes involving orbitofrontal cortex (Timms 1997) and orexin neurons discussed in Chapter1 section 1.4.

In Chapter 1, evidence was discussed indicating that habituation and sensitization can occur at the level of the hypothalamus via projections from lateral or anterior sigmoid gyri and anterior part of orbital gyrus of the prefrontal cortex, but they can also occur in the region of the RVLM via projections from the medial pre-frontal cortex. Complete habituation of the alerting response as reported by Edwards et al. (1998) could readily be explained by modulation of the whole pattern at the level of the hypothalamus. However, habituation and/or sensitization of individual components of the response as seen in the BA men and women of the present study, would seem more likely to be explained by interactions at the level of RVLM where the individual components of the vascular response are determined. Further studies are needed to elucidate the neural processes involved in habituation and sensitization.

4.4.3 Conclusions

Lack of habituation of the components of the alerting response is considered to predispose individuals to raised blood pressure when exposed the repeated stressful stimuli experienced as part of daily living and to predispose them to development of hypertension (Brod, 1982; Folkow, 1991). Thus, the present results are consistent with the hypothesis that young BA men and women are at particularly high risk of stress-induced hypertension and that BA women are at greater risk than BA men because they generally show forearm vasoconstriction as well as the other vasoconstrictor

components of the alerting response and show sensitization on repetition of the stimulus.

An obvious question that arises from the studies described in the present Chapter and Chapter 3, is why so few BA women showed the forearm vasodilator component of the alerting response as the first response to a stress stimulus, or on repetition. Given the evidence discussed in Chapter 1 that the forearm vasodilatation is largely endothelium-dependent, it seems likely that young BA women may be particularly likely to show indications of endothelial dysfunction. This issue is considered in Chapter 5.

4.4.4 Limitations

It would have been a better design if it had been possible to directly compare WE and BA men and women in the present study, rather than relying on published data. The fact that the published studies were performed in Birmingham by individuals involved in, or known to those involved in the present study, makes the comparisons easier that they might otherwise have been. It would also have been good to perform similar experiments on SA men and women to all more complete comparisons between ethnicities on habituation/sensitization.

The small number of subjects in this study precluded separation into vasodilator (VDs) and vasoconstrictor (VCs) groups. As indicated above, it would have been helpful to determine baroreceptor reflex sensitivity during mental stress, using regression of muscle sympathetic nerve activity with diastolic blood pressure and R-R interval. This would have given better insight into whether the cardiac and vascular components of the baroreceptor reflex are differentially affected during mental stress in BAs and/or WEs. Determining the heart rate variability of the subjects would also have given more information on differences in cardiac autonomic regulation.

Table 4.1 Baseline values of anthropometric and cardiovascular variables on day 1 in men and women.

	Men (n=7)	**Women (n=7)**	**P value**
Age (years)	21.14±1.06	23.71±0.87	0.08
BMI (kg/m^2)	23.48±0.91	20.18±1.01*	0.03
Waist circumference (cm)	78.50±3.71	69.36±1.82*	0.04
FAC (cm)	26.29±0.81	21.86±0.75*	0.00
SBP (mmHg)[1]	115.71±3.33	100.64±4.54*	0.02
DBP (mmHg)[1]	69.43±2.54	64.76±2.79	0.24
HR (bpm) [1]	71.00±4.40	70.86±3.47	0.98
MAP (mmHg)[1]	84.86±2.64	76.86±3.30	0.08
Perceived stress score (PSS)	19.86±2.99	14.83±2.24	0.22
Salt intake score (SIS)	44.57±2.09	46.00±2.78	0.69
Response to S1(VD/VC)	5/7(71%)	4/7(57%)	1.00
FH+	4/7(57%)	4/7(57%)	1.00

Values are shown as mean ± SEM, except for parental hypertension (FH+) and vasodilatory response to S1 which are shown as number (n) and % of total. Values of ABP[1] in upper part were recorded by sphygmomanometer.*$p<0.05$: men vs women.

Tables 4.2 Baseline values of cardiovascular variables in BA men (n=7) and women (n=7) on days 1, 2 and 3.

	Day 1			Day 2			Day 3		
	Men	**Women**	**P**	**Men**	**Women**	**P**	**Men**	**Women**	**P**
MeanABP[2] (mmHg)	92.37±4.60	81.71±5.14	0.15	84.34±6.12	86.95±6.83	0.78	80.43±3.37	78.10±3.44	0.43
HR[2] (bpm)	70.59±4.44	70.06±3.83	0.93	70.16±3.80	69.35±4.59	0.89	65.70±3.09	76.47±4.92	0.08
FBF (ml/100ml/min)	5.43±0.80	3.98±1.13	0.32	5.75±1.09	3.64±0.76	0.14	5.18±1.48	4.00±0.81	0.59
FVC (CU)	0.06±0.01	0.04±0.01	0.41	0.08±0.02	0.04±0.01	0.16	0.08±0.02	0.05±0.01	0.30
DCRCF (PU)	120.35±29.06	122.37±37.61	0.97	140.25±53.92	128.08±33.15	0.86	111.06±41.58	204.00±56.27	0.08
DCVC (CU)	1.34±0.32	1.59±0.52	0.69	2.23±0.80	1.14±0.37	0.27	1.97±0.52	1.61±0.94	0.60
FCRCF (PU)	32.32±9.10	20.58±5.84	0.30	25.34±4.63	15.28±2.72	0.09	17.12±4.95	17.69±4.09	0.13
FCVC (CU)	0.37±0.10	0.25±0.07	0.36	0.34±0.08	0.19±0.04	0.13	0.23±0.07	0.23±0.06	0.24

Values are shown as mean ± SEM, Values of ABP[2] were recorded by Finapres *p<0.05: men vs women.

Table 4.3 Test of habituation within each daily session; Effect of repetition of sound (S1-S5) on each day.

	F	Degrees of freedom	P value	Partial Eta Squared (η^2)
Males				
Day 1				
ΔFVC	1.757	4,24	0.171	0.227
Δ MABP	3.183		0.031	0.347
ΔHR	1.855		0.151	0.236
ΔDCVC	1.174		0.347	0.164
ΔFCVC	0.485		0.747	0.75
Day 2				
ΔFVC	0.547	4,24	0.703	0.084
Δ MABP	7.785		<0.0001	0.565
ΔHR	1.361		0.277	0.186
ΔDCVC	1.031		0.354	0.147
ΔFCVC	2.296		0.088	0.277
Day 3				
ΔFVC	2.301	4,24	0.088	0.277
Δ MABP	3.652		0.018	0.378
ΔHR	0.367		0.830	0.058
ΔDCVC	2.483		0.135	0.293
ΔFCVC	1.755		0.171	0.226
Females				
Day 1				
ΔFVC	0.242	4,24	0.912	0.039
Δ MABP	0.060		0.993	0.010
ΔHR	0.301		0.874	0.075
ΔDCVC	0,780		0.549	0.155
ΔFCVC	0.304		0.872	0.048
Day 2				
Δ FVC	0.270	4,24	0.895	0.043
Δ MABP	0.139		0.966	0.023
ΔHR	2.032		0.122	0.253
ΔDCVC	0.266		0.897	0.042
ΔFCVC	0.739		0.575	0.110
Day 3				
Δ FVC	3.109	4,24	0.034	0.341
Δ MABP	5.216		0.004	0.465
ΔHR	1.350		0.295	0.184
ΔDCVC	2.972		0.099	0.331
ΔFCVC	0.330		0.855	0.052

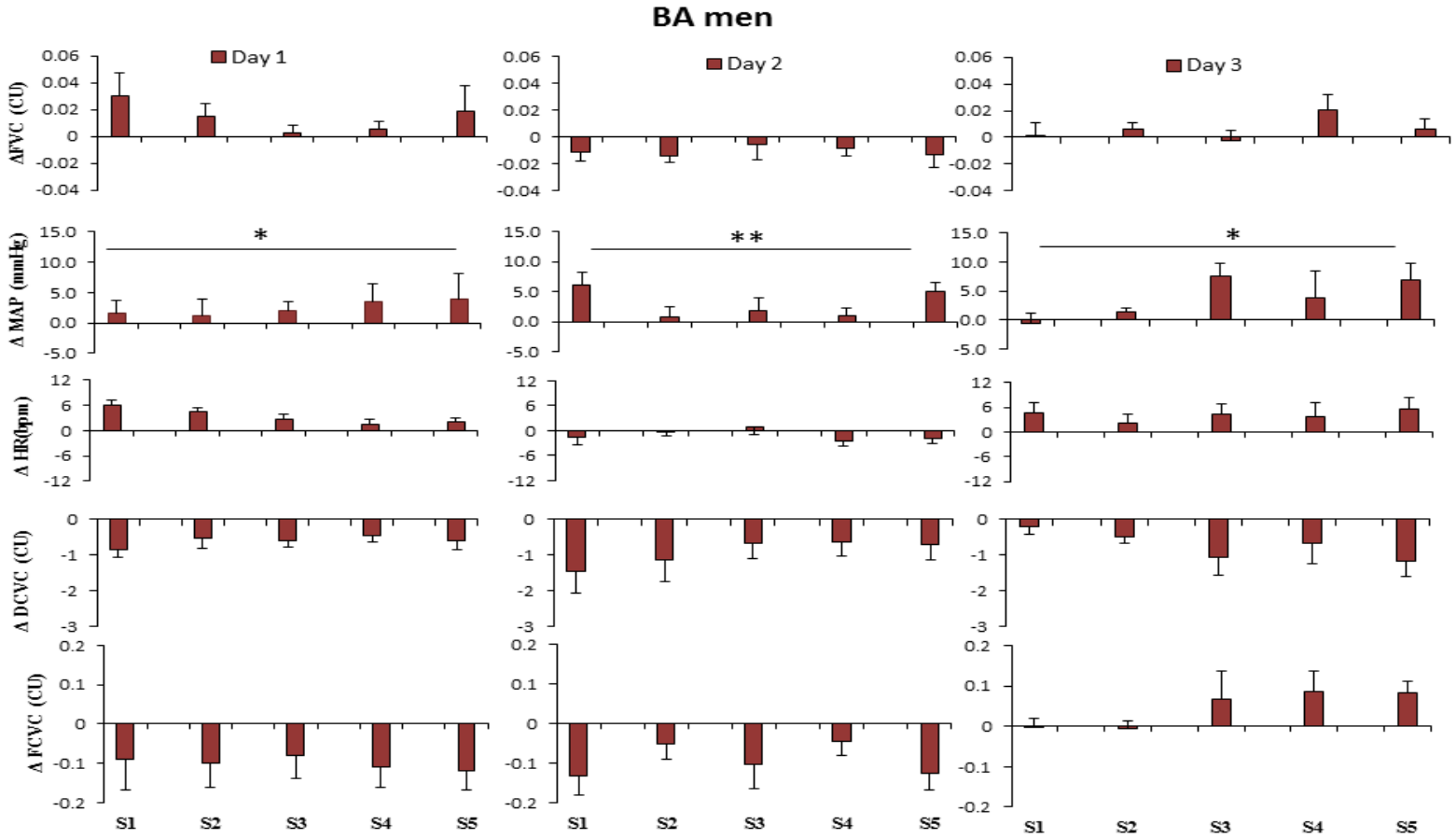


Figure 4.2: Change from baseline values of FVC, MAP, HR DCVC and FCVC during sound 1-5 at 15s into each sound on each of 3 alternate days (Day 1-3) in BA men. Values are mean ± SEM. * p<0.05, **0.005: one way repeated measures ANOVA for main effect of sound stimulus within each day.

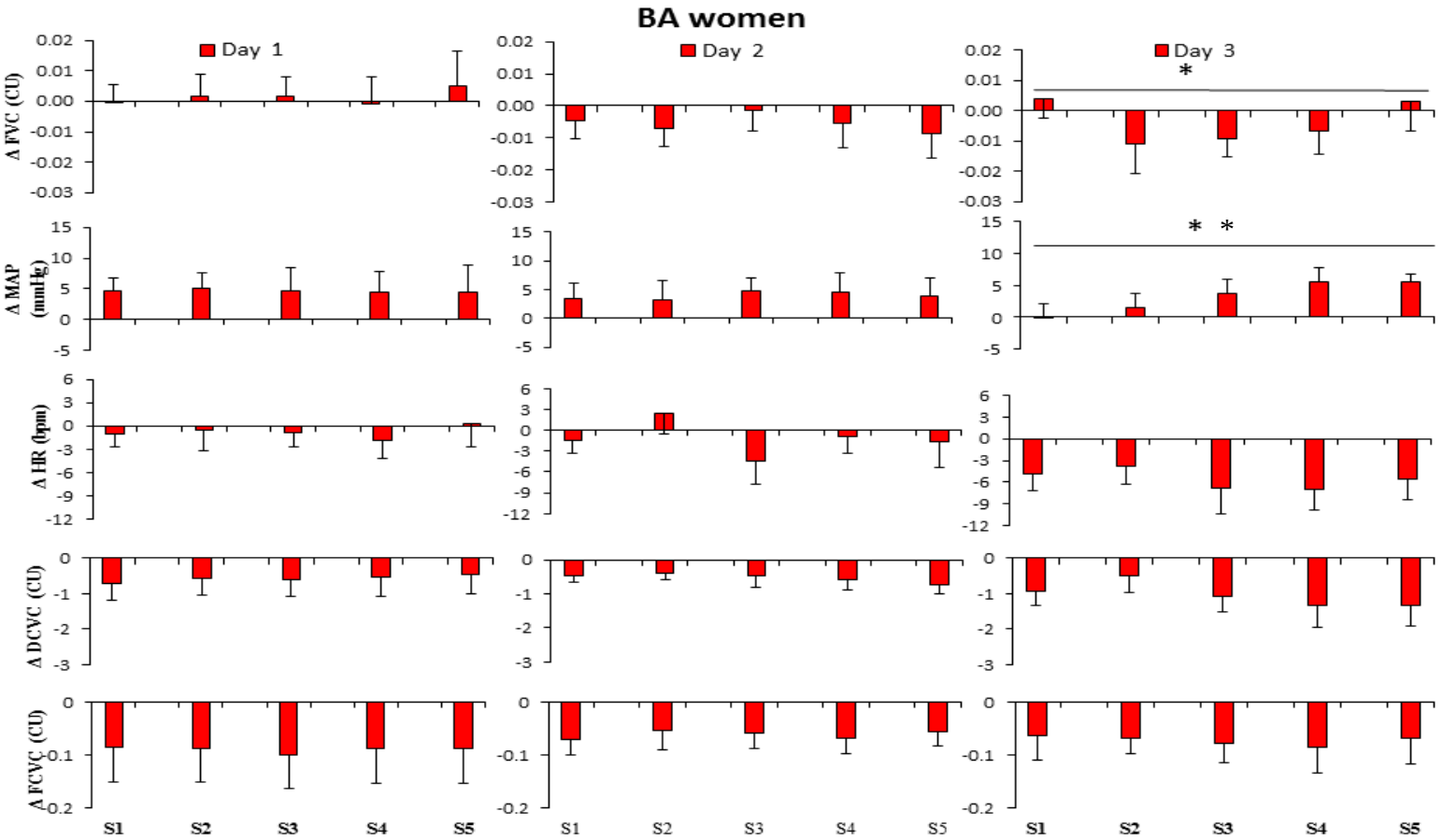


Figure 4.3: Change from baseline values of FVC, MAP, HR DCVC and FCVC during sound 1-5 at 15s into each sound on each of 3 alternate days (Day 1-3) in BA women. Values are mean ± SEM. * p<0.05, **p<0.005: one way repeated measures ANOVA for main effect of sound stimulus within each day.

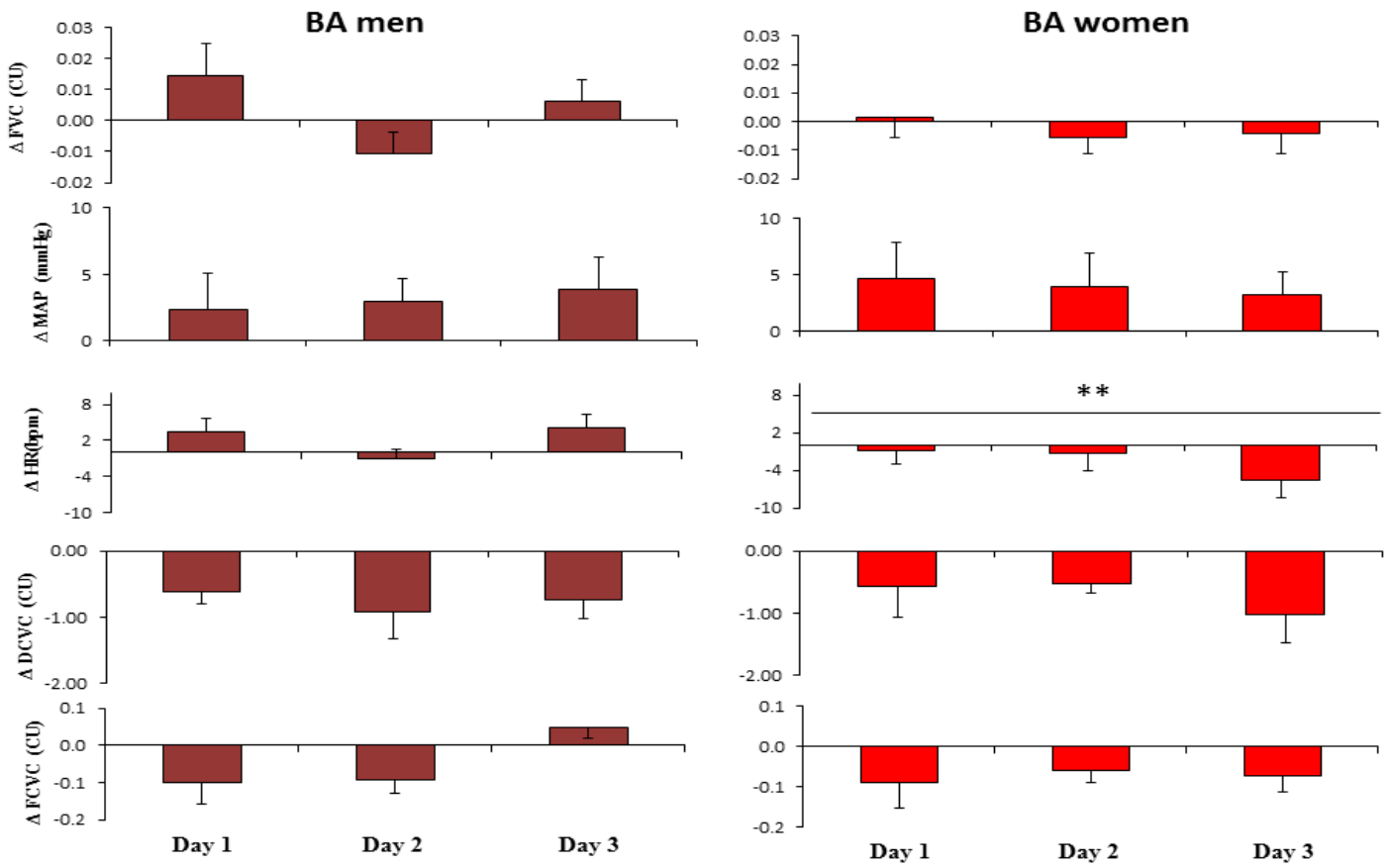


Figure 4.4: Mean changes from baseline values of FVC, MABP, HR, DCVC and FCVC on each of 3 alternate days (Day 1-3) in BA men and women. Values are mean ± SEM. *p<0.05, **p<0.005: effect of sound stimuli over the 3 days

CHAPTER 5

Ethnic And Sex Differences In Contributions Of Prostaglandins To Reactive Hyperaemia And Vascular Responses To Acute Mental Stress

5.1 Introduction

There is evidence as discussed in Chapter 1 (Section 1.3), that forearm vasodilatation evoked by mental stress is at least partly attributable to endothelium-dependent dilatation. Further, as discussed below, reactive hyperaemia is considered to be an endothelium-dependent dilatation and prostaglandins (PGs) have been implicated in reactive hyperaemia. Whether there are ethnicity- or sex-dependent differences in the contributions PGs make to reactive hyperaemia has not been tested. Further, to the best of our knowledge, whether or not PGs play a role in the forearm vasodilator, or the vasoconstrictor responses to mental stress has not been reported. Therefore in the study described in this chapter, the role of PGs in reactive hyperaemia and the pattern of responses evoked by mental stress were explored by using the cyclooxygenase (COX) inhibitor, Acetyl-salicylic acid (Aspirin), to inhibit PG synthesis. The sections below review the relevant literature that is not covered in Chapter 1 (General introduction).

5.1.2 Reactive hyperaemia

5.1.2.1 Mechanism of reactive hyperaemia

Reactive hyperaemia is the rapid increase in blood flow that occurs after release of arterial occlusion (Patterson & Whelan, 1955). The hyperaemia is transient and blood flow returns to baseline by 1.5-2 minutes (min) after release of occlusion. The peak of reactive hyperaemia increases with increasing duration of vascular occlusion (Wascher *et al.*, 1998). Reactive hyperaemia is an endothelium-dependent phenomenon, hence impaired reactive hyperaemia is an index of endothelial dysfunction and has been associated with cardiovascular risks such as hypertension (Mattei *et al.*, 1997; Taddei *et al.*, 1997b).

During arterial occlusion, the diminished pressure in the blood vessels causes myogenic relaxation. When the occlusion is released, there is rapid influx of blood into the relaxed vessels (Bayliss, 1902; Wennmalm & Sandgren, 1991). Consequent upon this increased flow, it has been suggested that shear stress increases and the endothelium is stimulated to release local mediators of vasodilatation (Koller & Bagi, 2002; Pyke & Tschakovsky, 2005). However, whilst shear stress can explain flow mediated dilation (FMD) of the brachial artery, there is no direct evidence that it plays an important role in the increase in blood flow (see Pyke and Tschakovsky (2005). On the other hand, there is substantial evidence indicating that hypoxia induced by vascular occlusion, leads to accumulation of vasodilator substances that contribute to the increased flow after release of occlusion (Carlsson *et al.*, 1987). Interestingly, Carlsson *et al.* (1987) concluded that the myogenic response is more important than the influences of vasoactive substances because when forearm blood flow was prevented by venous occlusion rather than arterial occlusion, reactive hyperaemia did not occur on release of the occlusion, even when it was extended to 20 minutes.

5.1.2.2 Role of prostaglandins in reactive hyperaemia

Formation of PGs is mediated by COX found in smooth muscles and endothelium of blood vessels. There are two types of COX: COX1 and COX 2. The major COX product in endothelium is prostacyclin (PGI_2) (Feletou *et al.*, 2011) which has a half-life of 4 minutes at near body temperature of 37^0C (Gryglewski, 2008).

The role of PGs in reactive hyperaemia was first demonstrated in animals. Firstly, release of PG following vascular occlusion was shown to accompany reactive hyperaemia in dog hind limb (Herbaczynska-Cedro *et al.*, 1974). In addition, COX inhibition attenuated vasodilator response to arachidonic acid and reactive hyperaemia

in dog hind limbs (Beaty & Donald, 1979). Subsequently, the role of PGs in reactive hyperaemia in humans was demonstrated. Arterial occlusion of the forearm for 5 minutes elicited reactive hyperaemia, which lasted for 1.5-2 min as assessed by venous occlusion plethysmography (VOP) in a group of men and women (5/5 respectively) aged 23-39 years and was accompanied by increased release into plasma of a PGE-like substances quantified by bio-assay. In addition, COX inhibition with Indomethacin attenuated the release of into plasma of PGE-like substances as well as the peak of reactive hyperaemia by 25% (Kilbom & Wennmalm, 1976). Subsequently, COX inhibition attenuated peak forearm and calf reactive hyperaemia and total reactive hyperaemia following 5 min of vascular occlusion in men and women (5/5 respectively) aged 20-40 years. The resting calf, but not forearm blood flow was decreased by COX inhibition suggesting PGs contributed a tonic dilator influence (Nowak & Wennmalm, 1979). Further in a group of 10 men and 10 women, release of PGs was demonstrated after 3 min of vascular occlusion by using the Vane superfusion technique *in vitro*. Interestingly, there was exclusive release of PGI_2 in the first minute of reactive hyperaemia but repeated vascular occlusion depleted PGI_2, providing evidence of locally formed PGs during reactive hyperaemia and a depletable pool of arachidonic acid (Serneri *et al.*, 1980).

In further experiments on a group of 5 men and 5 women aged 18-48 years, the highest doses of different COX inhibitors such as Indomethacin, Aspirin, Diclofenac, Piroxicam, Ibuprofen, Naproxen attenuated total reactive hyperaemia to varying extents, while, Aspirin, Diclofenac and Ibuprofen attenuated the peak of reactive hyperaemia (Carlsson & Wennmalm, 1983). In these experiments, since COX inhibitors did not attenuate resting forearm blood flow, they concluded that local formation of

prostaglandins occurred within the blood vessels during the period of occlusion. Ibuprofen also attenuated peak reactive hyperaemia and shortened its duration in a mixed gender group of 16 men and women, aged 19-41 years (Carlsson *et al.*, 1987). However, in a group of 18 men and 5 women, aged 18-42 years, Ibuprofen attenuated peak reactive hyperaemia but not total blood flow; in fact, there was an augmentation of blood flow during the second minute of reactive hyperaemia after COX inhibition suggesting the influence of a COX vasoconstrictor product may have been removed (Engelke *et al.*, 1996). By contrast, reactive hyperaemia in the forearm was attenuated by oral aspirin (500 mg) (Cameron *et al.*, 2012) and a higher dose of oral aspirin (1g) attenuated peak reactive hyperaemia and tended to blunt its duration (Addor *et al.*, 2008). Taken together, these results indicating that COX inhibition attenuates the peak of reactive hyperaemia are, consistent with PGs being generated during vascular occlusion and contributing to the beginning of reactive hyperaemia.

Increasing the duration of vascular occlusion increases the magnitude of peak and duration of reactive hyperaemia (Carlsson *et al.*, 1987; Wascher *et al.*, 1998). This finding is consistent with increased release of PGs during the period of ischaemia. Thus, accumulated PGs contribute to the peak of reactive hyperaemia. In addition, increased shear stress, occurring following release of occlusion may also stimulate release of PG (Koller & Kaley, 1990) and contributes to the early phase of reactive hyperaemia.

However, not all studies have demonstrated attenuation of reactive hyperaemia by COX inhibition. In fact, in 6 men (mean age of 25 years), oral indomethacin (1800mg) or ibuprofen (225mg) augmented peak reactive hyperaemia following 5 minutes of vascular occlusion but had no effect on peak forearm vascular conductance or that recorded during recovery (Naylor *et al.*, 1999). Therefore, they argued that PGs do not

contribute to the vasodilatation that underlies reactive hyperaemia. On the other hand, COX inhibition with oral Indomethacin augmented both peak reactive hyperaemia and forearm vascular conductance following 5 minutes of vascular occlusion in a group of 7 men and 6 women (aged 18-34 years), whereas in older men and women (aged 55-77 years) there was attenuation of peak reactive hyperaemia (Taylor *et al.*, 2014). The authors suggested that the earlier studies mentioned above (of Carlsson and others) had missed this augmenting effect of COX inhibition on reactive hyperaemia because they had relied on blood flow rather than calculating vascular conductance. Augmentation of a recognised endothelium-dependent vasodilatation following COX inhibition is rather surprising for COX inhibition has generally been shown to improve endothelial dilator function when endothelial dysfunction is present, and to be an indication that that vasoconstrictor COX products play a role in endothelial dysfunction (Husain *et al.*, 1998). There is no obvious reason to suppose that the young adults in the studies of Taylor et al., (2014) and Naylor et al., (1999) had endothelial dysfunction, for they were in the normal BMI range, were normotensive and had no known pathology.

Thus, these opposing sets of results raise the possibility that healthy young individuals may fall into two groups with respect to the effect of COX inhibition; those who show attenuation and those who show augmentation of reactive hyperaemia. It has been suggested that in healthy people inhibition of PG generation with COX inhibitors may enhance production of vasodilator eicosanoids (epoxyeicosatrienoic acids; EETs) via the cytochrome P450 pathway and thereby cause augmentation of vasodilator responses (Cohen & Vanhoutte, 1995; Ozkor & Quyyumi, 2011). Thus, EETs, which are known as endothelium derived hyperpolarizing factors (EDHFs) may have contributed to the

augmentation of reactive hyperaemia after COX inhibition (Naylor *et al.*, 1999; Taylor *et al.*, 2014).

5.2.3 Other mediators of reactive hyperaemia

It has generally been reported that NO contribute modestly to total reactive hyperaemia, i.e. to its maintenance, rather than to the peak (Tagawa *et al.*, 1994; Engelke *et al.*, 1996; Nugent *et al.*, 1999). It was also shown that the combination of COX and NOS inhibition attenuated the later phase of reactive hyperaemia by ~35%, but tended to augment the peak (Engelke *et al.*, 1996; Crecelius *et al.*, 2013). If shear stress contributes to reactive hyperaemia then the effect of shear stress on NO synthesis may synergise with PG (Gryglewski, 2008) to contribute to the early phase of reactive hyperaemia.

On the other hand, there is evidence that adenosine makes a substantial contribution to reactive hyperaemia and the fact that adenosine receptor inhibition and COX inhibition together had no greater effect than either independently suggested that their contributions are interdependent (Carlsson *et al.*, 1987). This is consistent with the evidence that PGs and adenosine act interdependently under conditions of hypoxia (Marshall & Ray, 2012; Mortensen et al., 2012).

Further, it has been shown that K^+ channel opening makes a major contribution to both peak reactive hyperaemia and total hyperaemia in the forearm, for inhibition of inwardly rectifying potassium channels with barium chloride ($BaCl_2$) attenuated the peak and total reactive hyperaemia and the combination of $BaCl_2$ and Oaubain, a Na^+/K^+-ATPase inhibitor nearly abolished reactive hyperaemia (Crecelius *et al.*, 2013). Since inhibition of these mechanisms would block hyperpolarisation of the endothelial cells and vascular smooth muscle, these results potentially implicate EETs whose

release has been attributed to transient receptor potential channels activation on endothelial cells which cause intracellular calcium to increase. This, in turn can open calcium activated K^+ channels to cause efflux of potassium from endothelial cells and cause hyperpolarisation, which spreads via myoendothelial junctions to the vascular smooth muscle (Crecelius *et al.*, 2013; Garland & Dora, 2017). Moreover, K^+ channel opening is implicated in the mechanisms for other recognised EDHFs and in NO-, PG- and adenosine-mediated dilatation (Garland & Dora, 2017). There is also evidence that ATP-sensitive potassium channels contribute to total reactive hyperaemia, but not to the peak response (Banitt *et al.*, 1996) although this has been debated for Glibenclamide, an ATP-sensitive K channel inhibitor did not attenuate peak reactive hyperaemia or the later phase of the hyperaemia (Farouque & Meredith, 2003a); opening of K_{ATP} channels would also have been prevented by the inhibitors used by Crecelius *et al.* (2013).

5.1.2.4 Reactive hyperaemia in cardiovascular disease (CVD)

Blunted reactive hyperaemia has been demonstrated in many patient groups with CVD including hypertension, coronary artery disease, atherosclerosis and old age (Kroese, 1977; Romanovska *et al.*, 1980; Strano *et al.*, 1985). Blunted reactive hyperaemia in young healthy people has also been shown to predict future cardiovascular risk and to be a better predictor than depressed FMD (Anderson *et al.*, 2011). Of relevance in the present context, a selective Thromboxane receptor (TP) antagonist augmented FMD and endothelium-dependent dilatation induced in the forearm by ACh in patients with coronary artery disease (Belhassen *et al.*, 2003). TP receptors respond to Thromboxane (TXA2) and PGH2, but are also known to respond to PGI_2 in CVD disease states (Feletou *et al.*, 2011). Unfortunately, reactive hyperaemia was not tested in the study of

Belhassen *et al.* (2003), but others showed that the same TP receptor antagonist had no significant effect on reactive hyperaemia in young healthy subjects with no CVD (Pasche *et al.*, 2013). Taken together, these results raise the possibility that prostanoids acting on TP receptors may modulate reactive hyperaemia even in young people without CVD, but at risk of CVD.

5.1.2.5 Ethnic differences in reactive hyperaemia and role of PGs in reactive hyperaemia

None of the studies mentioned above in which the characteristics of reactive hyperaemia as an endothelium-dependent dilatation were explored considered the ethnicity of the subjects. This is relevant because young SA men in their 20s showed blunted reactive hyperaemia following 2 minutes of vascular occlusion relative to WE men (Ormshaw *et al.*, 2018). Likewise, young BA men in their 20s showed blunted peak and total reactive hyperaemia relative to similar aged WEs (Heffernan *et al.*, 2008). In these two studies VOP was used; other studies showed FMD was blunted in BAs (Campia *et al.*, 2002a; Diaz *et al.*, 2012; Cooper *et al.*, 2018). However, not all studies indicated endothelial dysfunction in BAs. Thus, hypertensive, but not normotensive BAs showed blunted forearm vasodilation to the endothelium-dependent agonist Methacholine (Kahn *et al.*, 2002). Moreover, middle-aged BAs showed greater responses to the NO donor (Nitroglycerin) but similar FMD to age-matched WEs, while in both ethnicities, these responses were depressed in hypertensives relative to normotensives (Gokce *et al.*, 2001).

There is paucity of information on reactive hyperaemia in SAs: Ormshaw *et al.* (2018) recently showed reactive hyperaemia was blunted in young SA men relative to young WE men. There is also evidence of blunted FMD following 5 minutes of vascular

occlusion in 24 SA men (aged 20-40 years) (Murphy *et al.*, 2007) and in 26 SA men aged 35-61 years (Chambers *et al.*, 1999) relative to age matched WE men. But, in another study, 25 SA men aged 20-65 years, did not show blunted FMD relative to WEs (Raji *et al.*, 2004). On the other hand, blunted forearm vasodilation to acetylcholine was reported in SAs suggesting impairment of endothelium-dependent dilatation (Murphy *et al.*, 2007). Thus, the evidence available generally suggests that SAs who have no obvious CVD have endothelium dysfunction.

Whether ethnicity affects the role of PGs in reactive hyperaemia has not been explored. Nitric oxide suppresses endothelial derived hyperpolarisation factor (EDHF), when NO is impaired, EDHF mediate vasodilation (Bauersachs *et al.*, 1996). Therefore, NO impaired BAs have preserved EDHF which mediates vasodilation during exercise hyperaemia (Ozkor *et al.*, 2014). Further, redundancy between NO and PG has been reported such that when one is suppressed the other vasoactive substance compensates and mediates vasodilation. Therefore, in the NO impairment, PGs may play a greater role in RH. It is therefore probably that during RH, BAs and SAs may use more of vasodilatory COX products, which when inhibited will unveil the role of EDHF.

5.1.2.6 Sex difference in reactive hyperaemia and role of prostaglandins in reactive hyperaemia

The role of sex in determining the magnitude of reactive hyperaemia is not clear, with reports of no sex difference, higher reactive hyperaemia in men, or higher reactive hyperaemia in women. Briefly, using VOP to assess reactive hyperaemia, 1 minute vascular occlusion did not lead to any sex-dependent difference in reactive hyperaemia between 7 men and women, who were presumably WEs (ethnicity was not mentioned).

However, following 2, 5 and 10 minutes of vascular occlusion, men showed greater peak reactive hyperaemia than women (Wascher *et al.*, 1998). On the other hand, there was attenuated reactive hyperaemia in men relative to women following 10 mins of vascular occlusion (Vyden *et al.*, 1977). Further, there was no sex difference in peak reactive hyperaemia following 1 minute of vascular occlusion, but, following occlusion for 3 or 5 minutes, the peak reactive hyperaemia was attenuated in men relative to women (Webb *et al.*, 1981). By contrast, no sex difference was observed in reactive hyperaemia or in the effect of COX inhibition following 5 mins of vascular occlusion assessed by VOP (Carlsson *et al.*, 1987).

There have so far been no attempts to test whether the contributions of PGs to reactive hyperaemia are different in men and women. Moreover, whether sex alters the role of COX inhibition in BAs and SAs has not been tested.

5.1.3 Role of prostaglandins in vascular responses to acute mental stress

As reviewed in the general introduction (Chapter 1), acute emotional stressors evoke the alerting response, which includes increase in arterial pressure (ABP), systemic vasoconstriction, but skeletal muscle vasodilation. The muscle vasodilation during the alerting response has been attributed to NO, which is released by nNOS rather than eNOS stimulated by locally released acetylcholine (Ach) and shear stress (Dietz *et al.*, 1994; Cardillo *et al.*, 1997b; Khan *et al.*, 2017), and by adrenaline (Lindqvist *et al.*, 1996b) which occurs whether MSNA decreases or increases (Carter *et al.*, 2005). Adrenaline which contributes ~30% of stress-induced vasodilation is mediated by NO and PGs as well as by direct stimulation beta-adrenoreceptors on vascular smooth muscle, while shear stress can stimulate release of PGs as well as NO, discussed in chapter 1.

Thus, as proposed in Section 1.3.5, PGs may play a role in the forearm vasodilatation of the alerting response. Although the role of NO has become established, there is paucity of information on role of PGs or COX products. Interestingly, Ozkor *et al.* (2014) demonstrated that BAs (mean age of 39/40 years) showed blunted exercise hyperaemia and blunted endothelium-dependent dilatation to ACh in the forearm relative to WEs. The effect of NOS inhibition on these responses was greatly attenuated in BAs, but inhibition of K^+ channels had much greater attenuating effect in BAs than WEs. Thus, these results indicated that impairment of NO availability in BAs may unveil the role of vasodilator role of EDHFs, which as indicated above, include the arachidonic acid metabolites, EETs (Bauersachs *et al.*, 1996). They also raise the possibility that vasodilator PGs might help to maintain the forearm vasodilatation of the alerting response in BAs and SAs in who NO availability is impaired.

Since dilator PGs have also been shown to moderate the vasoconstrictor effects of sympathetic activation (see Section 1.3.5). The question also arises as to whether PGs modulate the renal, splanchnic and cutaneous vasoconstriction of the alerting response and thereby moderate the pressor response. Given the results described in Chapter 3 showed that repeated sound stress evoked forearm vasoconstriction in some individuals, particular in WE men and in BA and SA women, the question also arises as to how PGs might modify these responses.

5.1.4 Aim of study

Aim of this study was to determine the role of prostaglandins in reactive hyperaemia and the cardiovascular components of the alerting response in WEs, BAs and SAs.

5.1.5 Hypotheses

1. Prostaglandins contribute to reactive hyperaemia in WEs as well as in BAs and SAs: as such COX inhibition will blunt reactive hyperaemia in WEs, BAs and SAs.
2. In the WE group, there will be no sex differences in the contribution of PGs to reactive hyperaemia, but BAs and SAs may show sex-related differences.
3. Forearm vasodilatation during the alerting response evoked by repeated sound will not be blunted by COX inhibition in WEs, but will be blunted in BAs and SAs.
4. Repetition of mental stress will alter the modulatory contribution PGs make to the vasoconstrictor components of the alerting response: cutaneous vasoconstriction, forearm vasoconstriction which occurs in some individuals and the pressor response will be accentuated on repetition.

5.2 Methods

52 men and women aged 18-26 years of WE (19), BA (19) and SA (14) ethnic groups were recruited into the study. All of them were included in the study reported in Chapter 3. After the familiarization visit (see Chapter 2), experiments were done on 2 separate days with at least a two-week interval for the men and a month interval for the women. Subjects were experimented on 30 minutes after consumption of orange squash drink with and without 600 mg of soluble aspirin; Orange squash alone served as the placebo. Aspirin experiments were done following placebo experiments. The questionnaires completed by the subjects, the recordings and protocol were those described in Chapter 2 and 3. In brief, resting ABP was recorded by sphygmomanometer. Subsequently pulsatile arterial blood pressure (ABP), heart rate (HR), forearm and digital cutaneous red cell flux and vascular conductances (FRCF, FCVC, DRCF and DCVC) were recorded continuously, while forearm blood flow (FBF) and forearm vascular conductance (FVC) were recorded by VOP at intervals. Recordings were made before and during reactive hyperaemia and before and during each of 5 repeated sounds (S1-S5), after placebo and after aspirin. The complete protocol started 30 minutes after consumption of aspirin containing orange drink and lasted approximately 60/70 min within the period of effectiveness of COX inhibition (Heavey *et al.*, 1985).

5.2.2 Data Analysis

All variables are presented as mean ± SEM except where indicated otherwise. Calculations were made of ΔFVC, ΔMABP, ΔHR, ΔDCVC and ΔFCVC during reactive hyperaemia and during each of S1-S5 as the value recorded at different time points minus the appropriate baseline, after placebo (control) and after COX inhibition (aspirin). Reactive hyperaemia was assessed at peak (time 0s) and at 15s, 30s, 45s, 60s,

90s and 120s after release of occlusion, while responses to S1-S5 were measured at 5s, 15s, and 30s during each sound. The effect of COX inhibition on resting variables was determined using paired Student's T test. Within each ethnicity, reactive hyperaemia in individuals was categorised on the basis of the effect of aspirin: subjects who showed decrease in the peak ΔFVC following COX inhibition were categorised as positive COX responders (COX+) as they showed the expected reduction in peak hyperaemia, while subjects who showed increase in peak ΔFVC were categorised as negative responders (COX-). The effect of COX inhibition on peak reactive hyperaemia in each subject was presented in Table 5.3. Effect of COX inhibition on peak reactive hyperaemia in each subject was plotted in scattergraphs in each ethnic group (Figure 5.3) and in COX responder groups (Figure 5.5). Subjects were categorised as vasodilators if FVC increased from baseline or vasoconstrictors if FVC decreased from baseline at 15s during S1. Within each ethnicity, effect of COX inhibition on reactive hyperaemia was also considered on the basis of whether the individual showed forearm vasodilatation or constriction in response to S1. Scattergraph of change in FVC in response to COX inhibition at 15s during S1 of vasodilators and vasoconstrictors was plotted. Difference in proportions of COX responders and differences in proportions of vasodilators and vasoconstrictors within ethnic groups was tested using Fishers exact test. Mixed factorial ANOVA was used to test effect of COX inhibition on reactive hyperaemia and effect of sound 1-5 at 15s into each sound. Bonferroni post hoc tests were done as appropriate. A single group comprising of 6 levels with ethnicity and COX responder groups combined (WE COX+, WE COX-, BA COX+, BA COX-, SA COX+, SA COX-) or ethnicity and sex group combined (WE males, WE females, BA

males, BA females, SA males, SA females) were used as between factors where appropriate. In all cases, $p < 0.05$ was taken as significant.

5.3 Results

5.3.1 Baseline

In the full group of WEs and in WE men and women, COX inhibition with aspirin had no effect on any of the cardiovascular baselines (Table 5.2 A). Similarly, aspirin did not affect most of the cardiovascular baselines in the full group of BAs or in the BA men and women. However, in the full BA group and in BA men, aspirin decreased baseline DCRCF, while in BA women, aspirin decreased FCRCF (Table 5.2 B). Aspirin also had no effect on the majority of baselines in the full group of SAs or in males or female SAs: the exception was FCRCF which tended to be reduced in SA men (15.53±2.50 vs 9.51±0.41 PU, p =0.07 see Table 5.2 C).

5.3.2 Effect of COX inhibition on reactive hyperaemia

When considered in whole ethnic groups, there were no significant 3-way or 2-way interactions between aspirin, time and ethnicity on reactive hyperaemia. There was no significant effect of aspirin on reactive hyperaemia in WEs and BAs ($p>0.05$, Figure 5.1), however in SAs, aspirin attenuated total reactive hyperaemia ($F(1,11)=5.31$, $p=0.04$, partial $\eta^2=0.33$). The effect was significant at 30s -120s ($p<0.05$ in each case) but not at the peak (Figure 5.1). There was a significant 3-way interaction between aspirin, time and sex during reactive hyperaemia ($F(6,31)=7.69$, $p=0.002$, partial $\eta^2=0.31$). There was a significant simple two-way interaction between time and aspirin for WE males ($F(2,17)=7.91$, $p<0.001$, partial $\eta^2=0.41$) but not for WE women, BA or SA men or women ($p>0.05$). There was a significant simple main effect of aspirin on reactive hyperaemia for WE men, COX inhibition attenuated peak reactive hyperaemia in WE men ($p=0.003$, Figure 5.1).

By contrast, aspirin did not attenuate total or peak reactive hyperaemia in the full group of BAs, in BA men or women ($p>0.05$, Figure 5.1). On the other hand, aspirin attenuated total reactive hyperaemia in the whole SA group ($p=0.04$) and in SA women at 45s -120s ($p<0.05$ in each case) but not in SA men. However, aspirin had no effect on peak reactive hyperaemia in SA men or women (Figure 5.1).

Figure 5.2 shows individual responses to COX inhibition at peak of reactive hyperaemia within each ethnic group. In each ethnic group, following COX inhibition, peak reactive hyperaemia decreased (COX+ responders) or increased (COX- responders).

5.3.3 Effect of COX inhibition in COX responder groups

In WEs, as shown on Table 5.4, there was no effect of aspirin on resting FBF, FVC, DCRCF, DCVC, FCRCF or FCVC in COX+ or COX-; those whose RH was attenuated or augmented respectively after COX inhibition (see section 5.1.2.2). Individual responses to COX inhibition at the peak of reactive hyperaemia in COX responder groups are shown in Figure 5.3. Peak reactive hyperaemia was blunted in WE and SA COX- responders relative to COX+ responders ($p<0.005$, 0.05 in each case). There were no differences between BA COX+ and COX-responders ($p>0.05$, Figure 5.4, Figure 5.5).

Aspirin attenuated peak reactive hyperaemia (COX+) in 10/10 WE men (100%) and in 4/9 WE women (44.4%), while reactive hyperaemia was accentuated in 5 WE women (55.6%, COX). The difference in proportions of COX responders between the gender groups was significant ($p =0.01$).

In the whole BA group, 10/19 (53%) were COX+ and 9/19 (47%) were COX-. Of the BA men, 6/11 (54.50%) were COX+ and 5/11 (45.50%) were COX–, while of the BA women, 4/8 (50%) were COX + and 4/8 (50%) were COX-. There were no differences

in proportion of COX+ between BA men and women (p >0.05). In the COX+ BAs, aspirin reduced resting FBF, FVC and FCVC in COX+ (p=0.04, 0.03, 0.03, respectively, Table 5.3). In the COX- BAs, aspirin did not affect resting FBF, FVC, DCRCF, DCVC, FCRCF or FCVC.

In whole SA group, 9/16 (56%) were COX+ and 5/16 (44%) were COX-. Of the SA men, 4/6 (66.67%) were COX+ and 2/6 (33.33%) were COX-, while of the SA women, 5/8 (62.5%) were COX+ and 3/8 (37.5%) were COX-. There were no sex differences in the proportions of COX+ (p >0.05). There was no significant effect of aspirin on resting FBF, FVC, DCRCF, DCVC, FCRCF or FCVC in COX+ or COX- SAs (p>0.05, Table 5.4). Considering effect of COX inhibition on total reactive hyperaemia, there was a significant 3-way interaction between COX inhibition aspirin, time and COX responder groups during reactive hyperaemia ($F(10,82)= 5.84$, $p<0.001$, partial $\eta^2= 0.42$). There was a significant simple two-way interaction between aspirin and COX responder groups ($F(5,40)=3.20$, $p=0.02$, partial $\eta^2=0.29$). There was a significant simple main effect of aspirin on reactive hyperaemia in WE, BA and SA COX+ responders, aspirin attenuated peak reactive hyperaemia in the WE, BA and SA COX+ responders ($p<0.001$, 0.01 in each case, see Figure 5.4). Further, there was significant simple main effect of aspirin on peak reactive hyperaemia in WE and SA COX negative responders who showed accentuation of peak reactive hyperaemia ($p=0.01$ respectively, Figure 5.4).

Considering effect of COX inhibition on only the peak of reactive hyperaemia, there was a significant 2-way interaction between COX inhibition aspirin and COX responder group at the peak of reactive hyperaemia ($F(1,46)=53.58$, $p<0.0001$, partial $\eta^2= 0.53$). There was a significant simple two-way interaction between aspirin and COX responder

groups (F(5,40)=3.20, p=0.02, partial η^2=0.29). There was a significant simple main effect of aspirin on reactive hyperaemia in the COX+ responders. Aspirin attenuated peak reactive hyperaemia in the WE, BA and SA COX+ responders (F(1,46)=15.64, p=0.0001, partial η^2= 0.25): (F(1,46)=6.59, p<0.001, partial η^2= 0.13): (F(1,46)=13.76, p=0.001, partial η^2= 0.14), respectively). Further, in WE, BA and SA COX negative responders there was accentuation of peak reactive hyperaemia (F(1,46)=7.88, p=0.007, partial η^2= 0.15): (F(1,46)=7.73, p=0.008, partial η^2= 0.14): (F(1,46)=7.61, p=0.008, partial η^2= 0.14), see Figure 5.5.

5.3.3 Effect of COX inhibition on reactive hyperaemia in vasodilator and vasoconstrictor groups

As explained in Section 5.2.2 in each ethnicity, some individuals showed forearm vasodilatation in response to the 1st sound stimulus, while others showed vasoconstriction; on this basis they were separated into vasodilator and vasoconstrictor groups. The effects of COX inhibition on peak reactive hyperaemia in the each individual vasodilator and vasoconstrictor within each ethnic group are shown in Figure 5.9.

Aspirin had no effect on total reactive hyperaemia in WE vasodilators or vasoconstrictors, There was no effect of aspirin on reactive hyperaemia in BA vasodilators or vasoconstrictors. Finally, there was no effect of aspirin on reactive hyperaemia in SA vasoconstrictors, but in vasodilator SAs, there was a significant effect on reactive hyperaemia reflecting attenuation of peak reactive hyperaemia (p=0.04, Figure 5.6).

5.3.4 Effect of COX inhibition on the responses evoked by sound.

Aspirin had no effect on the pattern of response evoked by S1 or repetition of sounds (S1-S5) in WEs whether the group was considered as a whole or as men and women groups (Figures 5.7 and 5.8), with the exception of the forearm cutaneous vasoconstrictor response to S1 in WE women which was attenuated, (p=0.004). Similarly, aspirin had no effect on the pattern of response evoked by S1 or S1-S5 in the whole group of BAs or SAs, or in BA men or SA men (Figure 5.7). Notably, in the 3 ethnicities, men showed net forearm vasodilatation which was not altered by aspirin. The pattern of response evoked by S1-S5 in BA and SA women included net forearm vasoconstriction, which was not significantly affected by aspirin (Figure 5.8). Aspirin also had no effect on the other components of the response to S1-S5 in BA women except for progressive pressor responses in BA women, which were attenuated after aspirin ($F(1,18)=11.03$, $p=0.004$, partial $\eta^2=0.38$,Figure 5.8). The effect of aspirin was significant at S2-S5 ($p<0.05$ in each case),

5.3.4 Effect of COX inhibition on the responses evoked by sound in vasodilators and vasoconstrictors.

In response to S1, 12/19 (63.16%) WEs showed forearm vasodilation (VD) to S1, while 7/19 (36.84%) showed forearm vasoconstriction (VC). In the BA group, 10/19 (52.63%) showed forearm vasodilation and 9/19 (47.37%) showed vasoconstriction to S1 and similarly, 7/14 (50%) SAs showed forearm vasodilation and 7/14 (50%) showed vasoconstriction.

In the WE vasodilators, aspirin had no effect on the ΔFVC evoked by S1-S5 (p=0.05). However, in BAs vasodilators, aspirin attenuated forearm vasodilation to S1 (ΔFVC = +0.017±0.002 vs -0.005 ±0.007 CU, $F(1,9)=6.51$, $p=0.03$, partial $\eta^2=0.42$) , see Table

5.6) and to S1-S5 ($F(1,8)=6.85$, $p=0.03$, partial $\eta^2=0.46$, Figure 5.10, middle). Similarly, in the SA vasodilators, aspirin attenuated forearm vasodilation during S1-S5 ($F(1,6)=20.47$, $p=0.004$, partial $\eta^2=0.77$, see Figure 5.10). In addition, aspirin attenuated the tachycardia evoked by S1-S5 in WE vasodilators, but had no effect on the HR response in BA or SA vasodilators (Figure 5.10). The attenuation of the forearm vasodilatation seen after aspirin in BA vasodilators was accompanied by a trend for the pressor responses and the forearm cutaneous vasoconstrictor responses to be augmented ($p=0.06$; 0.08 respectively, Figure 5.10 middle).

In the vasoconstrictor WEs, aspirin reversed forearm vasoconstriction to vasodilation during S1 ($\Delta FVC= -0.011\pm0.004$ vs $+0.012\pm0.008$, $p=0.03$, Table 5.5) and forearm vasodilation was sustained during S2-S5 Figure 5.11). Aspirin had no other effect on WE vasoconstrictors. Aspirin also tended to reverse forearm vasoconstriction to dilatation in BA vasoconstrictors ($F(1,7)=4.14$, $p=0.08$, partial $\eta^2=0.37$, Figure 5.11, middle). This was accompanied by attenuation of the pressor responses evoked by S1-S5 in BA vasoconstrictors ($F(4,14)=5.56$, $p=0.046$, partial $\eta^2=0.10$), Figure 5.11, middle). There was no significant effect of COX inhibition on any component of the alerting response in SA vasoconstrictors (Figure 5.11 right hand side, RHS).

5.4 Discussion

The main findings of the present study are that in young men and women, COX inhibition with aspirin decreased baseline digital cutaneous RCF in the full BA group but had no significant effect on other baselines in BAs or in WEs, or SAs. However, when the ethnic groups were considered according to whether COX inhibition attenuated or augmented reactive hyperaemia (COX+, COX- respectively), baseline FVC, FCVC and DCVC was decreased after COX inhibition in BA COX+, but not in BA COX- or in WE, or SA COX+ or COX-. When considered as the full ethnic groups, COX inhibition had no effect on reactive hyperaemia in WEs, BAs or SAs. However, within WE ethnic group, COX inhibition attenuated reactive hyperaemia in WE men only. Interestingly, this relative lack of effect on reactive hyperaemia disguised the fact that within each ethnicity, there were individuals in whom reactive hyperaemia attenuated, as well as others in whom reactive hyperaemia was augmented.

As far as the responses evoked by repeated sounds, are concerned, the main findings are that COX inhibition had no effect on the patterns of response evoked in the WE, BA or SA men, and attenuated the pressor responses in BA women, but with no other significant effect on any variable in WE, BA or SA women. However, when considered according to whether the first sound stimulus evoked forearm vasodilatation or vasoconstriction, COX inhibition attenuated forearm vasodilator responses to S1-S5 in BA and SA but not WE vasodilators, but did attenuate the repeated tachycardia evoked in WEs. On the other hand, COX inhibition also attenuated forearm vasoconstrictor responses and pressor responses to S1-S5 in BA vasoconstrictors, with no other significant effect in vasoconstrictors.

5.4.1 Tonic influences of COX products

The finding that COX inhibition with aspirin did not reduce resting forearm blood flow or vascular conductance in any of 3 ethnic groups is consistent with some previous reports in which COX inhibitors were used (Carlsson & Wennmalm, 1983; Carlsson *et al.*, 1987; Engelke *et al.*, 1996), and contrasts with the reports of others that resting forearm blood flow was reduced (Duffy *et al.*, 1999; Farouque & Meredith, 2003b).

Further, the present findings that COX inhibition reduced resting skin blood flow (skBF) in the finger of BA and SA men as well as in forearm skin of BA women, contrasts with findings that COX inhibition did not affect basal skin blood flow in young men and women whose ethnicity was not mentioned, and so may well have been WE (Noon *et al.*, 1998; Dalle-Ave *et al.*, 2004; Kellogg *et al.*, 2005). On the other hand, recent studies in young BAs also showed no effect on toe or finger skin blood flow following COX inhibition (Maley *et al.*, 2017). Such findings suggest that at most PGs may contribute a tonic vasodilator effect on forearm cutaneous vasculature of BA women. However, the present finding that COX inhibition reduced baseline FVC, DCVC and FCVC in BA COX+ suggests that PGs contribute a tonic dilator influence in these particular BA individuals that was disguised when all BAs were considered together.

As discussed in Chapter 1 and in the introduction to this Chapter, the stimuli for synthesis of PGs include shear stress and hypoxia. Therefore at rest, it would seem shear stress has a significant influence on vasodilator PG synthesis particularly in BAs. It may be that the contribution of PGs to vasodilator tone is pronounced in young BA men and women because the tonic dilator influence of NO is impaired, as has been shown in older BAs (Ozkor *et al.*, 2014).

5.4.2 Effect of COX inhibition on reactive hyperaemia.

5.4.2.1 Ethnic and sex-related difference

The present finding that the whole group of WEs did not show attenuation of reactive hyperaemia following COX inhibition, although the WE men did, contrasts with previous reports on mixed gender groups of subjects whose ethnicity was not mentioned and were therefore most likely to be WEs, for in them various different COX inhibitors attenuated peak and total reactive hyperaemia (Kilbom & Wennmalm, 1976; Carlsson *et al.,* 1987). In the present study, the lack of attenuation of reactive hyperaemia after COX inhibition in the whole WE group was attributable to lack of effect in WE women, for WE men showed attenuation of the response. Indeed, when subjects were categorized as COX+ or COX- responders, the COX+ WEs showed significant attenuation of peak a reactive hyperaemia, whereas COX- WEs showed augmentation of both peak reactive hyperaemia. It seems that amongst WE men, the COX+ dominated, whereas amongst WE women, the contributions of COX- and COX+ were more evenly balanced. On this basis it seems likely that differences between studies in the balance of those who were COX- and COX+ may also explain some of the discrepancies in earlier studies where augmentation, or no alteration of reactive hyperaemia following COX inhibition in groups who were probably WEs were reported (Naylor *et al.,* 1999; Taylor *et al.,* 2014).

It might be that vasoconstrictor PGs limited reactive hyperaemia in the COX- group of WEs consistent with the finding of increased Thromboxane A2 metabolite released from the hand of young men who showed augmentation of reactive hyperaemia in the finger following COX inhibition (Tooke *et al.,* 1982). The more probable reason for the augmentation of reactive hyperaemia following COX inhibition in some WEs is

increased cytochrome P450 mediated conversion of Arachidonic acid to EET when the COX pathway was inhibited (Ozkor & Quyyumi, 2011).

At first glance, the finding that in the mixed male/female group of BAs, and in BA men and women, COX inhibition did not attenuate reactive hyperaemia would suggest that BAs who have been reported to have impaired NO bioavailability (Stein *et al.*, 1997) also do not release PGs following release of occlusion. However, when considered as COX+ and COX- groups, there were as many BAs who showed attenuation of reactive hyperaemia after COX inhibition as there were those who showed augmentation, explaining why there was no net effect of COX in the whole group. In contrast to the WEs, there was no obvious difference between BA men and women on the contributions of COX+ and COX- to the full group. However, it can be proposed that as COX inhibition attenuated peak but not total reactive hyperaemia in the COX+ BAs, this provides some evidence for release of vasodilator PGs following release of occlusion in some BAs. On the other hand, since the COX- BAs showed significant accentuation of peak reactive hyperaemia, it would seem there may have been release of vasoconstrictor PGs or that COX inhibition skewed the Arachidonic acid pathway towards production of EETs in these BA individuals. It is important to note that there was no difference in the magnitude of reactive hyperaemia between the COX- and COX+ BAs in the absence of COX inhibition, thus there is no obvious reason to suppose that COX- BAs had greater endothelial dysfunction than COX+ BAs. Whether vasoconstrictor PGs or enhancement production of EETs was more important could be tested in future studies.

Finally, considering the full group of SAs and SA women, COX inhibition attenuated total reactive hyperaemia with no effect on the peak, whereas in SA men reactive hyperaemia was not affected by COX inhibition. This again reflected the presence of COX+ and COX- and in this case, the two subgroups must have made similar contributions amongst both SA men and women. The fact that COX- SAs showed blunted peak reactive hyperaemia relative to the COX+ SAs in the absence of COX inhibition suggests that they already show evidence of endothelial dysfunction relative to other SAs. This being the case, in COX- SAs, the increase in reactive hyperaemia following COX inhibition could have been due to inhibition of vasoconstrictor PGs; this should be tested in future studies.

It should be noted that some previous studies done to test effect of COX inhibition on reactive hyperaemia used 5 minutes of vascular occlusion rather than 2 minutes as in the present study. It is possible that a longer duration of occlusion, would lead to more PG release as a consequence of hypoxia and that the effect of COX inhibition would be more prominent. However the duration of occlusion would be more likely to affect total reactive hyperaemia rather than peak for Carlsson *et al.* (1987) demonstrated, that increasing the duration of occlusion from 1-3 minutes increased the peak of reactive hyperaemia but increasing the duration of occlusion from 3 to 20 minutes affected total hyperaemia, but did not increase the peak. The effect of a longer duration of occlusion on the effects of COX inhibition on reactive hyperaemia in different ethnic groups should be tested.

5.4.3 Effect of COX inhibition on responses to mental stress

The patterns of response evoked by repeated sounds before COX inhibition in the present study are comparable to those reported in the study of Chapter 3 and are not

discussed in any detail here, indeed many of the same individuals were involved in both studies. Briefly, before COX inhibition, repeated sound (S1-S5) evoked the characteristic pattern of the alerting response in men of all 3 ethnicities with net forearm vasodilatation, accompanied by pressor responses, tachycardia and cutaneous vasoconstriction. The responses evoked in women were similar except that BA and SA women showed net forearm vasoconstriction rather than dilatation and the pressor responses were more pronounced, particularly in BA women. In all 3 ethnicities some individuals showed forearm vasodilatation, others vasoconstriction, the latter being more common amongst BA and SA women.

The primary hypothesis tested in the present study with regard to the response to mental stress is that COX inhibition would attenuate forearm vasodilatation in BAs and SAs. In fact, the results supported this hypothesis. COX inhibition had no effect on the forearm vascular responses in male or female WEs, BAs, or SAs, but attenuated forearm vasodilatation in the BA and SA vasodilators, with no effect on the WE dilators. This novel finding suggesting that PGs do not contribute to the forearm vasodilator response to mental stress in WEs is consistent with previous evidence that that NO was a major contributor to mental stress in WEs (Dietz *et al.*, 1994). Moreover, the present findings provide the first evidence that vasodilator prostaglandins make an important contribution to mental stress-induced forearm vasodilation in both BAs and SAs. It may be noted that there was no correlation between the effect of COX inhibition on the forearm vasodilator response to mental stress and the effect on reactive hyperaemia: in the WE and BA vasodilators, COX inhibition had no effect on reactive hyperaemia, whereas in the SA dilators, reactive hyperaemia was attenuated by COX inhibition. This

suggests that the stimuli responsible for vasodilator PG release in the forearm are different during and after occlusion as compared with mental stress.

In previous studies, the blunted forearm vasodilation evoked by mental stress in hypertensives (Cardillo *et al.*, 1998a) and BAs (Cardillo *et al.*, 1998b) was attributed to NO impairment and more recently, to impaired NO generated by nNOS, at least in hypertensives (Khan *et al.*, 2015). The present findings raise the possibility that NO availability is reduced in both young BAs and SAs (see Murphy *et al.* (2007) and that under such conditions, release of dilator PGs replaces the normal contribution of NO. As discussed in Chapter 1 section, 1.3.6, PG release that contributes to the forearm vasodilatation is most likely to be due to shear stress caused by the raised ABP and/or by the consequences of adrenaline or ACh acting on endothelial receptors, whereas the vasodilatation of reactive hyperaemia may include shear stress, but is more likely to reflect PGs released as a consequence of tissue hypoxia (see Introduction to this Chapter). If this is the case it is not surprising there was no correlation within individuals between the effects of COX inhibition on reactive hyperaemia and the forearm vasodilator response to mental stress.

Interestingly, in the BA vasodilators, COX inhibition also reversed forearm cutaneous vasodilatation to vasoconstriction suggesting that vasodilator PGs contributed to this response. How this happened is unclear: it could be that PGs contributed to cutaneous dilatation induced in forearm skin by sweating triggered by mental stress (Donadio *et al.*, 2012). Future studies could test whether sweating is more prevalent in the BA vasodilators than vasoconstrictors, given forearm cutaneous vasodilatation was not apparent in vasoconstrictor BAs.

The fact that the pressor responses to S1-S5 tended to be augmented after COX inhibition in BA vasodilators could be at least partly attributed to attenuation of vasodilatation not only in forearm muscle, but also in the legs as is typical of the alerting response (Chapter 1).

In fact, in the BA vasoconstrictor group, not only was there a trend for forearm vasoconstriction to be attenuated but the pressor responses to S1-S5 were also substantially decreased. These findings suggest that vasoconstrictor PGs contributed to the altered alerting response seen in these individuals, who were predominantly BA women (see Chapter 3). This is the first report to show that vasoconstriction and the pressor response evoked during mental stress is attributable to COX products.

Endothelium-dependent contractions in response to ACh and arachidonic acid were demonstrated in isolated canine arteries (De Mey & Vanhoutte, 1982) and in isolated arteries from in spontaneously hypertensive rats and in diabetic rodents as well as female animals that lacked oestrogen as a consequence of ovariectomy (Vanhoutte *et al.*, 2005). This vasoconstrictor effect was proposed to be due reduced NO release as well as release of endothelium-derived contracting factors. Such contractions were not altered by TXA2 synthase inhibitors but by antagonist of TB receptor (Yang *et al.*, 2003), suggesting the role of COX-dependent products, but excluding TXA2. In the presence of oxidative stress, COX stimulation converts arachidonic acid into endoperoxides which bind to the TXA2 receptors on vascular smooth muscle to elicit endothelium-dependent contraction (Vanhoutte *et al.*, 2005). Moreover, PGI_2 has also been shown to evoke endothelium-dependent contraction in spontaneously hypertensive rats by acting on TXA2 receptors (Feletou *et al.*, 2009). Accordingly, in hypertensive

humans and in ageing, blunted ACh-mediated forearm vasodilation associated with NO impairment was reversed by COX inhibition (Taddei *et al.*, 1997a; Taddei *et al.*, 1997b). In the present study, the trend for reversal of forearm vasoconstriction to vasodilatation and attenuation of the pressor responses to mental stress was observed only in BA vasoconstrictors. Thus it is possible that vasoconstrictor arachidonic acid products or PGI_2 bound to TXA2 receptors to elicit vasoconstriction in muscle in the presence of endothelial dysfunction occurring particularly in BA women.

Somewhat surprisingly, COX inhibition attenuated the tachycardia of the alerting response or reversed it to bradycardia in the WE vasodilators. This suggests that COX inhibition removed an effect of PGs that was contributing to the sympathetically mediated effects on the heart. Whether this reflected a local effect of PGs at the sinoatrial node or within the central nervous system that altered the inhibition of baroreflex that occurs during the alerting response cannot be deduced. This could be tested in future studies.

5.4.4 Conclusions

The results of the present study suggest that vasodilator PGs released during vascular occlusion contribute to reactive hyperaemia in WEs, BAs and SAs, but particularly in WE men. In all three ethnicities, the ability to discriminate an effect of COX inhibition on reactive hyperaemia is impaired by the fact that there were individuals in whom reactive hyperaemia was augmented by COX inhibition. It is proposed that this may reflect release of vasoconstrictor PGs, but more likely, generation of vasodilator EET when COX was inhibited. Although reactive hyperaemia was blunted in BAs relative to

WE as shown in Chapter 3, the results of the present study indicate that the contribution of COX products does not explain this difference.

In addition, the present study has shown for the first time that vasodilator COX products contribute to the muscle vasodilator response to mental stress in BAs and SAs, but not in WEs. It is proposed that in BAs and SAs, vasodilator PGs take over in the presence of a reduced contribution of NO to the muscle forearm vasodilatation. On the other hand, the present study also provides evidence that COX products contribute to forearm vasoconstriction and exaggerated pressor responses to mental stress that occur particularly in BA women. It is proposed that these effects may reflect release of vasoconstrictor COX products or PGI2 acting on Thromboxane A2 (TXA2) receptors in the presence of endothelial dysfunction.

5.4.5 Limitations.

Firstly, the study was limited by the number of individuals in each ethnicity; larger group sizes particularly when ethnic groups were divided on the basis of sex, effects of COX inhibition or direction of forearm vascular responses to sound would have allowed more secure tests of differences within and between groups and subgroups. Secondly, as no assays were conducted for PG metabolites before or after COX inhibition, there was no direct measure of the efficacy of the COX inhibition induced by aspirin under the conditions of the present study, nor of the range of PGs and other arachidonic acid products released during reactive hyperaemia or mental stress. Thirdly, it was a limitation that NO bioavailability and release of NO generated by eNOS and nNOS was not tested in the subjects from the different ethnic groups. This would have required intra-arterial administration of selective nNOS and non-selective NOS inhibitors in order to avoid effect on systemic arterial blood pressure and was not technically

possible. In future studies, it would be good to rectify these limitations and to test the effect of COX inhibition on reactive hyperaemia following different durations of vascular occlusion and to compare the contributions of other vasoactive mediators to reactive hyperaemia and forearm vascular response to mental stress in WEs, BAs and SAs. COX responder groups or vasodilator and vasoconstrictors were determined using zero as the cut-off point. The results obtained in the experiments were consistent during S1-5 and provide the basis for the arguments in the discussion, however in the future, percentage change will be used to validate these findings.

Table 5.1 Baseline values of anthropometric and cardiovascular variables in WE, BA and SA men and women.

	White Europeans (WE)			Black Africans			South Asians		
	All WE (n=19)	**WE men (n=10)**	**WE Women (n=9)**	**All BAs (n =19)**	**BA men (n= 11)**	**BA women (n=8)**	**All SAs (n =19)**	**SA men (n= 11**	**SA women (n=8)**
Age (years)	21.05±0.55	21.50±0.93	20.56±0.53	20.89±0.43	20.00±0.43	22.13±0.64	20.79±0.63	21.00±1.03	20.63±0.84
BMI (kg/m^2)	22.95±0.57	23.79±0.87	22.03±0.61	22.59±0.58	22.96±0.74	22.10±0.97	22.69±0.91	22.41±0.62	22.90±1.56
WC (cm)	79.95±2.24	87.00±2.34	72.11±1.58	75.55±1.39	76.55±1.26	74.19±2.86	75.04±3.58	79.33±1.80	71.81±6.04
FAC (cm)	24.47±0.45	25.72±0.61	23.22±0.29	24.92±0.50	26.23±0.52	23.13±0.43	23.57±0.59	24.83±0.40	22.63±0.86
PSS	15.47±1.07	16.00±1.44	14.89±1.67	18.29±1.89	15.55±1.75	23.33±3.64	15.58±1.33	16.20±2.27	15.14±1.75
SIS	43.06±2.20	46.20±1.66	39.13±4.24	45.61±2.36	46.64±2.64	44.00±4.64	42.33±3.22	39.80±6.54	44.14±3.31
SBP (mmHg)	103.16±1.97	108.40±1.48	97.33±2.77	108.74±3.07	114.36±4.45	101.00±2.02	101.79±3.37	106.33±3.26	98.38±5.22
DBP (mmHg)	62.79±1.34	63.60±1.96	61.89±1.88	67.24±1.78	68.18±2.58	65.94±2.43	62.50±1.91	60.83±1.42	63.75±3.21

Values are shown as mean ± SEM.*$p < 0.05$: WEs vs BAs or WEs vs SAs.

Table 5.2 Baseline values of cardiovascular variables in WE, BA and SA men and women before and after COX inhibition with Aspirin.

	All WE (n=19)			WE men (n=10)			WE women (n=9)		
	Control	**Aspirin**	**P**	**Control**	**Aspirin**	**P**	**Control**	**Aspirin**	**P**
Table 5.2 A			**value**			**value**			**value**
MABP (mmHg)	78.05±2.31	80.19±2.59	0.47	77.50±3.21	79.48±3.14	0.68	78.65±3.54	80.98±4.41	0.69
HR (bpm)	65.61±2.04	65.68±1.94	0.97	67.05±2.61	65.50±2.14	0.64	64.02±3.27	65.89±3.49	0.70
FBF (ml/dl/min)	4.35±0.30	4.32±0.41	0.95	4.86±0.36	4.19±0.47	0.12	3.78±0.45	4.47±0.71	0.42
FVC (CU)	0.06±0.00	0.06±0.01	0.71	0.07±0.00	0.05±0.01	0.14	0.05±0.01	0.06±0.01	0.48
DCRCF (PU)	159.58±30.60	123.81±16.22	0.18	206.45±57.13	138.39±18.07	0.21	123.12±28.92	124.19±25.79	0.98
DCVC (CU)	2.08±0.42	1.74±0.25	0.25	2.86±0.83	1.99±0.40	0.29	1.48±0.31	1.50±0.32	0.96
FCRCF (PU)	21.69±2.99	20.67±3.49	0.63	20.31±5.47	18.88±6.42	0.84	22.77±3.48	22.24±3.73	0.92
FCVC (CU)	0.28±0.04	0.22±0.03	0.11	0.28±0.09	0.16±0.02	0.25	0.29±0.04	0.26±0.04	0.67
	All BAs (n =19)			**BA men (n= 11)**			**BA women (n=8)**		
Table 5.2 B	**Control**	**Aspirin**		**Control**	**Aspirin**		**Control**	**Aspirin**	
MABP (mmHg)	84.88±3.55	85.49±2.80	0.87	89.53±3.95	89.82±3.62	0.95	77.63±2.14	79.54±3.67	0.87
HR (bpm)	65.11±1.43	64.68±2.23	0.83	63.75±1.95	60.87±2.03	0.21	78.48±6.01	69.92±3.92	0.41
FBF (ml/dl/min)	4.52±0.41	3.99±0.41	0.34	4.30±0.56	4.34±0.54	0.96	4.81±0.64	3.51±0.63	0.18
FVC (CU)	0.06±0.01	0.05±0.01	0.39	0.05±0.01	0.05±0.01	0.95	0.07±0.01	0.05±0.01	0.26
DCRCF (PU)	**184.59±45.05**	**107.17±33.24***	**0.03**	**228.33±51.50**	**135.53±51.81***	**0.02**	114.60±80.07	61.78±16.27	0.49
DCVC (CU)	2.37±0.60	1.71±0.58	0.29	2.75±0.62	2.29±0.89	0.55	1.76±1.27	0.79±0.24	0.43
FCRCF (PU)	16.15±1.76	13.40±2.52	0.28	16.96±2.59	15.75±3.84	0.66	**14.69±1.83**	**9.65±1.62***	**0.04**
FCVC (CU)	0.20±0.02	0.16±0.03	0.25	0.20±0.03	0.19±0.05	0.67	0.20±0.03	0.12±0.02	0.05
	All SAs (n=14)			**SA men (n=6)**			**SA women (n=8)**		
Table 5.2 C	**Control**	**Aspirin**		**Control**	**Aspirin**		**Control**	**Aspirin**	
MABP (mmHg)	80.20±2.93	75.33±4.16	0.23	80.08±2.80	80.96±6.30	0.90	80.30±4.87	71.11±5.39	0.08
HR (bpm)	59.65±2.65	60.10±3.07	0.80	55.69±4.65	55.74±5.04	0.99	59.65±2.65	63.38±3.07	0.77
FBF (ml/dl/min)	4.36±0.38	5.04±0.59	0.31	4.89±0.46	4.72±0.94	0.82	3.97±0.55	5.27±0.80	0.22
FVC (CU)	0.05±0.004	0.07±0.01	0.12	0.06±0.004	0.06±0.01	0.88	0.05±0.006	0.08±0.02	0.09
DCRCF (PU)	135.53±32.75	145.87±30.59	0.82	130.69±54.48	95.76±35.84	0.69	138.77±45.01	179.27±41.67	0.49
DCVC (CU)	1.86±0.51	2.08±0.42	0.74	1.75±0.80	1.25±0.51	0.69	1.94±0.72	2.63±0.53	0.40
FCRCF (PU)	23.42±7.08	14.48±2.42	0.41	15.53±2.50	9.51±0.41	0.07	27.93±10.95	17.79±3.46	0.92
FCVC (CU)	0.29±0.08	0.22±0.05	0.97	0.20±0.04	0.12±0.02	0.09	0.35±0.13	0.28±0.08	0.58

Values are shown as mean ± SEM. * $p < 0.05$: control vs aspirin.

Table 5.3 Baseline values of cardiovascular variables in WE, BA and SA COX+ and COX- responder groups before and after Aspirin.

	WE COX + (n=14)			WE COX- (n=5)		
	Control	**Aspirin**	**P value**	**Control**	**Aspirin**	**P value**
FBF (ml/dl/min)	4.37±0.35	4.19±0.44	0.71	4.28±0.68	4.69±1.02	0.61
FVC (CU)	0.06±0.00	0.05±0.01	0.51	0.06±0.01	0.06±0.01	0.63
DCRCF (PU)	179.23±40.87	138.56±14.87	0.28	116.35±36.96	89.27±44.02	0.46
DCVC (CU)	2.39±0.58	1.87±0.30	0.34	1.71±0.32	1.35±0.60	0.54
FCRCF (PU)	21.34±3.54	20.23±4.48	0.82	25.41±7.05	21.91±5.30	0.26
FCVC (CU)	0.28±0.05	0.20±0.02	0.18	0.32±0.09	0.27±0.07	0.18
	BA COX + (n=10)			**BA COX- (n=9)**		
	Control	**Aspirin**		**Control**	**Aspirin**	
FBF (ml/dl/min)	**4.83±0.65**	**3.37±0.34***	**0.04**	4.17±0.50	4.67±0.74	0.56
FVC (CU)	**0.06±0.01**	**0.04±0.00***	**0.03**	0.05±0.01	0.06±0.01	0.52
DCRCF (PU)	161.60±58.92	86.17±31.11	0.06	211.41±73.64	131.66±64.55	0.25
DCVC (CU)	2.04±0.74	0.91±0.31	0.06	2.76±1.03	2.65±1.14	0.93
FCRCF (PU)	18.20±2.98	11.84±2.87	0.14	14.10±1.78	14.66±3.85	0.89
FCVC (CU)	**0.23±0.03**	**0.13±0.03***	**0.03**	0.17±0.03	0.19±0.06	0.76
	SA COX+ (n=9)			**SA COX - (n=5)**		
	Control	**Aspirin**		**Control**	**Aspirin**	
FBF (ml/dl/min)	4.96±0.40	5.03±0.58	0.90	3.28±0.53	5.06±1.41	0.32
FVC (CU)	0.06±0.00	0.07±0.01	0.07	0.04±0.01	0.08±0.03	0.28
DCRCF (PU)	152.99±54.26	147.50±34.74	0.90	118.08±41.60	144.23±54.80	0.77
DCVC (CU)	2.10±0.86	2.01±0.51	0.89	1.63±0.63	2.15±0.74	0.68
FCRCF (PU)	15.14±3.97	10.27±1.72	0.13	18.14±2.38	18.69±3.81	0.91
FCVC (CU)	0.20±0.07	0.14±0.03	0.18	0.23±0.04	0.30±0.09	0.55

Values are shown as mean ± SEM, *$p < 0.05$ (control vs aspirin).

Table 5.4 Change from baseline values of forearm vascular conductance (FVC) at the peak of reactive hyperaemia in groups of WEs, BAs and SAs and in COX+ and COX- responder groups before and after COX inhibition with Aspirin.

	Control	**Aspirin**	P value
All WE (n=19)	0.43±0.04	0.37±0.03	0.11
WE men (n=10)	**0.54±0.06**	**0.39±0.05***	0.003
WE women (n=9)	0.30±0.03	0.34±0.03	0.39
WE COX + (n=14)	**0.48±0.05§**	**0.36±0.04***	0.001
WE COX- (n=5)	0.27±0.04	0.38±0.03	0.14
All BAs (n=19)	0.39±0.03	0.39±0.04	0.97
BA men (n=11)	0.40±0.03	0.37±0.03	0.43
BA women (n=8)	0.38±0.06	0.43±0.09	0.52
BA COX + (n=10)	**0.44±0.03**	**0.32±0.02***	0.001
BA COX- (n=9)	**0.34±0.05**	**0.48±0.08***	0.006
All SAs (n=14)	0.38±0.03	0.34±0.02	0.40
SA men (n=6)	0.45±0.07	0.36±0.02	0.33
SA women (n=8)	0.33±0.02	0.32±0.03	0.88
SA COX+ (n=9)	**0.44±0.04§**	**0.30±0.03***	0.01
SA COX- (n=5)	**0.26±0.01**	**0.40±0.03***	0.02

Values are shown as mean ± SEM. *p<0.05: control vs aspirin tested with paired Student's T tests.

Table 5.5 Change from baseline values of FVC, Mean ABP, HR, DCVC and FCVC at 15s during first sound stimulus (S1) in groups of White Europeans (WEs) before and after COX inhibition with Aspirin.

		All WEs (n=19)	**Men (n=10)**	**Women (n=9)**	**Vasodilators (n=12)**	**Vasoconstrictors (n=7)**
Δ FVC	Control	0.007±0.005	0.009±0.007	0.005±0.007	0.019±0.005	**-0.011±0.004**
(CU)	Aspirin	0.016±0.005	0.015±0.007	0.016±0.008	0.019±0.006	**0.012±0.008***
	p value	0.20	0.22	0.47	0.82	**0.03**
Δ MABP	Control	0.92±1.51	2.20±2.40	-0.48±1.80	-0.74±2.03	3.78±1.90
(mmHg)	Aspirin	-0.99± 1.58	0.67±2.06	-2.84±2.24	-1.16±2.23	-0.71±2.15
	p value	0.36	0.66	0.89	0.35	0.06
Δ HR	Control	1.03±0.95	**0.27±1.51**	1.88±1.11	**3.00±0.90**	-2.35±0.09
(bpm)	Aspirin	-1.61±1.21	**-3.14±1.43***	0.08±1.94	**-0.69±1.26***	-3.18±2.51
	p value	0.06	**0.03**	0.49	**0.049**	0.72
Δ DCVC	Control	-0.72±0.25	-0.89±0.47	-0.57±0.26	-0.54±0.30	-1.17±0.51
(CU)	Aspirin	-0.53±0.18	-0.67±0.16	-0.40±0.31	-0.35±0.25	-0.89±0.14
	p value	0.52	0.70	0.57	0.72	0.60
Δ FCVC	Control	-0.09±0.03	-0.08±0.06	**-0.10±0.02**	-0.11±0.04	-0.04±0.03
(CU)	Aspirin	-0.02±0.02	-0.03±0.02	**-0.02±0.03***	-0.02±0.02	-0.01±0.04
	p value	0.07	0.48	**0.004**	0.11	0.52

. Values are shown as mean ± SEM. * $p < 0.05$: control vs aspirin tested with paired Student's T tests

Table 5.6 Change from baseline values of FVC, Mean ABP, HR, DCVC and FCVC at 15s during first sound stimulus (S1) in groups of Black Africans (BAs) before and after COX inhibition with Aspirin.

	Intervention	All BAs (n=19)	Men (n=10)	Women (n=9)	Vasodilators (n=10)	Vasoconstrictors (n=9)
Δ FVC	Control	0.003±0.004	0.009±0.006	-0.005±0.006	**0.017±0.002**	**-0.012±0.002**
(CU)	Aspirin	-0.004±0.004	-0.002±0.006	-0.007±0.005	**-0.005±0.007***	**-0.002±0.003***
	p value	0.24	0.22	0.84	**0.03**	**0.02**
Δ MABP	Control	1.31±1.75	-2.65±1.60	6.76±2.52	-2.85±1.54	**5.93±2.56**
(mmHg)	Aspirin	-0.21±1.49	-1.00±1.98	0.88±2.34	-0.05±2.21	**-0.38±2.09***
	p value	0.45	0.47	0.10	0.22	**0.05**
Δ **HR**	Control	-0.01±1.32	-0.94±1.49	1.26±2.43	-1.10±1.57	1.20±2.21
(bpm)	Aspirin	-1.40±1.84	1.78±2.35	-5.76±2.28	1.87±2.60	-5.02±2.13
	p value	0.60	0.45	0.07	0.45	0.07
Δ **DCVC**	Control	-0.86±0.53	-0.62±0.60	-1.25±1.06	-0.63±0.82	-1.06±0.74
(CU)	Aspirin	-0.06±0.22	0.12±0.34	-0.34±0.12	0.12±0.47	-0.21±0.13
	p value	0.16	0.32	0.40	0.46	0.25
Δ **FCVC**	Control	-0.02±0.02	-0.001±0.03	-0.03±0.02	0.01±0.04	-0.05±0.02
(CU)	Aspirin	-0.05±0.06	-0.06±0.10	-0.06±0.03	-0.09±0.13	-0.01±0.02
	p value	0.72	0.58	0.51	0.50	0.26

Values are shown as mean ± SEM. * $p < 0.05$: control vs aspirin tested with paired Student's T tests.

Table 5.7 Change from baseline values of FVC, mean ABP, HR, DCVC and FCVC at 15s during first sound stimulus (S1) in groups of South Asians (SAs) before and after COX inhibition with Aspirin.

	Intervention	All SAs (n=14)	Men (n=6)	Women (n=8)	Vasodilators (n=7)	Vasoconstrictors (n=7)
Δ FVC (CU)	Control	-0.003±0.006	0.003±0.010	-0.008±0.007	0.021±0.010	-0.021±0.005
	Aspirin	-0.016±0.005	0.012±0.005	-0.038±0.005	-0.003±0.002	-0.041±0.007
	p value	0.21	0.43	0.06	0.07	0.33
Δ MABP (mmHg)	Control	0.58±2.58	-0.42±4.65	1.33±3.14	-2.98±4.29	4.14±2.47
	Aspirin	4.30±1.71	-0.47±2.77	7.87±1.04	4.39±2.13	4.21±2.84
	p value	0.25	0.99	0.08	0.16	0.99
Δ HR (bpm)	Control	0.21±0.83	-1.25±0.56	1.30±1.29	-0.71±0.77	1.12±1.45
	Aspirin	-1.59±0.77	-1.73±1.00	-1.49±1.19	-2.36±0.92	-0.82±1.25
	p value	0.19	0.55	0.25	0.17	0.46
Δ DCVC (CU)	Control	-1.88±0.28	0.07±0.11	-1.34±0.53	-1.27±0.84	-0.45±0.33
	Aspirin	-1.15±0.36	-0.40±0.50	-1.65±0.41	-1.76±0.71	-0.74±0.28
	p value	0.17	0.40	0.36	0.40	0.38
Δ FCVC (CU)	Control	-0.04±0.03	-0.02±0.01	-0.05±0.05	-0.06±0.05	-0.02±0.03
	Aspirin	-0.04±0.03	0.03±0.04	-0.09±0.03	-0.04±0.04	-0.04±0.04
	p value	0.96	0.30	0.52	0.71	0.73

Values are shown as mean ± SEM. * $p < 0.05$: control vs aspirin tested with paired Student's T tests.

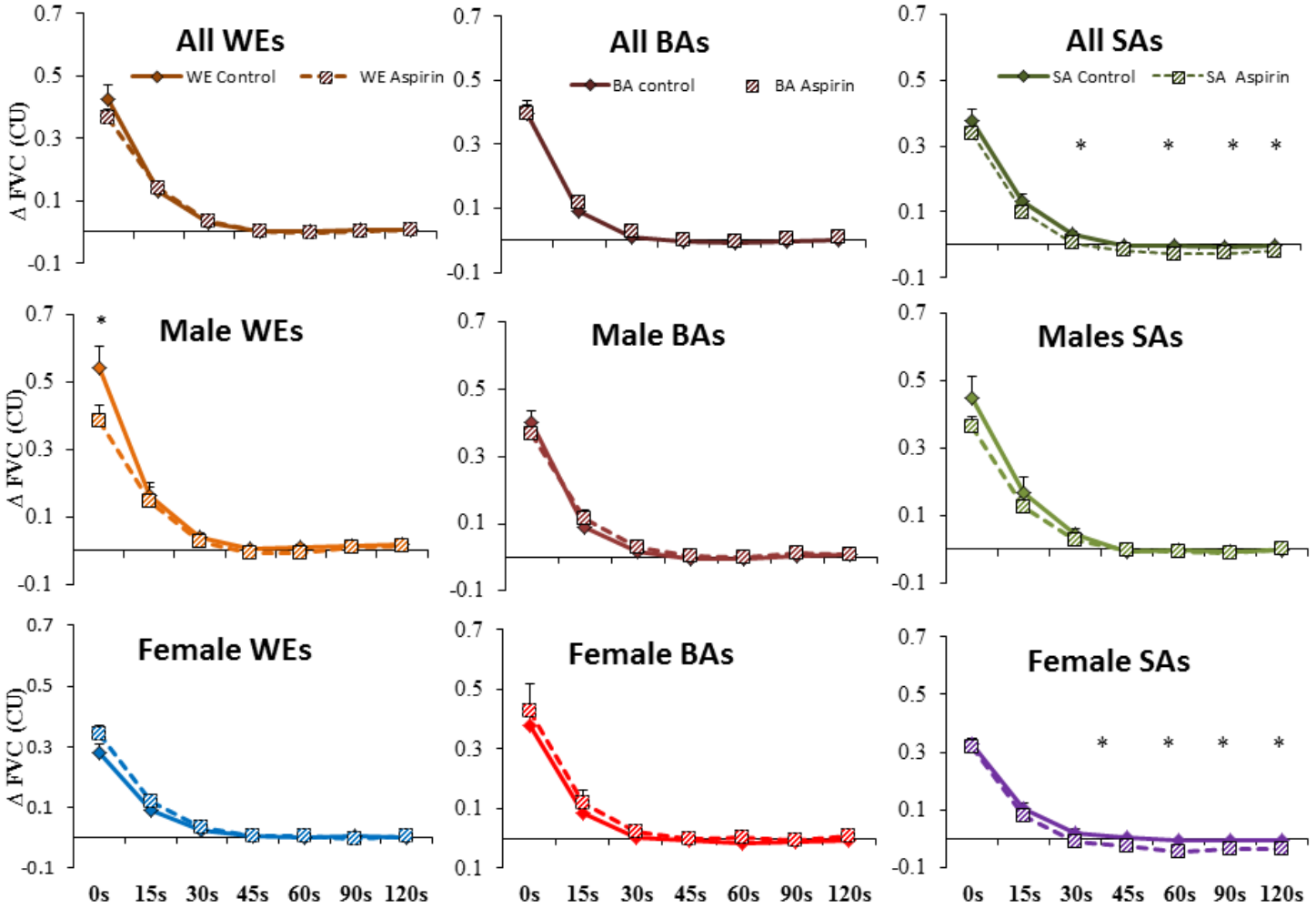


Figure 5.1: Change from baseline values of forearm vascular conductance (ΔFVC) during reactive hyperaemia before and after COX inhibition with aspirin in WEs, BAs and SAs. Values are mean ± SEM. * p<0.05: control vs aspirin with 3-way mixed ANOVA with Bonferroni post hoc tests.

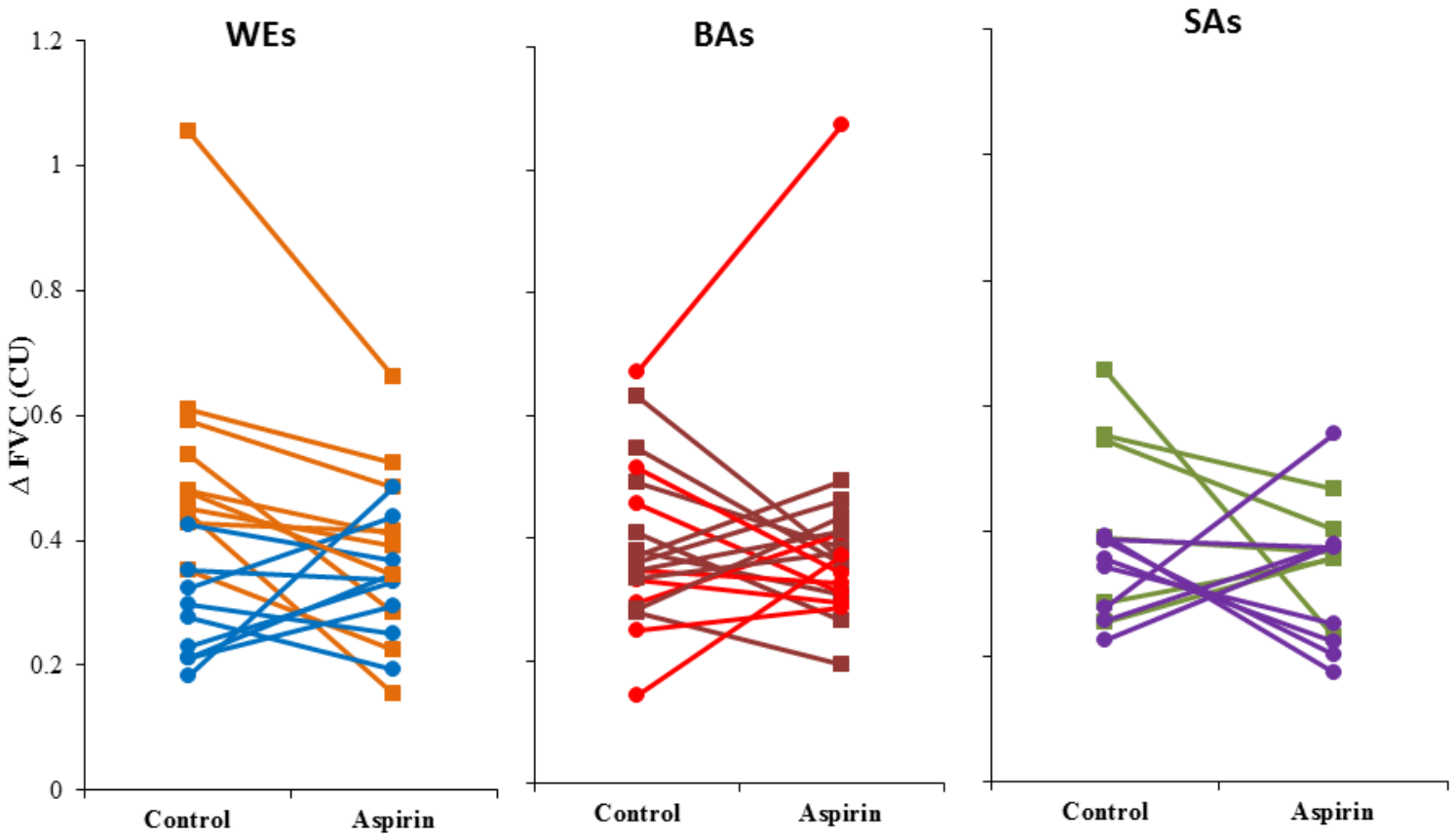


Figure 5.2: Change from baseline values of FVC at peak of reactive hyperaemia before and after COX inhibition with Aspirin in WEs, BAs and SAs. WE males are represented by orange lines and WE females in blue lines. BA males are represented in brown lines and BA females in red lines. SA males are represented in green lines and females in purple lines. Values are mean ± SEM.

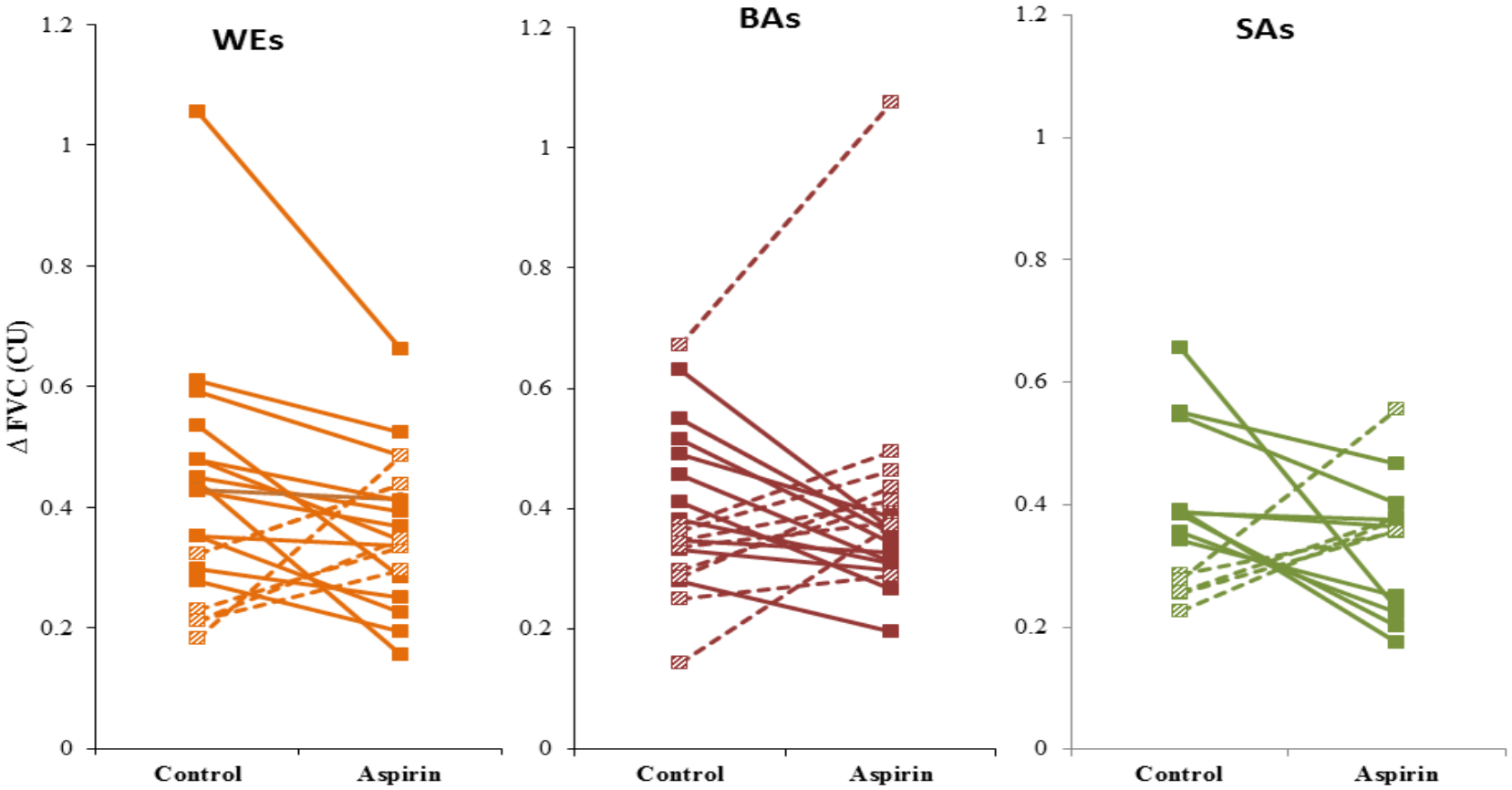


Figure 5.3: Change from baseline values of FVC at peak of reactive hyperaemia before and after COX inhibition with Aspirin in COX+ and COX- responder groups of WEs, BAs, SAs. COX+ responders are represented in unbroken lines and COX- responders are represented in broken lines. Values are mean ± SEM.

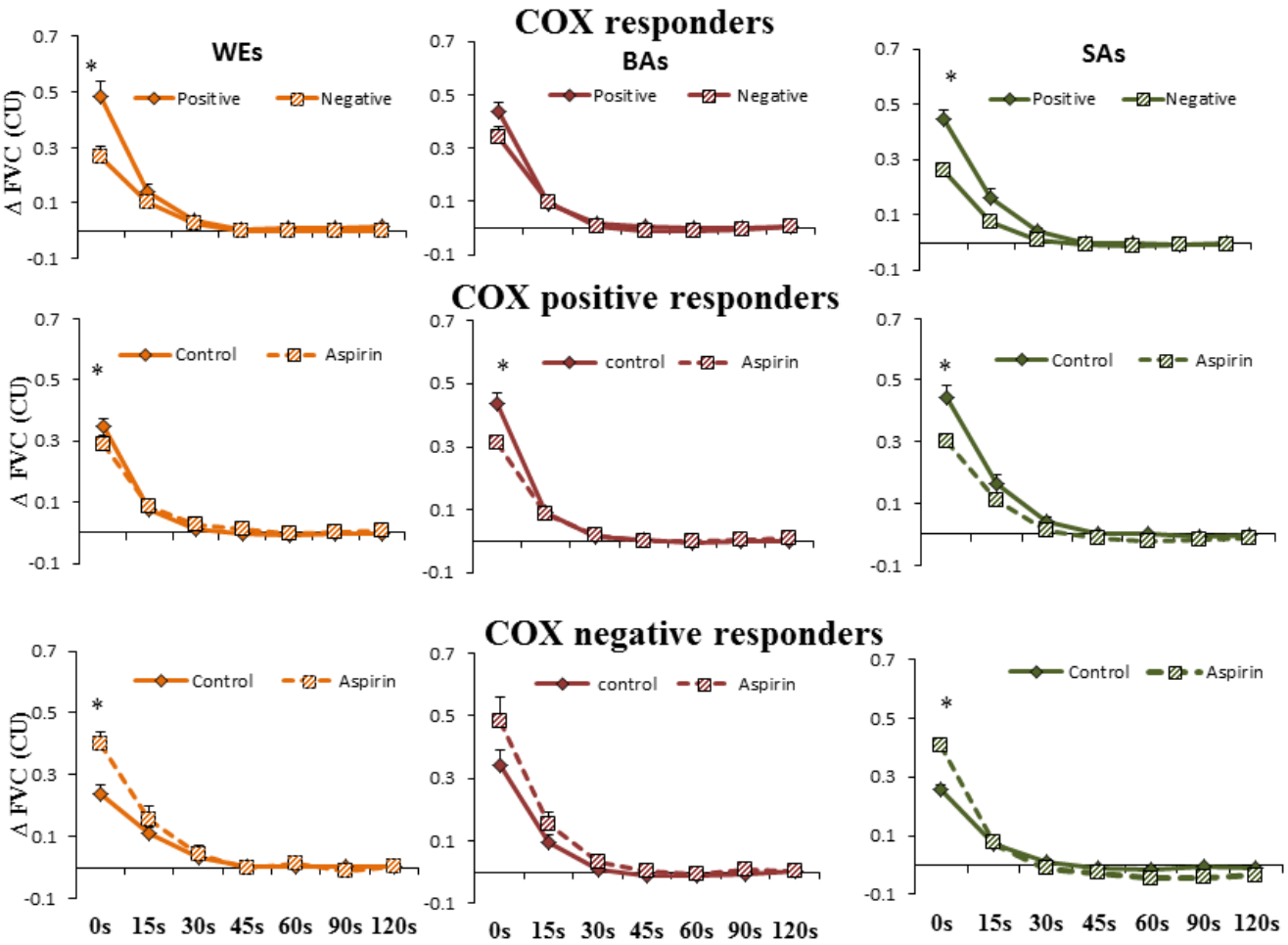


Figure 5.4: Change from baseline values of FVC during reactive hyperaemia before and after COX inhibition with Aspirin in COX+ and COX- responder groups of WEs, BAs, SAs. Values are mean ± SEM. *p<0.05: COX+ vs COX- responders. * p<0.05, ** p<0.005: control vs aspirin, 3-way mixed ANOVA with Bonferroni post hoc tests.

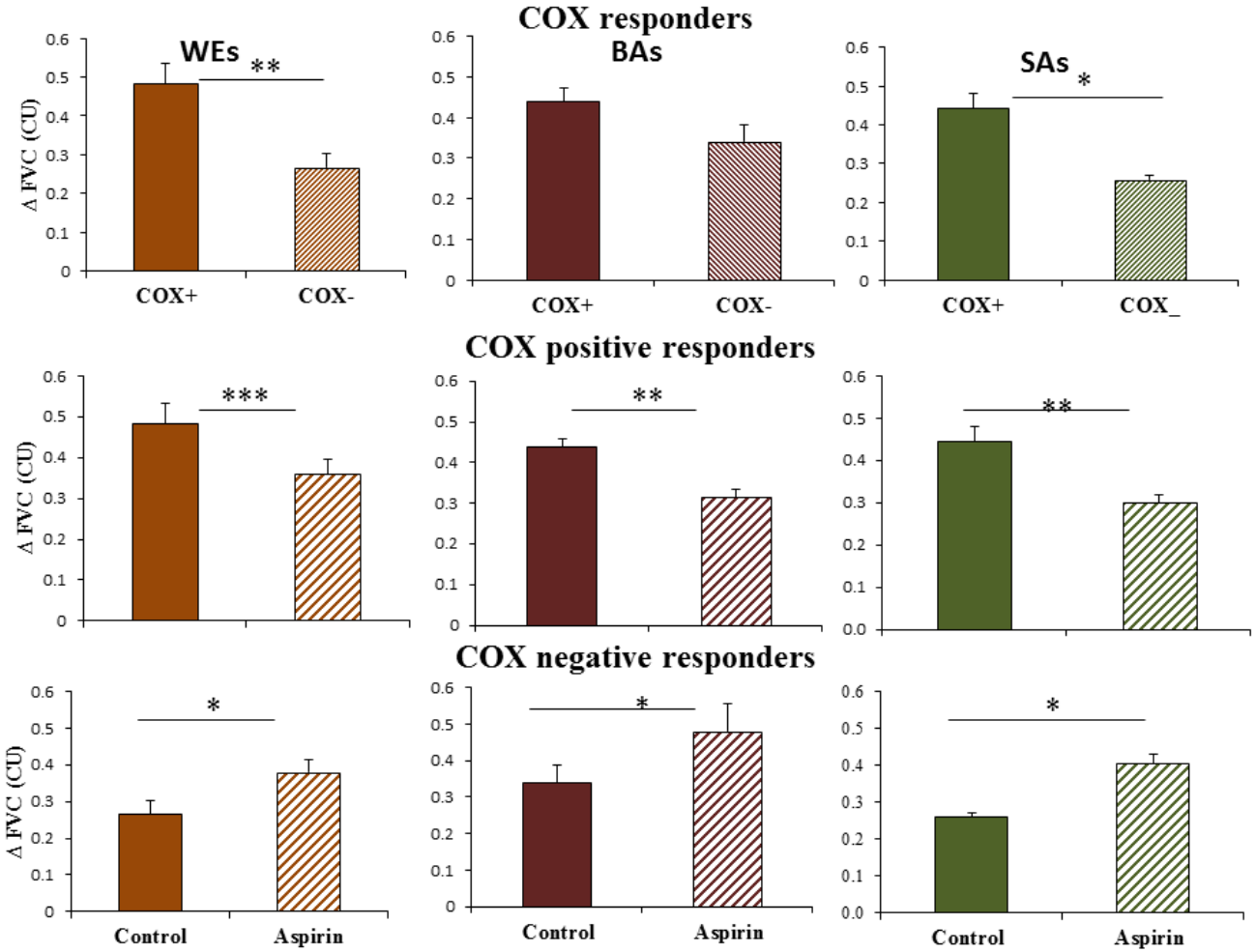


Figure 5.5: Change from baseline values of FVC during at peak of reactive hyperaemia in COX responders, before and after COX inhibition with Aspirin in COX+ and COX- responder groups of WEs, BAs, SAs. Values are mean ± SEM. *p<0.05: COX+ vs COX- responders. * p<0.05, **p<0.005: control vs aspirin, 3-way mixed ANOVA with Bonferroni post hoc tests.

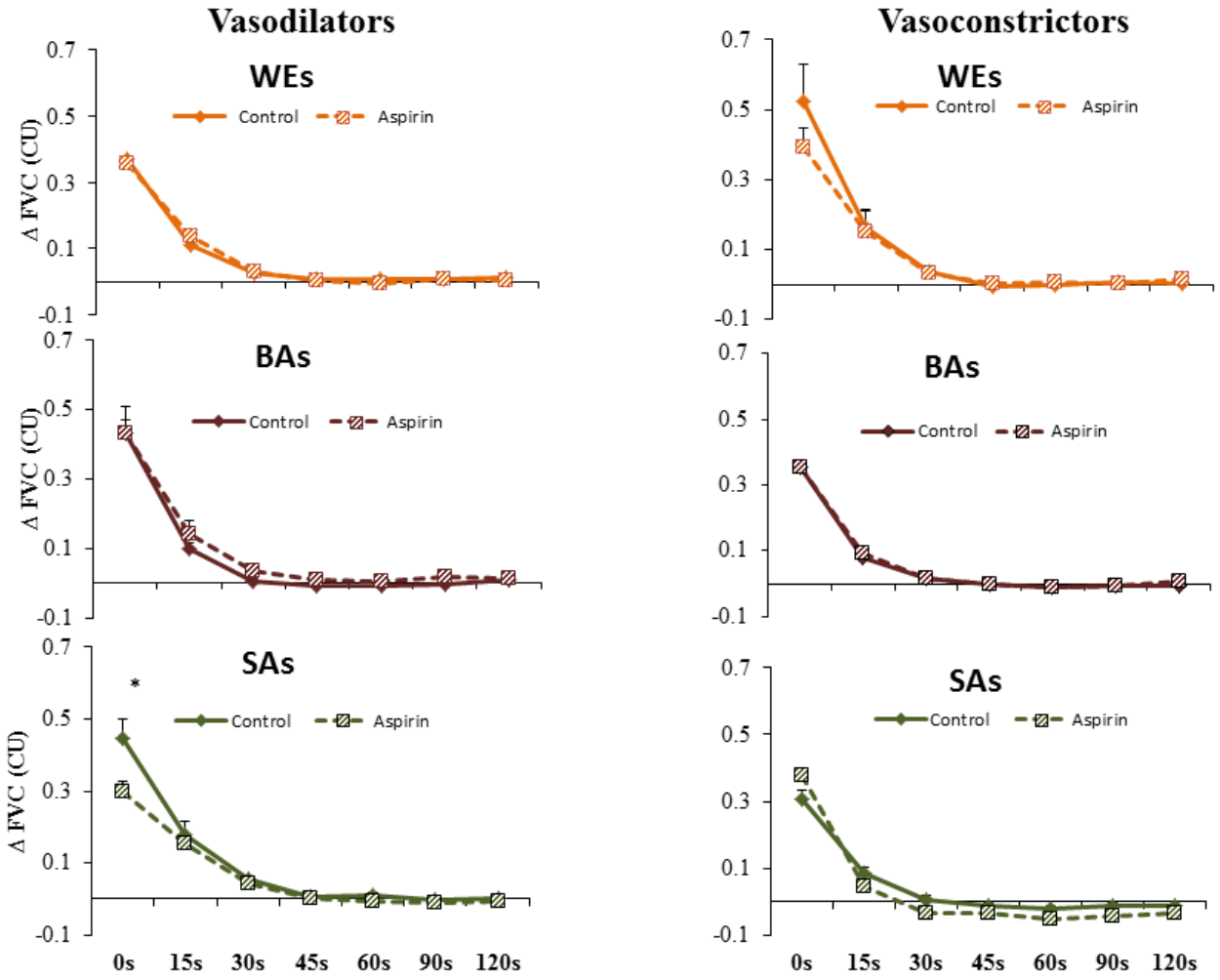


Figure 5.6: Change from baseline values of FVC during reactive hyperaemia before and after COX inhibition with Aspirin in WE, BA, SA vasodilators and vasoconstrictors. Values are mean ± SEM. * $p<0.05$: control vs aspirin, 3-way mixed ANOVA with Bonferroni post hoc tests.

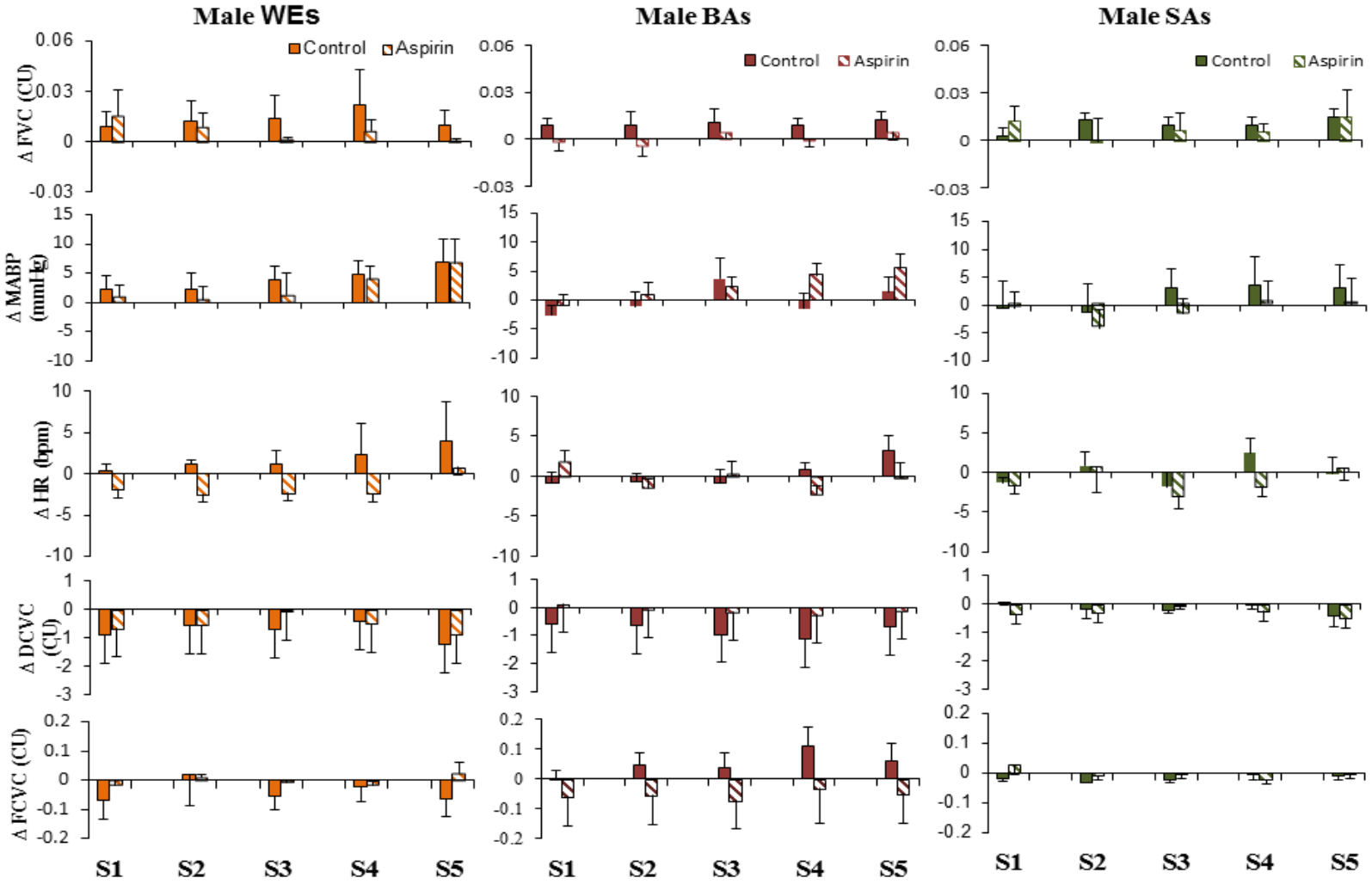


Figure 5.7: Change from baseline values of FVC, MABP, HR, DCVC and FCVC during S1-5 at 15s into each sound before and after COX inhibition with Aspirin in male WEs, BAs and SAs. Values are mean ± SEM.

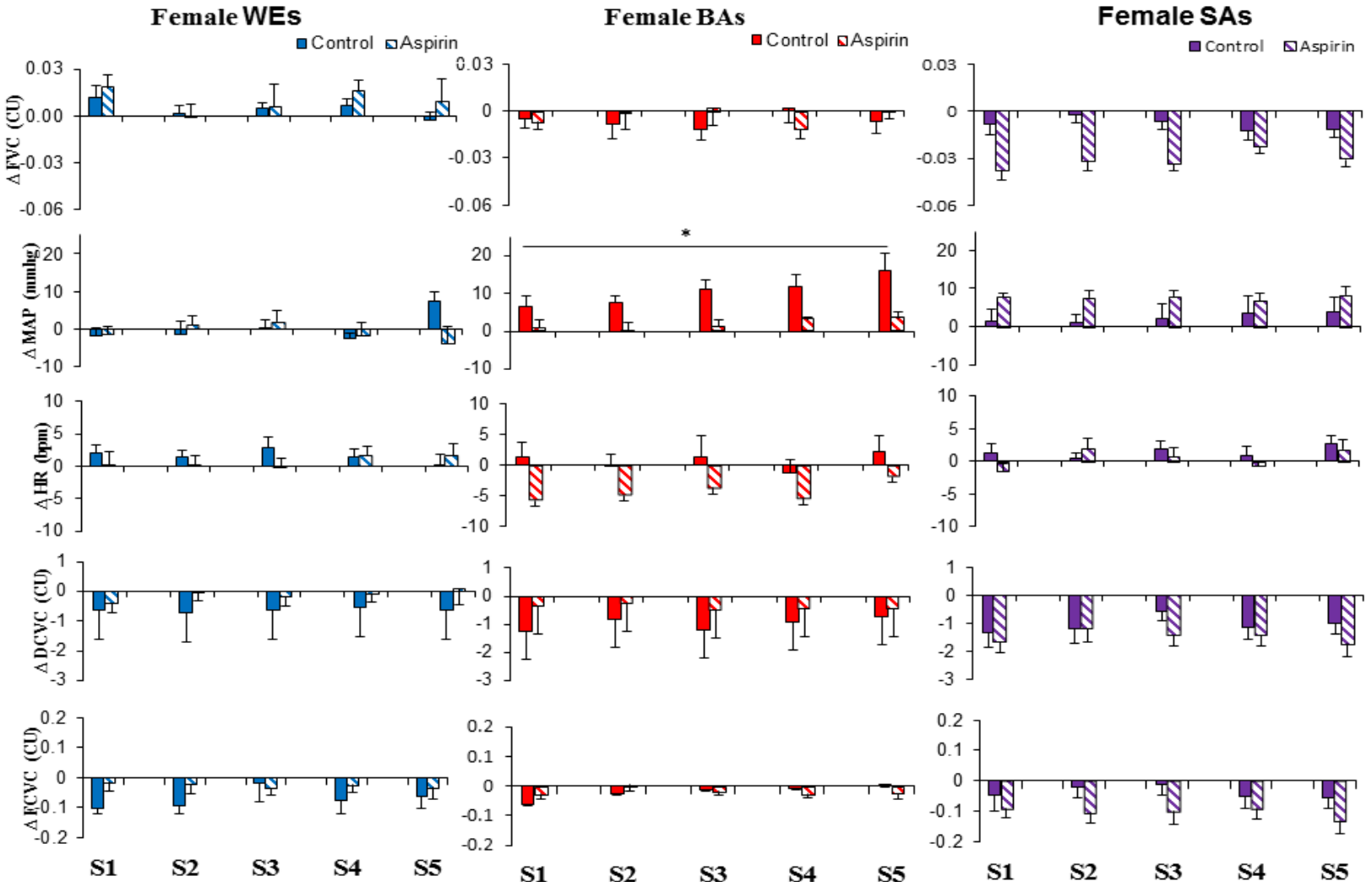


Figure 5.8: Change from baseline values of FVC, MABP, HR, DCVC and FCVC during S1-5 at 15s into each sound before and after COX inhibition with Aspirin in female WEs, BAs and SAs. Values are mean ± SEM. * $p<0.05$: control vs aspirin, 3-way mixed ANOVA with Bonferroni post hoc tests.

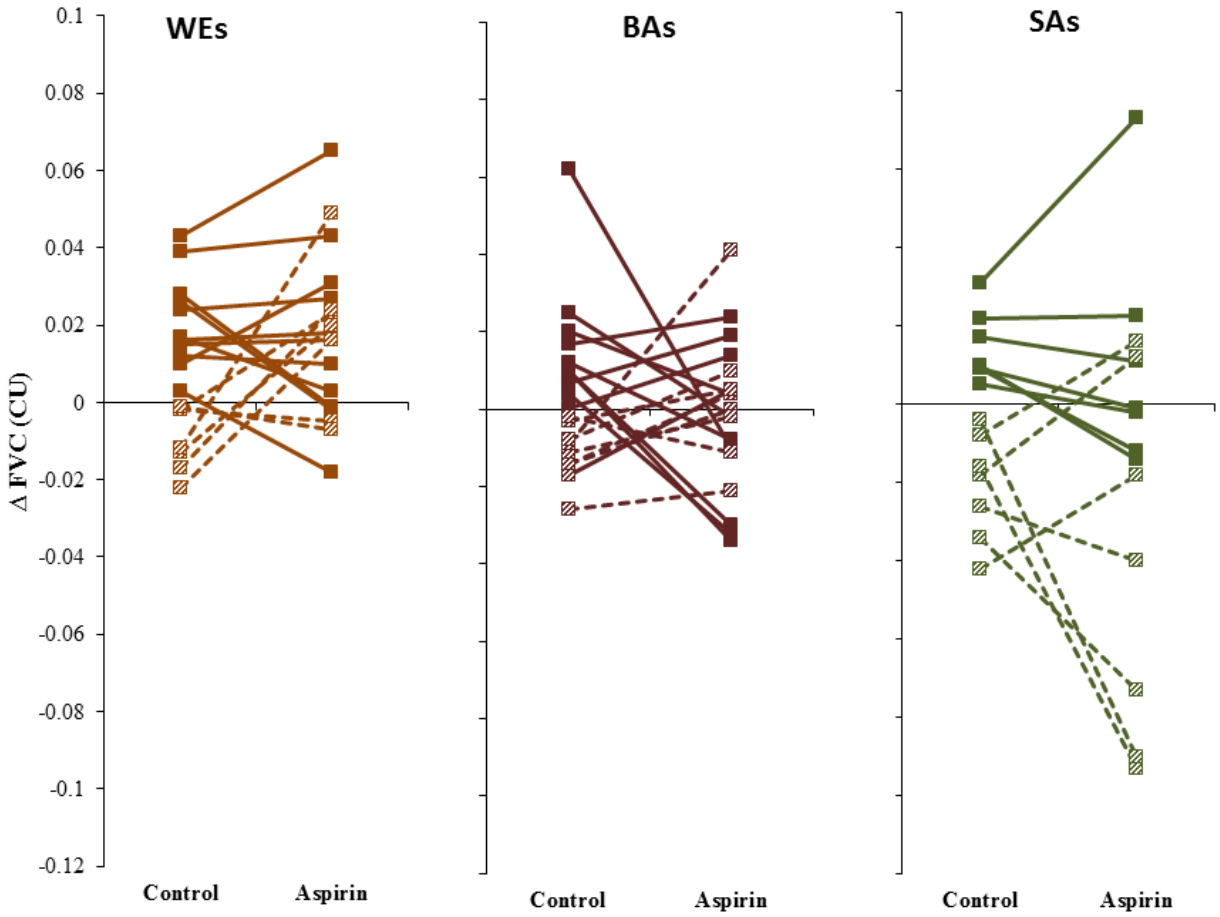


Figure 5.9: Change from baseline values of FVC at peak of reactive hyperaemia before and after COX inhibition with Aspirin in vasodilators and vasoconstrictors in each ethnic group. Vasodilators are represented in unbroken lines and vasoconstrictors represented in broken lines. Values are mean ± SEM.

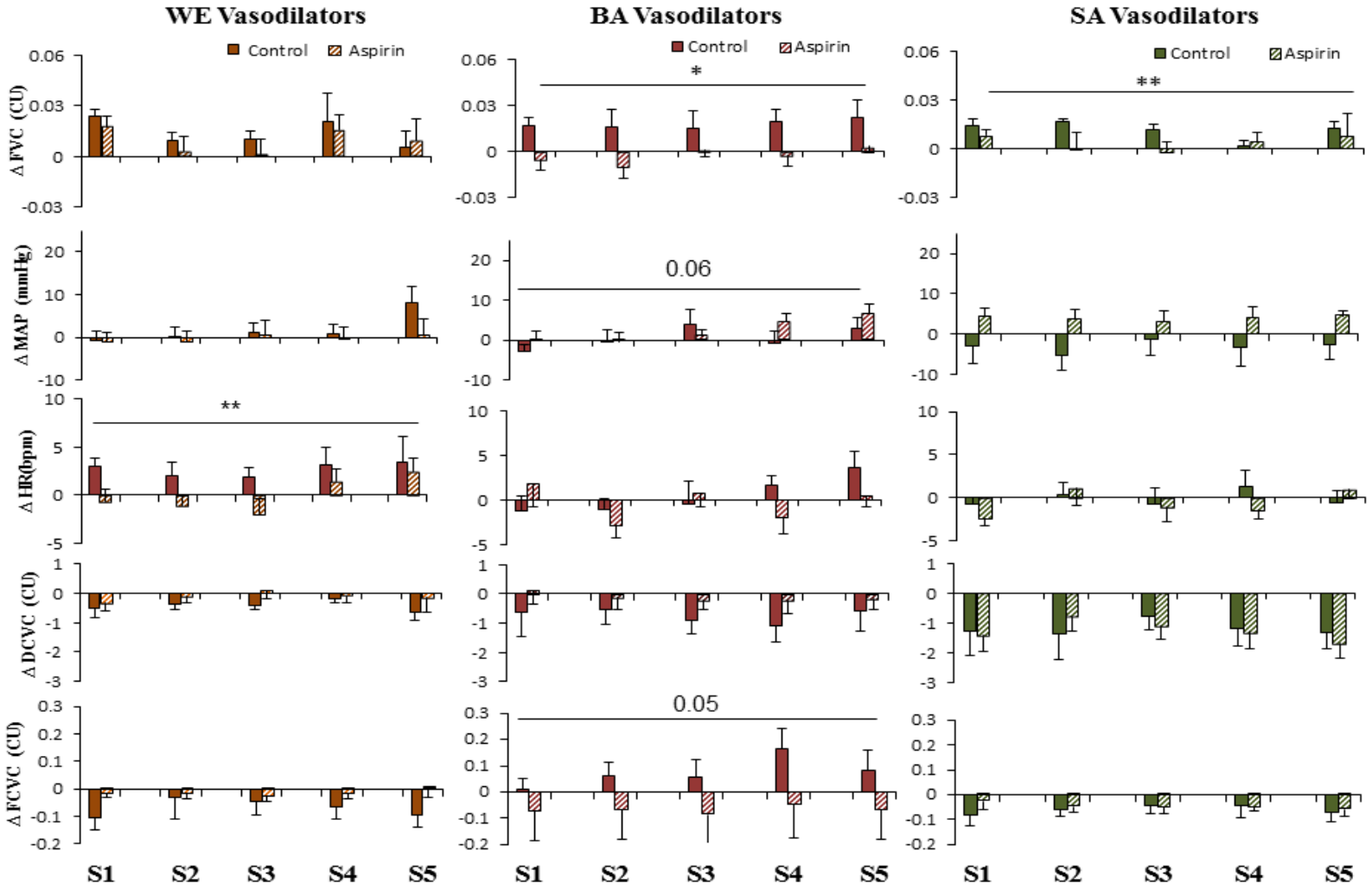


Figure 5.10: Change from baseline values of FVC, MABP, HR, DCVC and FCVC during S1-5 at 15s into each sound before and after COX inhibition with Aspirin in WE, BA and SA vasodilators. Values are mean ± SEM. * $p<0.05$, ** $p<0.005$: control vs aspirin, 3-way mixed ANOVA with Bonferroni post hoc tests.

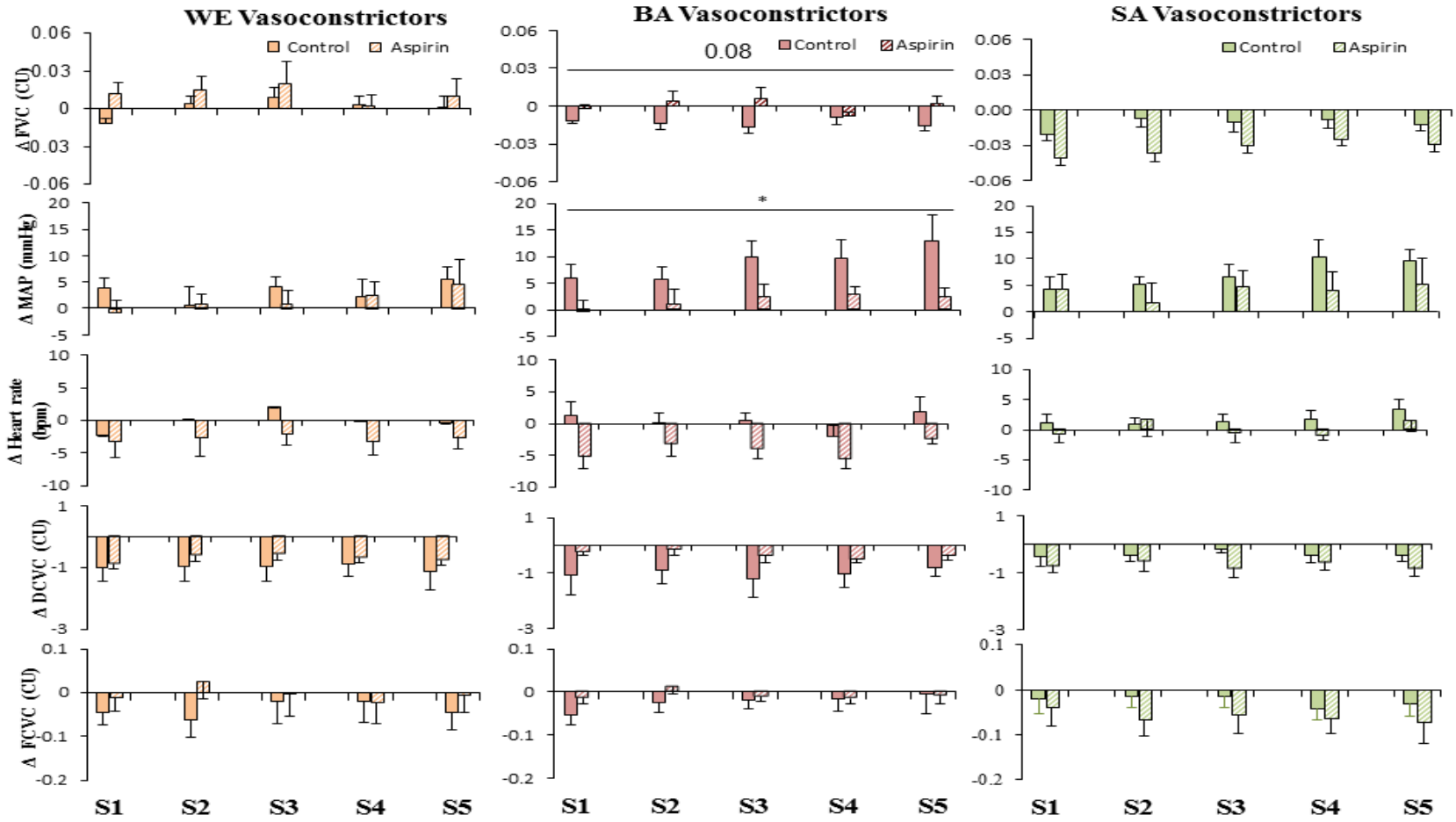


Figure 5.11: Change from baseline values of FVC, MABP, HR, DCVC and FCVC during S1-5 at 15s into each sound before and after COX inhibition with Aspirin in WE, BA and SA vasoconstrictors. Values are mean ± SEM. * $p<0.05$: control vs aspirin, 3-way mixed ANOVA with Bonferroni post hoc tests.

CHAPTER 6

Contribution Of Prostaglandins To Exercise Hyperaemia: Ethnic And Sex Differences

6.1 Introduction

The studies described in Chapter 3 and 5, demonstrated ethnic- and sex-dependent differences in endothelial-dependent reactive hyperaemia as well as the pattern of cardiovascular response evoked by repeated mental stress in young apparently healthy individuals. Reactive hyperaemia was blunted in Black Africans (BAs) relative to White Europeans (WEs), particularly in BA men and was blunted in WE, and South Asians (SA) but not BA women relative to their male counterparts. On the other hand, forearm vasodilatation in response to mental stress was blunted or reversed to vasoconstriction particularly in BA and SA women. The results also showed that vasodilator prostaglandins (PGs) contribute to peak reactive hyperaemia in some individuals within both sexes of each of the 3 ethnic groups (COX+) as judged by the effects of COX inhibition, but this was most evident in WE men who showed a substantial role for PG reactive hyperaemia. On the other hand, neither men nor women in any of the 3 ethnic groups showed a role for PGs in the vascular responses to mental stress. However, when grouped according to whether individuals showed forearm dilatation or constriction to the 1st sound (vasodilators/constrictors respectively), BA and SA vasodilators showed evidence of PGs contributing to the forearm dilatation evoked by mental stress, whereas BA vasoconstrictors showed evidence of COX products contributing to their forearm constriction.

As discussed in Chapter 1 section 1.3, and Chapter 5 section 5.1.2, the endothelium contributes to the muscle vasodilation evoked by mental stress as well as to reactive hyperaemia. Thus, the blunted reactive hyperaemia observed in whole groups of Black Africans and especially in BA men, as well as the blunted forearm vasodilator responses

to mental stress in BAs and SAs (see Chapter 3) may be due to impaired ability of the endothelium to produce dilators.

Exercise hyperaemia that follows handgrip exercise and reactive hyperaemia that follows release of vascular occlusion increase blood flow in forearm muscle through different mechanisms: reactive hyperaemia primarily involves mediators which are released from the endothelium as a consequence of hypoxia and possibly, shear stress (Chapter 5, Introduction), whereas exercise hyperaemia involves, vasodilators which are released from the exercising muscle fibres as well as from the endothelium (Clifford & Hellsten, 2004; Marshall & Ray, 2012). Exercise also increases sympathetic nerve activity (SNA) leading to an increase in heart rate (HR), cardiac contractility with concurrent vasoconstriction in the viscera and inactive skeletal muscle leading to increased arterial blood pressure (ABP). However, within the active skeletal muscles, the increased muscle sympathetic nerve activity (MSNA) is blunted by local dilator influences: functional sympatholysis (Clifford & Hellsten, 2004).

It is known that blunted vasodilator responses to stressors such as novel stimuli, or exercise are associated with endothelial dysfunction, which is a major contributor to cardiovascular diseases (CVD) (Chapter 1, section 1.4.2). It is also known that exaggerated pressor responses to stressors in young adult life are associated with development of hypertension (Chapter 1, section 1.4). Given that BAs and SAs are at high risk of developing CVD and given the findings outlined above, it seems they have impaired endothelial dilator function even before they develop symptoms of CVD. Thus, we considered it important to examine whether there are differences in the

cardiovascular responses evoked by handgrip exercise between WEs and BAS and between WEs and SAs and in contributions of PGs to exercise hyperaemia.

We have recently published an invited review titled "Contribution of Prostaglandins to exercise hyperaemia: ethnicity and sex matter" in the Journal of Physiology as part of the output of this study (See Appendix 7). The sections below provide a shortened version of the background to the study, while the results are provided in more detail.

6.1.2 Role of prostaglandins in exercise hyperaemia

The first evidence for the role of PGs in exercise hyperaemia was documented in dogs by the finding that the release of PGs elicited during and after muscular work was abolished by the COX inhibitor, Indomethacin (Herbaczynska-Cedro *et al.*, 1976) and by the demonstration of graded release of PGE during graded intensity contractions of calf muscles (Young & Sparks, 1980).

Further, in men and women, post-exercise hyperaemia assessed by venous occlusion plethysmography (VOP) following isometric and rhythmic forearm exercise was attenuated by COX inhibition (Kilbom & Wennmalm, 1976) and increased PGE in venous effluent following high intensity leg exercise was demonstrated (Nowak & Wennmalm, 1978). In addition, COX inhibition attenuated post-exercise hyperaemia in the leg following treadmill exercise at 50% maximum intensity (Cowley *et al.*, 1985), as well as in the arm following low intensity rhythmic hand grip exercise and high intensity isometric hand grip exercise (Duffy *et al.*, 1999).

By contrast, other studies have shown no role of PGs in exercise hyperaemia. Briefly, Beaty and Donald (1979) showed that although COX inhibition attenuated reactive

hyperaemia, there was no effect on exercise hyperaemia in dogs. Likewise, Shoemaker *et al.* (1996), found that COX inhibition did not attenuate exercise hyperaemia as measured by Doppler ultrasound during low intensity (10% maximum voluntary contraction (MVC) rhythmic forearm exercise. Similarly, COX inhibition did not attenuate leg blood flow or vascular conductance measured using the constant infusion thermodilution method during 20% intensity knee extensor exercise (Mortensen *et al.*, 2007). These studies led to the conclusion that PGs do not contribute to exercise hyperaemia. Moreover, COX inhibitor infused during hyperaemia evoked by rhythmic forearm exercise at 15% MVC produced a transient attenuation of the hyperaemia in a group of men and women (Schrage *et al.*, 2004).

However, in recent studies from our laboratory, Junejo *et al.* (2015) showed that breathing 40% O_2 or COX inhibition with 600 mg oral aspirin had comparable attenuating effects on post-exercise hyperaemia evoked by rhythmic and static handgrip at 60% MVC for 2 min in young and older men. They also assayed venous efflux of PGs by ELISA following rhythmic and static handgrip contractions, during air breathing, 40% O_2, and after COX inhibition in a cross over design and showed there was release of both PGI_2 and PGE_2 in an O_2-dependent manner. Given that release of PGI_2 is attributable to endothelium (Feletou *et al.*, 2011) and PGE_2 is mainly attributable to skeletal muscle (McLennan & Macdonald, 1991), they proposed that the PGs that contribute to exercise hyperaemia are released from skeletal muscle fibres as well endothelium.

Taken together these results suggest that the disparities between studies as to whether PGs play a role in exercise hyperaemia may reflect different intensities of exercise: they

are more likely to contribute at high intensity of rhythmic or isometric handgrip exercises (at 40-60% MVC), but not at light to moderate intensity (10-15% MVC) (Junejo, 2017).

6.1.3 Ethnic differences in Exercise hyperaemia

Young American BAs showed blunted arterial stiffness during maximal aerobic exercise (Heffernan *et al.*, 2007). However studies on endothelial function during exercise have been equivocal. While Kappus *et al.* (2017b) did not demonstrate blunting of exercise hyperaemia following rhythmic hand grip exercise at 10% and 20% MCV in BAs compared with WEs. Barbosa *et al.* (2018) reported blunted exercise hyperaemia following moderate intensity rhythmic handgrip exercise at 30%, 45% MCV. Further, Ozkor *et al.* (2014) did not demonstrate blunted exercise hyperaemia in BAs following low intensity exercise (15%) but there was a trend for a reduction in exercise hyperaemia in BAs at higher intensities of 30% and 45% MCV. In all these studies BA men were compared with WE men. Considering SAs, there is a complete paucity of information on exercise hyperaemia within the ethnic group or compared with others.

Turning to whether PGs contribute to exercise hyperaemia in different ethnic groups, to the best of our knowledge, there are no previous studies. Resting forearm blood flow (FBF) was similar between BAs and WEs, but the contribution of nitric oxide (NO) was smaller in BAs than WEs as judged by the effect of NOS inhibition, while the contribution of endothelium derived hyperpolarising factor (EDHF) was similar in BAs and WEs as judged by the effect of a K^+ channel inhibitor. However, during exercise, EDHF made a greater contribution to exercise hyperaemia in BAs than in WEs (Ozkor

et al., 2014). Since BAs are well known to have NO impairment (Stein *et al.*, 1997; Cardillo *et al.*, 1999), the possibility is raised that in BAs, the contributors to exercise hyperaemia may be similar to that reported in eNOS knockout mice (Sun *et al.*, 1999; Huang *et al.*, 2001): EDHF contributed to exercise hyperaemia in female eNOS knocked out mice, whereas PGs contribute in eNOS knock out male mice.

6.1.4 Sex differences in exercise hyperaemia

Oestrogen upregulates cyclooxygenase (COX) expression in endothelial cells by increasing the expression of phospholipase A2, COX-1, and PGI_2 synthetase (Farhat *et al.*, 1996; Tostes *et al.*, 2003). But whether or not women show greater exercise hyperaemia is controversial as studies involving young premenopausal women have showed divergent results. In one study, women showed greater muscle vasodilatation during graded knee extensor exercise than men (Parker *et al.*, 2007). In another study, young women showed similar FBF to dynamic handgrip exercise as men, however due to greater fall in mean ABP, the forearm vascular conductance (FVC) was higher in the women (Gonzales *et al.*, 2007). On the other hand, during intermittent maximal handgrip exercise, mean brachial blood flow was higher in women than in men (Saito *et al.*, 2008). Further, women showed greater forearm vasodilation in response to exercise at 15% MVC (Kellawan *et al.*, 2015), whereas it was reported that at 15% and 30% MVC, forearm vascular conductance was similar between men and women (Limberg *et al.*, 2010). But in other studies, women demonstrated lower absolute FBF and FVC at 10% and 20% MVC and during graded dynamic handgrip exercise at 20%, 40%, 60% and 80% MVC compared with men (Hunter *et al.*, 2006a; Casey *et al.*, 2014).

Thus, in spite of early evidence suggesting a role for prostaglandins in exercise hyperaemia, since that time there has been controversy over whether it actually plays a role and if so, at what magnitude. The disparities may have been due to different methods of assessment of exercise hyperaemia, different exercise protocols, limbs and different types of exercise may have added to the confusion. Overall, there is evidence for modest contribution of prostaglandins to exercise hyperaemia at medium to high intensity, but there has so far been no study on the influence of ethnicity on the contribution of PGs; indeed, none of the studies published on the contribution of PGs to exercise hyperaemia have mentioned the ethnicity of the subjects.

6.1.2 Aim of the study

The aim of this study is to determine the ethnic differences between WEs, BAs and SAs in cardiovascular responses evoked by 2 min rhythmic hand grip exercise at 60% MVC and to determine the role of PGs.

6.1.3 Hypotheses

1. BAs and SAs will show blunted peak and or total post exercise hyperaemia and the contributions of PGs will be greater than in WEs.
2. Women will show greater peak and or total post exercise hyperaemia than men and the contributions of PGs will be greater in women than men.

6.2 Methods

6.2.1 Study participants

47 young adults: 18 WEs (10 men and 8 women), 18 BAs (10 men and 8 women) and 11 SAs (6 men and 5 women) were recruited.

6.2.2 Procedure

The methodology and recording equipments are described in detail in Chapter 2. In brief, following recruitment to the study, a familiarization visit took place during which the subject was requested to lie semi reclined in the experimental position and use the dominant hand to grip the handgrip dynamometer (Lafayette 70718, Loughborough, UK) with maximum strength(see Figure 6.8). They were asked to maintain the grip for about 5 seconds: this was repeated 3 times. An average of the 3 readings was used as the 100% MCV, 60% MCV was derived from this value by multiplying 100% MCV value by 0.6.

The handgrip dynamometer was connected to a visual display unit, which was placed in front of the subject in order that they could regulate the strength of contraction. Before experiment started, calibration was done such that the visual unit displayed 1.0 and 0.6 volts when 100% and 60% MVC respectively were achieved by each subject. During the familiarization visit a trial of the rhythmic handgrip exercise was done to accustom participants with the study procedure. On the day of the experiment following 10 mins of rest, ABP and HR was recorded using automated blood pressure monitor (OMRON 4, UK): 3 measurements were done at 1-2 min interval, and mean of the 3 recordings was used as baseline value. Before exercise, baseline forearm blood flow was recorded

as described in chapter 2. Subjects performed rhythmic hand grip exercise for 2 mins at 1s on : 1s off to the beat of an online web based metronome accessed on https://www.webmetronome.com/. Following exercise, FBF was recorded by VOP within 0-5s following cessation of exercise (peak exercise hyperaemia) and at 30s intervals for 2 minutes and thereafter, every 1 minute for another 5 minutes.

Orange juice containing 600mg of aspirin dissolved was then consumed and the exercise protocol was repeated 30 minutes after consumption of aspirin. The protocol is shown below (Figure 6. 1).

Figure 6.1 Exercise experiment Protocol . Baseline period of 15 min followed by 2 min period of handgrip exercise of the dominant arm, followed by 7 min period of FBF recording at 0-5s, 30s, 60s, 90s , 120s,3, 4, 5, 6 and 7 mins

6.2.3 Data analysis

BP and HR was extracted offline within 2s before onset of exercise to determine anticipatory responses (A). Thereafter, extractions of BP and HR were done at onset of exercise (0s), then at 30s, 60s, 90s and 120s during the exercise. During exercise hyperaemia data were extracted at intervals as FBF was recorded highlighted above. All data are presented as mean ± standard error of mean (SEM) except where stated otherwise. Comparisons between ethnic groups and between men and women for anthropometric measurements and cardiovascular baselines were done using independent Student's T tests.

Responses to exercise were recorded as absolute values and are presented as FBF, FVC, MABP and HR. Three-way mixed factor ANOVA was done with ethnicity (WE, BA

and SA) and sex as between subjects factors, for exercise hyperaemia (FBF, FVC), time (10 points) was the within subjects factor and time (17 time points) was the within subjects factor for MABP, HR. Bonferroni post hoc tests were done as appropriate. To assess effect of COX inhibition, change from baseline values of FVC before and after COX inhibition during exercise hyperaemia were calculated. Effect of aspirin on exercise hyperaemia was determined using 3-way mixed ANOVA with Bonferroni post hoc tests Effect of aspirin on the peak exercise hyperaemia only was tested with 3-way ANOVA with treatment (2), control vs aspirin, as the within subjects factor and ethnicity and sex as the between subjects factors. Bonferroni post hoc tests were done as appropriate. In all cases, $p < 0.05$ was taken as significant.

6.3 Results

6.3.1 Baseline characteristics

6.3.1.1 Baseline characteristics in whole ethnic groups

As shown in Table 6.1, there were no differences in baseline anthropometric and baseline cardiovascular variables between WEs and BAs or SAs ($p>0.05$).

6.3.1.2 Sex differences in baseline characteristics within each ethnic group

Within the WE and BA but not SA group, men had higher forearm circumference (FAC) relative to women (WE: $p=0.04$; BA: $p=0.000$; SAs: $p=0.21$, see Table 6.2). BA men also had higher waist circumference relative to BA women ($p=0.01$, see Table 6.2). Men had higher 100% MCV (WE: $p=0.000$; BA: $p=0.000$; SAs: $p=0.06$) and higher 60% MCV (WE: $p=0.000$; BA: $p=0.000$; SAs: $p=0.02$) relative to respective women. BA men had higher SBP and mean ABP relative to BA women ($p=0.000$, 0.01 respectively. SA women had higher resting HR ($p =0.03$), however, during experimental session, resting cardiovascular variables were not different between men and women in each ethnic group (Table 6.2).

6.3.1.3 Baseline characteristics between in ethnicities for sex specific groups

6.3.1.3.1 Comparisons between men

There were no significant differences in baseline and anthropometric measurements between WE and BA men, or WE and SA men except for a higher FAC in BA men ($p=0.000$) relative to WE men and lower HR in SA men relative to WE men ($p=0.01$). However, during resting phase of experimental session, there were no differences between WE and BA men or between WE and SA men ($p>0.05$ for all cardiovascular variables, see Table 6.3).

6.3.1.3.2 Comparisons between women

BA women had smaller FAC relative to WEs women (p=0.02) while SA women had lower 100% and 60% MCV relative to WE women (p=0.03 and 0.01 respectively; Table 6.3). In addition, DBP and MABP tended to be higher in BA women than WEs (p=0.07, p=0.09 respectively). There were no significant differences between WE and SA women (Table 3.2).

6.3.2 Exercise hyperaemia

6.3.2.1 Exercise hyperaemia in whole ethnic groups

Forearm blood flow increased from +5.76±0.63 at baseline to 52.25±6.42 ml/100ml tissue/min at peak in WEs, from +5.43±0.41 to 38.84±3.50 ml/100ml tissue/min in BAs and from +6.57±0.93 to 36.31±3.52 ml/100ml tissue/min in SA ($p<0.000$ in each case). FVC values also increased in each ethnicity (Figure 3.1), from +0.07±0.01 at baseline to +0.50±0.05 CU at peak in WEs, from +0.07± 0.01 to + 0.37±0.04 CU in BAs and from +0.08±0.00 to +0.40±0.03 CU in SAs ($p<0.001$ in each case).

The highest increase in blood flow occurred immediately after cessation of exercise and the increase in blood flow persisted throughout the 7 minutes of recovery. Blood flow declined slowly towards baseline at the end of the 7 minute recovery period.

There was no significant 3-way interaction between time, ethnicity and gender for FBF, FVC, MABP, or HR ($p>0.05$ in each case see Table 6.5), Further there was no significant 2-way interaction between ethnicity and gender for FBF, FVC, MABP, or HR ($p>0.05$ in each case, see Table 6). There was significant main effect of ethnicity FBF ($F(2,40)=6.66$, $p=0.003$, partial $\eta^2 =0.25$, as well as for FVC ($F(2,40)=3.68$, $p=0.03$, partial $\eta^2=0.25$). There was significant main effect of sex for FBF ($F(1,40)=$

16.11, p<0.001 partial η^2 =0 .29) and FVC (F(1,40)=12.66, p=0.001, partial η^2=0.24). Bonferroni post hoc test done showed, that during the post exercise period, BAs showed attenuated FBF and FVC relative to WEs (p=0.003 and 0.04, respectively. Although the difference between WEs and SAs for FBF was significant (p=0.031) but FVC did not reach statistical significance (p>0.05, Figure 6.2).

There was no statistically significant difference in the MABP and HR during anticipation or peak of exercise or during and after exercise between WEs and BAs or between WEs and SAs (p>0.05). Although change in HR during anticipation of exercise tended to be higher in BAs (p=0.08, Figure 6.2).

6.3.2.2 Sex-related differences

Relative to men, WE and BA but not SA women showed blunted FBF (F(1,16)=8.27, p =0.01, partial η^2=0.34 and F(1,16)=22.08, p<0.001, partial η^2=0.58 respectively) as well as blunted FVC (F(1,16)=8.11, p=0.01, partial η^2=0.34 and F(1,16)=13.40, p=0.002, partial η^2=0.46 respectively). There were no differences in other variables (Figure 6.5, Table 6.5).

6.3.2.3 Comparison between men

Considering men, the forearm blood flow increased following rhythmic handgrip exercise from +5.66±0.78 to 59.42±8.88 and from +6.08±0.60 to 44.54±5.17 ml/100ml tissue/minute in WE males and BA males and from +6.59±1.49 to 40.40±5.24 ml/100ml tissue/minute in SA at peak of exercise hyperaemia (p=0.000 respectively). There were no changes in ABP (values not reported). Forearm vascular conductance increased from +0.09±0.01 to +0.55 ±0.07 in WE males and from +0.08±0.01 to + 0.50

±0.07 CU in BA males at the peak of exercise hyperaemia (p=0.000 respectively). Therefore, FVC values also increased in each ethnicity (Figure 3.1), from +0.09±0.01 at baseline to +0.55 ±0.07 CU at peak in WEs, from +0.08 ± 0.01 to +0.50 ± 0.74 CU in BAs and from +0.09 ± 0.02 to +0.42 ± 0.05 CU in SAs (p <0.000 in each case).

Relative to WEs, BA men showed blunted FBF and FVC ($F(1,18)=8.52$, $p=0.01$, partial $\eta^2=0.32$ and ($F(1,18)=5.12$, $p=0.04$, partial $\eta^2=0.22$ respectively). There were no differences in MABP and HR tended to be higher in BA men (p=0.07). Relative to WE men, SA men showed smaller FBF ($F(1,14)=5.54$, $p=0.03$, partial $\eta^2=0.28$), however the difference in FVC did not reach statistical significance (Figure 6.4).

6.3.2.4 Comparison between women

Although BA women tended to show blunted post exercise FBF ($F(1,14)=3.64$, $p=0.08$, partial $\eta^2=0.21$), there were no significant differences in all the variables between the women groups.

6.3.3 Effect of COX inhibition with Aspirin

COX inhibition did not attenuate basal forearm blood flow or vascular conductance in all ethnic groups (Table 6.4).

There was no significant interaction between ethnicity and sex on effect aspirin during exercise hyperaemia. There was no significant 2-way interaction between treatment with aspirin and sex or between treatment and ethnicity or between sex and ethnicity ($p>0.05$ in each case). There was significant main effect of sex ($F(1,37)= 6.78$, $p= 0.01$, partial $\eta^2= 0.16$) and ethnicity ($F(2, 37)= 4.37$, $p= 0.02$, partial $\eta^2= 0.19$).

COX inhibition attenuated exercise hyperaemia in whole groups of WEs at the peak, 60s, and 90s ($F(1,17)=9.05$, $p=0.008$, partial $\eta^2=0.35$) but not in whole groups of BAs

or SAs (p>0.05, see Figure 6.6). COX inhibition attenuated exercise hyperaemia in WE men (F(1, 9)=5.68, p=0.04, partial η^2=0.39). In WE women, exercise hyperaemia tended to be attenuated by COX inhibition (F(1,7)=4.11, p=0.08, partial η^2= 0.37, see Figure 6.6).

There was no significant 3-way interaction between aspirin, ethnicity and gender at the peak of exercise hyperaemia (p>0.05). In addition, there was no interaction between ethnicity and gender (p>0.05). The peak of exercise hyperaemia was attenuated in WE men and WE women (F(1,9)=14.29, p=0.004, partial η^2=0.61; (F(1,7)=7.36, p=0.03, partial η^2=0.51, Figure 6.7). In BA men, COX inhibition attenuated exercise hyperaemia at peak, 30s, 90s, 3 mins post exercise (F(1,9)=5.27, p=0.047, partial η^2=0.37: post hoc, p=0.015, 0.017, 0.049, 0.039 respectively), on the other hand, COX inhibition did not attenuate exercise hyperaemia in BA women (p>0.05). SA men and women showed no attenuation of peak exercise hyperaemia following COX inhibition (p>0.05).

6.4 Discussion

6.4.1 Ethnic differences in exercise hyperaemia

The main findings of the present study were that COX inhibition with aspirin attenuated the forearm vasodilatation and the increase in FBF evoked by rhythmic handgrip contractions at 60% MVC in WE men and women and in BA men, but there was no effect on BA women. Any effects of COX inhibition in SA men and women did not reach significance.

Considering the magnitude of exercise hyperaemia in the 3 ethnic groups, the present finding of blunted exercise hyperaemia following high intensity exercise at 60% MVC in BA men relative to WE men is consistent with previous studies that showed blunted exercise hyperaemia in BAs during maximal aerobic exercise (Heffernan et al., 2007) and moderately high intensity rhythmic handgrip exercise (Ozkor *et al.*, 2014; Barbosa *et al.*, 2018). Hyperaemia evoked by low intensity exercise was not blunted in BAs relative to WEs (Ozkor *et al.*, 2014; Kappus *et al.*, 2017a). Increasing intensity of exercise causes greater compression of blood vessels within muscles and allows greater local hypoxia, increased release of metabolites from muscles and greater deoxygenation of red blood cells than at lower intensity of exercise, indeed, there is evidence that skeletal muscle fibre hypoxia increases with intensity of exercise (Richardson et al., 2001). This suggests that at lower levels of muscle contraction and tissue hypoxia, BA men are capable of dilating muscle blood vessels to the same extent as WEs, but at higher levels of muscle contraction and hypoxia they do not.

Similarly, exercise hyperaemia tended to be blunted in the whole group of SAs relative to WEs and the increase in FBF was significantly attenuated in SA men compared with

WE men, although the increase in FVC was not. This suggests that SA men may, like BA men show smaller exercise hyperaemia than WE men, but that due to the relatively small number of subjects and the variability of the data, we could not demonstrate an effect on vascular conductance. Future studies with larger sample size will be needed to provide more information.

Considering women, the present finding that young BA women tended to show smaller exercise hyperaemia than WE women and a smaller increase in FVC at peak (Figure 6.4) suggests their ability to dilate in response to the local dilator influences associated with muscle contraction may already be blunted in young adulthood, consistent with their earlier onset of CVD than WE women (Geronimus et al., 2007, Hertz et al., 2005). This proposal would need to be tested with larger groups of BA and WE women.

In the present study, the finding of blunted post-exercise hyperaemia in BA and WE women, but not in SA women, relative to their male counterparts is contrary to findings of greater increases in muscle vascular conductance in women during graded knee extensor (Parker *et al.*, 2007) or dynamic handgrip exercise (Gonzales *et al.*, 2007) or (Kellawan *et al.*, 2015) and contrary to finding of similar exercise hyperaemia between men and women during low intensity exercise (Limberg *et al.*, 2010). On the other hand, our findings are consistent with the smaller post-exercise hyperaemia reported in women following dynamic handgrip exercise at high intensity (Casey *et al.*, 2014). Lower post-exercise hyperaemia in women may be attributable to the smaller compressive force exerted on blood vessels during exercise causing lesser vascular occlusion (Russ & Kent-Braun, 2003). Certainly, post-exercise hyperaemia was found

to be higher in men when absolute MVC was greater in men than women (Hunter *et al.*, 2006b). Moreover, in the present study, WE and BA men showed higher absolute value of 100% MVC and therefore, of 60% MVC than WE and BA women concomitant with their greater exercise hyperaemia. This could be tested in future studies by comparing post-exercise evoked in men and women of different ethnicities at the same absolute MVC.

6.4.2 Role of prostaglandins

The fact that COX inhibition had no effect on baseline FBF or FVC in men or women within any of the 3 ethnic groups indicates PGs have no significant effect on vascular tone in skeletal muscle. This finding is consistent with much evidence in the literature when COX inhibitor is given systemically as in the present study; this issue is described in Chapter 5. The evidence that COX inhibition then attenuated exercise hyperaemia, at least in some sub-groups of these ethnicities provides evidence of increased formation during exercise. This is consistent with previous reports that COX inhibition caused attenuation of hyperaemia from the beginning of post exercise period, and throughout the recovery period (Kilbom & Wennmalm, 1976).

The present finding that COX inhibition attenuated exercise hyperaemia throughout the post-exercise period in whole WE ethnic group of men and women and in WE men, is consistent with previous studies which have provided evidence for role PGs in exercise hyperaemia in mixed gender and in male groups of individuals whose ethnicity was not mentioned and who may have been WEs (see Introduction to this Chapter). The present finding that COX inhibition not only attenuated the peak vasodilatation of exercise hyperaemia in WE men, but also in WE women, is novel as far as we are aware as there

have been no previous studies focussed on women. In both WE men and women, COX inhibition attenuated peak FVC by ~30%. The role of PGs in the exercise hyperaemia that occurs during muscle contractions was modest as COX inhibition attenuated it by about 10-20% (Wilson & Kapoor, 1993; Schrage *et al.*, 2004), while Duffy *et al.* (1999) demonstrated 18-20% attenuation of post-exercise hyperaemia following medium intensity contractions.

However, in previous studies on men, exercise hyperaemia following hand grip exercise at 60% MVC as in the present study was reduced by 30-40% by COX inhibition with Aspirin and by breathing 40% O_2, while the combination of the two interventions did not have any additive effect (Win & Marshall, 2005). Further studies showed that the release of PGI_2 and PGE_2 into the venous efflux following 60% MVC was similarly attenuated by 40% O_2 and COX inhibition on exercise hyperaemia following rhythmic handgrip exercise (Junejo, 2017). The present findings are consistent with these and indicate that PGs released during handgrip exercise at 60% MVC in both WE men and women is dependent on the fall of tissue O_2 levels that occurs during exercise and that they make a substantial contribution to the post-exercise hyperaemia.

To the best of our knowledge there are no previous studies on role of COX inhibition on exercise hyperaemia in BAs or SAs. Thus, the present finding that COX inhibition attenuated peak FVC in BA men, but not in BA women demonstrates a role PG in exercise hyperaemia in BA men, but not in BA women. In a previous study on WE and BA men (mean age of 39, 40 year respectively), the contribution of NO to baseline tone in BAs was blunted relative to WE men and NOS inhibition had attenuated effect on exercise hyperaemia evoked by graded forearm contractions at 15-45% MVC (Ozkor *et*

al., 2014). Thus, the authors concluded that the availability of NO was impaired in BA men. Since inhibition of K^+ channels had greater attenuating effect on exercise hyperaemia in BA than WE, they proposed that EDHF replaces to some extent at least, the contribution of NO in BA men. If NO availability is also blunted in young BA men such as those who took part in the present study, then the present results indicate that the ability of PGs to be released and contribute to exercise hyperaemia is still preserved in young BA men.

By contrast, the present finding that COX inhibition attenuated peak FVC by ~30% in WE women, with no effect on peak FVC in BA women and the finding that peak FVC after COX inhibition in WE women was comparable to peak FVC in BA women before and after COX inhibition, suggests not only that PGs do not contribute to exercise hyperaemia in BA women but that the reason exercise hyperaemia is blunted in BA women relative to WE women is explained by an impaired contribution of PGs. Whether, it is the release or action of PGs that is impaired during exercise in BA women cannot be deduced from the present results but could be tested in future studies by assaying for PGI_2 and PGE_2. If NO availability is impaired in young BA women as in middle-aged BAs, it may be that EDHF plays a greater role in exercise hyperaemia in BA women than in men who are more dependent on PGs: this would be consistent with conclusion drawn from NO-knockout male and female mice (Sun *et al.*, 1999; Huang *et al.*, 2001). Unfortunately, the effects of COX inhibition on exercise hyperaemia in SA men and women in the present study do not provide conclusive evidence for the role of PGs in exercise hyperaemia in SAs. This may be explained by the relatively small group

sizes and/or may suggest that other mediators play a bigger role in exercise hyperaemia in SAs.

6.4.3 Conclusion

The present findings are limited by relatively small group sizes, but allow the novel conclusions that dilator PGs contribute to exercise hyperaemia in WE men *and* women and in BA men, but *not* in BA women. The last finding is of particular interest and is consistent with other evidence presented in Chapters 3-5, that vasodilator mechanisms contributing to the responses evoked by mental stress and during reactive hyperaemia are particularly impaired in BA women and may reflect impaired PG-induced dilatation. Blunted exercise hyperaemia in BA women and SA women with impaired PG release may be further evidence of their increased risk of CVD.

6.4.4 Limitations:

Assessment of release of prostaglandins in venous efflux from exercising muscle was not done in the present study although it has been established in previous studies that PGs are released into the venous efflux and into interstitial fluid during rhythmic contractions at 60% MVC (Kilbom & Wennmalm, 1976; Karamouzis *et al.*, 2001; Junejo, 2017). It would be particularly interesting to establish whether there are ethnicity and/or sex related differences in PG efflux given the present results. However, previous studies using similar exercise protocol have demonstrated release of prostaglandin (Junejo, 2017). Since the effects of COX inhibition on exercise hyperaemia were tested in the same experimental session, the order of the without and with COX inhibition arms of the protocol could not be randomised. However, a rest

period of 45 mins was allowed between periods of rhythmic contraction for 2 min, all subjects managed the task requested without fatiguing, and the baseline FBF and FVC were fully recovered before the 2nd rhythmic contractions began.

The number of South Asians who participated in the study was low despite the effort put into recruitment, thus splitting on basis of sex further reduced numbers in each gender subgroup, although effort was made to increase recruitment. This may account for the lack significant differences in comparisons involving SA men or women and effects of COX inhibition. Thus a larger cohort of South Asians will be needed in order to determine conclusively whether or not COX products play a role in exercise hyperaemia in gender groups of SAs.

Table 6.1 Baseline characteristics and cardiovascular variables in whole groups of White Europeans (WEs), Black Africans (BAs) and South Asian (SAs).

	WEs (n=18)	BAs (n=18)	SAs (n=11)	WEs vs BAs P value	WEs vs SAs P value
Age (years)	21.94 ± 0.62	22.00 ± 0.60	20.82±0.58	0.95	0.23
Men:Women	10:8	10:8	6:5		
Body mass index, BMI (kg/m^2)	22.44 ± 0.67	21.69 ± 0.67	21.56±2.76	0.44	0.70
Waist circumference (cm)	76.56 ± 1.34	73.89 ± 1.67	78.35±3.25	0.22	0.55
Forearm circumference, FAC (cm)	24.17 ± 0.24	24.61 ± 0.71	25.18±0.69	0.55	0.11
100% Maximum Voluntary Contraction (MCV)	23.11 ± 1.58	24.78 ± 2.36	19.70±3.21	0.56	0.29
60% MCV	14.00 ± 0.94	14.83 ± 1.40	10.75±2.05	0.62	0.11
Systolic blood pressure, SBP^1 (mmHg)	103.39 ± 1.99	105.22 ± 3.12	108.64±4.60	0.62	0.24
Diastolic blood pressure, DBP^1 (mmHg)	64.28 ± 1.64	64.39 ± 1.76	65.55±2.13	0.96	0.64
HR rate, HR^1 (bpm)	71.41 ± 2.35	69.00 ± 2.37	64.18±4.52	0.48	0.13
Mean arterial pressure, $MABP^1$ (mmHg)	77.31 ± 1.54	78.00 ± 2.07	79.91±2.69	0.79	0.38
Cardiovascular variables at baseline of experimental protocol					
$MABP^2$ (mmHg)	82.77±3.92	78.81 ±0.44	79±2.64	0.44	0.51
HR^2 (bpm)	68.10±2.25	69.26±1.99	65±3.90	0.70	0.54
Forearm blood flow, FBF (ml/100ml/min)	5.76 ± 0.63	5.43 ± 0.41	6.57±0.93	0.66	0.46
Forearm vascular conductance, FVC (ml/100ml/min/mmHg)	0.07 ± 0.01	0.07 ± 0.01	0.08±0.01	0.87	0.40

Values are mean ± SEM. Except for sex (n). Values of ABP^1 in upper part were recorded by sphygmomanometer, those in lower part ($ABP^{2)}$ by Finapres. *$p<0.05$: WEs vs BAs or WEs vs SAs, with unpaired Student's T test

Table 6.2 Baseline characteristics and cardiovascular variables in White European (WE), Black African (BA) and South Asian (SA) men and women

	White Europeans			Black Africans			South Asians		
	Men(n=10)	**Women(n=8)**	**P value**	**Men(n=10)**	**Women (n=8)**	**P value**	**Men (6)**	**Women (5)**	**P value**
Age (years)	21.40 ± 0.73	22.63 ± 1.07	0.34	20.70 ± 0.67	23.63 ± 0.75*	0.01	20.83±0.95	20.80±0.73	0.98
BMI (kg/m^2)	22.45 ± 0.77	22.42 ± 1.23	0.98	22.54 ± 0.58	20.63 ± 1.29	0.17	18.62±3.89	25.09±3.68	0.26
WC (cm)	78.90 ± 1.82	73.63 ± 1.49	0.05	77.50 ± 1.89	69.38 ± 2.07*	0.01	80.60±5.84	76.10±3.30	0.52
FAC (cm)	24.60 ± 0.31*	23.63 ± 0.26	0.04	26.85 ± 0.42	21.81 ± 0.65*	0.00	26.00±1.00	24.20±0.80	0.21
100% MVC	28.30 ± 1.11*	16.63 ± 0.91	0.00	32.00 ± 2.14	15.75 ± 1.47*	0.00	24.50±4.31	12.50±1.44	0.06
60% MVC	17.10 ± 0.66*	10.03 ± 0.54	0.00	19.10 ± 1.26	9.50 ± 0.89*	0.00	14.70±2.59*	6.00±1.64	0.02
SBP^1 (mmHg)	106.30 ± 1.71	99.75 ± 3.68	0.10	112.90 ± 3.68	95.63 ± 2.77*	0.00	113.67±5.58	102.60±7.27	0.25
DBP^1 (mmHg)	63.70 ± 1.48	65.00 ± 3.33	0.71	67.30 ± 2.42	60.75 ± 2.02	0.06	64.33±2.49	67.00±3.83	0.56
HR^1 (bpm)	70.40 ± 2.10	72.67 ± 4.76	0.65	67.50 ± 3.44	70.88 ± 3.29	0.50	55.83±4.69*	74.20±5.79	0.03
$MABP^1$ (mmHg)	77.90 ± 1.11	76.58 ± 3.30	0.68	82.50 ± 2.64	72.38 ± 2.05*	0.01	80.78±3.41	78.87±4.69	0.74
Cardiovascular variables at baseline during experimental protocol									
$MABP^2$ (mmHg)	85.88±6.75	78.87±2.63	0.39	82.99±5.02	73.58±2.55	0.14	82.77±3.92	78.01±3.17	0.73
HR^2 (bpm)	67.57±2.10	68.76±4.53	0.80	67.19±2.98	71.85±2.40	0.26	68.10±2.25	71.38±6.41	0.18
FBF (ml/100ml/min)	5.66 ± 0.78	5.88 ± 1.09	0.87	6.08 ± 0.57	4.62 ± 0.47	0.08	7.14±1.49	5.88±1.10	0.53
FVC (CU)	0.07 ± 0.01	0.07 ± 0.01	0.97	0.08 ± 0.01	0.06 ± 0.01	0.24	0.09±0.02	0.07±0.01	0.56

Values are mean ± SEM. Values of ABP^1 in upper part were recorded by sphygmomanometer, those in lower part ($ABP^{2)}$ by Finapres. * $p < 0.05$:men vs women, with unpaired Student's T test

Table 6.3 Baseline characteristics in White European (WE), Black African (BA) and South Asian (SA) men and women groups.

	Men			Women		
	WEs (n=10)	**BAs (n=10)**	**SAs (n=6)**	**WEs (n=8)**	**BAs (n=8)**	**SAs (5)**
Age (years)	21.40 ± 0.73	20.70 ± 0.67	20.83±0.95	22.63 ± 1.07	23.63 ± 0.75	20.80±0.73
BMI (kg/m^2)	22.45 ± 0.77	22.54 ± 0.58	18.62±3.89	22.42 ± 1.23	20.63 ± 1.29	25.09±3.68
WC (cm)	78.90 ± 1.82	77.50 ± 1.89	80.60±5.84	73.63 ± 1.49	69.38 ± 2.07	76.10±3.30
FAC (cm)	24.60 ± 0.31	26.85 ± 0.42*	26.00±1.00	23.63 ± 0.26	21.81 ± 0.65*	24.20±0.80
100% MVC	28.30 ± 1.11	32.00 ± 2.14	24.50±4.31	16.63 ± 0.91	15.75 ± 1.47	12.50±1.44§
60% MVC	17.10 ± 0.66	19.10 ± 1.26	14.70±2.59	10.03 ± 0.54	9.50 ± 0.89	6.00±1.64§
SBP[1] (mmHg)	106.30 ± 1.71	112.90 ± 3.68	113.67±5.58	99.75 ± 3.68	95.63 ± 2.77	102.60±7.27
DBP[1] (mmHg)	63.70 ± 1.48	67.30 ± 2.42	64.33±2.49	65.00 ± 3.33	60.75 ± 2.02	67.00±3.83
HR[1] (bpm)	70.40 ± 2.10	67.50 ± 3.44	55.83±4.69§	72.67 ± 4.76	70.88 ± 3.29	74.20±5.79
MABP[1] (mmHg)	77.90 ± 1.11	82.50 ± 2.64	80.78±3.41	76.58 ± 3.30	72.38 ± 2.05	78.87±4.69
Cardiovascular variables at baseline during experimental protocol						
MABP [2] (mmHg)	85.88±6.75	82.99±5.02	82.77±3.92	78.87±2.63	73.58±2.55	78.01±3.17
HR [2] (bpm)	67.57±2.10	67.19±2.98	68.10±2.25	68.76±4.53	71.85±2.40	71.38±6.41
FBF (ml/100ml/min)	5.66 ± 0.78	6.08 ± 0.57	7.14±1.49	5.88 ± 1.09	4.62 ± 0.47	5.88±1.10
FVC (CU)	0.07 ± 0.01	0.08 ± 0.01	0.09±0.02	0.07 ± 0.01	0.06 ± 0.01	0.07±0.01

Values of ABP[1] in upper part were recorded by sphygmomanometer, those in lower part (ABP[2)] by Finapres. Values are mean ± SEM. * $p < 0.05$: WEs vs BAs and § $p < 0.05$:WEs vs SAs with unpaired Student's T test

Table 6.4 Effect of COX inhibition on baseline forearm blood flow and vascular conductance in groups of WEs, BAs and SAs.

	FBF (ml/100ml/min)			FVC (CU)		
	Control	**Aspirin**	**P value**	**Control**	**Aspirin**	**P value**
All WEs	5.53±0.66	6.03±0.73	0.44	0.08±0.01	0.09±0.01	0.22
WE men (n=10)	5.66±0.78	6.14±0.95	0.41	0.07±0.01	0.05±0.01	0.44
WE women (n=6)	5.31±1.31	5.84±2.40	0.39	0.07±0.02	0.08±0.02	0.58
All BAs	5.62±0.43	6.25±0.73	0.29	0.07±0.01	0.07±0.01	0.96
BA men (n=11)	6.34±0.58	7.96±0.90	0.36	0.08±0.01	0.09±0.01	0.37
BA women (n=8)	4.62±0.48	3.91±0.56	0.36	0.06±0.01	0.05±0.01	0.18
All SAs	6.62±1.03	7.72±1.18	0.13	0.08±0.01	0.09±0.01	0.22
SA men (n=6)	7.14±1.49	8.37±1.55	0.30	0.09±0.02	0.10±0.02	0.57
SA women (n=4)	5.82±1.41	6.76±1.98	0.25	0.07±0.02	0.07±0.03	0.25

All values are mean ±SEM. Control vs aspirin analysed with paired Student's T test.

Table 6.5 Effect of ethnicity and sex on exercise hyperaemia in WEs, BAs and SAs.

	F ratio	Degrees of freedom	P value	Partial Eta squared
FBF(ml/100ml/min)				
Time*Ethnicity *Sex	0.136	4,80	0.968	0.007
Ethnicity*Sex	1.064	2,40	0.355	0.051
Sex	16.107	1,40	<0.0001 (Males>Females)	0.250
Ethnicity	6.661	2,40	0.003 WEs>BAs(0.003) WEs>SAs(0.031)	
FVC(CU)				
Time*Ethnicity *Sex	0.392	5.99	0.850	0.019
Ethnicity*Sex	0.950	2.40	0.393	0.046
Sex	12.658	1,40	0.001 (Males>females)	0.240
Ethnicity	3.681		0.034 WEs>BAs(0.035)	0.155
MABP(mmHg)				
Time*Ethnicity *Sex	1.027	11,195	0.424	0.054
Ethnicity*Sex	0.860	2,36	0.432	0.046
Sex	0.002	1,36	0.965	0.000
Ethnicity	1.546	2,36	0.227	0.079
HR(bpm)				
Time*Ethnicity *Sex	0.788	11,22	0.656	0.039
Ethnicity*Sex	0.876	2,40	0.424	0.043
Sex	0.679	1,39	0.415	0.017
Ethnicity	1.546	2,36	0.227	0.079

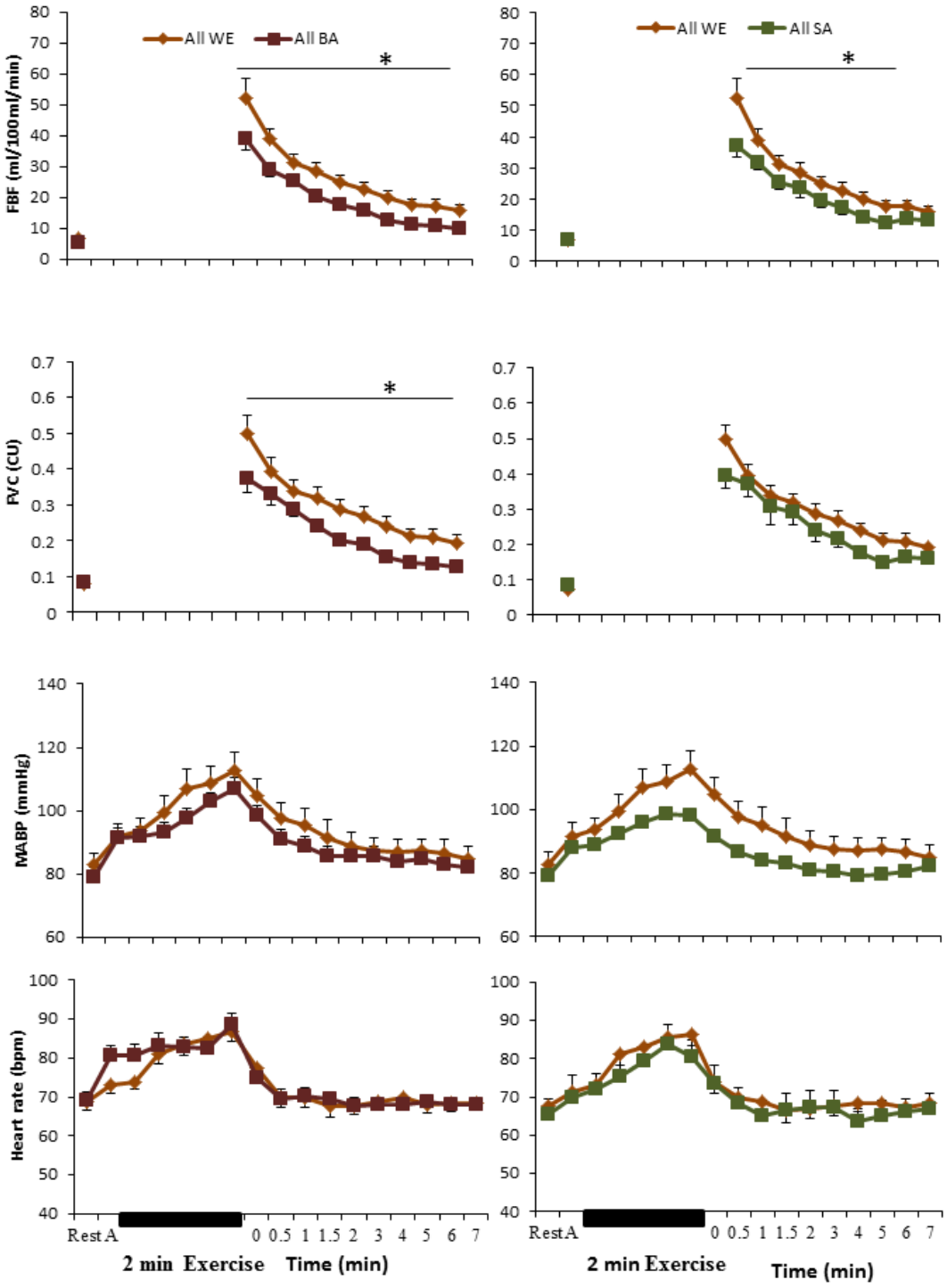


Figure 6.2: Forearm blood flow (FBF), Forearm vascular conductance (FVC), Mean ABP (MABP) and Heart rate (HR) before, during and after exercise in whole WEs, BAs and SAs. Values are mean ± SEM. * $p<0.05$: WEs vs BAs or WEs vs SAs. 3-way mixed ANOVA with Bonferroni post hoc test.

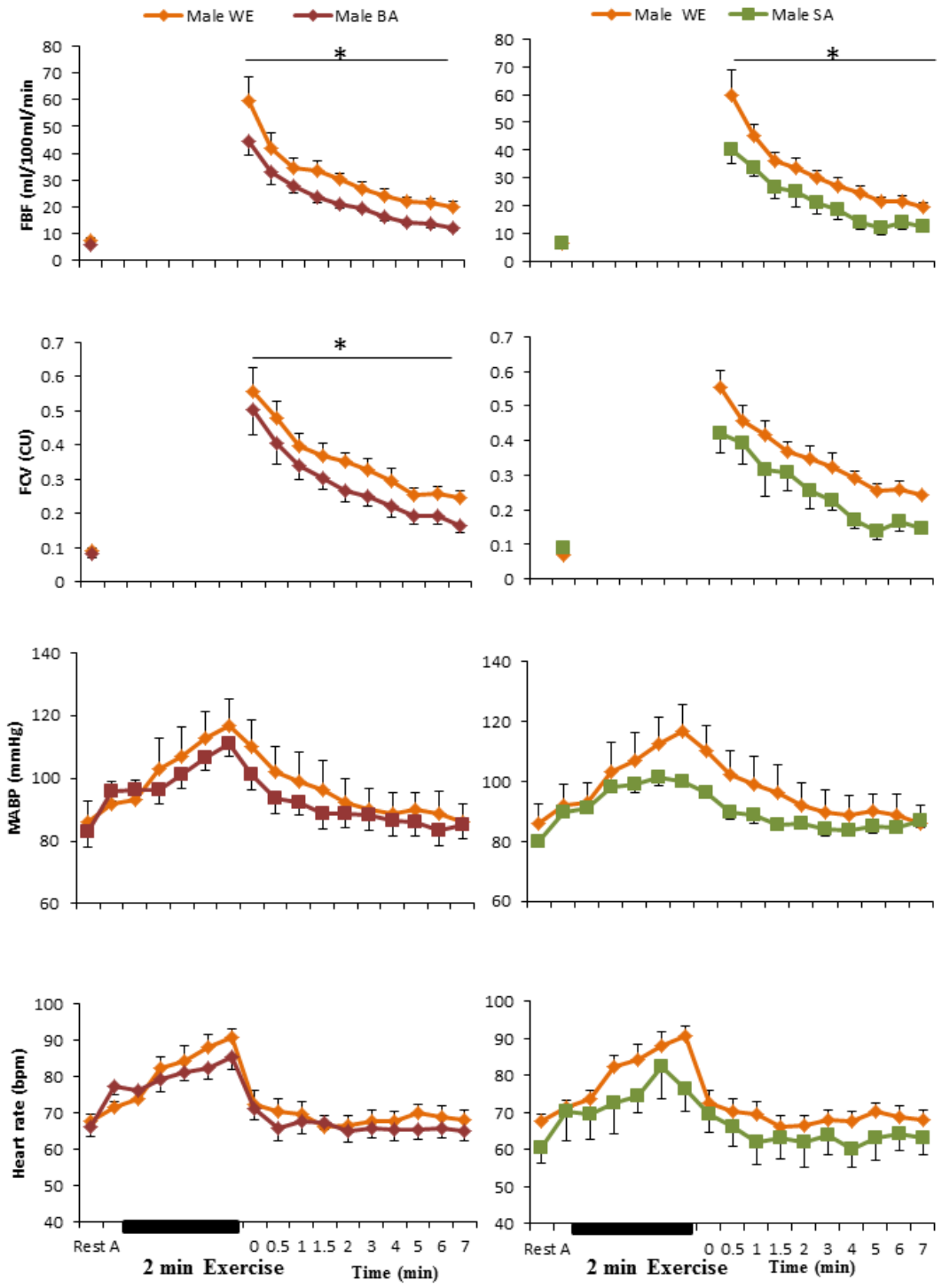


Figure 6.3: Forearm blood flow (FBF), Forearm vascular conductance (FVC), Mean ABP (MABP) and Heart rate (HR) before, during and after exercise in male WEs, BAs and SAs. Values are mean ± SEM. * p<0.05: WEs vs BAs or WEs vs SAs. 3-way mixed ANOVA with Bonferroni post hoc test.

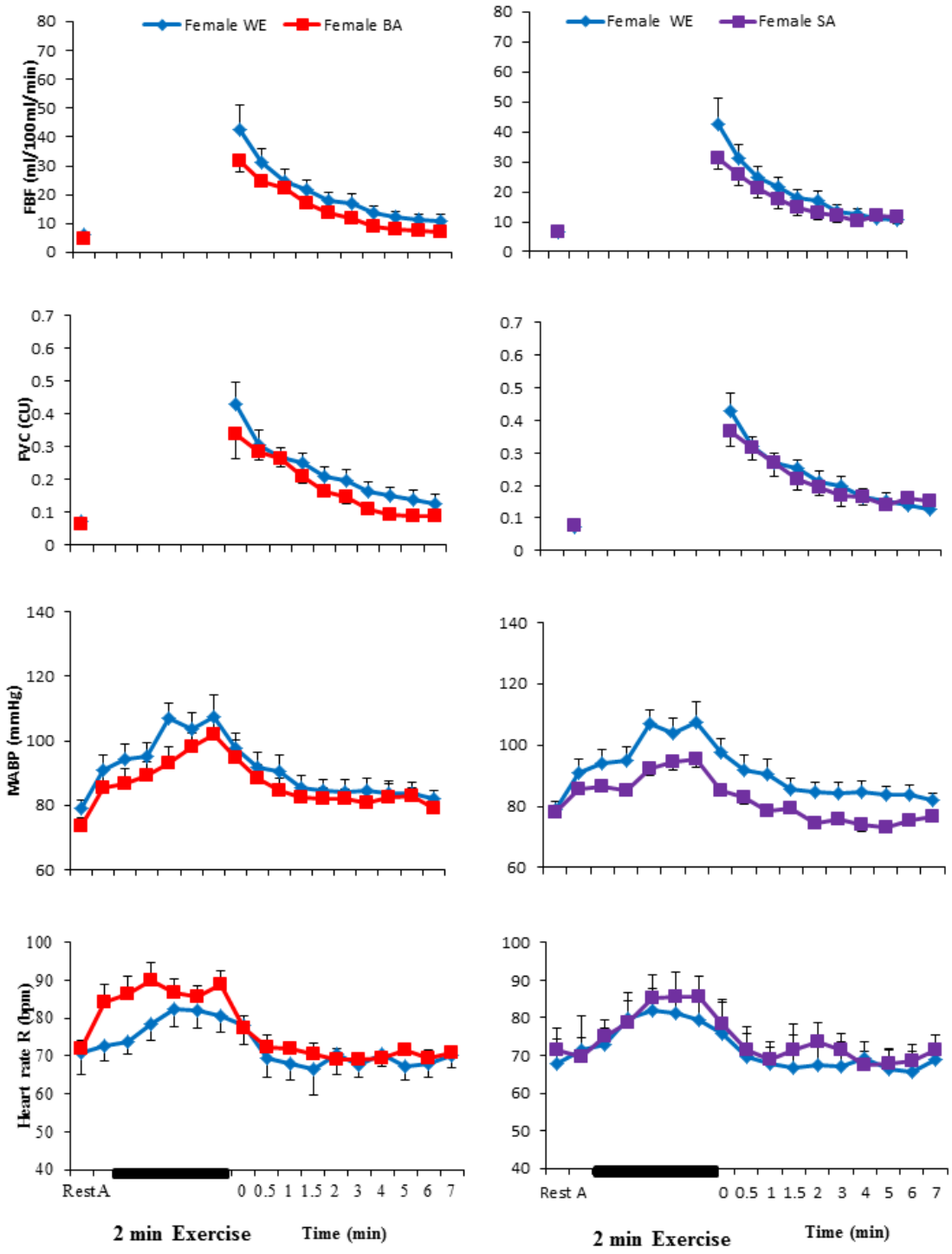


Figure 6.4: Forearm blood flow (FBF), Forearm vascular conductance (FVC), Mean ABP (MABP) and Heart rate (HR) before, during and after exercise in female WEs, BAs and SAs. Values are mean ± SEM. * $p<0.05$: WEs vs BAs or WEs vs SAs

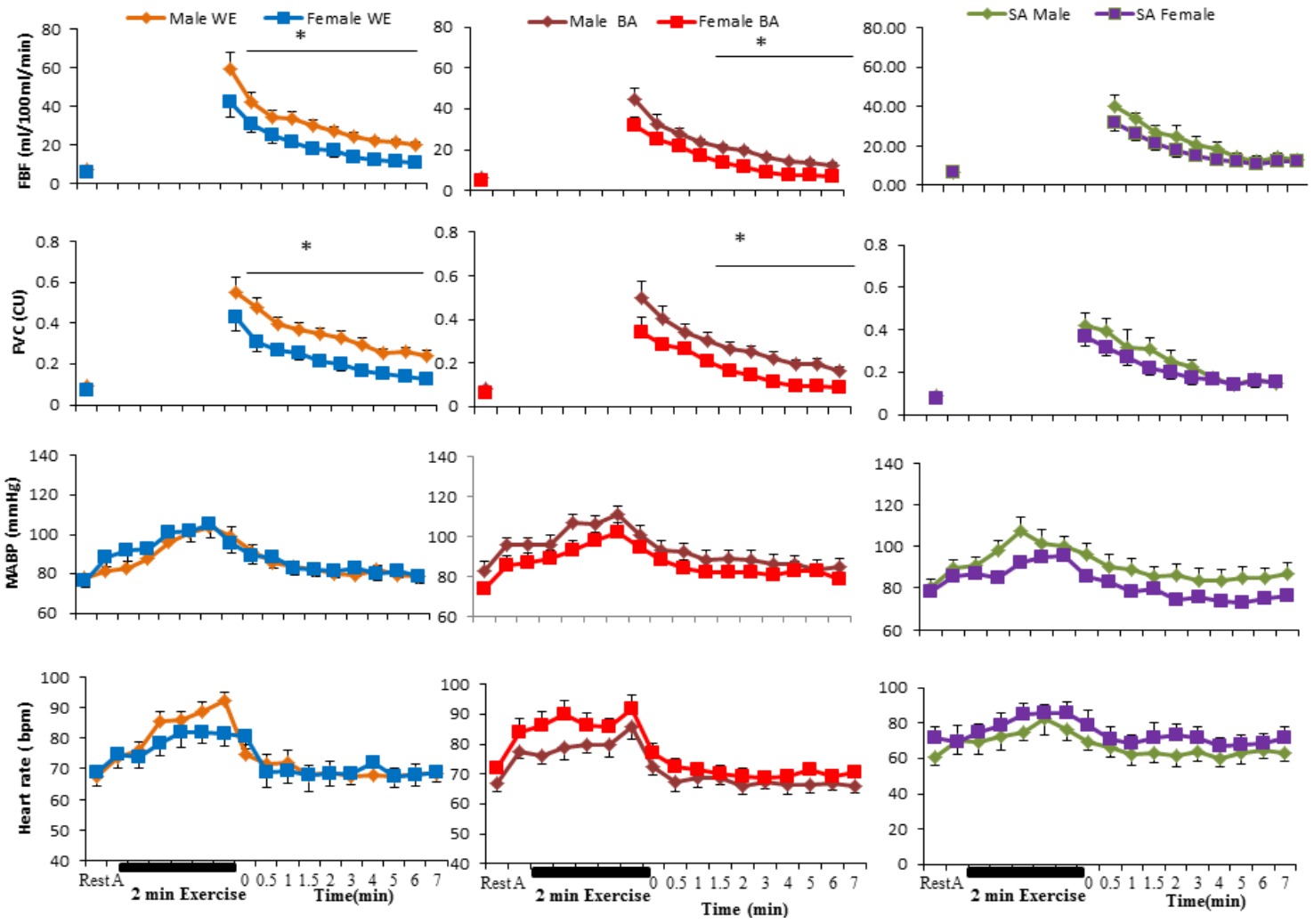


Figure 6.5: Forearm blood flow (FBF), Forearm vascular conductance (FVC), Mean ABP (MABP) and Heart rate (HR) before, during and after exercise in WE, BA and SA males and females. Values are mean ± SEM. * $p<0.05$: males vs females, 3-way mixed ANOVA with Bonferroni post hoc test.

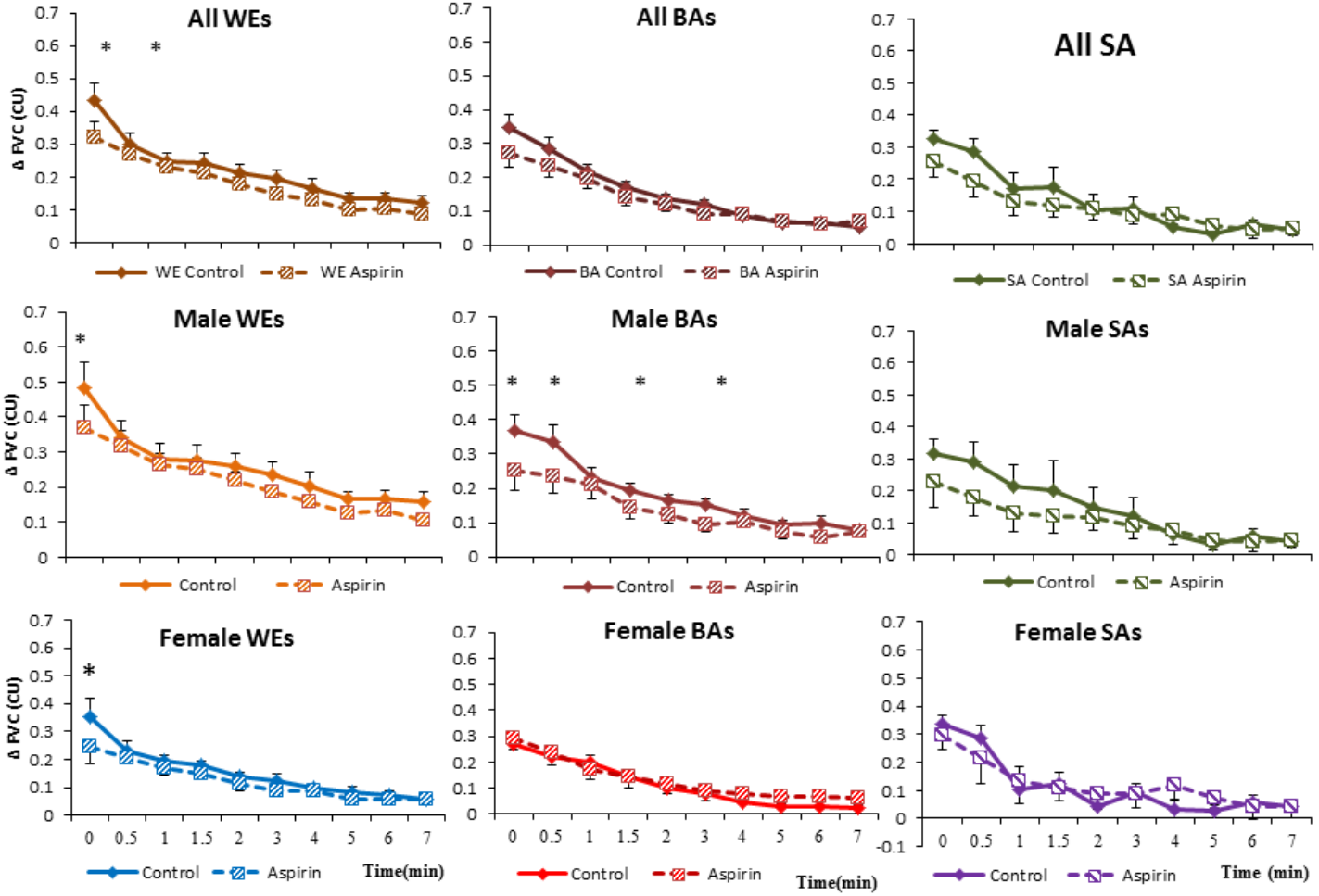


Figure 6.6: Change from baseline values of forearm vascular conductance (FVC) during exercise hyperaemia in WE, BA and SA males and females before and after COX inhibition with Aspirin. Values are mean ± SEM.
* $p<0.05$: control vs aspirin, 3-way mixed ANOVA with Bonferroni post hoc test.

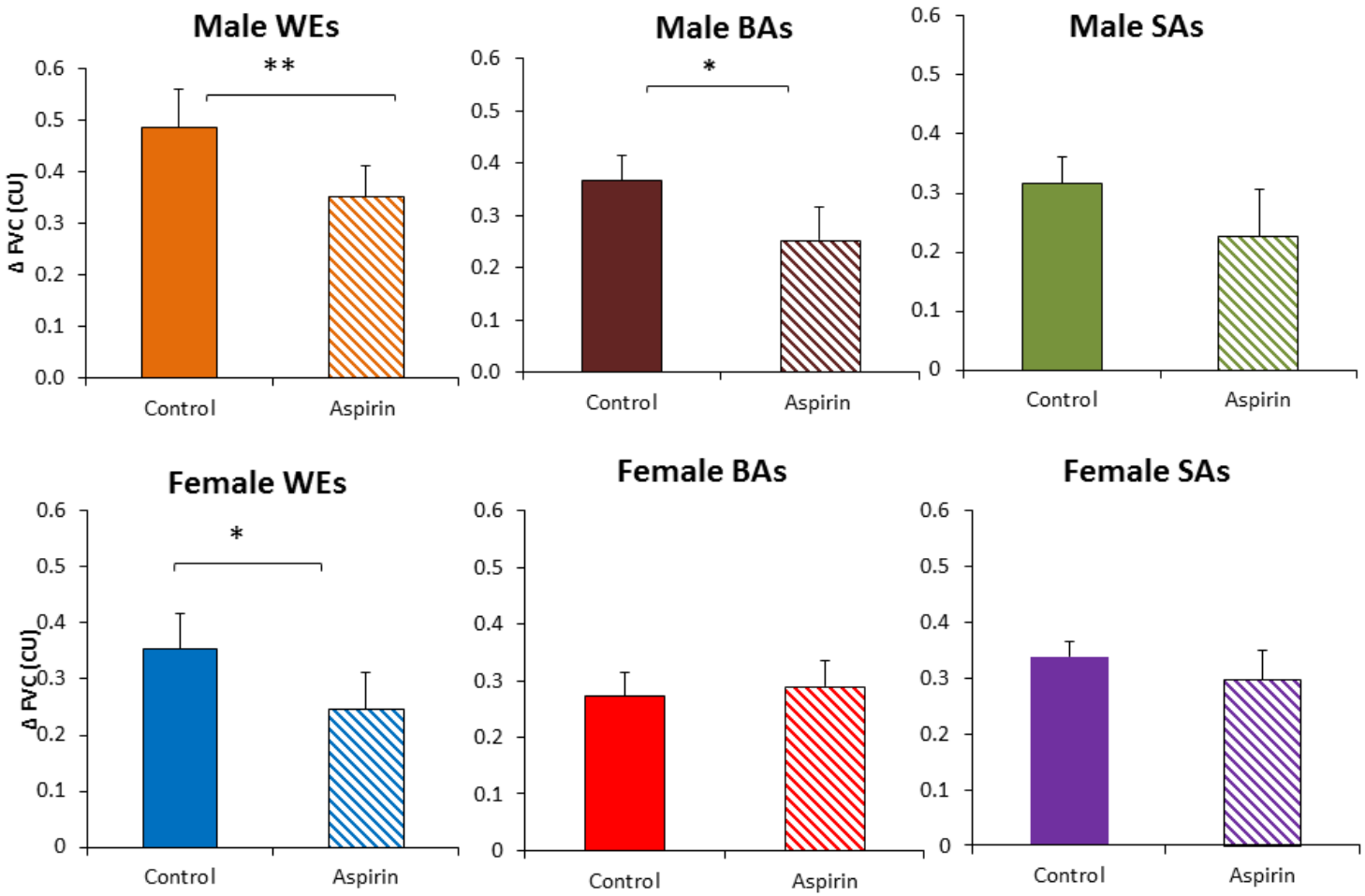


Figure 6.7: Change from baseline values of forearm vascular conductance (FVC) at peak of exercise hyperaemia in groups of WEs, BAs and SAs before and after COX inhibition with Aspirin. Values are mean ± SEM. * $p<0.05$, ** $p<0.005$: control vs aspirin, 3-way mixed ANOVA with Bonferroni post hoc test.

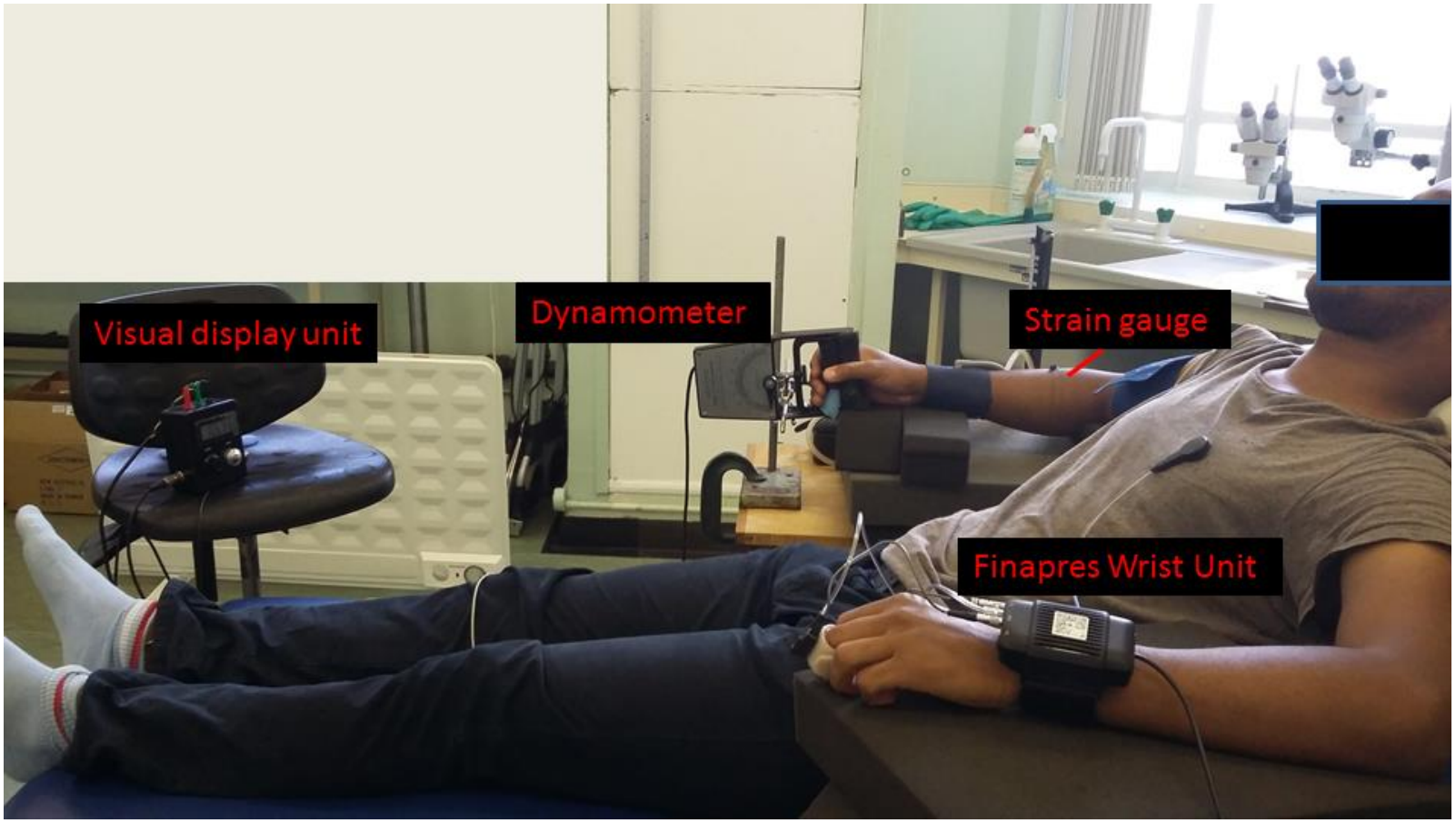


Figure 6.8: Handgrip exercise experimental set up.

CHAPTER 7

Relationship between Blood Pressure Variability and Cardiovascular Responses Evoked By Environmental Stress

7.1 Introduction

7.1.1 Background

Blood pressure (BP) varies over 24 hours (diurnal or circadian rhythm) as well as from beat to beat. The changes in BP occur continuously during the day and night in response to the environment as well as to activities. As discussed in Chapter 1, there is evidence that acute emotional stress alters BP and repetition of stress could subsequently lead to sustained increases in BP. Thus, mental stress altered short term BP variability. In addition, endothelial function differed by ethnicity and sex as reported in Chapter 3, 4 and 5. The fluctuations in BP over the 24-hour cycle reflects the influence of the baroreceptors and elasticity of arteries (Parati *et al.*, 2013a). Thus, a reduction of the ability of the baroreceptor reflexes to buffer changes in BP will enhance short-term BP variability. We therefore explored the association between 24-hour patterns of BP variability and endothelial function in response to environmental stress in some of the subjects who participated in the study of acute mental stress and handgrip exercise. The sections below review the relevant literature.

7.1.2 The day-night blood pressure variability

Evidence that BP falls during sleep recorded hourly using the Hill-Barnard sphygmomanometer was shown as early as the 18th century (Hill, 1898). The circadian rhythm of BP was demonstrated by Millar-Craig *et al.* (1978) using continuous intra-arterial monitoring on a group of 20 hypertensive (aged 34-72 years) and 5 normotensive men (aged 19-43 years). They showed fall in BP during night sleep signifying nocturnal dip and rise before waking indicating a morning surge. Thus high day-time BP and low night-time BP characterise the circadian rhythm. There is variation in BP during the stages of sleep. At the onset of sleep, during the non-rapid

eye movement sleep (NREM), a small fall in BP occurs, with a further fall occurring during the rapid eye movement (REM) sleep (Coccagna *et al.*, 1971). The factor attributed to the nocturnal BP decline in BP is night-time sleep as the onset of sleep was associated with a rapid fall in systolic blood pressure (SBP) which reaches its lowest level with the first 2 hours of sleep and then increases throughout the rest of sleep (Snyder *et al.*, 1963).

Several intrinsic and extrinsic factors modulate the circadian rhythm. Exogenous factors such as physical activity, posture, emotional stress may also influence the rhythm (James and Pickering, 1993, Pickering and James, 1993). Among the intrinsic factors is the endogenous biological clock, the hypothalamic suprachiasmatic nucleus (SCN). Evidence for the role of SCN was provided by lesions of the SCN leading to abolishment of the circadian rhythm in rats while minute to minute variations persisted (Janssen *et al.*, 1994). Production of melatonin (N-acetyl-5-methoxytryptamine) by the pineal gland occurs at night and follows the circadian rhythm under control of the SCN (Brzezinski, 1997). Melatonin acts via G-coupled receptors in a calcium dependent process to increase nitric oxide (NO) production in endothelial cells to cause vasodilation and reduction in blood pressure (Arangino *et al.*, 1999; Paulis & Simko, 2007).

In addition, during sleep, there is down-regulation of sympathetic nervous activity (SNA) and upregulation of the peripheral nervous activity (PNA) such that heart rate (HR), stroke volume and cardiac output (CO) decrease with a reversal upon awakening in the morning (Furlan *et al.*, 1990; Veerman *et al.*, 1995). In addition, release of catecholamines reduces at night and rises during the day following the BP rhythm (Linsell *et al.*, 1985; Sherwood *et al.*, 2002). During sleep, the concurrent reduction in

HR, BP and sympathetic activity suggests modulation of baroreceptor activity (Somers *et al.*, 1993). Moreover, decline in baroreflex sensitivity has been demonstrated during NREM sleep (Shamsuzzaman *et al.*, 1994).

Further, vascular dilation was first demonstrated within minutes of onset of sleep thereafter (Howell, 1897). Brachial artery flow mediated dilatation (FMD) reaches the highest level at midnight due to effect of melatonin and reduces early in the morning (Ringqvist *et al.*, 2000; Al Mheid *et al.*, 2014). This vasodilation is mediated by melatonin induced increases in nocturnal NO (Cagnacci *et al.*, 2001) and contributes to reduction in nocturnal blood pressure (Arangino *et al.*, 1999). Thus, the decline in systemic vascular resistance which occurs during sleep parallels the BP rhythm of a fall during sleep and rise during wakefulness (Casiglia *et al.*, 1996; Sherwood *et al.*, 2018).

Although, the nocturnal fall in BP is well documented, O'Brien *et al.* (1988) first reported that all individuals do not exhibit the similar magnitude of nocturnal decline in BP. They grouped the hypertensive subjects studied as dippers or non dippers using a difference of 10 or 5mmHg difference between day-time and night-time BP. Subsequently the classification was defined in more detail: those who show decline in BP during sleep that is ≥10% of wake-time BP are referred to as "dippers" while those who experience less fall (<10%) are referred to as "non-dippers" (Pickering, 1990). Individuals who show >20% decline are referred to as "extreme dippers" and those whose nocturnal BP rises above day-time BP are referred to as a "reverse dippers" or "risers" (Salwa *et al.*, 2014).

As discussed above, nocturnal release of NO contributes to vasodilation and reduction in nocturnal BP, consequently, the non-dipping BP pattern (NDBP) has been associated

with endothelial dysfunction and increased risk of cardiovascular disease (Hermida *et al.*, 2013). Briefly, in addition, in non-dippers there is reduced forearm vascular conductance (Rizzoni *et al.*, 1992; Hoshide *et al.*, 2003; Mezue *et al.*, 2016) and higher nocturnal vascular resistance relative to dippers (Casiglia *et al.*, 1996). Further, non-dippers were more likely to have carotid artery plaque and increased intimal-medial thickness (Roman *et al.*, 1997). Moreover, non-dippers display higher daytime forearm vascular resistance (Rizzoni *et al.*, 1992; Pierdomenico *et al.*, 1997) as well as blunted brachial artery FMD (Rekhviashvili *et al.*, 2015). Further, non-dipper hypertensives showed elevated vascular resistance following isometric hand grip exercise (Porro *et al.*, 1995). Not only do non-dippers show impaired responses to endogenous stimuli, impaired endothelium dependent vasodilation in response to intra-arterial infusion of acetylcholine (Ach) attributable to decreased NO (Higashi *et al.*, 2002) and to increased oxidative stress (Maio *et al.*, 2012) has been documented in non-dipper hypertensives. Therefore altered endothelial function due to increased oxidative stress and reduced NO bioavailability occurs in hypertensive non-dippers.

Ethnic differences in nocturnal blood pressure pattern

As discussed in Chapter 1, relative to White Europeans (WEs), Black Africans (BAs) show higher prevalence of cardiovascular disorder. In addition, differences in circadian blood pressure between BAs and WEs include higher 24-hour, day-time and night-time BPs documented in BA children and adolescents (Wang *et al.*, 2006), BA young adults (Chase *et al.*, 1997) as well as BA teenagers with family history of hypertension (Treiber *et al.*, 1994). Whereas other studies showed similar day-time BPs but higher night-time BP in BA children of both sexes (Harshfield *et al.*, 1993), young women aged 20s-30 years (James, 1991), and in American blacks but not in African BAs

(aged 20-40 years) (Fumo *et al.*, 1992). Further, a systematic review of 18 studies showed that BAs have higher SBP and DBP, both at night and during the day compared to WEs (Profant & Dimsdale, 1999).

In addition, there is higher prevalence of NDBP in American young BAs aged 18-28 years (Mellman *et al.*, 2015), older BAs (aged 40-60 years) (Sherwood *et al.*, 2011) and in BA hypertensives (Prather *et al.*, 2011) relative to WEs. A systematic review further confirmed this as BAs had lower mean percentage nocturnal BP fall and higher prevalence of non-dipping compared with WEs (Agyemang *et al.*, 2005). However, unlike these studies which demonstrate higher prevalence of non-dipping in BAs, Fumo *et al.* (1992) did not demonstrate blunted fall in nocturnal BP in South African blacks living in South Africa, in spite of non-dipping demonstrated in American blacks, this suggested that the difference in prevalence of NDBP could be environmental rather than genetic. Further, Osei and Schuster (1996) did not find any difference in day, night and 24-hour BPs between the African migrants to United States and African Americans and both groups of BAs showed higher BP and HR than in Whites. The blunted nocturnal declines in American Blacks and recent African migrants but not Blacks in African regions suggests that environment and gene interaction plays a role in NDBP (Harshfield & Treiber, 1999). Environmental factors causing sodium retention, as well as stress-induced sodium retention leading to a need for a higher nocturnal pressure to excrete the sodium load may be the mechanism for nocturnal hypertension in BAs (Harshfield & Treiber, 1999).

It is also probable that the lack of nocturnal dipping is due to stress as lower socioeconomic status has been associated with reduced nocturnal dipping (Stepnowsky *et al.*, 2004; Spruill *et al.*, 2009; Rodriguez *et al.*, 2013) and peak pressor responses

during laboratory stressors were found to correlate with mean day-time and night-time SBP (Treiber *et al.*, 1994), providing indication of relationship between stress and circadian BP pattern. Although there are evidences suggesting correlation of greater pressor response to laboratory stress and greater 24-hour BP (Meininger *et al.*, 1999) or peak BP response to mental stress and peak 24-hour BP (Guasti *et al.*, 1998), however, no association was found between a 24-hour ambulatory arterial stiffness index, (AASI) and nocturnal dipping (Liu *et al.*, 2009). This suggests there may be no relationship between BP dipping pattern and arterial dysfunction. However, the increase vascular resistance observed in non-dippers highlighted earlier in this chapter suggests otherwise. It is therefore probable that AASI calculated as 1 minus the regression slope of 24-hour diastolic on systolic pressure (Li *et al.*, 2006), used in the study by (Liu et al., 2009) was not robust enough to detect vascular dysfunction.

NDBP may be regarded as an early marker of cardiovascular risk, since hypertensive non-dippers show evidence of endothelial dysfunction and impaired NO (Higashi *et al.*, 2002), however, whether normotensive young non-dippers also show any evidence of endothelial dysfunction relative to young normotensive dippers is not known. Since BAs have a higher prevalence of NDBP (Sherwood *et al.*, 2002) and reduced endothelial function due to attenuated NO bioavailability (Stein *et al.*, 1997), this raises the question of whether the high prevalence of non-dipping in BAs is associated with attenuated endothelial function. However, there are no studies demonstrating endothelial dysfunction in young healthy normotensive non-dippers.

Moreover, altered responses to stressful stimuli have been associated with endothelial dysfunction and increased risk of CVD as discussed in Chapter 1. It is not known whether young normotensive non-dippers also show altered alerting response to mental

stress. In addition, hypertensive non-dippers demonstrated greater vascular resistance index during isometric handgrip (Porro *et al.*, 1995), however, whether or not normotensive young non- dippers show similar changes is not known. This study was done as an explorative study to determine association between responses to laboratory stressors and circadian BP patterns in young adults, the findings of which will be used in future research.

7.1.3 Aim of study: To explore ethnic differences in 24-hour BP pattern and BP variability as well as to determine relationship between responses to environmental stressor and nocturnal dipping pattern.

Therefore we aimed

1. To determine ethnic or sex differences in 24-hour BP pattern and BP variability.
2. To determine association between responses to vascular occlusion, mental stress and rhythmic handgrip exercise and NDBP.

7.2 Methods

7.2.1 Participants

All the subjects who participated in the mental stress (Chapter 3-5) and hand grip exercise (Chapter 6) experiments were invited to take part in the 24-hour ambulatory BP monitoring (ABPM) as an optional study. 17 WEs, 16 BAs undertook the 24-hour ABPM, but 1 BA female completed only day time recordings.

Procedure

24-hour ABPM was done using the Spacelabs monitor (Model 90217) as described in Chapter 2. The monitor measured BP and HR half hourly during day-time (7 am-11 pm) and hourly during night-time (11 pm-7 am). From these recordings, 24-hour, day- and

night-time averages of systolic, diastolic, mean arterial and pulse pressures (SBP, DBP, MABP, PP) and heart rate (HR) as well as respective standard deviations (SD) were provided as output. The output also provided percentage nocturnal dipping as well as maximum and minimum pressures during the 24-hour recordings. The pre-set recording time for day-time was 7 am-11 pm and night-time was from 11 pm-7 am, however, some subjects slept and woke up at different times. Thus, using each participant's diary the actual wake-time (day-time) and sleep-time (night-time) BPs and HR as well as nocturnal dipping were recalculated. Three indices of the nocturnal BP drop were calculated. Firstly, absolute difference calculated as the difference between actual day-time and night-time values. Secondly, the percentage night-time BP drop (dipping) expressed as a proportion of the actual day-time value (mean day-time minus mean night-time) / mean day-time x 100). Thirdly, the subjects were classified into two categories: those whose night-time BP fell by more than 10% of the day-time values were categorized as "dippers", while those with less than 10% were categorized as "non-dippers" (Mancia & Parati, 2000). BP and HR short term variability were determined using the 24-hour SD of the mean of ABP and HR recordings as well as 24-hour coefficient of variation (CV) derived by dividing the average SD by the corresponding mean arterial pressure (SD / mean BP * 100). Day-time and night-time SD and CV of ABP and HR recordings were also determined (Parati *et al.*, 2003; Parati *et al.*, 2013a).

Data analysis

Baseline anthropometric and cardiovascular variables as well as 24-hour BP, day- and night-time BP and HR were presented as mean ± SEM. Groups of WEs and BAs were compared using independent Student's T. Subjects with day-time SBP >120 mmHg

were excluded from analysis of relationship between nocturnal dipping and responses to environmental stress. Thus, 6 male BA dippers and 1 BA woman who did not complete night-time BP recording due to nuisance from cuff inflation were excluded. For the comparisons, the normotensive subjects were grouped into mixed ethnic groups of dippers and non-dipper groups. Proportions of dippers and non-dippers were compared using Chi squared or Fischer's exact test. SD and CV were compared between whole ethnic, sex and dipper groups using independent Student's T test. Responses to 2 minutes of vascular occlusion, 5 sets of sound and to 2 minutes of rhythmic hand grip exercise were compared between dippers and non-dippers using 2-way mixed ANOVA ,with time as the within subjects factor and dipping status as the between subject factor. In all cases $p > 0.05$ was taken as significant.

7.3 Results

7.3.1 Baseline

Baseline characteristics in whole ethnic groups

BPs and HR recorded with automated BP monitor, were similar between whole group of WEs and BAs. There were no significant differences in anthropometric values or other cardiovascular variables between WEs and BAs (Table 7.1).

Comparing sex based ethnic groups, there were no differences in baseline values between WE and BA men. However, BA women were older, had higher forearm circumference (FAC) and higher salt intake relative to WE women ($p<0.05$ respectively). BP and HR measured with the automated BP monitor did not differ between the groups of men or women (Table 7.2).

Sex differences in baseline characteristics

As displayed on Table 7.2, WE men had higher waist circumference (WC), SBP and salt intake relative to the WE women ($p<0.05$ respectively). BA women were older than BA men ($p=0.02$), but the BA men had higher body mass index (BMI), FAC, and WC relative to BA women ($p<0.05$ respectively). Further, SBP tended to be higher in BA men relative to BA women ($p=0.05$).

7.3.2 24-hour ambulatory blood pressure and heart rate

Whole ethnic groups

As displayed on Table 7.3, relative to WEs, BAs had higher 24-hour and nocturnal SBP ($p=0.04$) and nocturnal SBP ($p=0.01$) however, day-time SBP only tended to be higher ($p=0.06$). There was a higher proportion of BAs who had 24-hour and day-time SBP exceeding 120 mmHg relative to WEs (37.5% vs 0% and 44% vs 0%, $p=0.007$ and

0.003 respectively). Of the BAs, 5 subjects had day-time SBP of 120-134 mmHg and 2 subjects had day-time SBP ≥ 135 mmHg.

Comparison in sex groups

As shown on Table 7.4, BA men showed higher 24-hour, higher day-time and night-time BP relative to WE men ($p < 0.05$ in each case) but HR was similar. Proportions of BA men with day-time or night-time SBP >120 mmHg or day-time DBP >80 mmHg were higher than WE men. There was no significant difference between WE and BA women.

Sex differences

There were no differences in 24-hour, day-time or night-time BP or HR between the WE men and women. Relative to BA women, BA men showed higher 24-hour SBP and PP, day-time SBP, MAP and PP as well as night-time PP ($p<0.05$ in each case) while night-time SBP tended to be higher in BA men ($p=0.05$). A higher proportion of BA men had day-time SBP 120-135 relative to BA women ($p=0.05$). Two BA men but none of the BA women had day-time SBP >135mmHg (Table 7.4).

7.3.3 Nocturnal blood pressure and heart rate dipping pattern

Whole ethnic groups

As shown on Table 7.5, the absolute nocturnal fall and percentage nocturnal dipping of BPs and HR were not different between WEs and BAs. The proportion of SBP dippers was higher in WEs relative to BAs (82% vs 60%), however, the difference was not significant ($p=0.24$).

Comparison in sex groups

Comparing sex groups, BA men showed smaller nocturnal fall in HR relative to WE men ($p=0.01$ Table 7.6) but there was no difference between BA and WE women**.**

Sex differences

There were no differences between the men and women in each ethnic group in absolute fall and percentage dipping of BP or HR. A higher proportion of BA women were non-dippers relative to BA men (67% vs 22%, p=0.14).

7.3.4 Variability in 24-hour blood pressure and heart rate

Variability in blood pressure and heart rate over 24-hr period

Whole ethnic groups

The whole group of BAs showed lower SD and CV of HR relative to WEs (p=0.02, 0.01 respectively, Table 7.11).

Sex groups

Comparing men, relative to WEs, BAs showed higher SD of DBP and PP (p=0.03, 0.00 respectively). In addition BA men showed lower SD and CV of HR (p=0.01 in each case). Comparing women, there were no significant differences in SD or CV of BP or HR (Table 7.12).

Sex differences

Comparing men and women, WE men showed higher SD of PP and SD of SBP tended to be higher relative to WE women (p=0.04, 0.06 respectively). Relative to BA women, BA men showed higher SD of SBP, DBP and PP ($p<0.05$ in each case) as well as tendency for SD of MAP to be higher (p=0.05). In addition, BA men showed higher CV of DBP relative to BA women (Table 7.12).

Variability in blood pressure and heart rate in day-time and night-time

Relative to WEs, the whole group of BAs showed lower SD and CV of HR in day time ($p<0.001$ and 0.05 respectively) but not in night-time ($p>0.05$). Relative to WEs, the

whole group of BAs showed higher CV but not SD of PP at night but not in day-time (Table 7.11).

Comparing men and women, WE men showed higher SD but not CV of MABP in day-time ($p=0.01$, >0.05 respectively). There were no differences at night-time ($p>0.05$). CV of PP was higher in men at night-time but not day-time ($p=0.01$, Table 7.12).

Relative to BA women, BA men showed higher SD but not CV of SBP, DBP, MABP and PP in daytime ($p<0.005$) as well as night-time ($p \leq 0.05$ in each case, Table 7.13). In addition, BA men showed higher SD of HR than women in day-time but not at night ($p=0.01$, 0.12).

7.3.7 Association between response to environmental stressors and dipping status

Baseline characteristics in dippers versus non-dippers

There were no differences in BPs and HR recorded with automated BP monitor between dippers and non-dippers. The non-dippers showed higher nocturnal BPs and HR relative to dippers however, 24-hr and daytime BPs and HR were not different between dippers and non dippers. Absolute nocturnal fall in BP and HR were lower in non-dippers ($p<0.05$ respectively, Table 7.10). Although % dipping of BPs were lower in non-dippers, % dipping of HR did not differ between dippers and non-dippers (Table 7.10). There were no differences in 24-hr SD and CV measures of BP or HR variability between the normotensive dippers and non-dippers (Table 7.13), however, normotensive non-dippers showed lower day-time but not night-time SD of HR relative to the dippers ($p=0.01$, >0.05, Table 7.14).

Reactive hyperaemia in dippers vs non-dippers

In both dippers and non-dippers, reactive hyperaemia was associated with an increase in FVC. There was no significant 2-way interaction between time and dipping status on

ΔFVC during reactive hyperaemia ($p>0.05$), There was no significant main effect of dipping status ($p>0.05$). The peak change from baseline in FVC during reactive hyperaemia was not significantly different in dippers vs non-dippers (+0.42 ± 0.05 vs +0.38 ± 0.04 CU, $p=0.61$, Figure 7. 1).

Responses evoked by mental stress in dippers vs non dippers

Mental stress evoked a pattern of net increase in forearm vascular conductance indicating vasodilation with increased heart rate, increased mean arterial pressure, forearm and digital cutaneous vasoconstriction in dippers during sound 1-5, but the non-dippers showed a pattern of net decrease in forearm vascular conductance indicating vasoconstriction with decrease in heart rate and smaller digital cutaneous vasoconstriction and pressor responses relative to dippers. There was no significant 2-way interactions between dipping status and time, or significant main effect of dipping status on ΔFVC, ΔMABP, ΔHR, ΔFCVC, ΔDCVC during mental stress ($p>0.05$). None of the responses was significantly different between dippers and non-dippers (Figure 7.2 and 7.3).

Response evoked by rhythmic hand grip exercise

Rhythmic hand-grip exercise evoked increases in FVC in the dippers and non-dippers. There was no significant interaction between dipping status and time, or significant main effect of dipping status on FBF, FVC, MABP, HR during post exercise period ($p>0.05$). Although, the increase FVC at the peak of exercise hyperaemia was greater in the dippers than non-dippers, the difference was not significant. Blood pressure and heart rate changes were similar between dippers and non-dippers (Figure 7.4).

7.4 Discussion

7.4.1 Baselines

At baseline, BP recorded with automated sphygmonameter was similar between WEs and BAs, however 24-hour ambulatory monitoring revealed ethnic differences. The higher nocturnal blood pressure in BAs is consistent with previous report (James & Pickering, 1993). The nocturnal hypertension in young BAs could be due to blunted nocturnal parasympathetic nervous activity (PNA) or increased cardiac sympathetic nervous activity (SNA) which would cause increased heart rate and cardiac output. However, HR did not differ between whole groups BAs and WEs, this suggests that heart rate did not contribute to the difference in nocturnal BP.

During sleep, there is down-regulation of SNA and upregulation of PNA (Furlan *et al.*, 1990) such that HR, stroke volume and cardiac output (CO) decrease with a reversal upon awakening in the morning (Veerman *et al.*, 1995). It is probable that increased peripheral resistance contributed to the nocturnal hypertension, there was lack of difference in day-time DBP but the difference in night- time DBP tended towards significance giving an indication that higher vascular resistance at night in BAs may contribute to the difference. The present study was done as an exploratory study, thus a planned study with higher number of participants is more likely to show significant differences in nocturnal DBP. Vascular NO release induced by melatonin contributes to reduction in peripheral resistance at night (Arangino *et al.*, 1999). Blunted NO availability in BAs has been reported (Stein *et al.*, 1997). Hence this may play a role in the higher night time DBP. However the lack of difference in day-time DBP was unexpected.

Day-time systolic pressure tended to be high whereas nocturnal SBP was significantly higher in BAs, SBP depends on cardiac output and arterial elasticity. This would suggest that a mechanism amplified at night was involved. It is likely that increased pressure natriuresis may contribute to the increased nocturnal BP in BAs who are more likely to be salt sensitive (Higashi *et al.,* 1997; Fukuda *et al.,* 2006). Pressure natriuressis enables persons with reduced daytime sodium excretion achieve greater level of excretion at night.

Poor sleep quality and sleep apnoea have been associated with nocturnal hypertension and could have played a role in the ethnic difference (Matthews *et al.,* 2008; Bowman *et al.*, 2019). However, we did not assess quality of sleep or sleep apnoea in this study.

2 BA young men in their 20s already showed hypertension relative to none in BA women, consistent with previous reports of male gender being a risk factor for hypertension. In comparison with WE men, BA men showed higher 24-hr, day-time and night-time BP but no difference in heart rate, whereas BA women did not differ from WE women suggests that the differences observed in whole groups of BAs were largely due to BP differences in men. The lack of difference in HR in spite of higher BP in BA men suggests altered cardiac autonomic responses contributed to increased BP in the BA men. Further, BA men showed smaller nocturnal HR decline which suggests greater cardiac sympathetic drive (Grassi *et al.,* 2015).

The finding of a higher proportion of non-dippers among BA women than BA men shows that BA women show BP dysregulation that is more evident at night than daytime. The reasons for this is unclear and would be focus of further studies, given that we have also observed higher proportions of vasoconstrictors during mental stress among BA women (See Chapter 3), it is probable that impaired nocturnal endothelial

function may contribute to non- dipping pattern in BA women, whereas in BA men, dysfunction in autonomic nervous regulation, sodium and volume regulation may play a role. The observed lower nocturnal HR dipping in non-dippers suggests blunted nocturnal PNA relative to the dippers in addition to increased peripheral vascular resistance evidenced by increased nocturnal DBP. These suggest altered nocturnal autonomic function in non-dippers.

Higher variability in 24-hr, day and night blood pressure in BA males in addition to lower 24-hr and day-time HR variability suggests impaired baroreceptor control of blood pressure as well as control of HR (Parati *et al.*, 2013b). Further, within each ethnic group, relative to women, men showed significantly higher variability of BP, suggesting sex differences in short term blood pressure control in both ethnic groups. High 24 hr variability of BP suggests higher likelihood of target organ damage in men (Parati *et al.*, 1987). These findings can be explored further in larger studies.

Whether dipping pattern was associated with endothelial responses to vascular occlusion, mental stress or handgrip exercise was tested. In the present study, the young normotensive non-dippers did not show blunted reactive hyperaemia contrary to findings in non-dipper hypertensives who showed blunted acetylcholine mediated dilation in forearm resistance vessels (Higashi *et al.*, 2002) and blunted FMD in brachial arteries (Rekhviashvili *et al.*, 2015).

The finding of forearm vasoconstriction rather than vasodilation in the normotensive non-dippers, in response to mental stress and smaller exercise hyperaemia suggests that altered endothelial function evoked by stress could contribute to CVD in non-dippers. This could be tested in a larger sample in future studies.

7.4.2 Conclusion

This was an exploratory study with limited sample size, however, the findings suggest that the high proportion of non-dippers among BA women and the tendency for non-dippers to show mental stress induced vasoconstriction may contribute to higher incidence of hypertension among BA women.

7.4.3 Limitations

The sample size was small due to subjects' unwillingness to undertake 24-hour BP monitoring, a larger sample size will be needed to further examine the differences in endothelial function between normotensive young dippers and non-dippers.

Table 7.1 Baseline values of anthropometric and cardiovascular variables in WEs and BAs.

	WEs (n=17)	BAs (n=16)	P value
Age (years)	21±0.64	22±0.77	0.42
Gender (M:F)	11(65): 6(35)	9(56): 7(44)	0.73
BMI, kg/m 2	22.62±0.61	21.93±0.70	0.46
FAC cm	24.35±0.39	24.17±0.74	0.82
SBP mmHg	105±2.03	106±3.22	0.66
DBP mmHg	64±1.21	66±1.87	0.63
HR, bpm	67±2.44	65±1.85	0.54
MABP mmHg	78±1.24	79±2.18	0.61
PSS	16±1.06	16±1.63	0.95
Salt intake score	41±2.20	47±1.95	0.05
FBF (ml/100ml /min)	4.37±0.32	4.65±0.55	0.66
FVC (CU)	0.06±0.00	0.06±0.01	0.67
DCRCF(PU)	167±32.90	224±36.85	0.26
DCVC (CU)	2.19±0.47	2.88±0.53	0.33
DCRCF(PU)	22.73±3.29	17.66±2.26	0.21
DCVC (CU)	0.29±0.05	0.24±0.03	0.35
SBP≥120mmHg, n (%)	0(0)	2(12.5)	0.23
FH+, n (%)	1(5.88)	5(31.25)	0.09

Values are shown as mean ± SEM, except for sex, pre-hypertension (SBP >120mmHg) and parental hypertension (FH+) which are shown as number (n) and % of total.
*p<0.05: WEs vs BAs, independent Student's T test.

Table 7.2 Baseline values of anthropometric and cardiovascular variables in WE and BA men and women

	White Europeans			Black Africans		
	Men (n=11)	**Women (n=6)**	**P value**	**Men (n=9)**	**Women (n=7)**	**P value**
Age (years)	21.36±0.90	20.50±0.81§	0.54	20.33±0.85	23.86±1.01*	0.02
BMI	23.06±0.80	21.82±0.89	0.34	23.44±0.78*	19.98±0.79	0.01
WC (cm)	80.09±2.35*	71.17±0.60	0.02	80.00±3.37*	69.42±2.52	0.03
FAC (cm)	24.91±0.50	23.33±0.36§	0.05	26.11±0.53*	21.25±0.57	0.00
SBP (mmHg)	108±1.66*	98±3.73	0.01	112±4.19	99±3.80	0.05
DBP (mmHg)	65±1.41	63±2.31	0.39	67±2.44	64±3.03	0.51
PR (bpm)	68±2.63	66±5.26	0.63	65±2.54	66±2.88	0.70
MABP (mmHg)	80±1.01	75±2.65	0.06	82±2.89	76±3.09	0.19
PSS	16±1.21	16±2.17	0.82	14±1.53	19±2.85	0.09
Salt intake score	45±2.01*	34.±4.16§	0.02	47±2.93	47±2.64	0.93
FBF(ml/dl/min)	4.73±0.36	3.71±0.58	0.13	4.87±0.62	4.37±1.03	0.67
FVC (CU)	0.06±0.00	0.05±0.01	0.08	0.06±0.01	0.06±0.02	0.80
DCRCF(PU)	197±49.47	127±37.99	0.31	288±41.39	159±52.85	0.08
DCVC (CU)	2.71±0.73	1.49±0.42	0.21	3.53±0.55	2.32±0.84	0.27
FCRCF (PU)	20.31±4.59	25.96±4.74	0.42	19.14±2.80	16.17±3.69	0.53
FCVC (CU)	0.27±0.07	0.32±0.06	0.63	0.25±0.03	0.23±0.05	0.69
SBP >120 mmHg	0(0%)	0(0%)	1.00	2(22%)	(0%)	0.15
FH+, n (%)	1(9%)	0(0%)	1.00	1(11%)	3(43%)	0.69

Values are shown as mean ± SEM, except for pre-hypertension and parental hypertension (FH+) shown as number (n) and % of total. Sex based comparison within each ethnic group *p<0.05: males vs females and between ethnic groups, § p<0.05: females WEs vs female BAs, independent Student's T test.

Table 7.3 Values of 24 hour BP and HR in Wes and BAs.

	WE n=17	BA (n=16)	P value
24 hour			
SBP (mmHg)	110±1.33	117±3.17*	0.04
DBP (mmHg)	68±1.15	71±1.33	0.09
MAP (mmHg)	82±0.96	86±1.63	0.05
Pulse Pressure (mmHg)	41±1.26	46±2.21	0.09
Heart rate (bpm)	73±1.87	72±2.14	0.84
Day-time			
SBP (mmHg)	113±1.34	120±3.29	0.06
DBP (mmHg)	71±1.23	74±1.46	0.18
MAP (mmHg)	86±1.15	89±1.95	0.23
Pulse Pressure (mmHg)	41±1.30	45±2.29	0.12
Heart rate (bpm)	75±1.79	73±2.15	0.47
SBP >121 mmHg, n (%)	0(0%)	7(43.8%)	0.003
SBP ≥121-135 mmHg, n (%)	0(0%)	5(31.25%)	
SBP >135 mmHg, n (%)	0(0%)	2(12.5%)	
Night-time			
SBP (mmHg)	99±1.36	108±3.05*	0.01
DBP (mmHg)	58±1.21	61±0.97	0.05
MAP (mmHg)	73±1.20	77±1.50	0.06
Pulse Pressure (mmHg)	41±1.18	47±2.69	0.07
Heart rate (bpm)	62±2.49	66±2.24	0.24
SBP>120 (mmHg)	0(0%)	2(12.5%)	0.21

Values are shown as mean ± SEM, except for proportion of SBP >120 mmHg shown as number (n) and % of total. *p<0.05: WEs vs BAs, independent Student's T test**.**

Table 7.4 Values of 24 hour BP and HR recordings in men and women in each ethnic group

	White Europeans			Black Africans		
	Men (n=11)	**Women (n=6)**	**P value**	**Men (n=9)**	**Women (n=7)**	**P value**
			24 hr			
SBP (mmHg)	111±1.66§	109±2.33	0.52	**123±4.02***	109±3.56	0.03
DBP (mmHg)	67±1.07§	70±2.56	0.23	73±2.03	70±1.49	0.31
MABP (mmHg)	82±0.88§	83±2.31	0.66	89±2.11	83±2.04	0.07
PP (mmHg)	43±1.73§	38±0.60	0.06	**50±2.64***	**40±2.41**	**0.01**
Heart rate(bpm)	72±2.45	74±3.00	0.55	69±1.96	75±4.02	0.17
			Day-time			
SBP (mmHg)	113±1.59§	111±2.54	0.46	126±4.14*	111±3.31	0.02
DBP (mmHg)	70±1.15§	73±2.81	0.30	76±2.18	72±1.61	0.19
MABP(mmHg)	85±1.02§	89±2.42	0.07	93±2.57*	85±2.05	0.03
PP (mmHg)	43±1.80§	38±0.58	0.07	50±2.84*	39±2.11	0.01
Heart rate(bpm)	75±2.39	76±2.80	0.77	71±2.38	77±3.66	0.19
SBP >135 mmHg, n (%)	0(0%)	0(0%)		2 (22%)	0(0%)	
			Night-time		**Females n=6**	
SBP (mmHg)	100±1.80§	99±2.20	0.68	113±3.18	101±4.91	0.05
DBP (mmHg)	57±1.56§	60±1.91	0.38	62±1.10	61±1.87	0.57
MABP (mmHg)	72±1.38§	76±2.13	0.18	79±1.50	74±2.78	0.13
PP (mmHg)	43±1.50§	39±1.61	0.15	51±2.83*	40±4.15	0.04
Heart rate(bpm)	61±2.99	66±4.46	0.33	65±2.06	68±4.87	0.54

Values are shown as mean ± SEM, except for proportion of subjects with SBP >120mmHg shown as number (n) and % of total. * $p<0.05$: BA men vs BA women. § $p<0.05$: WE men vs BA men, independent Student's T test.

Table 7.5 Dipping pattern showing absolute differences and percentage (%) dipping of BPs and HR in WEs and BAs.

Absolute differences	WEs (n=17)	BAs (n=15)	p value	Dipping (%)	WEs (n=17)	BAs (n=15)	p value
SBP (mmHg)	13.61±0.96	11.79±7.11	0.49	SBP	11.97±0.82	9.55±0.82	0.19
DBP (mmHg)	13.41±0.74	12.78±4.08	0.43	DBP	18.73±0.92	16.90±1.84	0.31
MABP (mmHg)	13.11±0.67	12.12±5.04	0.44	MABP	15.13±0.73	13.30±1.67	0.30
PP (mmHg)	0.06±0.83	-1.12±3.40	0.61	PP	-2.27±2.33	-2.00±4.51	0.96
HR (bpm)	12.91±1.41	6.81±5.37	0.72	HR	15.88±2.86	8.08±2.58	0.06
				Dipper, n (%)	14(82%)	9 (60%)	
				Non dippers n (%)	3(17%)	6(40%)	0.24

Values are mean± SEM except for proportion of dippers/non dippers shown as number (n) and % of total.. *p<0.05: WEs vs BAs, independent Student's T test. Comparison of proportions of dippers and non dipper by Chi squared Test

Table 7.6 Dipping pattern showing absolute differences in sex dependent groups of WEs and BAs

	WEs (n=17)			BAs (n=15)		
	Men (n=11)	Women (n=6)	P value	Men (n=9)	Women (n=6)	P value
SBP (mmHg)	13.94±1.29	13.02±1.44	0.66	13.70±2.85	8.68±2.91	0.26
DBP (mmHg)	13.26±0.93	13.69±1.31	0.79	14.03±1.90	10.88±2.49	0.33
MABP (mmHg)	12.79±0.92	13.69±0.92	0.54	13.85±2.04	9.54±2.52	0.21
PP (mmHg)	0.49±0.95	-0.74±1.66	0.49	-0.56±2.21	-2.20±2.82	0.65
HR (bpm)	14.35±1.56§	10.25±2.61	0.17	5.41±2.52	6.45±2.05	0.77

Values are mean± SEM. §: p=0.01: WE men vs women, independent Student's T test.

Table 7.7 Percentage of nocturnal dipping of BPs and HR in WEs and BAs.

	WEs			BAs		
	Men (n=11)	**Women (n=6)**	**P value**	**Men (n=9)**	**Women (n=6)**	**P value**
SBP dipping (%)	12.15±1.10	11.65±1.26	0.78	10.53±2.27	8.09±2.70	0.50
DBP dipping (%)	18.81±1.31	18.58±1.20	0.91	18.16±2.22	15.00±3.29	0.42
MABP dipping (%)	15.01±1.05	15.35±0.88	0.83	14.63±2.03	11.31±2.90	0.35
PP dipping (%)	-1.48±2.35	-3.53±5.14	0.69	2.13±3.89	-9.21±10.29	0.25
HR dipping (%)	17.60±3.95	13.13±4.12	0.47	9.18±3.59	6.17±3.73	0.60
SBP dippers, n (%)	9 (72%)	5 (83%)	1.00	7 (78%)	2 (33%)	0.14
SBP non dippers, n (%)	2 (18%)	1 (17%)		2 (22%)	4 (67%)	

Values are mean± SEM, except for proportion of dippers and non-dippers which are shown as numbers (n) and percentage (%). *p<0.05: men vs women, independent Student's T test.

Table 7.8 Baseline values of anthropometric and cardiovascular variables in normotensive dippers and non-dippers groups of WEs, BAs men and women being grouped together.

	Dippers (n=17)	Non-dippers (n=9)	P value
Age	21.18±0.65	23.00±0.96	0.12
Gender (M:F)	10/7	4/5	
Ethnicity (WE:BA)	14/3	3/6	
BMI (kg/m^2)	22.18±0.71	21.43±0.73	0.51
Waist (cm)	75.21±2.11	75.86±2.56	0.86
Forearm circumference (cm)	23.91±0.48	23.44±0.90	0.61
SBP (mmHg)	103.35±2.19	101.67±3.18	0.66
DBP (mmHg)	62.47±1.25	5.61±2.11	0.19
PR (bpm)	66.06±2.34	68.11±2.61	0.59
MABP (mmHg)	76.10±1.37	77.63±2.32	0.55
PSS	15.65±1.10	17.33±2.13	0.44
Salt intake score	44.00±2.37	40.89±2.97	0.43
FBF (ml/100ml/min)	4.49±0.45	4.06±0.51	0.56
FVC (CU)	0.06±0.01	0.05±0.01	0.60
DCRCF (PU)	184.15±31.20	173.54±55.15	0.86
DCVC (CU)	2.44±0.45	2.27±0.78	0.83
FCRCF (PU)	22.37±2.91	18.22±4.36	0.42
FCVC (CU)	0.29±0.04	0.23±0.06	0.36

Values are shown as mean ± SEM, *p<0.05: dippers vs non dippers, independent Student's T test.

Table 7.9 24 hour BP and HR in normotensive dippers and non-dippers groups of WE and BA men and women being grouped together.

	Dippers (n=17)	Non-dippers (n=9)	P value
	24-hour		
SBP (mmHg)	109±1.49	110±2.13	0.64
DBP (mmHg)	68±1.10	69±1.31	0.72
MABP (mmHg)	82±0.97	82±1.36	0.72
Pulse Pressure (mmHg)	41±1.41	41±1.60	0.78
Heart rate (bpm)	71±2.02	75±2.14	0.29
	Day-time		
SBP (mmHg)	112±1.45	111±2.37	0.64
DBP (mmHg)	71±1.16	71±1.66	0.81
MABP (mmHg)	85±1.11	87±1.79	0.47
PP (mmHg)	41±1.40	40±1.64	0.73
Heart rate (bpm)	74±1.99	75±2.07	0.58
	Night-time		
SBP (mmHg)	97±1.42*	106±2.34	0.00
DBP (mmHg)	58±0.96*	62±1.74	0.02
MABP (mmHg)	71±0.85*	79±1.47	0.00
PP (mmHg)	40±1.45	45±2.04	0.07
Heart rate (bpm)	61±2.48*	70±2.51	0.02

Values are shown as mean ± SEM, *$p<0.05$: dippers vs non dippers, independent Student's T test.

Table 7.10 Dipping pattern showing absolute differences between daytime and night-time and percentage dipping in dippers and non-dipper WE and BA men and women being grouped together.

	Absolute difference between day- and night-time				Percentage dipping		
	Dippers (n=17)	**Non-dippers (n=9)**	**P value**		**Dippers n=17**	**Non-dippers (n=9)**	**P value**
SBP (mmHg)	15±0.73	5±1.45*	0.000	SBP (mmHg)	13.34±0.64	4.28±1.31*	0.000
DBP (mmHg)	14±0.69	9±1.73*	0.005	DBP (mmHg)	19.28±0.80	12.53±2.35*	0.003
MABP (mmHg)	14±0.64	8±1.58*	0.000	MABP(mmHg)	15.93±0.62	8.60±1.75*	0.000
PP (mmHg)	+1±0.84	-4±1.85*	0.005	PP (mmHg)	0.40±2.75	-10.79±5.39	0.055
HR (bpm)	13±1.37	5±2.03*	0.004	HR (bpm)	16.30±3.01	7.89±5.39	0.096

Values are shown as mean ± SEM, *p<0.05: dippers vs non dippers, independent Student's T test.

Table 7.11 Standard deviation and coefficient of variability of 24-hr BP and HR in WEs and BAs.

	WEs	BAs	P value	WEs	BAs	P value	WEs	BAs	P value
				Standard deviation (SD)					
	24-hr SD			**Day-time SD**			**Night-time SD**		
SBP	10.59±0.37	11.58±0.81	0.27	9.03±0.38	7.94±1.31	0.40	8.10±0.51	8.26±1.14	0.89
DBP	9.35±0.30	9.83±0.49	0.41	7.70±0.33	6.22±0.96	0.14	7.35±0.37	7.66±1.08	0.77
MABP	8.60±0.29	8.89±0.49	0.61	7.35±0.34	5.62±0.89	0.07	6.69±0.48	6.37±0.89	0.74
PP	6.20±0.23	7.62±0.57*	0.03	6.35±0.33	5.68±0.93	0.48	5.17±0.40	6.61±1.00	0.16
HR	12.74±0.85*	9.96±0.69	0.02	12.40±0.97*	7.01±0.70	0.00	5.99±0.50	6.59±1.05	0.59
				Coefficient of variability (CV)					
	24-hr CV			**Day-time CV**			**Night-time CV**		
SBP	9.63±0.32	9.88±0.59	0.72	7.99±0.32	8.81±0.58	0.21	8.24±0.49	9.46±0.67	0.15
DBP	13.62±0.41	13.77±0.65	0.84	10.74±0.45	11.59±0.56	0.24	12.70±0.69	14.25±1.39	0.30
MABP	10.43±0.32	10.35 ±0.51	0.89	8.28±0.34	8.94±0.54	0.30	9.51±0.56	10.24±0.85	0.47
PP	15.15 ± 0.64	17.47 ± 1.03	0.06	15.42±0.79	16.73±1.22	0.37	12.43±0.90	16.52±1.91	0.05
HR	17.62 ± 1.08	13.60 ± 1.02*	0.01	16.35±1.10	13.33±0.99	0.05	11.14±1.23	11.73±1.63	0.77

Values are shown as mean ± SEM, *p<0.05: dippers vs non dippers, independent Student's T test.

Table 7.12 Standard deviation and coefficient of variability of 24-hr BP and HR in WEs (men vs women)

	Men	Women	P value	Men	Women	P value	Men	Women	P value
				Standard deviation (SD)					
	24-hr SD			**Day-time SD**			**Night-time SD**		
SBP	11.09±0.45	9.67±0.46	0.06	10.83±1.10	8.42±0.54	0.14	8.75±0.53	6.59±0.92	0.05
DBP	9.60±0.35§	8.90±0.54	0.28	8.07±0.43	7.16±0.62	0.24	7.41±0.48	6.36±0.82	0.26
MABP	8.74±0.40	8.35±0.37	0.53	8.23±0.54	7.84±0.62	0.66	7.56±0.51*	4.79±0.96	0.01
PP	6.55±0.24§	5.57±0.41*	0.04	7.64±1.21	5.66±0.45	0.26	5.92±0.42	4.28±0.97	0.09
HR	13.32±1.03§	11.67±1.51	0.37	12.90±1.17	11.47±1.63	0.48	5.60±0.55	6.09±0.85	0.63
				Coefficient of variability (CV)					
	24-hr CV			**Day-time CV**			**Night-time CV**		
SBP	10.04±0.41	8.91±0.43	0.10	8.30±0.44	7.42±0.39	0.20	8.88±0.56	7.08±0.77	0.08
DBP	14.24±0.47	12.66±0.69	0.07	11.40±0.48*	9.52±0.76	0.04	13.46±0.69	11.31±1.38	0.14
MABP	10.63±0.45	10.07±0.41	0.42	8.59±0.48	7.73±0.37	0.25	9.74±0.54	9.09±1.32	0.60
PP	15.46±0.85	14.56±0.96	0.52	15.86±1.09	14.63±1.06	0.47	14.10±1.04*	9.37±0.62	0.01
HR	18.60±1.24§	15.80±2.00	0.23	17.05±1.33	15.07±2.00	0.41	10.60±1.11	12.14±2.97	0.56

Values are shown as mean ± SEM. *p<0.05: men vs women, independent Student's T test.

Table 7.13 Standard deviation and coefficient of variability of 24-hr BP and HR in BAs (men vs women)

	Men	Women	p value	Men	Women	p value	Men	Women	p value
				Standard deviation (SD)					
	24-hr SD			**Day-time SD**			**Night-time SD**		
SBP	13.19±1.02	9.50±0.83*	0.02	10.94±1.47	3.44±0.11	0.00	10.54±1.22	4.17±0.13	0.00
DBP	10.91±0.44	8.43±0.68*	0.01	8.65±0.92	2.58±0.12	0.00	9.65±1.24	4.06±0.14	0.01
MABP	9.74±0.56	7.80±0.69	0.05	7.68±0.99	2.53±0.09	0.00	8.05±1.01	3.34±0.10	0.01
PP	8.72±0.66	6.21±0.70*	0.02	7.75±1.08	2.57±0.05	0.00	8.03±1.35	4.07±0.15	0.05
HR	9.29±0.83	10.81±1.15	0.29	8.45±0.84	4.85±0.47	0.01	7.81±1.50	4.38±0.17	0.12
				Coefficient of variability (CV)					
	24-hr SD			**Day-time CV**			**Night-time CV**		
SBP	10.76±0.74	8.75±0.82	0.09	9.15±0.82	8.31±0.80	0.50	10.15±0.94	8.21±0.59	0.18
DBP	15.06±0.59	12.11±0.99*	0.02	12.07±0.75	10.87±0.83	0.31	15.62±1.74	11.78±2.06	0.20
MABP	10.97±0.56	9.56±0.87	0.17	8.86±0.75	9.05±0.81	0.87	10.52±1.14	9.73±1.34	0.68
PP	17.76±1.59	17.11±1.28	0.76	16.77±1.81	16.66±1.62	0.97	17.79±2.51	14.24±2.89	0.39
HR	13.39±1.09	13.88±1.96	0.82	12.57±0.79	14.47±2.23	0.37	11.73±2.28	11.73±2.33	1.00

Values are shown as mean ± SEM. *$p<0.05$: men vs women, independent Student's T test.

Table 7.14 Standard deviation and coefficient of variability of 24-hr BP and HR in normotensive dippers and non-dippers.

	Dippers (n=17)	Non-dippers (n=9)	P value	Dippers	Non-dippers	P value	Dippers	Non -dippers	P value
				Standard deviation					
	24-hr SD			**Day-time SD**			**Night-time SD**		
SBP	11.46±0.54	10.03±0.62	0.15	8.50±0.63	6.86±1.28	0.21	7.52±0.54	9.13±1.60	0.24
DBP	9.77±0.34	9.08±0.47	0.28	7.25±0.52	6.01±1.15	0.27	6.62±0.44	7.78±1.37	0.31
MABP	8.93±0.34	8.24±0.42	0.27	6.55±0.48	6.25±1.12	0.78	6.35±0.45	6.91±1.31	0.62
PP	7.06±0.39	6.45±0.54	0.41	6.03±0.48	4.50±0.80	0.09	5.29±0.47	4.36±0.76	0.29
HR	11.64±0.74	10.72±0.94	0.50	12.07±1.08*	6.96±1.24	0.01	6.93±0.82	4.45±0.89	0.08
				Coefficient of variability					
	24-hr CV			**Day-time CV**			**Night-time CV**		
SBP	10.00±0.40	9.09±0.51	0.22	8.08±0.35	8.86±0.52	0.22	8.26±0.47	9.71±0.99	0.14
DBP	13.90±0.46	13.14±0.61	0.37	11.03±0.43	11.60±0.72	0.48	12.45±0.66	14.22±1.87	0.28
MABP	10.55±0.37	9.99±0.40	0.41	8.42±0.34	9.36±0.46	0.12	9.35±0.50	10.98±1.34	0.17
PP	16.04±0.74	16.91±1.20	0.54	15.70±0.77	16.45±0.54	0.79	13.11±1.02	13.82±2.03	0.73
HR	16.36±0.97	13.82±1.41	0.17	16.78±1.04	14.29±1.49	0.19	12.22±1.43	10.69±1.59	0.52

Values are shown as mean ± SEM. *p<0.05 dippers vs non dippers, independent Student's T test.

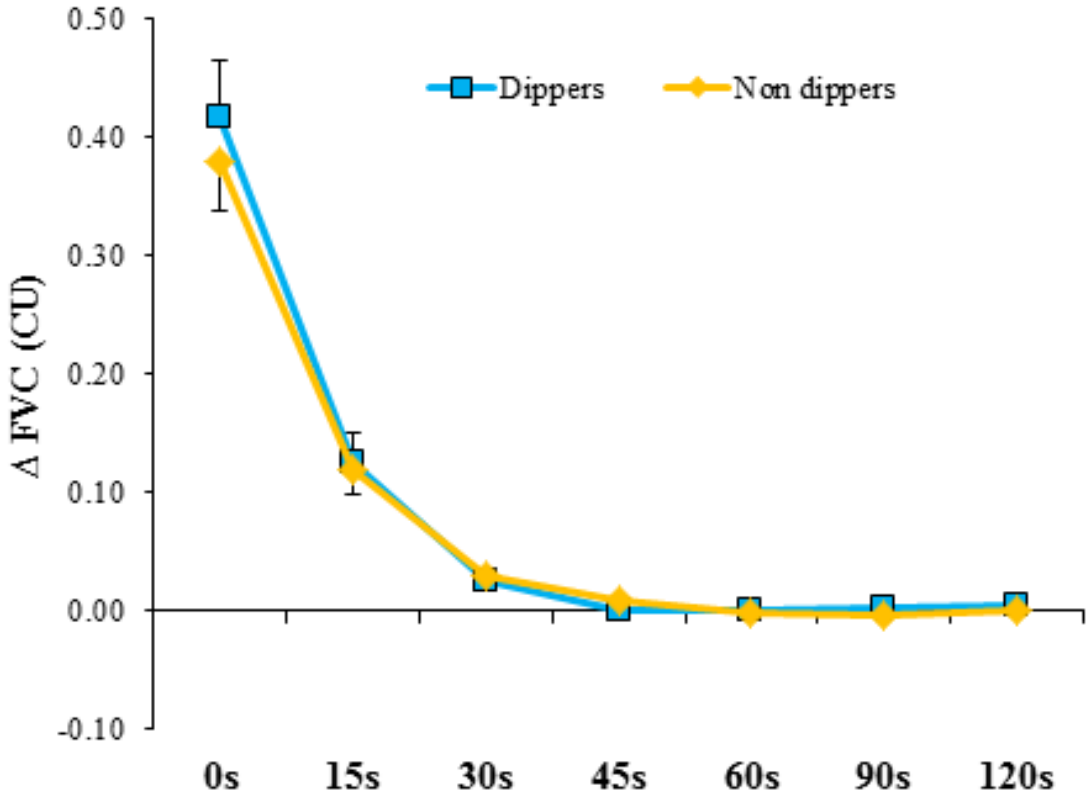


Figure 7.1 Change in forearm vascular conductance (FVC) in normotensive dippers and non-dippers. Values are mean ± SEM.

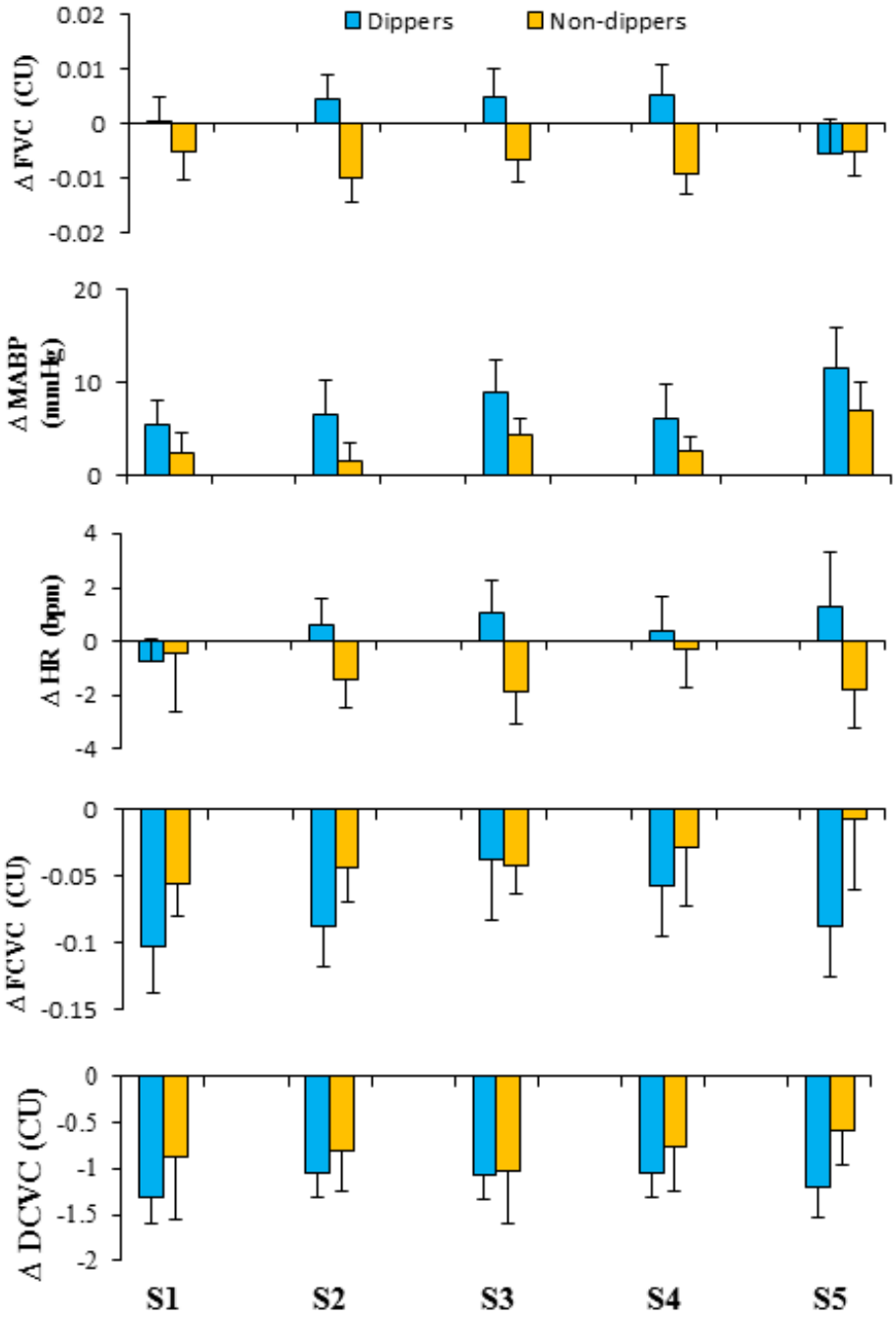


Figure 7.2: Change from baseline values of FVC, MAP, HR DCVC and FCVC during sound 1-5 at 15s into each sound in normotensive dippers and non-dippers. Values are mean ± SEM.

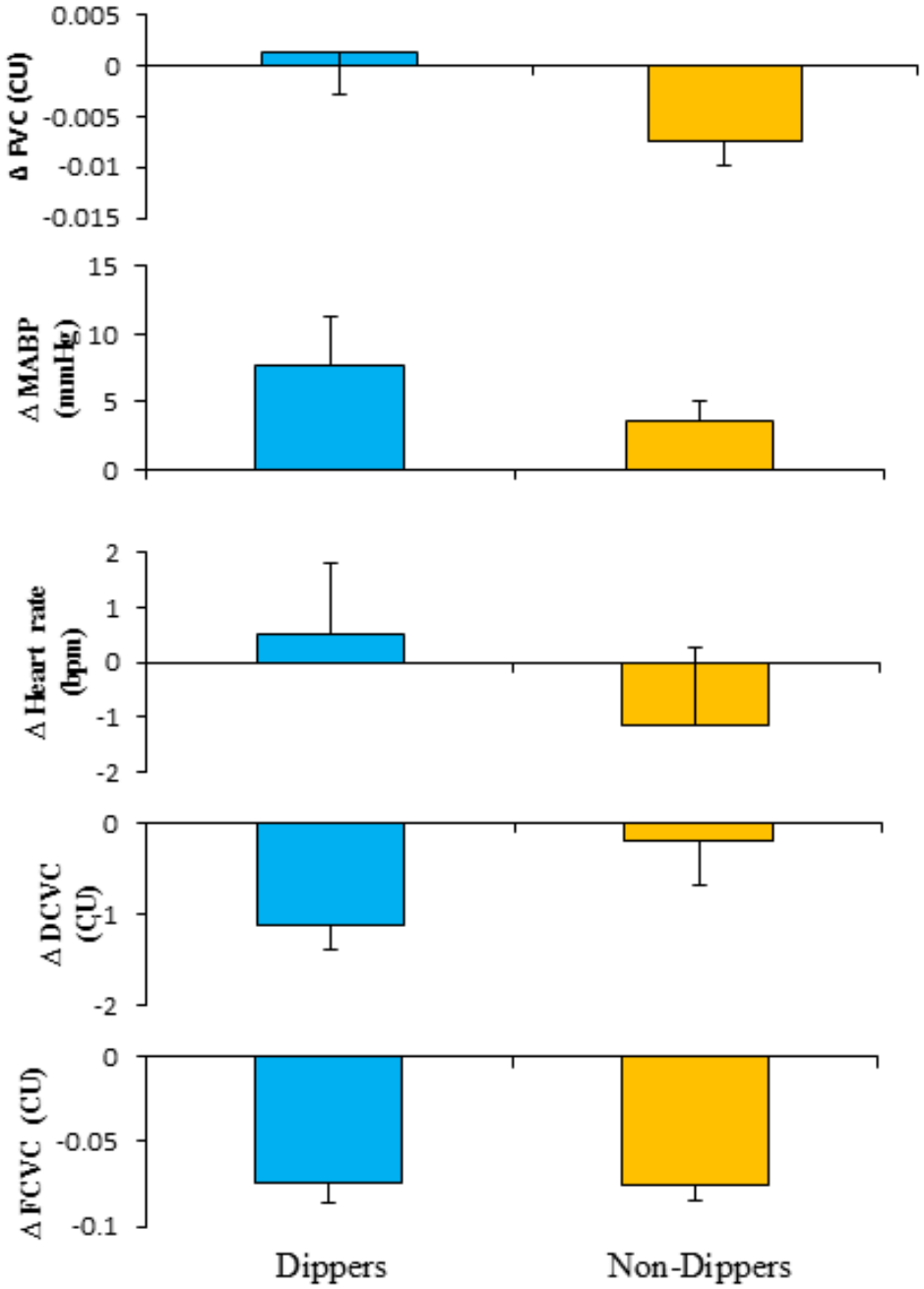


Figure 7.3: Mean of change from baseline values of FVC, MAP, HR DCVC and FCVC at 15s during sound 1-5 in normotensive dippers and non-dippers.
Values are mean ± SEM.

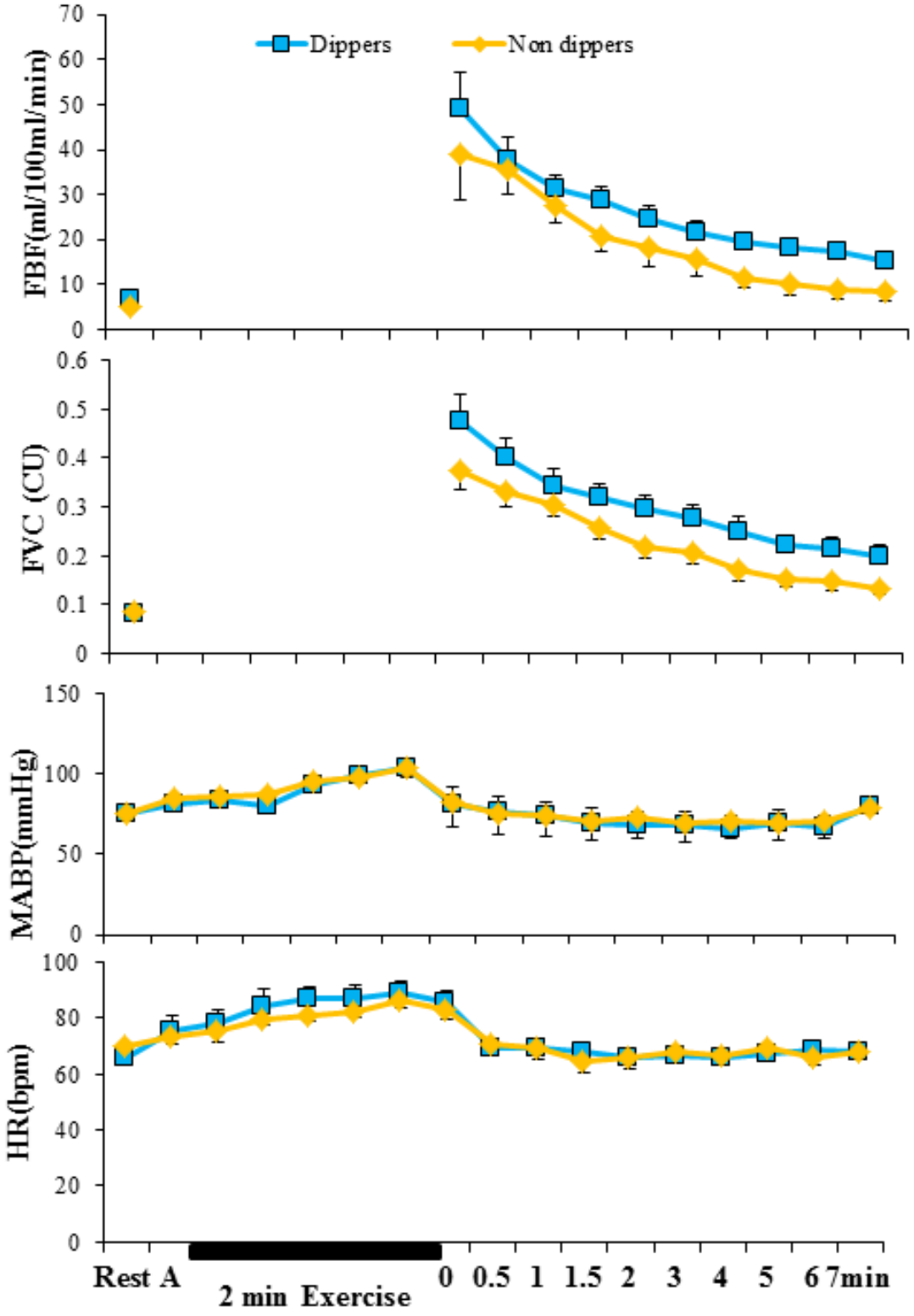


Figure 7.4: Forearm blood flow (FBF), Forearm vascular conductance (FVC), Mean ABP (MABP) and Heart rate (HR) before, during and after exercise in normotensive dippers and non-dippers. Values are mean ± SEM.

Chapter 8

Ethnic- and sex-dependent differences in reactive hyperaemia and responses evoked by Acetylcholine in cutaneous circulation

8.1 Introduction

The results of Chapter 3 showed blunted reactive hyperaemia in whole forearm circulation in BA men relative to WE men, but not in BA women relative to WE women. Chapter 5 indicated that in WE men only, but not in WE women, BA men or women, vasodilator PGs contribute to forearm reactive hyperaemia. However, whereas in some individuals of each ethnicity, COX inhibition attenuated reactive hyperaemia, in others, it was augmented implicating dilator and vasoconstrictor COX products respectively. Chapter 5 also indicated that vasodilator COX products contribute to both the forearm vasodilator and vasoconstrictor responses evoked by mental stress in BAs, but not in WEs; BA men predominantly showed forearm vasodilatation, whereas BA women mainly showed forearm vasoconstriction. Finally, Chapter 6 indicated that vasodilator PGs contribute to exercise hyperaemia in forearm in WE men and women, in BA men, but not in BA women. Thus, the contribution of COX products to various forearm vasodilator response differs both by ethnicity and sex.

Although reactive hyperaemia in forearm is widely used as a test of endothelial function (Anderson *et al.*, 2011). FBF assessed by venous occlusion plethysmography reflects behaviour mainly in resistance vessels of forearm muscle, but also cutaneous blood vessels. Indeed, reactive hyperaemia in forearm has been attributed to the release of vasoactive substances, including COX products, from skeletal muscle fibres as a consequence of ischaemia and from the endothelium, by ischaemia and shear stress (see Chapter 5). Further, forearm vascular responses to mental stress have been attributed mainly to substances released from the endothelium by the actions of shear stress, endothelium-derived acetylcholine (ACh) and circulating adrenaline. Accordingly, it was suggested in Chapter 5 that in BAs in particular, COX products are released from

the endothelium by these stimuli. On the other hand, exercise hyperaemia has been attributed to vasodilators, including PGs released from skeletal muscle fibres as well as endothelium. Thus, the results of Chapter 6 raised the possibility that BA women, either do not release PGs from these sites, or do not respond to them, whereas WE men and women and BA men do.

Aims:

The primary aim of the present study was to shed further light on the findings and proposals of previous Chapters by performing experiments on cutaneous microcirculation using laser Doppler fluximetry which allows endothelium dependent responses to be investigated more directly in the absence of significant influences from tissue parenchymal cells (Holowatz *et al.*, 2008). More specifically, the aims were to compare cutaneous vasodilator responses between BA and WE men and women during reactive hyperaemia and during dilatation evoked by endothelium-dependent dilator ACh, applied using iontophoresis. Furthermore, we aimed to assess the role of COX-dependent products in these responses by using oral aspirin**.**

Hypotheses:

1. Reactive hyperaemia and acetylcholine-induced vasodilation will be blunted in cutaneous circulation of BAs relative to WEs.
2. In WEs but not in BAs, men will show greater reactive hyperaemia and acetylcholine- induced vasodilation in cutaneous circulation relative to women.
3. In WE men and women, PGs will contribute to reactive hyperaemia and acetylcholine-induced vasodilation in cutaneous circulation.

   In BA men, but not BA women, PGs will contribute to reactive hyperaemia and acetylcholine vasodilation in cutaneous circulation.

4. The contribution of PGs to cutaneous reactive hyperaemia if present will be blunted in BAs relative to WEs.

8.2 Methods

Experiments were performed on 15 WE (7 men and 8 women) and 14 BAs (7 men and 7 women) aged 18-26 years. Questionnaires were completed by the subjects, and recordings were made as described in Chapter 2, except for iontophoresis: this is described below. The subject sat in a chair with arms rested on stable surfaces. Resting ABP and HR were recorded after 10 min of rest with an automated BP monitor. ABP and HR were then continuously recorded from a finger, by Finapres. Red blood cell flux (RCF) was recorded in perfusion units (PU), via a Laser Doppler probe placed on volar aspect of the forearm within the Perspex iontophoresis chamber (see Figure 8.11).

Cutaneous reactive hyperaemia

Following baseline recordings for 5 min, arterial occlusion of the forearm was induced by inflating a cuff wrapped around the upper arm to 200 mmHg for 2 minutes. Forearm skin RCF (FCRCF) was recorded immediately before and during reactive hyperaemia as peak RCF (the highest blood flow value after release of the cuff) and at 30s, 60s, 90s and 120s after release.

Acetylcholine iontophoresis protocol

Iontophoresis is a non-invasive method of transdermal delivery of charged molecules into the skin using a low-intensity electric current (Roustit & Cracowski, 2012). Iontophoresis was performed using a battery powered iontophoresis controller (MIC2, Moor Instruments, UK). The Perspex electrode chamber was attached to the volar surface of the forearm and connected to the anode. The chamber was filled with about 0.2 ml of 1% ACh prepared by dissolving 100 mg of ACh (Sigma-Aldrich, UK) in 10

ml of deionised water. Deionised water has minimal non-specific vasodilator effects (Loader *et al.*, 2017). The laser Doppler probe was inserted into the Perspex electrode chamber, an indifferent electrode was placed on the volar aspect of the forearm or wrist of the subject. A second Doppler probe was placed within 1-2 cm of the outer perimeter of the Perspex ring to assess whether the response evoked within the ring spread to the unstimulated area, see Figure 8.1 (Durand *et al.*, 2004). ACh was delivered using an anodal current: 6 pulses of 0.1 mA for 20 s, followed by one pulse of 0.2 mA for 20 s, with 60 s between each dose with total charge of 16 millicoulombs (mC) (Hendry & Marshall, 2004). FCRCF was recorded immediately before iontophoresis and after each pulse at40-60s following each pulse when the response to ACh was maximal. The protocol is shown below (Figure 8.1)

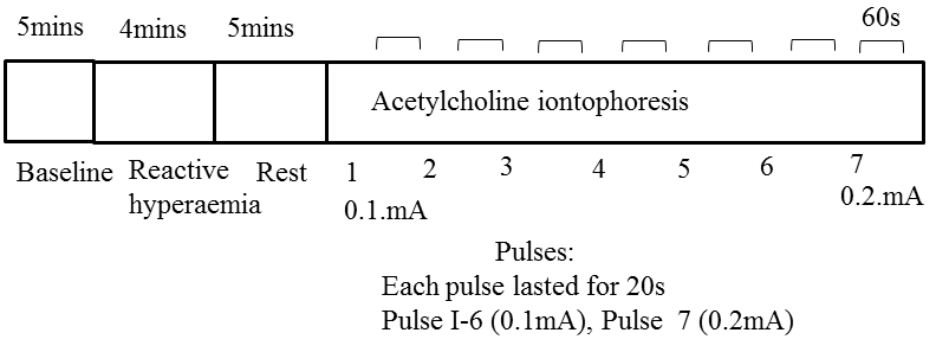


Figure 8.1 Baseline period was followed by 2 minutes of vascular occlusion and recording of reactive hyperaemia, after a period of rest, Ach was delivered by 7 pulses, each lasting for 20s with 60s interval.

Following the completion of the control experiment using the protocol above, the subject then consumed orange squash containing 600mg aspirin. The whole protocol was repeated on the same day, 30 min after aspirin drink was consumed, when COX inhibition is maximal (Heavey *et al.*, 1985). Both reactive hyperaemia and responses

evoked by ACh were recorded from different sites on the same forearm. Original recordings made during reactive hyperaemia and ACh-induced responses are shown in Figures 8.12- 8.14.

Data analysis

Anthropometric and baseline data were compared by Student's T tests as appropriate. For reactive hyperaemia, change in FCRCF was calculated by subtracting baseline FRCF from FCRCF recorded at peak of the response and at 30s, 60s, 90s and 120s following release of occlusion. Similarly, responses evoked by ACh were calculated as change from baseline FRCF to the maximal FRCF value following each iontophoretic pulse. In addition, a compacted mean was calculated for each subject as the average of the ΔFCRCF values recorded during the train of pulses (Hirst & Marshall, 2018). Absolute differences between ΔFCRCF before and after COX inhibition were determined, subjects with reduced FCRCF were categorised as COX+ responders and those with increased FCRCF were categorised as COX- responders. Scattergraphs of individual responses to COX inhibition at peak of reactive hyperaemia as well as for the compacted mean of Ach responses for each individual were plotted. Effect of COX inhibition between groups of responders was tested with univariate ANOVA.

To determine ethnic/sex differences, 3-way mixed ANOVAs with time as the within subjects factor with ethnicity and sex as the between subjects factors were done. For reactive hyperaemia there were 5 time points and for Ach mediated responses there were 7 time points. Bonferroni post hoc tests were done as appropriate.

To determine effect of COX inhibition on reactive hyperaemia and Ach mediated responses, 3-way mixed ANOVA with treatment and time as within subject factors and ethnicity with sex or COX responder groups as the between subjects factors. Bonferroni

post hoc tests were done as appropriate. Differences between the COX responder groups for peak of reactive hyperaemia and for compacted mean of Ach responses during control experiments were determined with 2-way ANOVA. Peak reactive hyperaemia and compacted means for ACh responses were compared between groups before and after COX inhibition by 3-way mixed ANOVA with ethnicity and sex as between factors and treatment as within subjects factor. In all cases, $p < 0.05$ was considered significant.

8.3 Results

Baseline anthropometric and cardiovascular variables were not different between WEs and BAs except that WE men were older than BA men (Table 8.1).

FCRCF values before aspirin were not significantly different between the full groups of WEs and BAs (14.05±2.16 vs 12.57±2.02 PU, p=0.62), between WE and BA males (18.92±3.75 vs 14.85±3.43 PU, p=0.44) or between WE and BA females (9.79±1.19 vs 10.30±2.03 PU, p=0.83). However, WE men showed higher FCRCF than women (18.92±3.75 vs 9.79±1.19 PU, p=0.03), while there was no difference between BA men and women (14.85±3.43 vs 10.30±2.03 PU, p=0.28).

Reactive hyperaemia

There was no significant 3-way interactions between time, sex and ethnicity (p>0.05). There was no significant interaction between time and sex or between ethnicity and sex (p>0.05). There was a significant interaction between effect of sex and ethnicity on ΔFCRCF ($F(1,25)=4.57$, p=0.04, partial $\eta^2=0.15$). Simple main effect of ethnicity on ΔFCRCF was significant at peak, 30s, 60s, 90s and 120s (p<0.05 in each case). Bonferroni corrections were made with comparisons within the simple main effect. The whole group of BAs showed smaller reactive hyperaemia than WEs (Figure 8.2); at peak reactive hyperaemia (68.95±9.58 vs 50.48±3.95, p=0.01) and whole of reactive hyperaemia ($F(1,27)=13.37$, p=0.001, partial $\eta^2=0.33$). Simple main effect was significant in men ($F(1,27)=15.68$, p=0.001, partial $\eta^2=0.39$) but not in women (p>0.05). BA men showed smaller total reactive hyperaemia than WE men (Figure 8.2), with smaller peak values in BA men (90.47±15.19 vs 51.25±5.99), whereas BA and WE women showed similar reactive hyperaemia (Figure 8.2). Considering sex differences, WE women showed smaller total reactive hyperaemia than WE men (p<0.05, Figure

8.1), peak values being smaller in WE women (90.47±15.19 vs 50.11±7.92), but there was no difference between BA men and women (Figure 8.1; peak values 51.25±5.99 vs 49.71±5.62; p= 0.67).

Effect of COX inhibition on cutaneous reactive hyperaemia

There was no significant 3-way, 2-way interaction or main effect of treatment with aspirin on reactive hyperaemia ($p>0.05$). COX inhibition did not attenuate the whole reactive hyperaemia or peak responses in full groups of WEs or BAs or in male and female groups whether comparisons were made within or between ethnicities (Figure 8.3). However, within each ethnicity, in some individual subjects COX inhibition attenuated reactive hyperaemia and in others it augmented the response (Figure 8.4).

Amongst WEs and BAs, there were some individuals in whom COX inhibition attenuated peak reactive hyperaemia (COX+ responders) and others in whom the response was augmented (COX- responders). Among the WEs 8 (61.5%) were COX+ and 5 (38.5%) were COX-. Among the BAs, 8 (61.5%) were COX+ and 5 (38.5%) were COX-. There were no significant differences in the proportions ($p>0.05$). When subjects were grouped as COX positive responders and COX negative responders respectively, WE COX- responders showed smaller reactive hyperaemia than WE COX+ responders ($F(1,22)=26.53$, $p<0.0001$, partial $\eta^2=0.55$, whereas there was no difference between BA COX+ and COX- responders (Figure 8.5).

Within ethnicity, both WE and BA COX+ responders showed significant attenuation of peak reactive hyperaemia following COX inhibition ($F(1,22)=68.45$, $p<0.0001$, partial $\eta^2=0.78$): $F(1,22)=4.62$, $p=0.04$, partial $\eta^2=0.174$). COX- responders showed significant accentuation of peak reactive hyperaemia in WE COX- responders from

($F(1,22)=27.08$, $p<0.0001$, partial $\eta^2=0.17$) as well as in BA COX- responders ($F(1,22)=27.08$, $p=0.02$, partial $\eta^2=0.24$), (Figure 8.5).

Responses evoked by ACh

There was no significant 3-way interactions between time, treatment, sex and ethnicity ($p>0.05$). There was no significant interaction between time and sex or between ethnicity and sex ($p>0.05$). There was significant 2-way interaction between ethnicity and sex on ΔFCRCF ($F(1,25)=4.676$, $p=0.04$, partial $\eta^2= 0.16$). The simple main effect ethnicity was significant in men ($F(1,25)=9.64$, $p=0.005$, partial $\eta^2=0.28$) but not women ($p>0.05$). Bonferroni corrections were made with comparisons within the simple main effect. As shown in Figure 8.7, the full group of BAs showed smaller increases in FCRCF in response to ACh than WEs ($F(1,27)=10.92$, $p=0.003$, partial $\eta^2=0.29$). In addition, the simple main effect sex was significant in BAs but not in WEs ($F(1,25)=5.59$, $p=0.03$, partial $\eta^2= 0.18$). Bonferroni post hoc test was significant for each pulse, $p<0.05$). BA men but not BA women showed smaller increases in FCRCF than WE men and women respectively ($p<0.05$ at each time point for men Figure 8.7). Whereas WE men and women showed similar ACh-induced dilator responses, BA men showed smaller ACh-induced dilatation than BA women ($p>0.05$ Figure 8.7).

Effect of COX inhibition on ACh-induced responses

There was no significant 3-way, 2-way interaction or main effect of treatment with Aspirin on Ach induced vasodilation during the 7 pulses ($p>0.05$). In the full groups of WEs and BAs and in men and women in each ethnic group, COX inhibition had no effect on ACh-induced vasodilation (Figure 8.8). Amongst the WEs and BAs, there were some individuals in whom COX inhibition attenuated the compacted mean ACh responses and others in whom the ACh response was augmented (COX-, COX+

responders respectively). Among the WEs, 8 (61.5%) were COX+ and 5 (38.5%) were COX-. Among the BAs, 6 (46.2%) were COX+ and 7 (53.8%) were COX-. There were no significant differences in the proportions ($p>0.05$). Figure 8.8 shows compacted mean responses in each individual before and after COX inhibition. In WEs and BAs, there were no significant differences in the responses to the train Ach pulses between the COX responders ($p>0.05$). There was significant 2-way interaction between treatment with aspirin and COX responder groups ($F(3,22)=7.58$, $p=0.001$, partial $\eta^2=0.51$). In WE and BA COX+ responders, COX inhibition significantly attenuated Ach mediated vasodilation during the train of 7 pulses ($p<0.05$) and accentuated the responses in WE COX- responders at pulse 3 and 4 only ($p<0.05$) but not in BAs ($p>0.05$, Figure 8.10).

When considered as compacted mean of responses to the Ach, COX inhibition had no effect on ACh-induced vasodilation in groups of WE men and women ($p>0.05$). WE and BA COX- responders did not showed blunted compacted mean ACh responses relative to the respective COX+ responders ($p>0.05$). When the compacted mean Ach responses were considered, there was significant 2-way interaction between treatment with aspirin and COX responder groups ($F(3,22)=7.78$, $p=0.001$, partial $\eta^2 =0.52$). In addition, COX inhibition attenuated the mean responses in WE and BA COX+ ($F(1,22)=6.63$, $p=0.02$, partial $\eta^2=0.23$; ($F(1,22)=11.37$, $p=0.003$, partial $\eta^2=0.34$ respectively). COX inhibition accentuated the mean responses in WE COX- ($F(1,22)=4.32$, $p=0.05$, partial $\eta^2 =0.16$) but in BA COX- responders the differences did not reach statistical significance.

8.4 Discussion

The premise of the present study was that investigation of cutaneous microcirculation would give insight into ethnic- and sex-dependent differences in endothelium-dependent responses and the contribution of COX products to these responses, with negligible interference from substances released from parenchymal cells. Indeed, it was argued that skin microcirculation provides a generalised model of microcirculation that can be used to study normal endothelial function and dysfunction (Holowatz *et al.*, 2008).

The main findings of the present study were that the full group of young BA men and women showed blunted reactive hyperaemia and blunted ACh-induced dilator responses in cutaneous circulation relative to young WE men and women. These findings were attributable to BA men showing blunted dilator responses relative to WE men; BA and WE women showed similar responses to both stimuli. This accords with the finding of Chapter 3 that BA men showed blunted reactive hyperaemia in whole forearm relative to WE men and substantiates our hypothesis that endothelial dysfunction occurs in young BA men but not young BA women. This is consistent with previous reports that young BA men show blunted reactive hyperaemia (Berardesca & Maibach, 1989) and blunted dilator responses to iontophoresis of ACh (Maley *et al.*, 2017). As far as we are aware, ours is the first evidence that young BA women show similar cutaneous reactive hyperaemia and ACh-induced responses as WE women.

8.4.1 Mechanisms underlying cutaneous vasodilatation

In the skin, nitric oxide (NO) does not contribute to reactive hyperaemia, evidenced by lack of attenuation of cutaneous reactive hyperaemia by inhibition of NOS (Wong *et al.*, 2003) and failure of arterial occlusion to release NO concentration into the skin (Zhao *et al.*, 2004). NOS inhibition did not attenuate ACh-induced vasodilatation in cutaneous

circulation (Zhao *et al.*, 2004; Holowatz *et al.*, 2005). Thus, it seems unlikely NO contributed to reactive hyperaemia or ACh-induced dilatation in cutaneous circulation of WEs or BAs, particularly as NO-dependent dilatation is impaired in young BAs (Stein *et al.*, 1997).

Whether or not PGs play a role in reactive hyperaemia in cutaneous circulation has been debated. COX inhibition with intravenous lysine acetylsalicylate did not attenuate cutaneous reactive hyperaemia in young men (Dalle-Ave *et al.*, 2004; Addor *et al.*, 2008), while others reported intradermal ketoprofen had no effect and that a meta-analysis of published studies revealed no effect of COX inhibition on peak reactive hyperaemia (Hellmann *et al.*, 2015). However, aspirin blunted cutaneous reactive hyperaemia in a mixed group of men and women (Binggeli *et al.*, 2003), and in young WE and SA men with normotensive parents, but not in those with hypertensive parents (Hirst & Marshall, 2018). Further, intradermal ketorolac augmented cutaneous reactive hyperaemia in 12 young men and women of unspecified ethnicity (Medow *et al.*, 2007). That aspirin did not attenuate cutaneous reactive hyperaemia in the full groups of BAs or WEs, nor in men and women of either ethnic group is consistent with these variable findings, particularly as more detailed analyses showed that COX inhibition attenuated peak reactive hyperaemia in some WEs and BAs (COX+) and that in some individuals reactive hyperaemia was augmented (COX-). These results suggest the apparent lack of effect of COX inhibition in the full groups of both WEs and BAs was attributable to the opposing effects of COX inhibition in individuals. Since in the absence of COX inhibition, reactive hyperaemia was markedly different between COX+ and COX- WEs, but not in COX+ and COX- BAs and given dilator PGs caused less attenuation in COX+ BAs than COX+ WEs (Figure 8.3), it seems that dilator PGs make less

contribution to reactive hyperaemia in BAs than WEs. Thus, the present findings support the proposals of Chapter 5 that PGs released from endothelium contribute to reactive hyperaemia in the whole forearm circulation of COX+ WEs and BAs and that dilator PGs released by shear stress contribute to the forearm vasodilator response to mental stress in BAs.

The reported effect of COX inhibition on ACh-induced vasodilation in cutaneous circulation is similarly variable. COX inhibition had no effect on cutaneous vasodilatation induced by ACh iontophoresis in men aged 34.8 ± 7.6 years (Morris & Shore, 1996), in men aged 19-31 years (Dalle-Ave *et al.*, 2004) or in a mixed group of men and women aged 19-28 years (Berghoff *et al.*, 2002). In contrast, responses evoked by ACh iontophoresis were attenuated by oral aspirin in young WE and SA men with normotensive parents but not in those with hypertensive parents (Hirst & Marshall, 2018), and by intravenous aspirin in men aged 23–36 years whose ethnicity and familial hypertension was not mentioned (Noon *et al.*, 1998). Against this background, since aspirin did not affect cutaneous vasodilatation induced by iontophoresis of ACh in the full groups of WE or BA subjects, or in men or women within each ethnicity, it could be suggested that COX products play no role in dilatation evoked by stimulation of endothelial receptors. However, there were individual WEs and BAs in whom COX inhibition attenuated or augmented ACh-induced responses (COX+; COX- respectively). Thus, in the COX+ BAs and indeed WEs, PGs contribute to ACh-induced dilatation (Figure 8.9). Thus it remains a possibility that the attenuating effect of COX inhibition on the forearm vasodilator responses to mental stress in BAs (Chapter 5) is partly attributable to locally released ACh, but it seems unlikely this is a

major contribution: PGs released by adrenaline acting on endothelial beta adrenoceptors (Chapter 5) or by shear stress (see above) may be more important.

8.4.2 Conclusions

The blunted cutaneous reactive hyperaemia as well as Ach mediated dilation in black men relative to WE men but not in BA women relative to WE women is consistent with findings on whole forearm reactive hyperaemia (Chapter 3) and exercise hyperaemia (Chapter 6). Thus, these findings provide evidence that support the view that BA women do not show evidence of endothelial dysfunction except during mental stress. This suggests that mental stress is a specific stimulus that evokes endothelial dysfunction in BA women which can predispose them to stress induced CVD. The present study did not demonstrate role of PGs in cutaneous reactive hyperaemia or Ach evoked vasodilation in whole groups of WEs or BAs, however within each ethnic group are subjects who show contribution of PGs consistent with the findings of different groups of responders to effect of COX inhibition on whole forearm reactive hyperaemia (Chapter 5).

8.4.3 Limitations

A larger number of WE and BA subjects would have been required to allow more rigorous statistical analyses of the effects of COX inhibition. Given parental history of hypertension predicted the contribution of COX products to cutaneous responses (Hirst & Marshall, 2018), further studies should be carried out on BAs and WEs with and without parental history of hypertension.

Table 8.1 Baseline values of anthropometric and cardiovascular variables in WEs and BAs.

				Men			Women		
	All WEs (n=15)	**All BAs (n=14)**	**p value**	**WEs (n=7)**	**BAs (n=7)**	**p value**	**WEs (n=9)**	**BAs (n=7)**	**p value**
Age (years)	22.86±0.56	21.33±0.78	0.13	24.29±0.64	20.00±0.47	0.00	21.43±0.53	22.33±1.19	0.51
BMI (kg/m^2)	22.81±0.82	22.68±0.71	0.91	22.81±0.82	22.68±0.71	0.91	22.81±0.82	22.68±0.71	0.91
SBP (mmHg)	114.32±3.07	112.99±2.82	0.76	119.64±4.12	119.86±3.39	0.97	109.00±3.81	104.97±1.33	0.37
DBP (mmHg)	75.02±1.82	70.06±1.75	0.07	75.42±2.62	71.76±1.93	0.30	74.62±2.73	68.08±2.83	0.14
HR (bpm)	72.13±2.52	66.64±1.28	0.07	69.69±3.96	65.48±1.83	0.36	74.57±3.13	68.00±1.62	0.11
MAP (mmHg)	88.12±2.84	84.37±2.47	0.16	90.16±3.96	87.79±2.82	0.51	86.08±4.06	80.38±2.00	0.12
FCRCF(ml/dl/min)	14.05±2.16	12.35±1.88	0.57	18.92±3.75	14.41±3.07	0.38	9.79±1.19	10.30±1.88	0.83

Values are shown as mean ± SEM, *p<0.05: WE vs BAs, independent Student's T test.

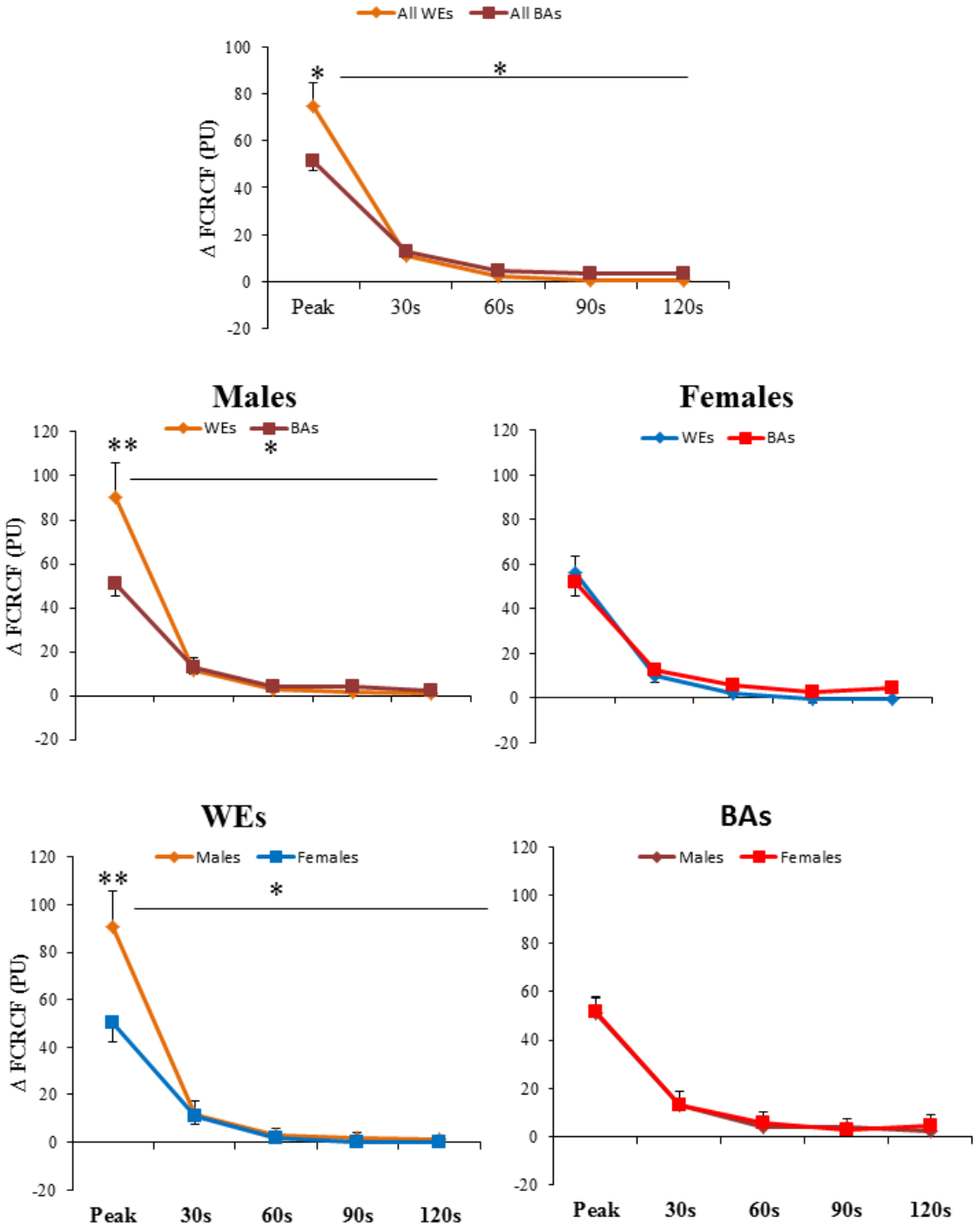


Figure 8.2: Change from baseline values of FCRCF during reactive hyperaemia in groups of White Europeans (WEs) and Black Africans (BAs). Values are mean ± SEM. * $p < 0.05$, **$p<0.005$: 3-way mixed ANOVA with Bonferroni post hoc test.

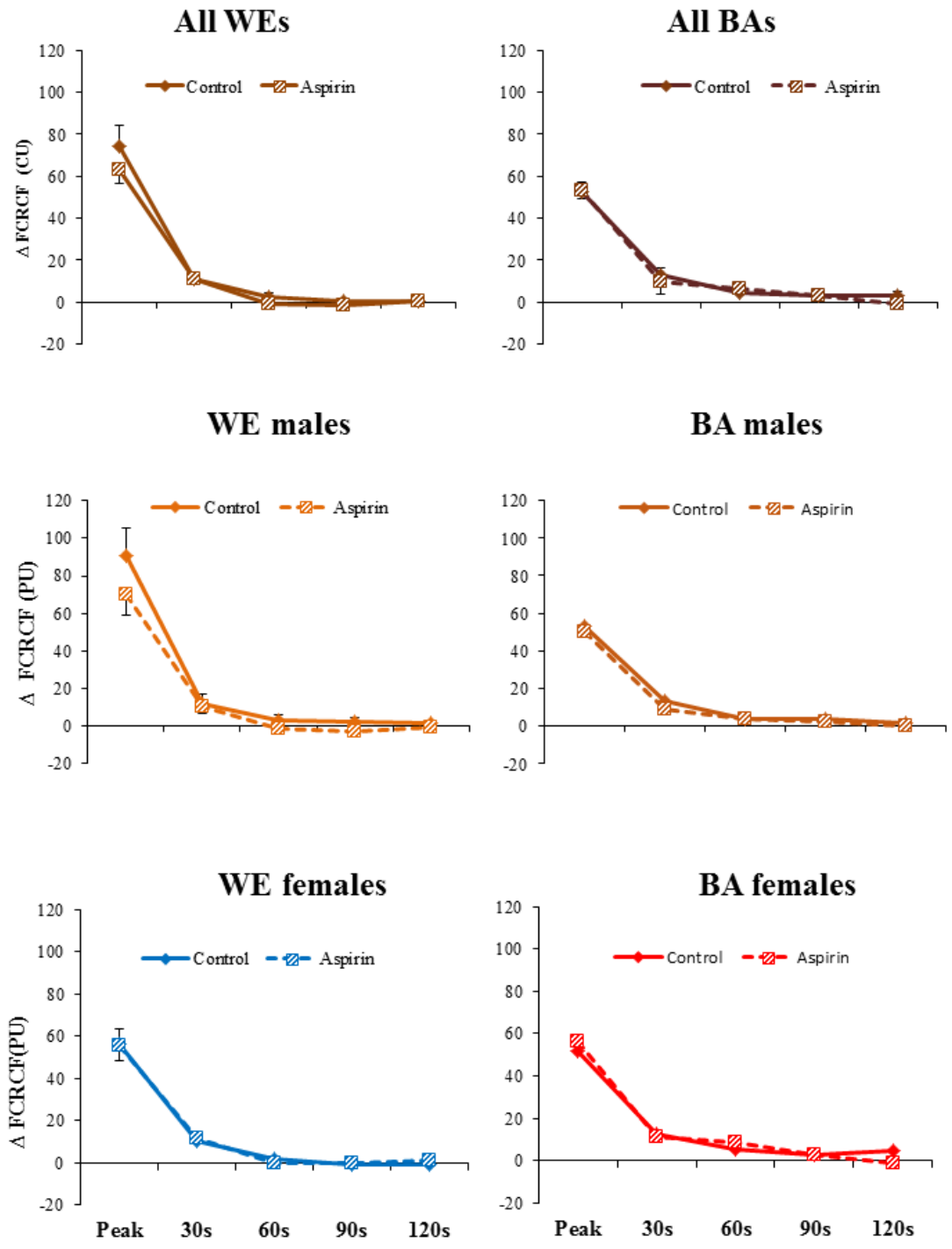


Figure 8.3: Change from baseline values of FCRCF before and following COX inhibition with Aspirin during reactive hyperaemia in groups of White Europeans (WEs) and Black Africans (BAs). Values are mean ± SEM.

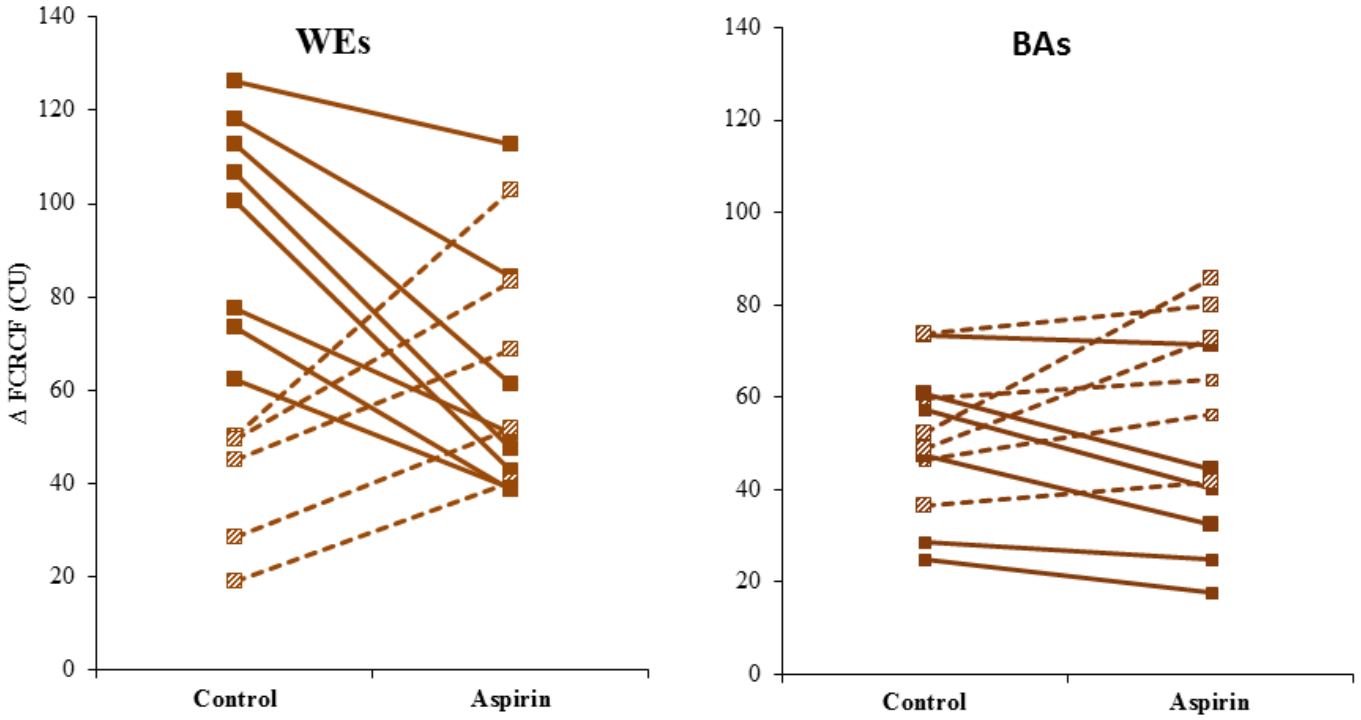


Figure 8.4: Change from baseline values of FCRCF at peak of cutaneous reactive hyperaemia before and after COX inhibition with Aspirin in WEs and BAs. Reduction in FCRCF (COX+ responders) represented by unbroken lines and accentuation in FCRCF (COX- responders) represented by broken lines. Values are mean ± SEM.

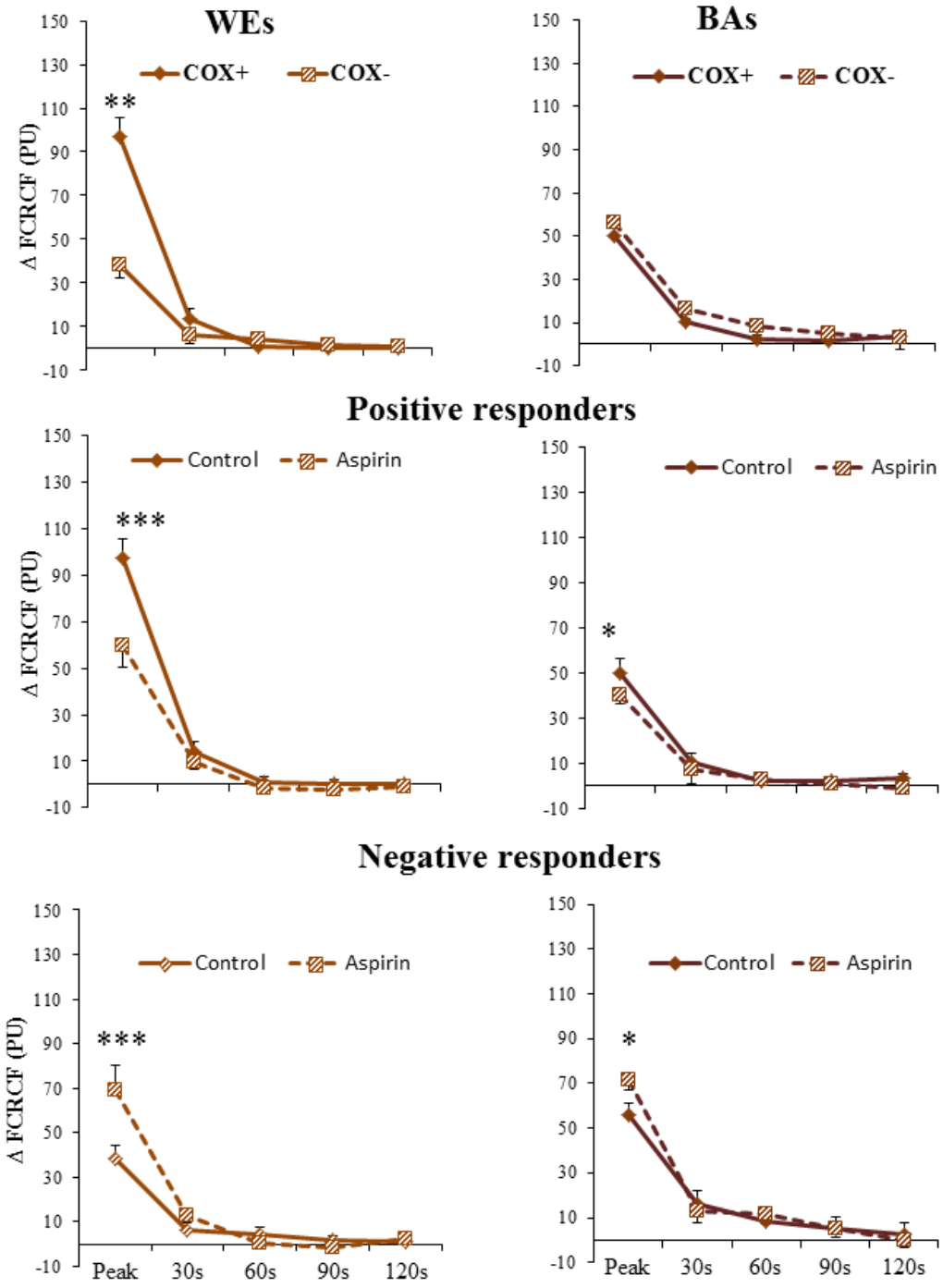


Figure 8.5: Change from baseline values of FCRCF before and following COX inhibition with Aspirin during reactive hyperaemia in COX+ and COX- groups of WEs and BAs. Values are mean ± SEM. * $p < 0.05$: control vs aspirin, 3 way mixed ANOVA with Bonferroni post hoc test.

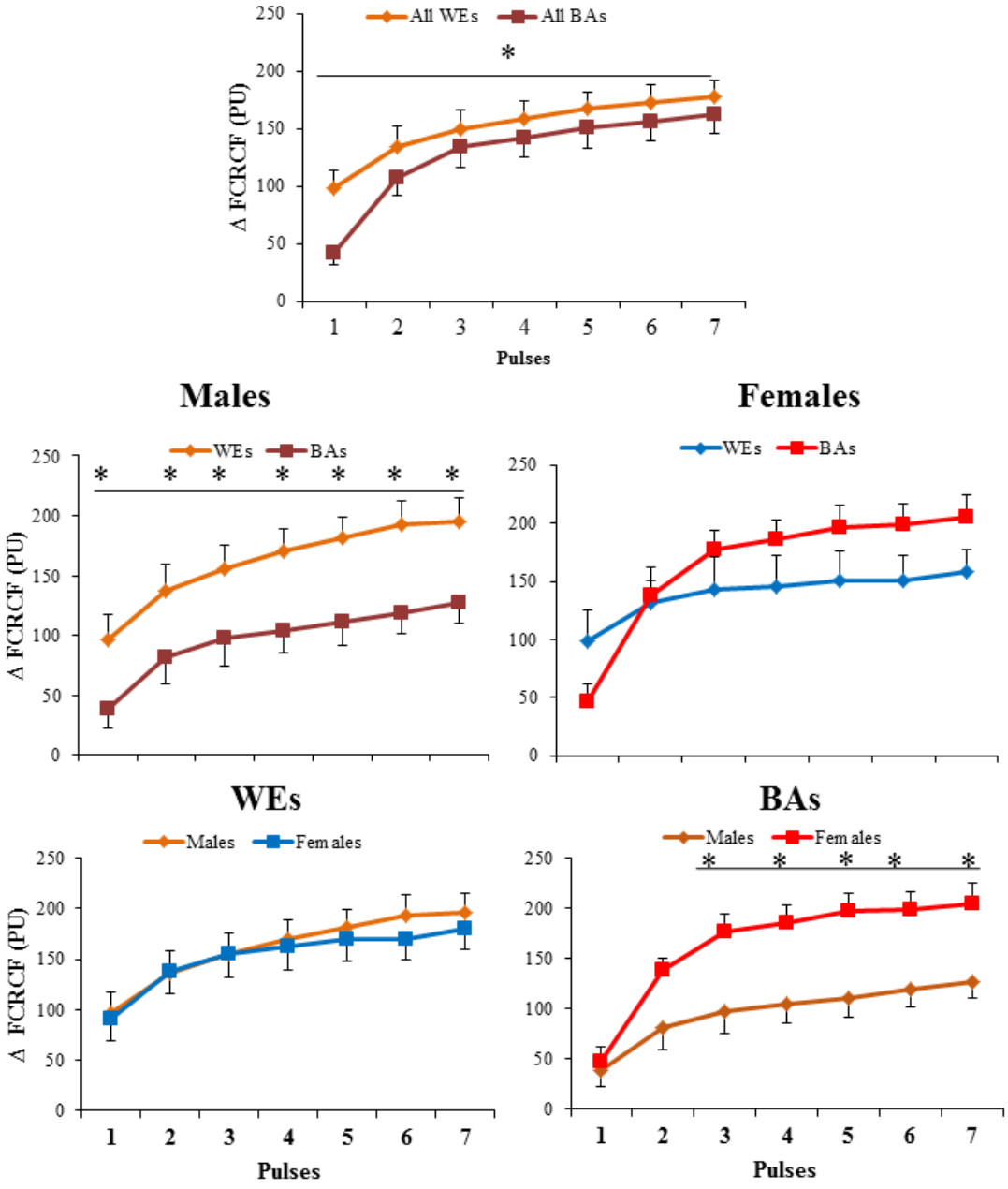


Figure 8.6: Change from baseline values of FCRCF during Acetylcholine iontophoresis in groups of White Europeans (WEs) and Black Africans (BAs). Values are mean ± SEM. * p <0.05, 3-way mixed ANOVA with Bonferroni post hoc test.

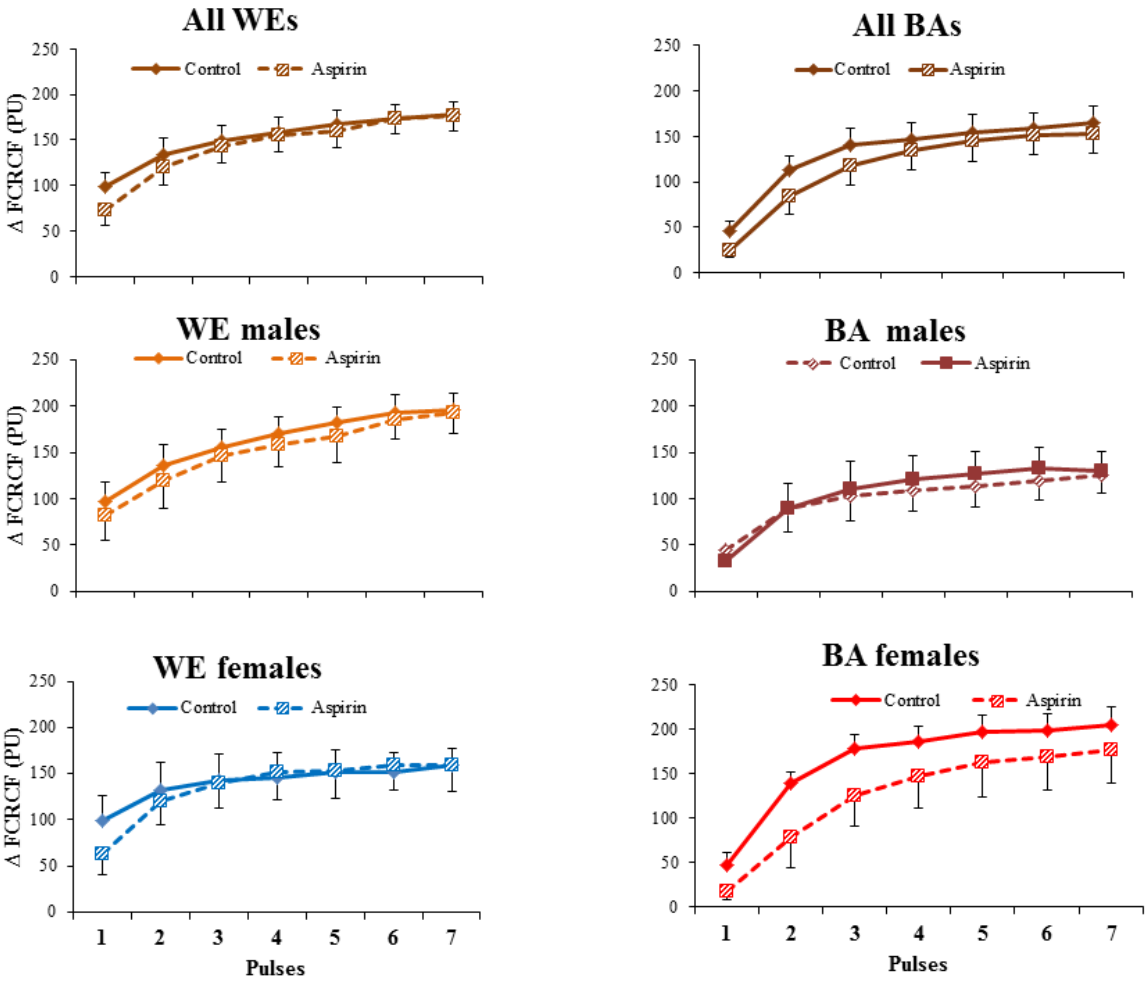


Figure 8.7: Change from baseline values of FCRCF before and after COX inhibition with Aspirin during iontophoresis of acetylcholine in groups of WEs and BAs. Values are mean ± SEM.

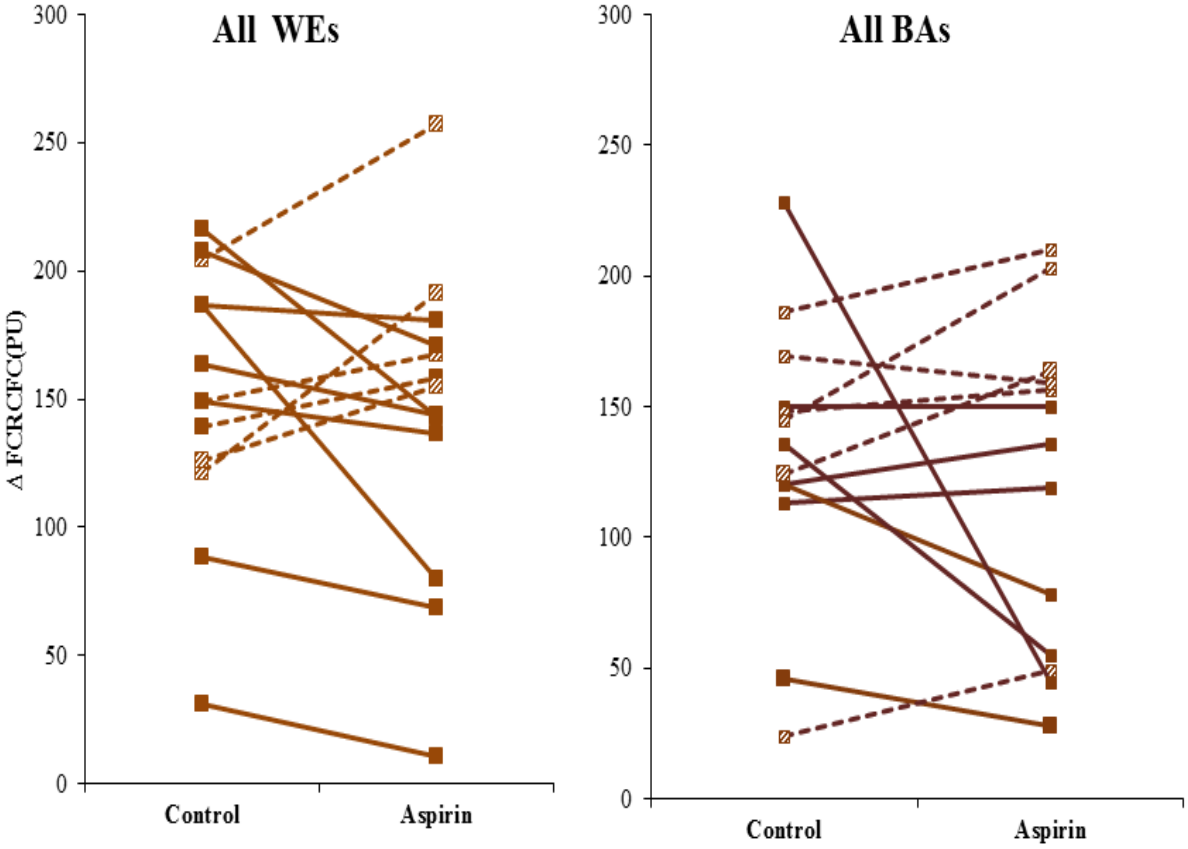


Figure 8.8: Compacted mean of change from baseline values of FCRCF responses to 7 pulses of Ach iontophoresis before and after COX inhibition with Aspirin in WEs and BAs. Reduction in FCRCF (COX+ responders) represented by unbroken lines and accentuation in FCRCF (COX- responders) represented by broken lines. Values are mean ± SEM.

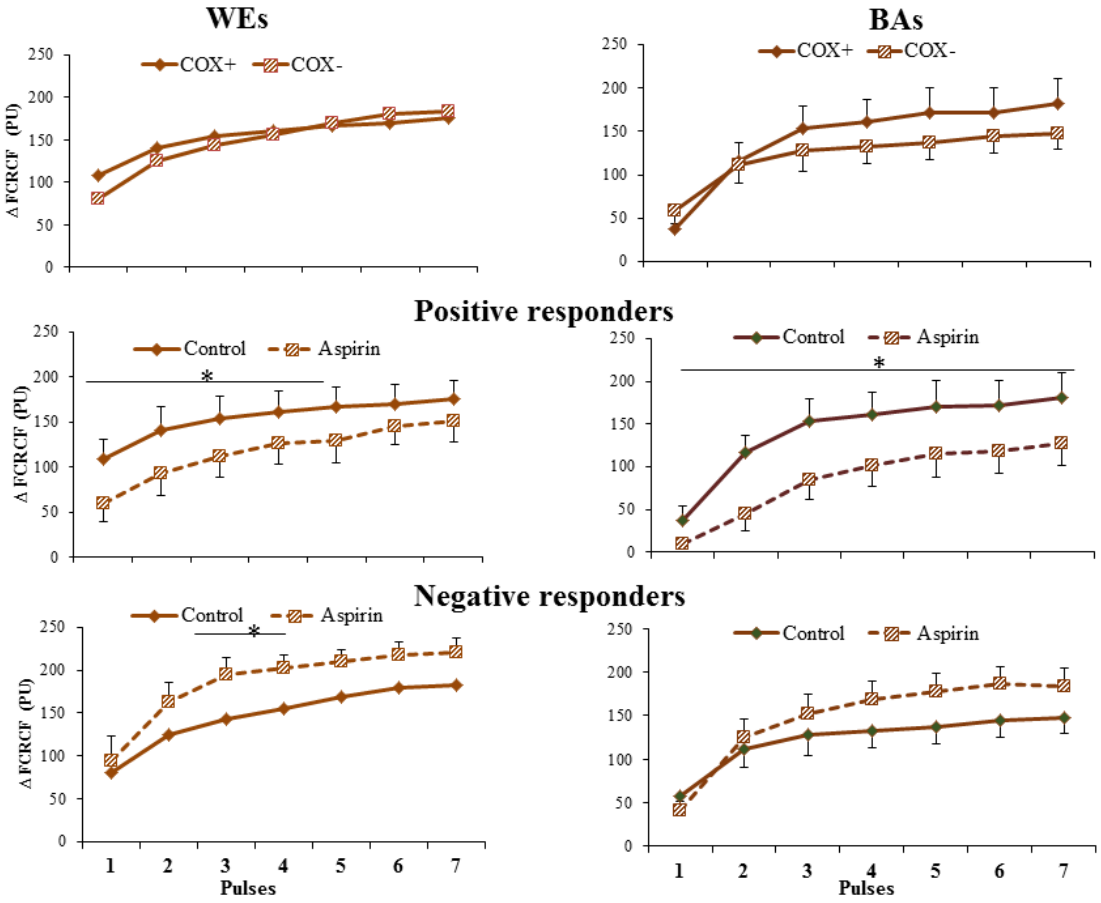


Figure 8.9: Change from baseline values of FCRCF before and after COX inhibition with Aspirin during iontophoresis of acetylcholine in COX responder groups of WEs and BAs. Values are mean ± SEM. *p <0.05: control vs Aspirin, 3-way mixed ANOVA with Bonferroni post hoc test:

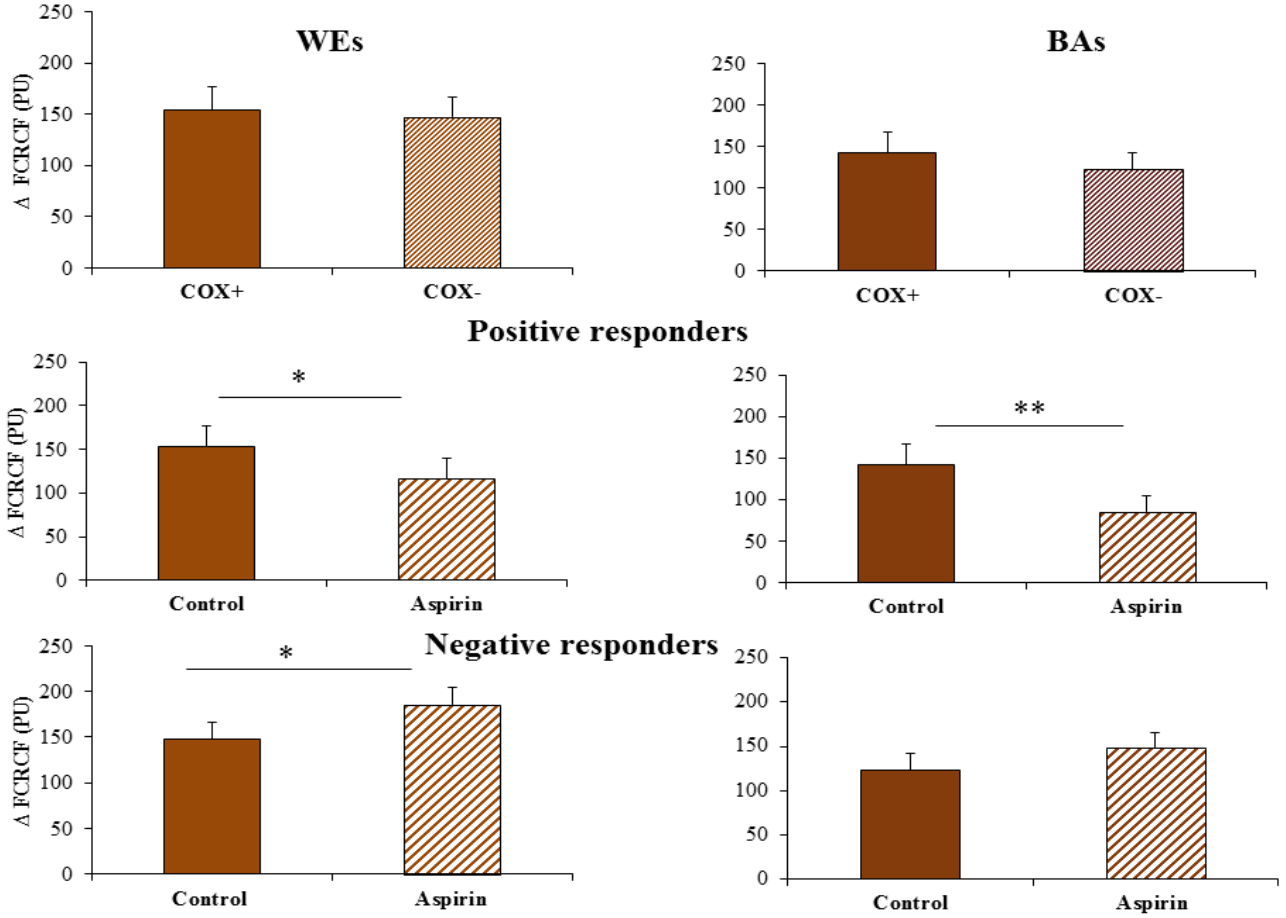


Figure 8.10: Compacted mean of change from baseline values of FCRCF during iontophoresis of acetylcholine in COX responder groups of WEs and BAs and before and after COX inhibition with Aspirin in COX responder groups. Values are mean ± SEM. *p <0.05: control vs Aspirin* p <0.05: 2 way ANOVA with Bonferroni post hoc test.

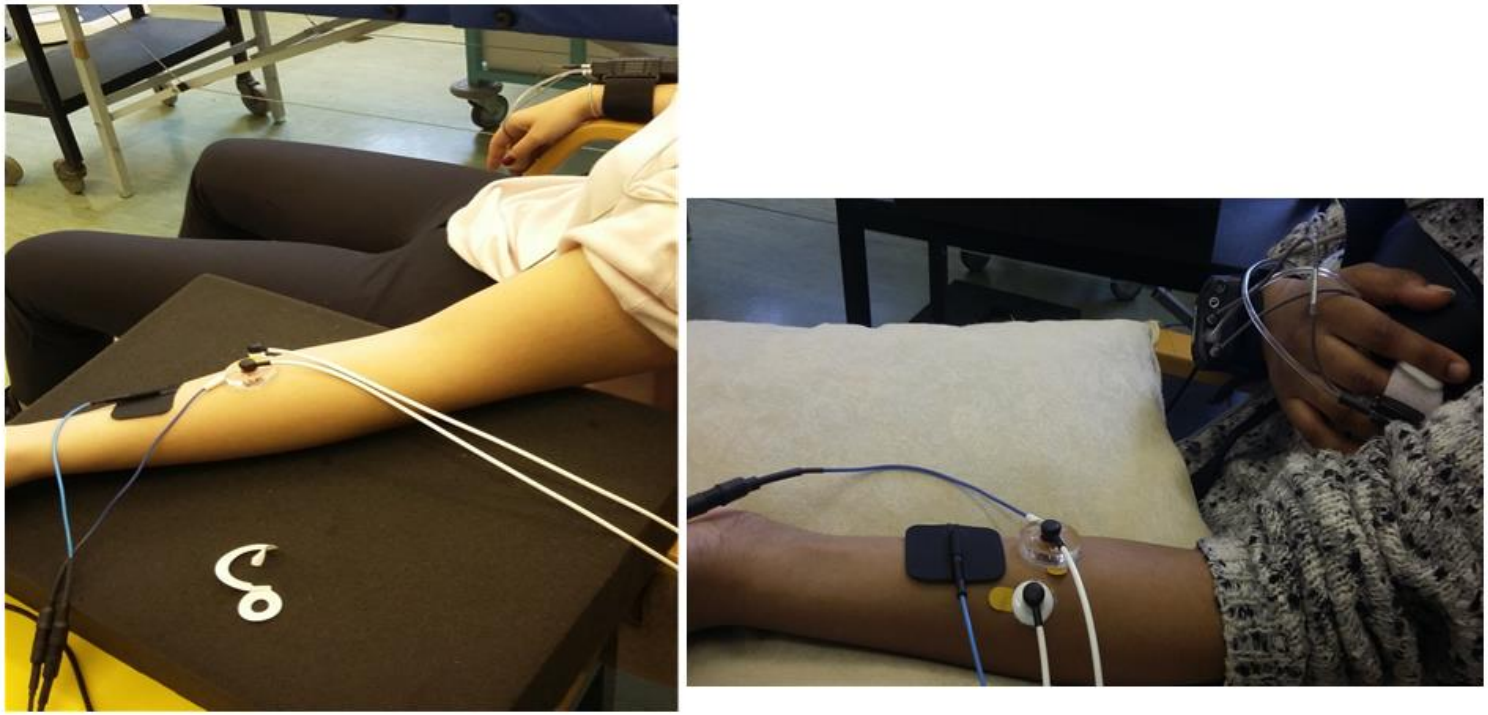


Figure 8.11: Subjects with iontophoresis chamber and electrodes on forearm during the experiments.

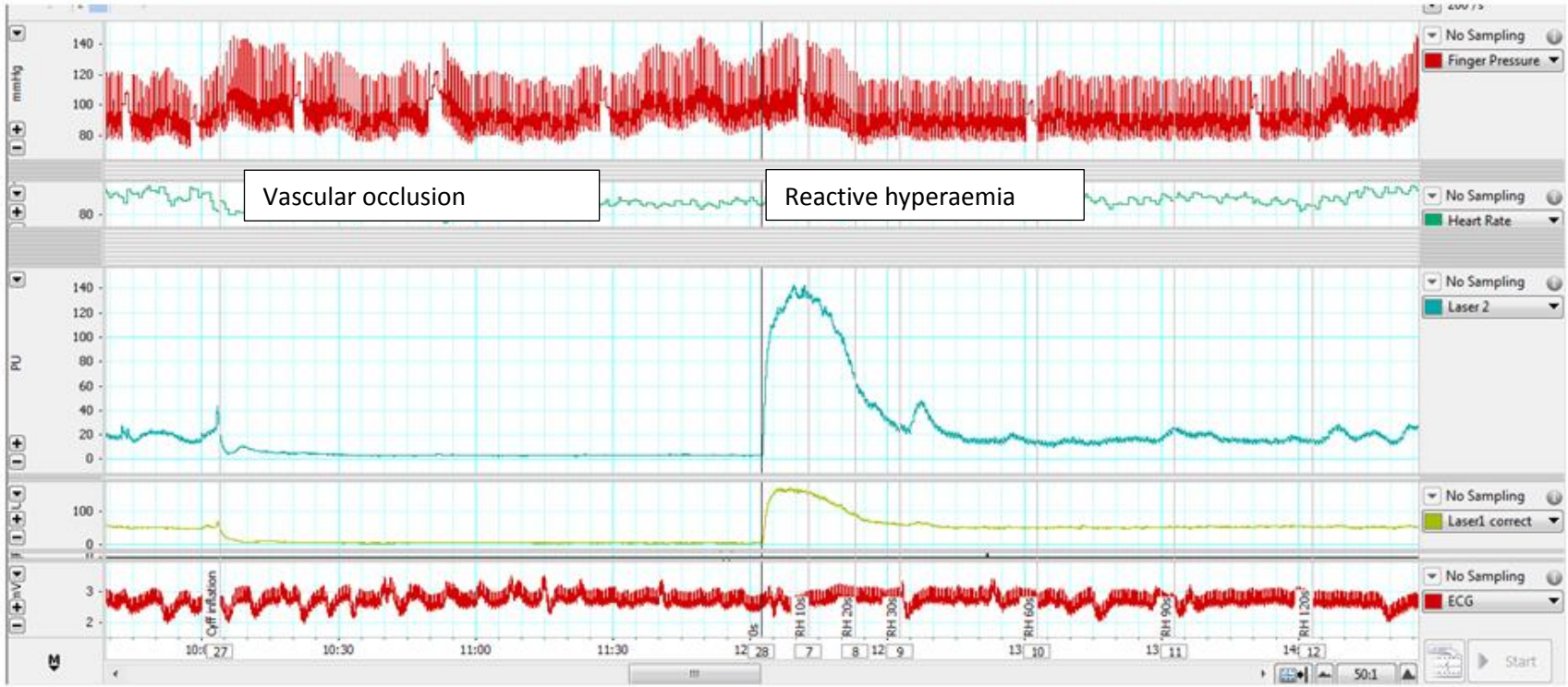


Figure 8.12: Original tracing showing cutaneous vascular responses to 2 minutes of arterial occlusion.

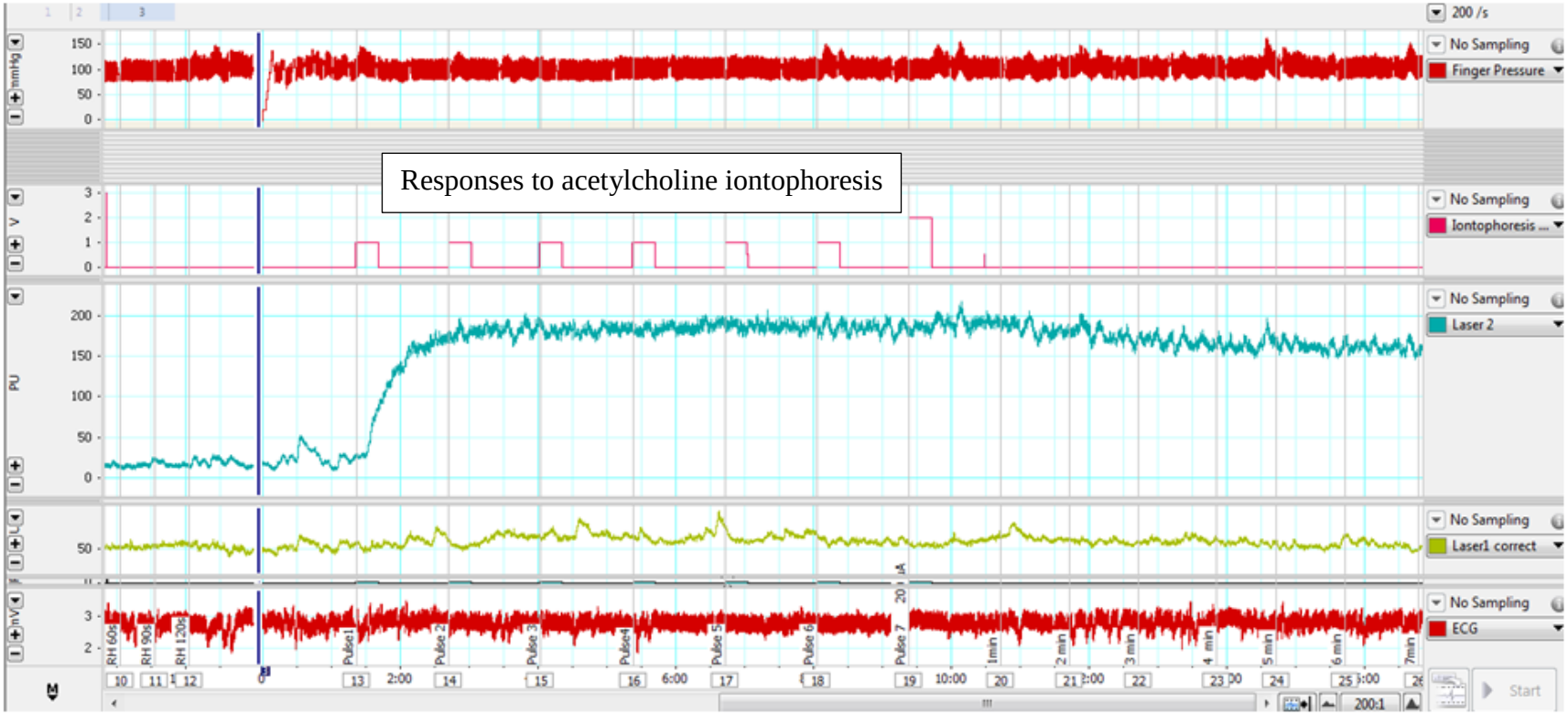


Figure 8.13: Original tracing showing cutaneous vascular responses to acetylcholine iontophoresis.

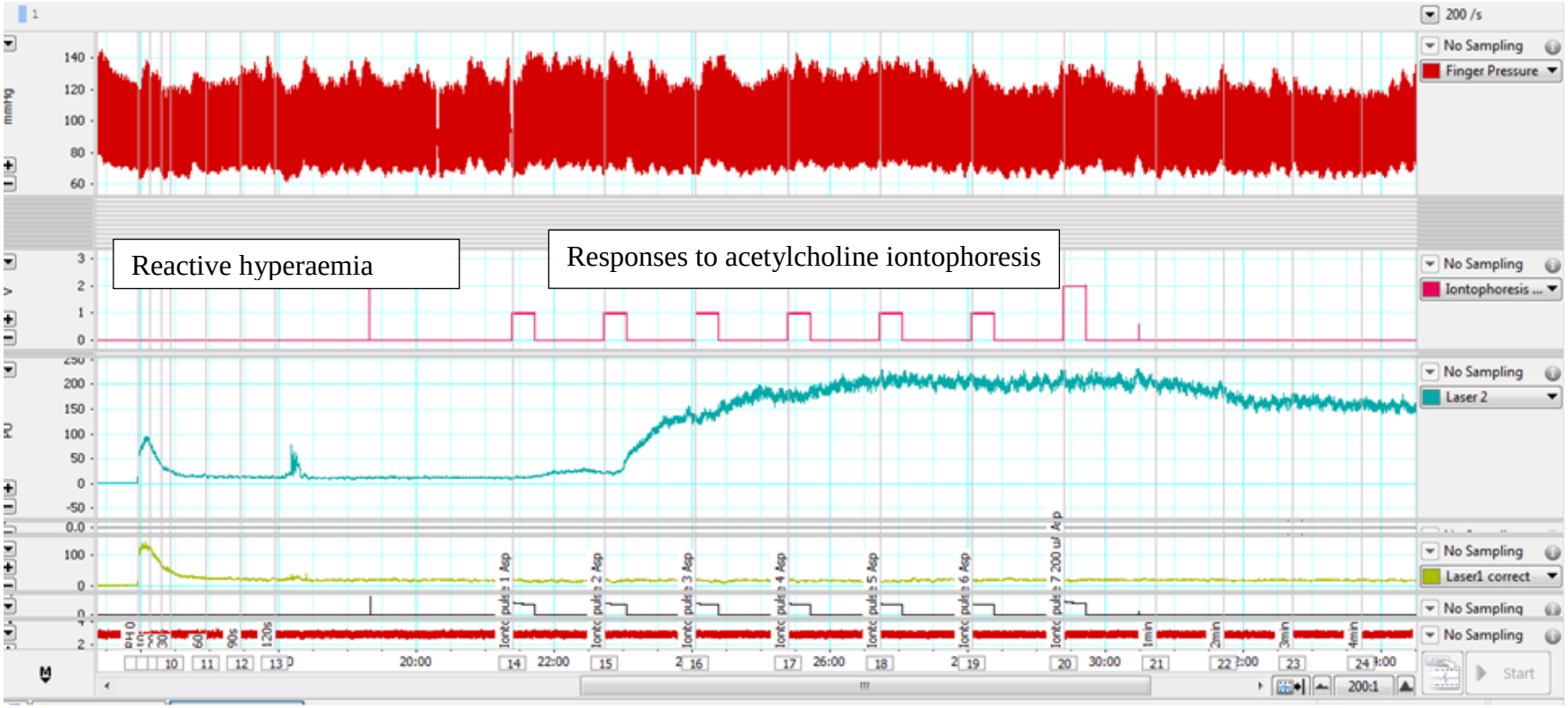


Figure 8.14: Original tracing showing cutaneous reactive hyperaemia and responses to acetylcholine iontophoresis following consumption of Aspirin.

CHAPTER 9

General Discussion

9.1 General discussion

The overall aim of this project was to determine the effect of ethnicity on cardiovascular responses to environmental stressors in young men and women and the role of PGs in the endothelial responses in the context that cardiovascular disease is of higher prevalence in BAs and SAs than WEs.

The study presented in Chapter 3 showed that whole groups of WEs and BAs respond to repeated sounds with a pattern consistent with the alerting response consisting of forearm vasodilation, increased ABP, forearm and digital cutaneous vasoconstriction (Brod et al, 1959; Hilton, 1980), whereas whole groups of SAs respond with increased ABP, forearm and digital cutaneous, but forearm vasoconstriction. Since skeletal muscle circulation makes a much larger contribution than cutaneous circulation to measurements made on the whole forearm, these findings indicate that in general, WEs and BAs show vasodilatation in limb skeletal muscle as expected of the alerting response, whereas in general SAs showed vasoconstriction in muscle as well as skin. Further, although WE men and women showed similar patterns of response consistent with the alerting response, within both BAs and SAs, women showed forearm vasoconstriction rather than vasodilation. Further, BA women showed greater pressor responses than all other subgroups.

As discussed in Chapter 3, section 3.4, in all 3 ethnic groups and within sex groups, there was no habituation of the alerting responses during 5 repetitions of sound. Rather BA women showed sensitization of pressor responses. Within SAs, although the women showed attenuated forearm vasodilation, there was no evidence of exaggerated sympathetic tone. This is the first study to show vasoconstrictor responses in limb

muscle to mental stress in BA or SA women and suggests that suggests that vasoconstriction in muscle as well as visceral circulation (Brod *et al.*, 1959) contributes to the exaggerated pressor responses to laboratory stressor that were found to predict development of hypertension (Chida & Steptoe, 2010).

It was interesting that short term habituation of alerting responses did not occur in any ethnic group or in the subgroups of men and women since it has been reported in mixed gender groups of WEs and in WE women (Zbrozyna & Westwood, 1988; Edwards *et al.*, 1998). Indeed, the study of Chapter 4 showed that BA men tended to show short-lived habituation of forearm vasodilator responses within the 1st of 3 sessions on alternate days, but not in the other sessions, while the pressor responses sensitized within each session. Further, in BA women, even medium term habituation over 3 alternate days did not occur as had been shown previously in middle-aged WE women; rather, the pressor and bradycardia components of the alerting response sensitized over 3 days of repetition. Lack of habituation of pressor responses with decline in muscle vasodilator and persistence of the pressor component of the alerting response occurred in young early hypertensives (Zbrozyna & Krebbel, 1985). Thus BA men and women show patterns to repeated aversive stimuli over several days consistent with pattern of response in early hypertensive.

Therefore, we conclude that in BAs and in BA women in particular, repeated stress would be likely to evoke intermittent sustained increases in ABP evidenced by lack of habituation and occurring as part of daily living experiences. This would be expected to contribute to vascular remodelling, eventually raising peripheral resistance and predisposing them to development of hypertension (Brod, 1982; Folkow, 1991). Although we did not record sympathetic nerve activity or cardiac output, the raised

ABP in BA women in response to mental stress was more likely dependent on increased peripheral resistance than increased cardiac output as heart rate declined during the tests. The results presented in this study are therefore consistent with the hypothesis that young BA men and women are at risk of stress-induced hypertension and that BA women in particular are at greater risk than BA men because they generally show forearm vasoconstriction as well as the other vasoconstrictor components of the alerting response and show sensitization on repetition of the stimulus. These findings are consistent with findings in large population studies of earlier onset and higher prevalence of hypertension stress in BA women compared with BA men and WE women and the finding that in this population hypertension in BA women was correlated with markers of daily stress (Hertz *et al.*, 2005; Geronimus *et al.*, 2007).

It is of interest that relative to WE women, BA and SA women did not show blunted reactive hyperaemia discussed in Chapter 3 or blunted exercise hyperaemia in forearm (Chapter 6), two endothelium-dependent responses, even though during mental stress these groups of women lacked forearm vasodilator responses to mental stress. Given the forearm vasodilator response to mental stress has been mainly attributed to endothelial NO, and that NO makes little contribution to reactive (Nugent *et al.*, 1999), this raised the possibility that endothelial NO synthesis is dysfunctional in BA and SA women. These findings raise the possibility that BA and SA women are not protected by oestrogen in the same way as WE women and may be .much more prone to stress-induced endothelial dysfunction (Ghiadoni *et al.*, 2000). They also raised questions about the role of the other endothelium derived vasodilators-PGs.

Thus, in the studies of Chapter 5, we investigated the role of COX products during reactive hyperaemia, and mental stress (see Chapter 5). This study provided the first

report of a role for PGs in endothelial dilator function in young BAs and SAs. In particular, it provided the first report of sex differences in role of PGs in reactive hyperaemia in all ethnic groups and the first report of a role for COX products in forearm vasodilator and vasoconstrictor responses to mental stress. The findings indicated that within each ethnic group, COX products contribute to muscle vasodilation in response to mental stress in some individuals and to muscle vasoconstriction in others.

Considering reactive hyperaemia, the evidence of Chapter 5 indicated that WE men use vasodilator PGs only, whereas in WE women, BA and SA men and women, 2 groups of people who use prostaglandins differently emerged. This should be seen against a background of published studies showing that COX inhibition has variable effects on reactive hyperaemia. This present study provides a clearer perspective on indicating that in some individuals, dilator PGs contribute to reactive hyperaemia, in others vasoconstrictor eicosanoids may be involved.

The role of PGs during mental stress also differed by ethnic groups. WEs showed no evidence of PGs contributing to forearm vasodilation or vasoconstriction, consistent with NO mediating forearm dilatation in WEs (Dietz *et al.*, 1994) (Figure 9.1). However, in BA and SA groups, forearm vasodilator responses to mental stress could be attributed to vasodilator PGs, while forearm vasoconstrictor responses were attenuated by COX inhibition suggesting a role for vasoconstrictor PGs in endothelial dysfunction in these individuals (Figure 9.1). Thus, we provide the first evidence of a role for PGs in the cardiovascular response to mental stress especially when NO bioavailability is blunted.

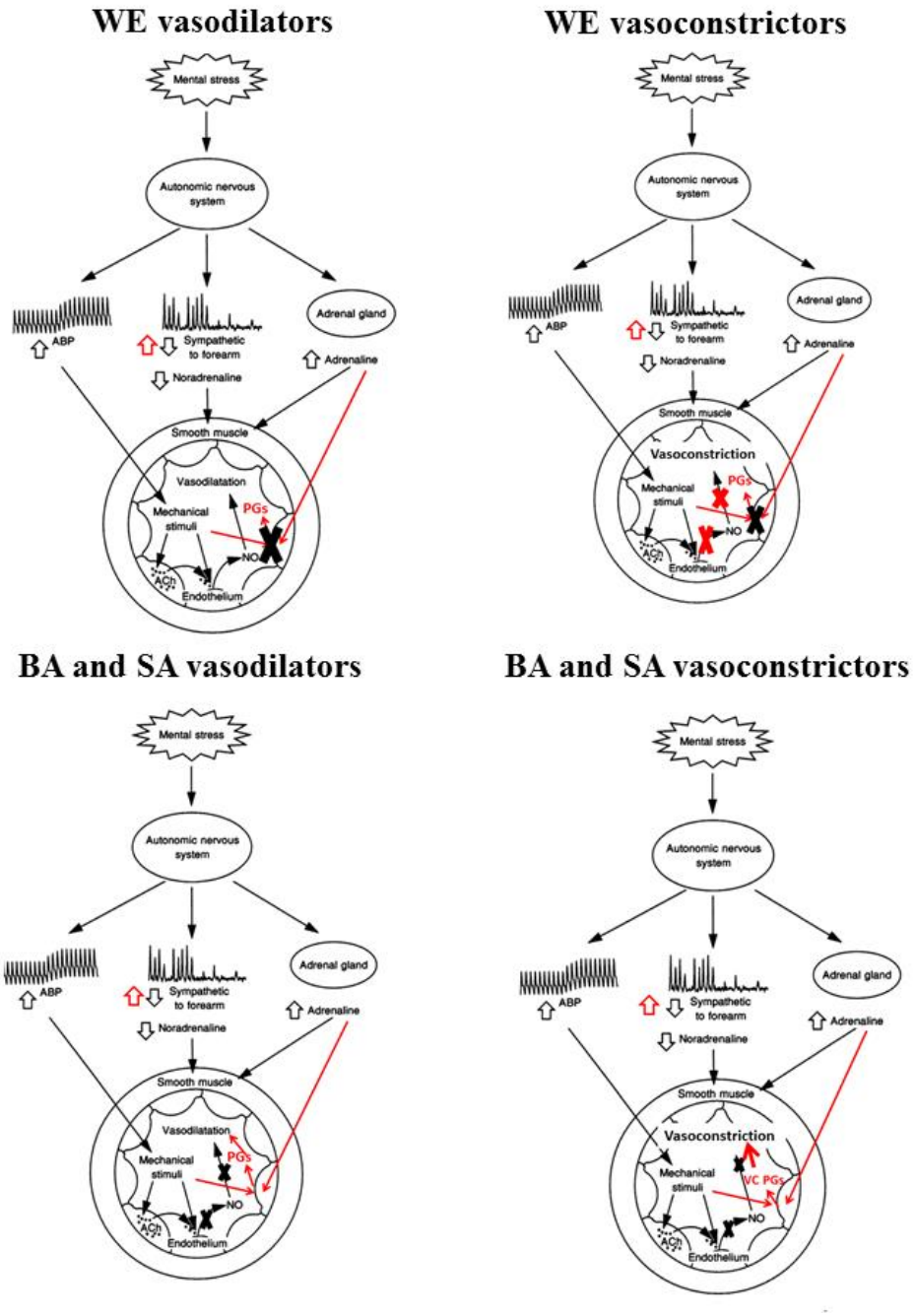


Figure 9.1: The proposed mechanisms of forearm vasodilation evoked by mental stress in each ethnic group – adapted from (Halliwill *et al.*, 1997). During metal stress, increases in systemic ABP and forearm flow stimulates NO but not PGs in WE vasodilators, in WE vasoconstrictors both NO and PGs may be inhibited. In BA and SA vasodilators, vasodilatory PGs but not NO mediates vasodilation, whereas in BA and SA vasoconstrictors, PGs EET/TXA-2 may mediate vasoconstriction.

Interestingly, Chapter 3 revealed that the blunted endothelium-dependent reactive hyperaemia seen in whole groups of BAs showed was largely attributable to BA men. Similarly only SA men showed blunted endothelial responses relative to WE men. Thus, BA and SA women did not show evidence of blunted reactive hyperaemia relative to WE women. This suggests that young BA and SA women do not show evidence of endothelial dysfunction unless they are subjected to mental stress whereas BA and SA men show early evidence of endothelial dysfunction during stimuli other than mental stressors. This dissociation of responses in the men and women is intriguing considering that women are generally considered to benefit from the cardioprotective effects of oestrogen on endothelial function.

NO impairment has previously been shown to play a role in blunted endothelial dilator function in BAs and SAs, and evidence was presented for a role of other mediators specifically, the EDHFs in compensating for lack of NO (Ozkor *et al.*, 2014). The possibility that PGs can compensate for inadequate availability of NO as suggested in the present study is high given the redundancy that exists between the two mediators. However in order to further investigate this idea, we tested the role of PGs in another endothelium-dependent response, exercise hyperaemia (presented in Chapter 6). When compared in sex groups, whereas only WE men showed attenuation of reactive hyperaemia with COX inhibition, WE women and BA SA men and women did not. However, WE men and women and BA men showed a role of PG in peak exercise hyperaemia whereas BA women and SA men and women did not. It therefore seemed that the contribution of PGs to forearm dilator responses is stimulus dependent (see Table 9.1)

Table 9.1 Summary of responses to COX inhibition on forearm vascular responses in WEs, BAs and SAs.

	All WEs		All BAs		All SAs	
			Whole Forearm response			
Reactive hyperaemia	No		No		Yes(D)	
Mental stress	No		No		No	
Exercise hyperaemia	Yes (D)		No		No	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
Reactive hyperaemia	Yes(D)	No	No	No	No	No
Mental stress	No	No	No	No	No	No
Exercise hyperaemia	Yes (D)	Yes (D)	Yes (D)	No	No	No
	VDs	**VCs**	**VDs**	**VCs**	**VDs**	**VCs**
Mental stress	No	No	Yes (D)	Yes(I)	Yes(D)	Yes(I)
	COX+	**COX-**	**COX+**	**COX-**	**COX+**	**COX-**
Reactive hyperaemia	Yes(D)	Yes(I)	Yes (D)	Yes(I) ?EET	Yes (D)	Yes(I) ? TBA

Yes: COX inhibition attenuated (D)/accentuated (I) response. No: COX inhibition failed to alter responses. TBA: Thromboxane A. EET: Eicosanoids.

In fact, as discussed in Chapters 5 and 6, the major stimulus for release of release of PGs from the endothelium during reactive hyperaemia is hypoxia (Carlsson *et al.*, 1987), while during mental stress the stimulus is most likely shear stress with additional influences of adrenaline or ACh released during the response acting on endothelial receptors (Halliwill *et al.*, 1997). However, during exercise and to an extent during reactive hyperaemia release of PGs may not only from endothelium in response to hypoxia and shear stress, but also from skeletal muscle and from deoxygenated red blood cells (Marshall & Ray, 2012). Therefore, the differential responses between sex and ethnicities may be due to differences in the relative importance of these sources of PGs: this issue remains to be investigated with assays of PG metabolites and NO.

As a way of more selectively investigating endothelium-dependent dilator function, we performed a final study on reactive hyperaemia and vasodilator responses induced by

the classic endothelium-dependent dilator ACh in cutaneous circulation of WEs and BAs (presented in Chapter 8), by using laser Doppler fluximetry. Since this technique records cutaneous perfusion at the level of the microcirculation and since skin has very low metabolic rate, responses in cutaneous vasculature mainly reflect the local interactions of endothelium and vascular smooth muscle (Holowatz *et al.*, 2008). Cutaneous reactive hyperaemia was blunted BA men relative to WE men but not in BA women relative to WE women, just as was found for reactive hyperaemia in whole forearm (Chapter 3). Moreover in cutaneous circulation also, COX inhibition had no effect on reactive hyperaemia in the the mixed sex groups of BA men and women. Similar findings were made for ACh-induced dilatation: it was blunted in BA men relative to WE men, similar in WE and BA women and not altered by COX inhibition. However, each ethnic groups, there were individuals in whom COX inhibition attenuated reactive hyperaemia and ACh-induced cutaneous dilatation and others in which the responses were augmented (Table 9.2). It seems likely that it is young individuals in whom COX products exert vasoconstrictor influences who are particularly at risk of future CVD; this remains to be tested.

Table 9.2 Summary of responses to COX inhibition on cutaneous vascular responses in WEs, BAs and SAs.

	All WE		**All BA**		**All SA**	
	Cutaneous Forearm response					
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
Reactive hyperaemia	No	No	No	No	NA	NA
Ach-vasodilation	No	No	No	No	NA	NA
	COX+	**COX-**	**COX+**	**COX-**	**COX+**	**COX-**
Reactive hyperaemia	Yes(D)	Yes(I)	Yes(D)	Yes(I)	NA	NA
Ach -vasodilation	Yes(D)	Yes(I)	Yes(D)	No	NA	NA

Yes.: COX inhibition attenuated (D)/accentuated(I) response. No: COX inhibition failed to alter responses. NA: data not available.

Finally, the study of Chapter 7 tested whether BP variability over 24 hours is associated with the pattern of response to laboratory stressors as these are independently known to be predictors of CVD. BAs are already known to show greater prevalence of nocturnal non-dipping as well as endothelial dysfunction (Mellman *et al.*, 2015), while hypertensive non-dippers, who were presumably WEs, are known to show endothelial dysfunction (Higashi *et al.*, 2002). In the present study, non-dippers did not demonstrate blunted reactive or exercise hyperaemia, but they showed a trend for forearm vasoconstriction rather than vasodilatation in response to mental stress, as well as a more prominent pressor response. There was also higher prevalence of non-dipping amongst BA women, providing further evidence of their predisposition to future development of hypertension. The reasons for BA women apparently being less protected by the beneficial effects of oestrogen on endothelial function are yet to be unravelled. There is a possibility that they also show exaggerated sympathetic activity and that beta-adrenoreceptor mediated vasodilation is blunted in BA women contrary to the normal pattern in WE women (Hart *et al.*, 2009a).

In conclusion, the outcomes of this project fulfil the main hypotheses of this PhD project extend the findings of Dietz *et al.* (1994), Cardillo *et al.* (1998b) and Khan *et al.* (2015), which showed an important role for NO in the forearm vasodilatation of mental stress in individuals whose ethnicity was unspecified and in whom gender was not considered. This PhD project has provided novel evidence firstly that young BAs and SAs, particularly women, are more likely to show forearm vasoconstriction than forearm dilatation and persistence of the pressor responses to repeated mental stress than WE men or women. This is significant in that forearm vasoconstriction and strong pressor responses to environmental stress in young adulthood is predictive of CVD.

Secondly, the present study shows a role for dilator and vasoconstrictor COX products in young BAs and SAs, but not WEs in the forearm vasodilatation or constriction to mental stress. This suggests that in young adulthood, some BAs and SAs may use dilator PGs as compensation for impaired NO availability in mediating forearm vasodilatation, thereby helping to offset the pressor response to stressors, while in others, vasoconstrictor PGs or other COX products such as TXA2 contribute to the forearm vasoconstriction (Figure 9.1). Overall, it would seem that endothelial dysfunction which is manifest as a change in the balance of the NO and COX-PG pathways that contribute to the response to mental stress predispose BA and SA men and women to earlier onset of hypertension, and that the risk is particularly high in BA and SA women.

Appendices

Appendix 1. Ethical approval

Dear Professor Marshall

Re: "The effect of ethnicity on vascular responses evoked by environmental stressors" Application for Ethical Review ERN_15-0714A

Thank you for the above application for amendment, which was reviewed by the Science, Technology, Engineering and Mathematics Ethical Review Committee.

On behalf of the Committee, I can confirm that this amendment now has full ethical approval.

I would like to remind you that any substantive changes to the nature of the study as now amended, and/or any adverse events occurring during the study should be promptly bought to the Committee's attention by the Principal Investigator and may necessitate further ethical review. A revised amendment application form is now available at https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Ethical-Review-Forms.aspx . Please ensure this form is submitted for any further amendments.

Please also ensure that the relevant requirements within the University's Code of Practice for Research and the information and guidance provided on the University's ethics webpages (available at https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Links-and-Resources.aspx) are adhered to and referred to in any future applications for ethical review. It is now a requirement on the revised application form (https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Ethical-Review-Forms.aspx) to confirm that this guidance has been consulted and is understood, and that it has been taken into account when completing your application for ethical review.

Please be aware that whilst Health and Safety (H&S) issues may be considered during the ethical review process, you are still required to follow the University's guidance on H&S and to ensure that H&S risk assessments have been carried out as appropriate. For further information about this, please contact your School H&S representative or the University's H&S Unit at healthandsafety@contacts.bham.ac.uk.

If you require a hard copy of this correspondence, please let me know.

Kind regards

Susan Cottam
Research Ethics Officer
Research Support Group

Web: https://intranet.birmingham.ac.uk/finance/accounting/research-support-group/Research-Ethics

Appendix 2. Consent form

UNIVERSITY OF BIRMINGHAM

Centre for Cardiovascular Science
School of Clinical and Experimental Medicine
College of Medical and Dental Sciences
University of Birmingham

The effect of ethnicity on vascular responses

Investigator: Abimbola Aiku ***Supervisors: Prof. Janice Marshall and Prof Una Martin***

Healthy Volunteer's Consent Form

Please read this form carefully and sign it once one of the above named, has explained fully the aims and procedures of the study to you.

- I have voluntarily agreed to take part in this study.
- I confirm that I have been given a full explanation by at least one of the above named and that I have read and understood the information sheet given to me.
- I have been given the opportunity to ask questions and discuss the study with one of the above investigators on all aspects of the study and have understood the advice and information given as a result.
- I agree to comply with the reasonable instructions of the investigators and will notify them immediately of any unexpected unusual symptoms.
- I understand that any data and/or samples collected will be strictly used for scientific research/study.
- I authorize the investigators to disclose the results of my participation in the study but not any details which could lead to my identification (e.g. name, contact details).
- I understand that information about me recorded during the study will be kept secure. If data is transferred to others it will be made anonymous.
- I understand that the data collected and/or results of the study may be published in a scientific journal and that if published, my identity will be protected.
- I authorize the investigators to disclose to me any abnormal test results.
- I understand that I can ask for further instructions and/or explanations at any time.

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- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing.
- If I have completed the first experimental session, I understand that the experimenter may ask my permission to retain my data so that they can be entered into the grouped results. Otherwise, the data will be destroyed following my withdrawal
- I confirm that I have disclosed all the relevant medical information before the study.

Please note that a copy of this form will be provided for you to keep. If you have any questions, require further instructions and/or explanations, please do not hesitate to contact Abimbola Aiku at [redacted]

Volunteer's Name:_____

Signature:_____

Date:_____

Investigator's Name:_____

Signature:_____

Date:_____

Subject Number:_____

PLEASE, DO NOT FORGET TO BRING THE CONSENT FORM WITH YOU ON THE PREARRANGED DATE OF EXPERIMENT.

Appendix 3. General participant questionnaire

UNIVERSITY OF BIRMINGHAM

School of Clinical and Experimental Medicine
College of Medical and Dental Sciences
University of Birmingham

The effect of ethnicity on vascular responses to environmental stressors

Investigator: Abimbola Aiku *Supervisors: Prof. Janice Marshall and Prof Una Martin*

Participant Questionnaire

All information will remain confidential and any information published for analysis will be kept anonymous, therefore please try to answer as honestly as possible.

Personal Details:

Participant Number (for investigator use only):

Name:

D.O.B:

Ethnicity:

Gender:

Date of experiment:

Address: _____

_____

Contact Number:

E-mail:

Height (cm):

Weight (kg):

BMI (kg/m2):

Waist Circumference (cm):

Heart rate (Beats/min):

Blood Pressure (mmHg):

Forearm Circumference (cm):

Medical History:

Are you aware of any long-term or current medical conditions that you suffer from? (E.g diabetes, respiratory disease, liver disease etc) If yes, please provide details (how long you have had the condition, medications etc):

_____

_____

Have you ever suffered from any of the conditions below that have now resolved?

Cardiovascular Disease **Yes/No**

Respiratory Disease **Yes/No**

Kidney Disease **Yes/No**

Liver Disease **Yes/No**

Metabolic disease (e.g. diabetes) **Yes/No**

Any other chronic (long-term) medical condition **Yes/No**

If you answered yes to any of the above conditions please provide details of the condition:

_____

_____

Are you currently on any medications? If so please provide details:

_____

Do you smoke? **Yes/No** Have you ever smoked? **Yes/No**

If you answered yes to the last question, please provide details (how long did you smoke for, on average how many per day and when did you stop?):

_____

Do you consume alcohol? **Yes/No**

If you drink alcohol, please indicate how often you drink in a week (circle)

Daily (7 days) 4-6days 3-1 days occasionally

If you drink alcohol, please indicate how many units of alcohol *on average* you consume per week (circle):

<5 units 6-10 units 10-14 units 15-20units 20-25 units >25units

Nb: A single shot of spirits (25ml) is one unit, a small glass of wine is 1.5units and a pint of higher-strength lager/beer/cider is 3 units

How many cups of caffeinated drinks do you drink on average per day? (E.g. coffee, red bull etc) _____ cups/day

Do you take any vitamin supplements?
If so please state what supplements you take and how much per week:

Indicate your average weekly intake of :

Citrus Fruits and soft fruit (not tinned)	None/1-3 days /4-6days/Daily
Fruit juice (Orange/ Grapefruit/ Tomato)	None/ 1-3 days /4-6days /Daily
Vitamin C enriched cordials	None / 1-3 days /4-6days /Daily
Potatoes (Incl. instant)	None /1-3 days /4-6days /Daily
Green Vegetables	None /1-3 days /4-6days /Daily
Other fruits (Incl. tinned)	None /1-3 days /4-6days /Daily

Indicate your average weekly intake of fish

Forage Fish (sardines, herring and anchovies)	None /1-3 days /4-6days /Daily
Pelagic fish (salmon, trout, tuna and mackerel)	None / 1-3 days /4-6days/Daily
Whitefish (cod, haddock and flatfish)	None / 1-3 days /4-6days/Daily

Do you exercise regularly? Yes/No
If yes, please list any regular types of exercise that you do (e.g. running, rowing, badminton, climbing, dancing etc):

How many hours of **moderate** exercise do you undertake each week? (E.g. walking, jogging) (Please circle your answers)

< 30 mins -<1 hr 1hr -3hrs 3-<5hrs 5-<7hrs 7-10hrs <10hrs

Do you undertake any **high intensity/ vigorous exercise?** (E.g. running, swimming, weight training) If so, how many hours a week on average?

< 30 mins - <1 hr 1hr -3hrs 3-<5hrs 5-<7hrs 7-10hrs <10hrs

Please estimate the percentage of your exercise that is aerobic as opposed to weight training/static exercise:

% cardio/ % weight training/static exercise

Ethnicity and Family History

Were you born in the UK? **Yes/No**

If not please provide details of your country of birth and how long you have lived in the UK:

_____

What ethnicity would you consider yourself? (Please sub-specify e.g. White-English/Scottish/Welsh/Northern Irish/British/European (sub-specify) or Asian-Pakistani/Asian-Indian /Asian–Bangladeshi or Black-African/Afro-Carribean/African-American etc)

_____

What ethnicity do your parents consider themselves? Please state their place of birth.

_____

Father's Ethnicity: _____ Place of Birth _____

Mother's Ethnicity: _____ Place of Birth _____

Are you aware of any cardiovascular conditions that your parents suffer from? (E.g. high blood pressure, angina, coronary artery disease)

Father: _____

Mother: _____

Are you aware of any long-term conditions that either of your parents suffer from and/or if they take any regular medications?

Father: _____

Mother: _____

Declaration

I declare that all of the information I have provided is correct to the best of my knowledge and I understand that all information collected will remain confidential.

Print Name: _____ Date: _____

Signed: _____

Thank you for taking the time to complete this questionnaire. Following a review of the information you will be informed if you are eligible to participate in the study.

Appendix 4. Cohen's Perceived Stress Questionnaire

UNIVERSITY OF BIRMINGHAM

School of Clinical and Experimental Medicine
College of Medical and Dental Sciences
University of Birmingham

The effect of ethnicity on vascular responses to environmental stressors

Investigator: Abimbola Aiku Supervisors: Prof. Janice Marshall and Prof. Una Martins

Participant Perceived Stress Questionnaire

Participant Number (for investigator use only):

All information will remain confidential and any information published for analysis will be kept anonymous, therefore please try to answer as honestly as possible

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?..... 0 1 2 3 4

2. In the last month, how often have you felt that you were unable to control the important things in your life? 0 1 2 3 4

3. In the last month, how often have you felt nervous and "stressed"? ... 0 1 2 3 4

4. In the last month, how often have you felt confident about your ability to handle your personal problems? 0 1 2 3 4

5. In the last month, how often have you felt that things were going your way?..... 0 1 2 3 4

6. In the last month, how often have you found that you could not cope with all the things that you had to do? 0 1 2 3 4

7. In the last month, how often have you been able to control irritations in your life?..... 0 1 2 3 4

8. In the last month, how often have you felt that you were on top of things? 0 1 2 3 4

9. In the last month, how often have you been angered because of things that were outside of your control?..... 0 1 2 3 4

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them? 0 1 2 3 4

Declaration

I declare that all of the information I have provided is correct to the best of my knowledge and I understand that all information collected will remain confidential.

Date: _____

Signed: _____

Thank you for taking the time to complete this questionnaire.

Appendix 5. Salt intake questionnaire

UNIVERSITY OF BIRMINGHAM

School of Clinical and Experimental Medicine
College of Medical and Dental Sciences
University of Birmingham

The effect of ethnicity on vascular responses to environmental stressors

Investigator: Abimbola Aiku Supervisors: Prof. Janice Marshall and Prof. Una Martin

Participant food and salt intake Questionnaire

All information will remain confidential and any information published for analysis will be kept anonymous, therefore please try to answer as honestly as possible.

Participant Number (for investigator use only):

This questionnaire asks you about the foods you eat. Please indicate how often using Always = (daily) Often=(4-6days) Sometimes= (2-3days) Rarely (≤1day) . Pick one option.

	Food item	Never	Rarely	Sometimes	Often	Always
1.	How often do you add salt to your food before you eat it or as you are eating it?	1	2	3	4	5
2.	How often is salt added in cooking or preparing foods in your household?	1	2	3	4	5
3.	How often do you eat processed food such as bacon, sausages, bacon, ham, salami and pâtés?	1	2	3	4	5
4.	Do you eat commercial sandwiches	1	2	3	4	5
5.	Do you eat breakfast cereals	1	2	3	4	5
6.	Do you eat Cheese	1	2	3	4	5
7.	Do you eat Tinned vegetables	1	2	3	4	5
8.	Do you eat Bread	1	2	3	4	5
9.	Do you eat Savoury snacks, such as crisps, tortilla	1	2	3	4	5
10.	Do you use condiments and seasonings such as Worcestershire sauce, soy sauce, onion salt, garlic salt, and bouillon cubes – Aromat, Knorr	1	2	3	4	5
11.	Do you eat Pizza	1	2	3	4	5
12.	Do you eat "convenience foods", such as microwave meals or ready meals	1	2	3	4	5
13.	Do you eat battered Chicken (KFC etc) and chicken burger	1	2	3	4	5
14.	Do you eat Pasta/noodle dishes with cheese sauces (macaroni cheese, lasagne, noodle salad etc.)	1	2	3	4	5
15.	Do you eat instant noodles	1	2	3	4	5
16.	Do you eat tinned fish in salted water	1	2	3	4	5

Print Name: _____ Date: _____

Signed: _____

Thank you for taking the time to complete this questionnaire.

Appendix 6. ABPM information sheet and diary

UNIVERSITY OF BIRMINGHAM

Centre for Cardiovascular Science
School of Clinical and Experimental Medicine
College of Medical and Dental Sciences
University of Birmingham

The effect of ethnicity on vascular responses

24 Hour Ambulatory Blood Pressure Monitor information sheet

Investigator: Abimbola Aiku ***Supervisors: Prof. Janice Marshall and Prof Una Martin***

The 24 hour Blood pressure Monitor

The blood pressure monitor is about the size of a large cell phone (generally worn at the waist) and is connected with a hose to a full size blood pressure cuff on your arm. The monitor will automatically obtain and record your blood pressure. You will be given a diary to record certain activities and symptoms experienced while wearing the monitor.

The aim of the blood pressure monitor is to collect accurate blood pressure recording over 24hour period.

Wearing the Blood pressure Monitor

- Please ensure you wear the ambulatory blood pressure monitor as shown by the investigator.
- The monitor is set to record your blood pressure:
  Every 30minutes between 7am and 11pm
  Every hour between 11pm and 7am
- Like a normal blood pressure machine, the cuff will blow tight on your arm to take a recording
- The monitor will bleep just before a recording.
- If possible sit down, keep your cuff at level of heart.
- It is important to keep your arm straight and still when the monitor is recording. If you move during a recording, the monitor will retake the reading and the cuff will be tighter than before. It is important to prevent this by keep the arm still during a recording.
- Please keep the monitor dry. However you may remove the cuff to have a bath or shower, in between recordings. When you reapply the cuff, line up the arrow with the pulse in your arm(as previously shown by the investigator)
- Please keep the cuff on overnight.
- REMOVE THE HOSE FROM YOUR NECK.
- Place the monitor under your pillow or on the floor at your bedside.
- After 24 hours remove the cuff and switch the small black button on the bottom of the monitor to the off position

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- The monitor may cause some discomfort to your arm during recordings
- Driving and operating electrical tools is not recommended while wearing the monitor
- If you need to remove the monitor quickly – pull open the Velcro fastening of the cuff and switch the monitor off.

Please return the monitor on agreed day to

Abimbola Aiku (Abi)

Room 37 East Lower Ground (ELG) Floor

The Medical School.

University of Birmingham

If you have any queries or problems with the monitor –Please telephone Abimbola Aiku on [redacted]

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The effect of ethnicity on vascular responses

24 Hour Ambulatory Blood Pressure Monitoring (ABPM) Participant Diary

Investigator: Abimbola Aiku ***Supervisors: Prof. Janice Marshall and Prof Una Martin***

Name: Study number:

DOB: Date of ABPM: Cuff size(S,M,L):

	Mid arm circumference	SBP	DBP	PR
Right arm				
Left Arm				
		Right arm	Left arm	
Dominant arm				
ABPM fitted to				

Please complete as accurately as possible					
First ABPM reading and time					
STARTED :			RETURNED :		
Time	Activity/Symptom	Time	Activity/Symptom	Time	Activity/Symptom
7am		3pm		10pm	
8		4pm		11pm	
9		5pm		12midnight	
10		6pm		1am	
11		7pm		2am	
12		8pm		3am	
1pm		9pm		4am	
2pm				5am	
				6am	
Went to bed at:		Woke up at:		Finished at	
Please tick when task is completed					
	Recorded Sleep/ wake time?		Switched off monitor?		

Appendix 7. Contribution of Prostaglandins to exercise hyperaemia: workload, ethnicity and sex matter!

J Physiol 597.19 (2019) pp 4887–4900

The Journal of Physiology

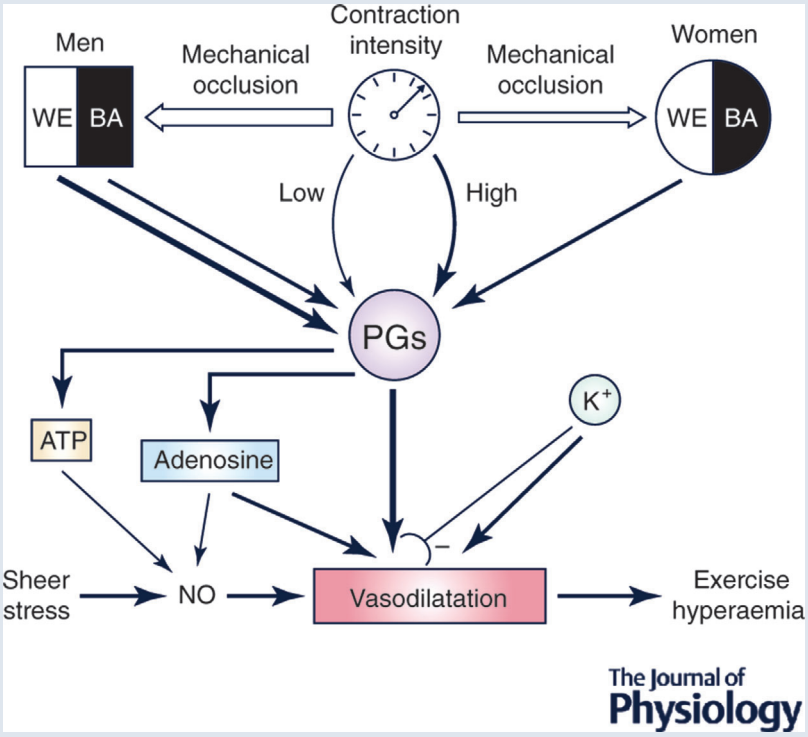
SYMPOSIUM REVIEW

Contribution of prostaglandins to exercise hyperaemia: workload, ethnicity and sex matter!

Abimbola O. Aiku and Janice M. Marshall

Institute of Clinical Sciences, College of Medical & Dental Sciences, University of Birmingham, Birmingham B15 2TT, UK

Edited by: Ole Petersen & Ylva Hellsten



Abstract The contribution of prostaglandins (PGs) to exercise hyperaemia is controversial. In this review, we argue this is partly explained by differences in exercise intensity between studies. The effects of cyclooxygenase (COX) inhibition and PG assays indicate that PGs contribute more

Abimbola Aiku graduated with MBBS from the University of Ibadan, Nigeria, where she subsequently obtained an MSc in Physiology and MPH. She was awarded MSc in Medical Parasitology by the University of London and recently gained a PGDip in Higher Education. She is undertaking a PhD at the University of Birmingham under the supervision of Janice Marshall on the role of ethnicity and sex in cardiovascular responses to environmental stressors. She hopes to complete her PhD soon and will return to her lectureship post at the University of Ibadan, where she plans to develop programmes in Cardiovascular Science and research on the role of infectious diseases and sex in determining endothelial function and baroreflex regulation of blood pressure. **Janice Marshall** is Bowman Professor of Physiology. She has a longstanding interest in reflex and local regulation of the cardiovascular system and has recently extended her work into ethnicity- and sex-related effects.

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at moderate to heavy than at light workloads and are mainly released by low tissue O_2. But, the release and actions of PGs also depend on other O_2-dependent dilators including ATP, adenosine and NO. K^+ may inhibit the action of PGs and other mediators by causing hyperpolarization, but contributes to the hyperaemia. Thus, at lighter loads, the influence of PGs may be blunted by K^+, while COX inhibition leads to compensatory increases in other O_2-dependent dilators. In addition, we show that other sources of variability are sex and ethnicity. Our findings indicate that exercise hyperaemia following rhythmic contractions at 60% maximum voluntary contraction, is smaller in young black African (BA) men and women than in their white European (WE) counterparts, but larger in men than in women of both ethnicities. We propose the larger absolute force in men causes greater vascular occlusion and accumulation of dilators, while blunted hyperaemia in BAs may reflect lower oxidative capacity and O_2 requirement. Nevertheless, COX inhibition attenuated peak hyperaemia by ∼30% in WE, BA men and WE women, indicating PGs make a substantial contribution in all three groups. There was no effect in BA women. Lack of PG involvement may provide early evidence of endothelial dysfunction, consistent in BA women with their greater risk of cardiovascular disease.

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Corresponding author J. M. Marshall: Institute of Clinical Sciences, College of Medical & Dental Sciences, University of Birmingham, Birmingham B15 2TT, UK. Email: j.m.marshall@bham.ac.uk

Abstract figure legend Muscle exercise leads to release of prostaglandins (PGs), which cause vasodilatation and contribute to exercise hyperaemia. PGs also release other known mediators of exercise hyperaemia, ATP and adenosine, to generate NO, whose release is tonically regulated by shear stress. Further, K^+ released from the onset of contraction causes vasodilatation, but also inhibits dilatation induced by other mediators. The release of PGs is graded with contraction intensity. However, strong muscle contraction also causes vascular occlusion, limiting vasodilatation during contraction, but allowing greater accumulation of PGs such that post-contraction hyperaemia is augmented. At the same relative force, these mechanical effects are greater in young men than in young women, both in those of white European (WE) and in those of black African (BA) ethnicity. However, the dilator effects of PGs are deficient in BA women implying endothelial dysfunction.

Prostaglandins (PGs) have been implicated in exercise hyperaemia since the 1970s. Despite this long association, the extent to which PGs contribute to exercise hyperaemia remains unclear. Review of the literature suggests the uncertainty arises, at least in part, from differences between experimental studies in the intensity of exercise, the sex, age range, ethnicity of the subjects or even in the techniques used to measure muscle blood flow. In this review we consider these issues, using them as a setting for our studies on the contributions of PGs to exercise hyperaemia in young men and women of white European (WE) and black African (BA) ethnicities.

Evidence for and against PG involvement

PGs were first reported to contribute to exercise hyperaemia by Kilbom and Wennmalm (1976), who used venous occlusion plethysmography (VOP) to record forearm blood flow (FBF). In men and women, post-contraction hyperaemia following rhythmic or isometric forearm contractions at moderate to heavy loads was attenuated by 30–50% after inhibition of cyclooxygenase (COX), which synthesizes PGs from arachidonic acid. Subsequently, Nowak and Wennmalm (1978) showed that cycling at 75% maximal workload increased venous efflux of prostaglandin E (PGE). Similarly, post-exercise hyperaemia recorded by VOP in the leg of young men following treadmill exercise at ∼50% maximum workload was attenuated by ∼50% after COX inhibition (Cowley *et al.* 1985). Further, Duffy *et al.* (1999) showed with VOP that post-exercise hyperaemia evoked by rhythmic forearm contractions at medium load in young men and women, was attenuated by ∼20% by COX inhibition. In addition, we showed by using VOP that COX inhibition attenuated post-exercise hyperaemia evoked by isometric exercise at 60% maximal voluntary contraction (MVC) by ∼40% (Win & Marshall, 2005).

The first attempt to determine the contribution of PGs *during* exercise was made by Wilson and Kapoor (1993). Since VOP cannot be applied reliably when muscles are contracted, FBF was measured during 4–5 s breaks in 5 min periods of graded rhythmic contractions. In young men and women, COX inhibition attenuated increases in FBF evoked during contractions at light and medium workload by ∼20% and abolished the 2- to 3-fold increase in prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) efflux (Wilson & Kapoor, 1993).

By contrast, Shoemaker *et al.* (1996), who used Doppler ultrasound recordings of brachial artery diameter and blood velocity to assess FBF in young men, found that COX inhibition had no effect on hyperaemia evoked during rhythmic forearm contractions at 10% MVC. Thus, they concluded PGs do not play an essential role in hyperaemia *during* exercise. A similar conclusion was drawn by Mortensen *et al.* (2007), who measured blood flow by thermodilution in young men performing knee extensor exercise at 20% maximum. In some contrast, Schrage *et al.* (2004), who used Doppler ultrasound in a group of men and women, found that infusion of COX inhibitor when hyperaemia evoked by rhythmic forearm contractions at 10% MVC was already established caused a short-lasting, 12% reduction in FBF. They proposed PGs do contribute to exercise hyperaemia, but when their influence is removed, other dilator(s) compensate (Schrage *et al.* 2004).

Resolving the discrepancies. The simplest explanation for these discrepancies is that PGs are more likely to be released and contribute to exercise hyperaemia associated with medium to strenuous exercise than with light exercise. Certainly, microdialysis samples showed PGE_2 concentration in the interstitium was unchanged during light knee extensor exercise, but increased during moderate workloads (Boushel *et al.* 2002). Further, graded cycling exercise in young men was accompanied by graded increases in interstitial PGE_2 and PGI_2 (Karamouzis *et al.* 2001).

An alternative explanation (see Shoemaker *et al.* 1996) is that PGs contribute to muscle vasodilatation during *recovery* from exercise rather than during exercise *per se*, and that VOP reveals this contribution even when used during breaks between rhythmic contractions (Wilson & Kapoor, 1993) because the technique essentially measures 'recovery flow'. However, Doppler ultrasound recordings during graded rhythmic calf contractions showed that only during weak contractions of 6–15% MVC did blood flow increase slightly *during* contraction and even then, blood flow increased further on relaxation. At intensities ≥15% MVC, blood flow *during* the contractions was progressively impaired and during relaxation phases, i.e. during 'recovery', blood flow increased to extents that were graded with contraction intensity (Green *et al.* 2011). Indeed, calf blood flow measured with VOP during the relaxation phases compared closely with that estimated by ultrasound (Green *et al.* 2011).

On this basis, it seems probable PGs do contribute to hyperaemia between contractions in rhythmic exercise, as well as during post-contraction hyperaemia following contractions, providing the PG concentrations reached during the period of contraction are sufficiently raised. Nevertheless, the possibility still remains that the influences of PGs may be difficult to reveal during light to medium exercise due to interaction with other factors; this is considered below ('Interactions between PGs and other factors').

Origin and stimuli for PG release during exercise

The PGs associated with exercise hyperaemia are PGI_2 and PGE_2. Endothelial cells predominantly release PGI_2 (Feletou *et al.* 2011). Microvessels of skeletal muscle were reported to release relatively more PGE_2 judging from assays performed on homogenates of rat cremaster muscle with main artery and vein removed: the ratio of PGI_2 : PGE_2 was 1 : 2 (Myers *et al.* 1985). However, the homogenates contained a high proportion of skeletal muscle fibres. Skeletal muscle fibres do not express PGI_2 synthase (McLennan & Macdonald, 1991), but they express COX, PGE_2 synthase and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) synthase, and release PGE_2 and $PGF_{2\alpha}$ in response to arachidonic acid and muscle contraction, PGE_2 being dominant (Testa *et al.* 2007; Trappe & Liu, 2013). Thus, it seems most likely the PGI_2 released into the interstitium and venous efflux of exercising skeletal muscle originates mainly from endothelial cells, whereas PGE_2 arises largely from skeletal muscle fibres (see Fig. 1).

In vitro, increased intraluminal shear rate, or graded fall in P_{O_2} dilated feed arteries and small resistance arteries of skeletal muscle by releasing PGI_2 (Hecker *et al.* 1993; Frisbee *et al.* 2002). Similarly, isolated muscle arterioles showed endothelium-dependent dilator responses to hypoxia and increased shear rate that were abolished by COX inhibition (Koller & Kaley, 1990; Messina *et al.* 1992). Shear stress-induced release of PGI_2 was attributed to phospholipase C activation (Berthiaume & Frangos, 1992), while hypoxia-induced PGI_2 release has been associated with influx of Ca^{2+}, activation of phospholipase A_2 (PLA_2) and mobilization of arachidonic acid (Berna *et al.* 2001). In skeletal muscle fibres, mechanical stretch and the increase in intracellular Ca^{2+} lead to PLA_2 activation and PGE_2 synthesis (Burkholder, 2007). The PG transporter (PGT) is inhibited by extracellular lactate, so augmenting net PG release (Chan *et al.* 2002), providing a mechanism by which reduced P_{O_2} could augment interstitial PGE_2 accumulation (Fig. 1).

Considering shear stress as a stimulus for PG release during exercise, Doppler ultrasound recordings of blood velocity and brachial artery diameter indicated that although COX inhibition did not affect the rate of increase in FBF evoked by rhythmic contractions at 10% MVC, the increase in brachial artery shear rate was exaggerated with a trend for the diameter to be smaller (Shoemaker *et al.* 1996). This suggested that endothelial release of PGs in downstream arterioles contributed to their dilatation so limiting further increases in brachial artery shear rate.

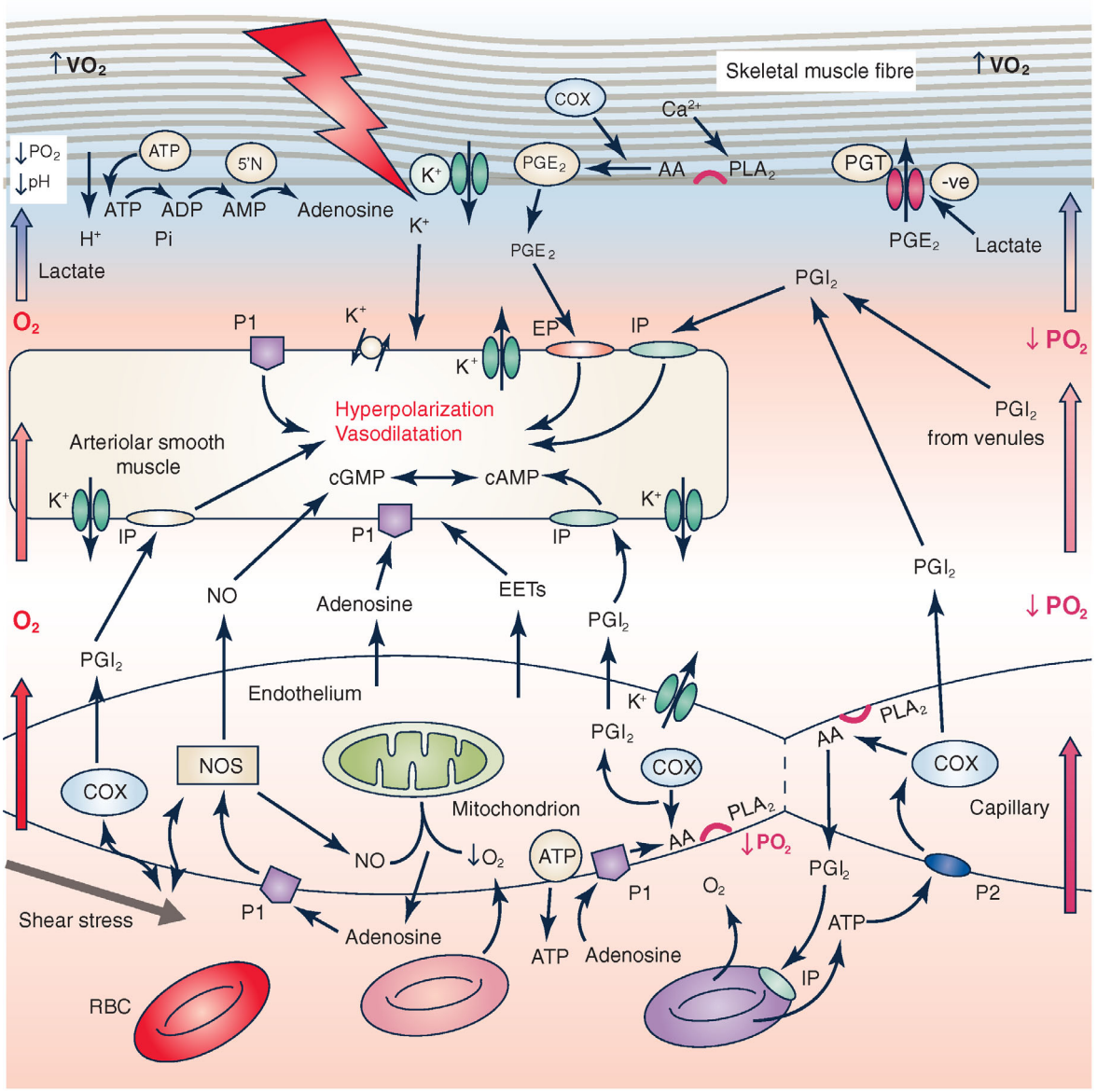

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Figure 1. Schematic diagram showing mechanisms by which prostaglandins (PGE_2 and PGI_2) are released during exercise and mechanisms by which PGs induce dilatation

Contraction and increased metabolism of muscle fibres leads to increased diffusion of O_2 from arterioles, capillaries and venules leading to steeper O_2 gradients from plasma to skeletal muscle fibres and from arterioles to venules, shown as pink to blue shading from bottom to top and left to right. PGE_2 is mainly released into interstitium from skeletal muscle fibres due to activation of arachidonic acid (AA) by raised intracellular Ca^{2+}; PGE_2 re-uptake occurs via PG transporter (PGT), which is inhibited by extracellular lactate. PGI_2 is released from endothelial cells into interstitum and plasma by activation of AA stimulated by increase in intracellular Ca^{2+} caused by the fall in intracellular O_2. PGI_2 is also released by shear stress acting on endothelial cells. PGE_2 and PGI_2 act directly on vascular smooth muscle via EP and IP receptors, respectively, to cause vasodilatation by increasing cyclic AMP (cAMP) levels and opening K^+ channels, causing hyperpolarization. PGI_2 also stimulates release of nitric oxide (NO) from endothelial cells and ATP from red blood cells (RBCs). In addition, muscle fibres release ATP, which is metabolized to adenosine by ectonuceotidases, and adenosine is released by endothelial cells as a consequence of a fall in P_{O_2}. ATP is released from red blood cells when haemoglobin is deoxygenated, and from endothelial cells by exocytosis. ATP and adenosine act via P2 receptors and P1 receptors, respectively, to stimulate PGI_2 and NO release from endothelial cells. For further details see text. 5′N, 5′-nucleotidase; cGMP, cyclic GMP; COX, cyclooxygenase; NOS, NO synthase; PLA_2, phospholipase A_2. Adapted from Marshall & Ray (2012).

Turning to P_{O_2}, arteriolar dilatation during muscle contractions was attenuated when tissue P_{O_2} was maintained by raising superfusate P_{O_2} (Gorczynski & Duling, 1978). Further, when young men breathed 40% O_2 during isometric contraction at 60% MVC to limit the fall in tissue P_{O_2}, post-contraction hyperaemia was attenuated to the same extent as with COX inhibition, while 40% O_2 and COX inhibition applied together had no greater effect (Win & Marshall, 2005). Moreover, 40% O_2 restricted to the period of contraction blunted post-contraction hyperaemia, whereas 40% O_2 given immediately post-contraction had no effect (Fordy & Marshall, 2012). These results suggest the PGs that contribute to post-contraction hyperaemia following isometric contraction accumulate as a consequence of the fall in tissue P_{O_2}.

Since muscle blood flow is limited throughout isometric contraction (Kagaya & Homma, 1997), it was possible isometric contraction accentuates O_2-dependent release of PGs. However, post-contraction hyperaemia evoked by rhythmic, or isometric contraction at 60% MVC were similarly attenuated by breathing 40%O_2, COX inhibition or their combination. Moreover, 40%O_2 greatly reduced venous efflux of PGI_2 and PGE_2: post-exercise efflux of PGI_2 was reduced by 75 ± 8.5% (mean ± SEM) and 70 ± 8.9% following rhythmic and isometric contraction, respectively, while PGE_2 efflux was reduced by 64 ± 10.0% and 67 ± 9.2%, respectively (Junejo, 2017; R. T. Junejo, C. J. Ray & J. M. Marshall, unpublished observation). Thus, it seems reasonable to propose that the release of PGI_2 and PGE_2 is largely dependent on the fall in tissue P_{O_2} during both rhythmic and isometric contractions.

Peri-arteriolar P_{O_2} falls transiently during muscle contraction, whereas P_{O_2} shows a sustained fall around capillaries and post-capillary venules (Lash & Bohlen, 1987). P_{O_2} close to skeletal muscle fibres falls gradually with increasing exercise intensity, to ∼3 mmHg during rhythmic contractions at 50–60% MVC (Richardson *et al.* 2001). Thus, the most likely sites for O_2-dependent release of PGs during exercise are terminal arterioles, capillaries, post-capillary venules and skeletal muscle fibres (Fig. 1). The increase in arterial P_{O_2} achieved with 40% O_2 must steepen the P_{O_2} gradients along the vascular tree and to the muscle fibres, raising P_{O_2} at these crucial sites; it certainly reduces lactate efflux (Fordy & Marshall, 2012).

Interactions between PGs and other factors

PGs released during muscle contraction can cause dilatation by a direct action on vascular smooth muscle (Murrant *et al.* 2014), PGI_2 and PGE_2 acting on IP and EP receptors, respectively (Feletou *et al.* 2011; Fig. 1). However, the release and actions of PGs also depend on other dilator factors whose release is O_2-dependent (see Marshall and Ray 2012).

PGs, ATP and adenosine. PGs released from post-capillary venules during muscle contraction cause dilatation of adjacent arterioles (McKay *et al.* 1998). This mechanism can be triggered by ATP (Hammer *et al.* 2001), which is released from red blood cells in proportion to O_2 unloading from haemoglobin; PGI_2 also releases ATP from red blood cells (Ellsworth *et al.* 2016). Further, a fall in P_{O_2} causes endothelial cells to release ATP by exocytosis (Lim To *et al.* 2015), and to release adenosine, by changing the balance between O_2 and NO, which compete for the same binding site on cytochrome oxidase (Edmunds *et al.* 2003). Both intra-arterially infused ATP and intra-arterially infused adenosine were shown to evoke muscle vasodilatation, which was attenuated by COX or NO synthase inhibition and accompanied by release of PGI_2 and NO into the interstitium (Ray *et al.* 2002; Mortensen *et al.* 2009; Nyberg *et al.* 2010). Since ATP and adenosine do not readily cross endothelium (Mo & Ballard, 2001), they can presumably act on adluminal P2 and P1 receptors, respectively, to release NO and PGI_2 from the abluminal surface of capillaries (see Fig. 1).

Skeletal muscle fibres also release ATP during contraction (Hellsten & Frandsen, 1997; Hellsten, 1999) by a mechanism dependent on lactic acid and indirectly on O_2 availability (Tu *et al.* 2010; Marshall & Ray, 2012). ATP is metabolized to adenosine by ectonucleotidases and 5′-nucleotidase whose activity is increased by hypoxia: both ATP and adenosine accumulate in interstitium in proportion to the level of exercise (Hellsten & Frandsen, 1997; Hellsten *et al.* 1998) (see Fig. 1). When delivered into interstitum by microdialysis, both ATP and adenosine increased interstitial PGI_2 and NO, while abluminal application of ATP caused dilatation of arterioles that was attenuated by inhibition of COX or nitric oxide synthase (NOS). Moreover, *in vitro* ATP and adenosine released NO from skeletal myocytes, and PGI_2 and NO from microvascular endothelial cells (Nyberg *et al.* 2010, 2013).

These results indicate the contributions of PGs to exercise hyperaemia must be partly mediated by PGs synthesized by ATP released from red blood cells, and/or by ATP or adenosine released from endothelium and skeletal muscle fibres (Fig. 1).

COX and NOS interactions. *In vitro* studies indicate that NO facilitates COX activity while products of the COX pathway may stimulate, or inhibit the NOS pathway (Salvemini *et al.* 2013). Further, PGs and NO interact synergistically in vascular smooth muscle via interaction between their second messengers: cAMP and cGMP, respectively. Thus, cGMP inhibits the catabolism of cAMP by phosphodiesterase, such that dilator responses evoked

by mediators that act via cAMP, including PGI_2, are facilitated by tonic NO synthesis, but attenuated by NOS inhibition (de Wit *et al.* 1994).

However, during knee extensor exercise at medium workload, NOS inhibition had no effect on PGI_2 or adenosine release into interstitium (Frandsen *et al.* 2000). Moreover, most studies report NOS inhibition decreased resting blood flow and vascular conductance, but when this was taken into account there was minimal effect on hyperaemia *during* exercise at light to maximal effort, although post-exercise hyperaemia was attenuated (Wilson & Kapoor, 1993; Endo *et al.* 1994; Gilligan *et al.* 1994; Duffy *et al.* 1999; Radegran & Saltin, 1999; Schrage *et al.* 2004). Thus, it appears newly synthesised NO makes little active contribution to exercise hyperaemia, and that inhibition of tonic NO synthesis and consequent reduction in cGMP cause little attenuation of dilatation induced by PGs or adenosine, which act via cAMP (de Wit *et al.* 1994).

Nevertheless, whilst exercise hyperaemia evoked by knee extensor exercise at 20% maximum load was not affected by COX inhibition alone, it was attenuated ∼30% by dual COX and NOS inhibition, accompanied by an increase in O_2 extraction, and increase in ATP efflux (Mortensen *et al.* 2007). Further, dual COX and NOS inhibition had no effect on exercise hyperaemia evoked by forearm contractions at 15% MVC, but progressively attenuated hyperaemia evoked at 30–60% MVC (Boushel *et al.* 2002). Moreover, hyperaemia during knee extensor exercise at 30% maximum load was attenuated by ∼30% with dual COX and NOS inhibition, by ∼14% with adenosine receptor inhibition alone, while triple blockade had no greater effect (Mortensen *et al.* 2009).

Thus, it seems likely that at light workloads, the individual dilator influences of PGs or NO are difficult to reveal because the greater fall in tissue P_{O_2} arising from attenuated exercise hyperaemia leads to compensatory increases in the release of ATP and adenosine (see Mortensen *et al.* 2007; Marshall & Ray 2012). At heavier workloads, or with both COX and NOS pathways blocked, the ability of adenosine or ATP, to cause dilatation and therefore maintain hyperaemia is limited because the mediators and second messengers by which they act, i.e. PGI_2 and NO, cAMP and cGMP, are severely depressed. By these arguments, interactions between ATP, adenosine, NO and PGs are fundamentally important in the much-discussed phenomenon of 'redundancy' that operates during exercise hyperaemia (Joyner & Wilkins, 2007; Murrant & Sarelius, 2015).

PGs and K^+. Interstitial K^+ rises rapidly at contraction onset and remains at levels related to workload (Vyskocil *et al.* 1983; Juel *et al.* 2000). K^+ released from muscle fibres initiates exercise hyperaemia by inducing hyperpolarization of capillaries and terminal arterioles (Fig. 1), which is conducted proximally to dilate arterioles and feed arteries (Bagher & Segal, 2011; Murrant & Sarelius, 2015). In addition, 'endothelium-dependent hyperpolarizing factors' (EDHFs) and specifically, epoxyeicosatrienoic acids (EETs) have been implicated in exercise hyperaemia (Hillig *et al.* 2003). EETs are released by endothelial cells in response to shear stress (Campbell & Fleming, 2010).

Consistent with these findings, dual inhibition of inwardly rectifying potassium (K_{IR}) channels and Na^+–K^+-ATPase, the mechanisms by which K^+ hyperpolarizes vascular smooth muscle (Armstrong *et al.* 2007; Campbell & Fleming, 2010), attenuated the onset *and* maintained phase of hyperaemia evoked by light forearm exercise at only 10% MVC (Crecelius *et al.* 2014). Moreover, addition of dual NOS and COX inhibition further attenuated both phases, even though NOS or COX inhibition alone or in combination had no effect during light exercise (Shoemaker *et al.* 1996; Radegran & Saltin, 1999; Boushel *et al.* 2002; Crecelius *et al.* 2014). Thus, these results suggest that hyperpolarization of endothelial and/or vascular smooth muscle cells blunts dilatation that might otherwise be induced by PGs and/or NO.

Accordingly, superfusion of hamster cremaster muscle with K^+ at 10 mM, as measured in interstitium during high workloads (Juel *et al.* 2000), attenuated arteriolar dilatation induced by graded concentrations of adenosine or NO donor, whereas neither NO donor nor adenosine affected dilatation induced by high K^+ (Lamb & Murrant, 2015). Given the mechanisms by which adenosine and NO evoke dilatation include opening of K^+ channels (Edwards *et al.* 2010; Marshall & Ray, 2012; Murrant & Sarelius, 2015), it is probable hyperpolarization induced by K^+ prevented adenosine and NO from producing their full effects. Since the actions of PGI_2 and PGE_2 also include opening of K^+ channels (Zhu *et al.* 2002; Edwards *et al.* 2010), K^+ would be expected to interfere with the dilator actions of PGs.

Towards a unifying hypothesis for PG involvement

Considering the evidence discussed so far, we suggest several factors contribute to the controversy over whether PGs contribute to exercise hyperaemia. There is experimental evidence indicating PGI_2 and PGE_2 are released from muscle in proportion to the level of exercise. Increased shear stress and reduced P_{O_2} are adequate stimuli for PGI_2 release from endothelial cells, and muscle contraction releases PGE_2 from skeletal muscle fibres. However, PGI_2 and NO are also generated as intermediates in the pathways by which two other O_2-dependent mediators – adenosine and ATP – make their contributions to exercise hyperaemia. Further, by generating cGMP, NO determines responsiveness to substances that act via cAMP, including PGs. On the other hand, K^+, which is released

Table 1. Baseline characteristics of male and female white Europeans (WE) and black Africans (BA)

	Male WE ($n = 10$)	Female WE ($n = 8$)	Male BA ($n = 10$)	Female BA ($n = 8$)
Age (years)	22.1 ± 0.7	22.7 ± 1.2	20.7 ± 0.7	24.2 ± 0.8*
Body mass index (kg/m^2)	22.5 ± 0.7	22.7 ± 1.4	22.5 ± 0.6	20.7 ± 1.5
Waist circumference (cm)	76.8 ± 1.4	73.7 ± 1.7	77.6 ± 2.1	69.7 ± 2.4*
Systolic blood pressure (mmHg)	102.5 ± 1.9	97.1 ± 3.0	112.9 ± 3.7	95.6 ± 2.8*
Diastolic blood pressure (mmHg)	63.2 ± 1.3	62.4 ± 2.4	67.3 ± 2.4	60.7 ± 2.0
Heart rate (beats/min)	71.8 ± 2.5	73.8 ± 5.4	67.5 ± 3.4	70.9 ± 3.3
Mean arterial pressure (mmHg)	76.3 ± 1.2	74.0 ± 2.4	82.5 ± 2.6	72.4 ± 2.1**
Forearm blood flow (ml/100ml/min)	5.8 ± 0.6	5.90 ± 1.1	6.1 ± 0.6	4.6 ± 0.5
Forearm vascular conductance (ml/100ml/min/mmHg)	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.0
Forearm circumference (cm)	24.2 ± 0.3	23.5 ± 0.3	26.9 ± 0.4	22.1 ± 0.7**
100% MVC (kg)	22.9 ± 2.0	16.3 ± 1.0*	32.0 ± 2.7	15.9 ± 1.7**

Values are shown as means ± SEM. $^{**}P < 0.01$, $^{*}P < 0.05$, male *vs.* female within ethnicity.

from the onset of contraction may initiate exercise hyperaemia but attenuate the influence of several key dilators, probably by opening K^+ channels. Thus, we propose that at light workloads, lack of effect of COX inhibition may be explained because K^+ attenuates the action of PGs, but also because there is reciprocal release of O_2-dependent adenosine and ATP. Nevertheless, single inhibition of PG synthesis by COX, *does* attenuate exercise hyperaemia by 20–40% during and following muscle contraction at medium to heavy workloads (Cowley *et al.* 1985; Wilson & Kapoor, 1993; Duffy *et al.* 1999; Schrage *et al.* 2004; Win & Marshall, 2005). Thus, higher concentrations of PGs overcome any inhibitory effects of K^+ and make a substantial direct contribution, by acting on IP and EP receptors to increase cAMP in vascular smooth muscle. However, COX inhibition may well partially attenuate the contributions of adenosine and ATP. Reciprocally, inhibition of their effects probably attenuates contributions of PGs.

Ethnicity and exercise hyperaemia

None of the studies discussed thus far have indicated the ethnicity of the subjects. This is important given endothelium-dependent dilatation is blunted in those of black African (BA) and South Asian descent relative to those of white European (WE) origin and associated with higher prevalence of cardiovascular disease (Hertz *et al.* 2005; Gupta *et al.* 2006).

It has already been reported that young BA men and women showed blunted endothelium-dependent dilatation compared to WEs in response to agonists (Kahn *et al.* 2002), reactive hyperaemia (Campia *et al.* 2002; Heffernan *et al.* 2008) and the forearm vasodilator response to mental stress (Cardillo *et al.* 1998). Blunted vasodilator responsiveness to NO (Stein *et al.* 1997), reduced NO bioavailability and impaired cGMP-dependent mechanisms have been implicated (Stein *et al.* 1997; Cardillo *et al.* 1999; Melikian *et al.* 2007). Few have compared vasodilator responses to exercise between ethnicities. In young BA and WE men, Doppler ultrasound recordings during rhythmic handgrip at 10 and 20% MVC or 15–45% MVC indicated the increases in FBF and vascular conductance were smaller in BAs (Kappus *et al.* 2017; Barbosa *et al.* 2018). Further, in early middle-aged men (mean age 39 years), NOS inhibition had greater attenuating effects in WEs than in BAs on resting FBF and forearm vasodilator responses to rhythmic contractions at 40% MVC, whereas K^+ channel inhibition had similar effects in BAs and WEs at rest, but greater attenuating effects in BAs during exercise. It was therefore suggested EDHF-mediated dilatation compensates for impaired NO availability during exercise in BA men (Ozkor *et al.* 2014). However, ageing may have complicated these findings: the effect of COX and NOS inhibition on exercise hyperaemia decreased with age (Schrage *et al.* 2007).

Against this background, we recently compared post-exercise hyperaemia responses in young WE and BA men and women (in each group: $n = 18$: 10 male, 8 female). Inclusion criteria were systolic/diastolic pressure <140/90 mmHg, normal BMI, recreationally active, but not trained (Table 1). Women were tested in the low oestrogen phase of the menstrual cycle. Subjects refrained from caffeine-containing beverages and alcohol for at least 12 h; none were taking medication. Experiments were performed in a temperature-controlled room at 21–23°C. The study was approved by the University of Birmingham Ethics Committee (ERN15-0714); all subjects gave informed consent. Rhythmic handgrip contractions were performed at 60% MVC for 2 min with the dominant hand by using a dynamometer, contractions being performed at 2 s intervals (1 s contraction/1 s relaxation). An audio signal and visual display of the output of the dynamometer

were used to ensure the subject achieved the required workload. FBF was recorded from the same arm by using VOP before, immediately after contractions ceased and at intervals thereafter (see Fig. 2). For each recording of FBF, the slope of the increase in forearm circumference was computed over the first one to two heart beats following venous occlusion at 50 mmHg to optimize the accuracy of the FBF measurement (Junejo *et al.* 2019). VOP was automatically calibrated and FBF was expressed per 100 ml tissue. Pulsatile arterial blood pressure (ABP) was continuously recorded by photoplethysmography via a finger cuff on the non-dominant hand: forearm vascular conductance (FVC) was calculated as FBF/ABP.

Considered as mixed male/female groups, BAs showed similar increases in ABP, but smaller increases in post-exercise FBF and FVC than WEs (Fig. 2*A*). Since all subjects achieved the task without fatigue, BA men and women considered together achieved this workload with lower blood flow and less vasodilatation than WEs.

Sex and exercise hyperaemia

So far, we have not considered how sex might affect exercise hyperaemia. This issue is complicated by men generally being stronger than women, exerting stronger compressive force, and causing more vascular occlusion during contraction (Russ & Kent-Braun, 2003). In studies in which men had a 1.6-fold greater absolute MVC than women, post exercise hyperaemia and vasodilatation were *greater* in men following isometric contractions at 20–80% MVC (Hunter *et al.* 2006). By contrast, when comparisons were made between men and women who

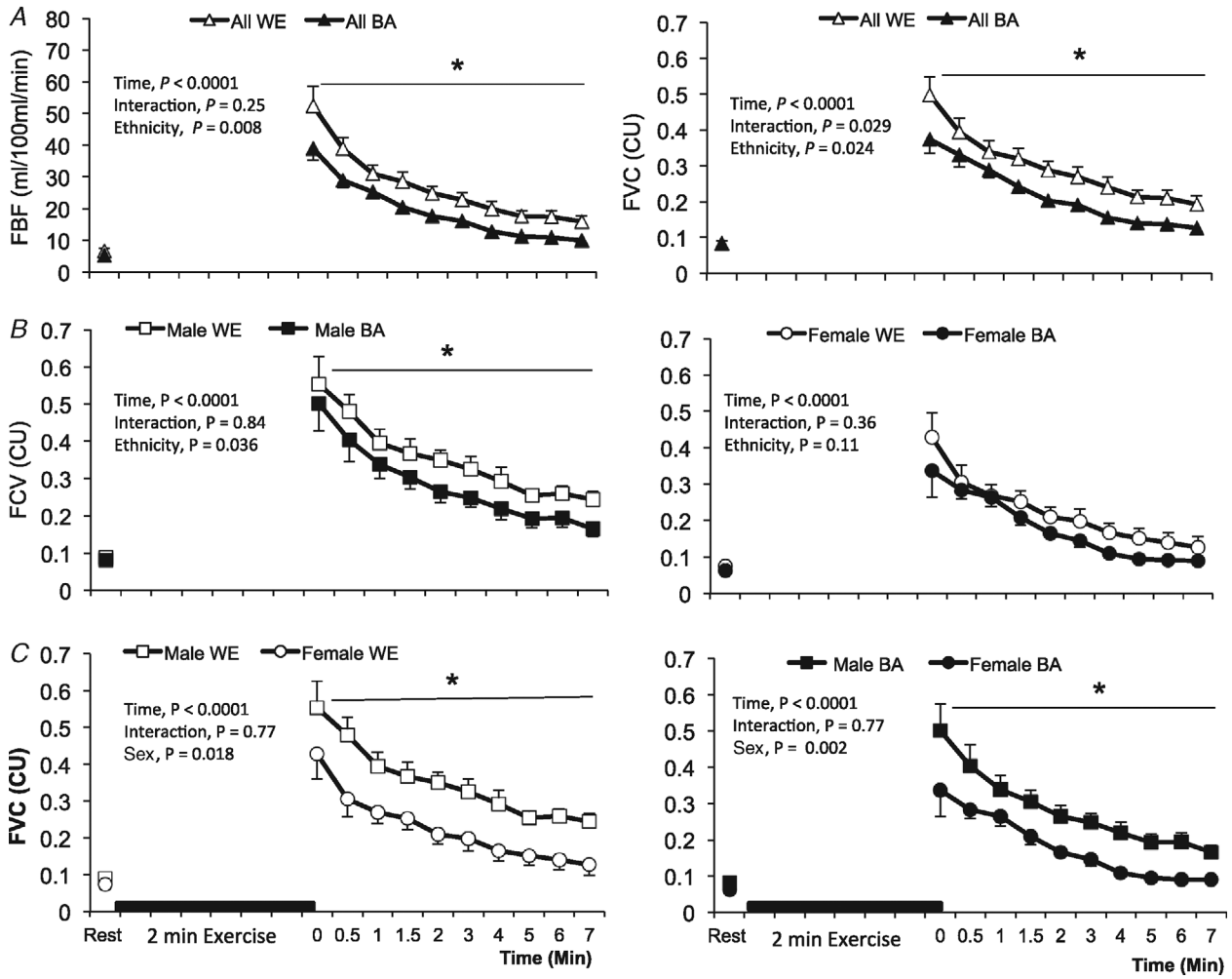

Figure 2. Effects of rhythmic contractions at 60% MVC for 2 min on forearm vasculature of young WE and BA men and women
A, comparisons between all WEs and all BAs for post-exercise forearm blood flow (FBF; left) and forearm vascular conductance (FVC in conductance units (CU); right). *B*, comparisons between WE and BA men (left) and WE and BA women (right) for post-exercise FVC. *C*, comparisons between WE men and women (left) and BA men and women (right) for post-exercise FVC. All data points are shown as mean ± SEM. Outcomes are provided for repeated measures ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$ from immediately contractions ceased (time 0) until 7 min.

were matched for muscle strength, post-exercise hyperaemia and vascular conductance were similar. These results suggest that when differences in compressive force are avoided, post-exercise blood flow is similarly coupled to workload and muscle metabolism in both sexes (Hunter *et al.* 2006).

If the *magnitude* of the compressive force and extent of vascular occlusion during contraction are the important factors, the findings of Kelly *et al.* (2004) are consistent with this idea: post-exercise hyperaemia and vascular conductance following *rhythmic* exercise at 15% MVC were similar in young men and women (Kelly *et al.* 2004). Similarly, Doppler ultrasound recordings *during* ramped, light rhythmic exercise, averaged over contraction and relaxation phases, indicated FBF was similar in men and women when compared at the same absolute workloads, but was greater in men at task failure (∼14% MVC) when absolute load was greater in men (Gonzales *et al.* 2007). However, other studies on light, rhythmic contractions yielded disparate results: FBF was similar in men and women during 15 and 30% MVC (Limberg *et al.* 2010), *smaller* in women than in men during 10 and 20% MVC (Casey *et al.* 2014), but *larger* in women than in men at 15% MVC (Kellawan *et al.* 2015).

Findings at higher workloads suggest additional factors are involved. During intense, rhythmic contractions (at MVC for 4 min) of forearm, Doppler ultrasound recordings in the relaxation phases, showed increases in FBF and vascular conductance were ∼25% *larger* in young women than men throughout exercise (Saito *et al.* 2008). Moreover, ultrasound recordings in young men and women during graded knee extensor exercise, showed increases in leg blood flow and vascular conductance were *greater* in women at the same absolute workloads whether compared as mean values over contraction and relaxation cycles, or during the relaxation phases. They were also greater in women when compared at the same relative workload, from 20–100% maximum (Parker *et al.* 2007). The authors suggested the disparity might reflect greater dependence on oxidative metabolism in women (Kent-Braun *et al.* 2002) and greater influence of O_2-dependent dilators, or facilitatory effects of oestrogen.

In our study, men had larger forearm circumference and greater MVC than women in both ethnic groups; there were no differences between WE and BA men, or WE and BA women (Table 1). Firstly, extending the findings of Barbosa *et al.* (2018) on BA and WE men at 45% MVC, post-exercise FVC following 60% MVC was lower in BA than in WE men. The trend for post-exercise FVC to be smaller in BA women than in WE women did not reach statistical significance (Fig. 2*B*). Secondly, within both ethnicities, women showed *smaller* post-exercise increases in FVC than men (Fig. 2C). Thus, it seems the facilitatory effects of being female on post-exercise vasodilatation following strenuous contractions is relatively weak in both ethnicities (Parker *et al.* 2007), at least, in the forearm. Rather, the greater occlusive effects of each contraction may have dominated in men (Hunter *et al.* 2006), such

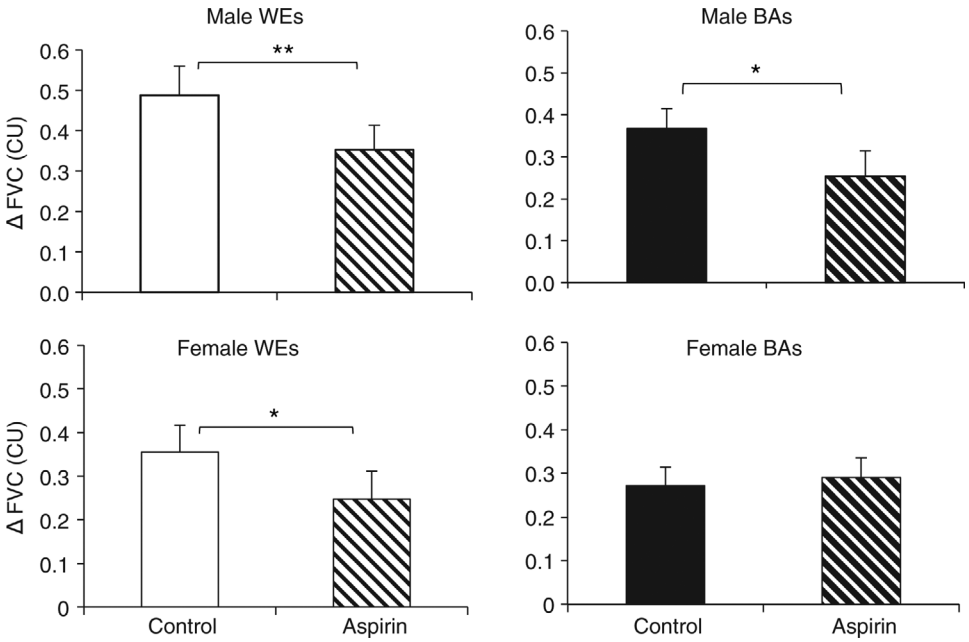

Figure 3. Effect of COX inhibition with aspirin on peak change in forearm vascular conductance (FVC) following rhythmic contractions at 60% MVC for 2 min in WE and BA men (above) and WE and BA women (below)
Values are shown as change in FVC in conductance units (CU): mean ± SEM. *$P < 0.05$, **$P < 0.01$ before *vs.* after aspirin.

that when exercise ceased, accumulated vasodilators had a greater influence, irrespective of BA or WE ethnicity.

Oestrogen, PGs and exercise hyperaemia. Raised levels of oestrogen increase NOS and COX expression in endothelial cells, while oestrogen facilitates NO and PGI_2 generation by agonists and shear stress. Oestrogen also relaxes vascular smooth muscle facilitating the cAMP pathway and increasing K^+ channel activity (Huang & Kaley, 2004). Thus, higher levels of oestrogen in premenopausal women might be expected to facilitate the component of exercise hyperaemia that is dependent on PGs and interactions with ATP, adenosine, K^+ and NO.

However, BA women show earlier onset and faster increase in prevalence of hypertension than BA men (Hertz *et al.* 2005; Geronimus *et al.* 2007). This was attributed to increased influences of psychosocial stressors amongst BA women (Geronimus *et al.* 2007), factors that may underlie the increasing prevalence of hypertension in sub-Saharan Africa with progressive urbanization (Opie & Seedat, 2005). Accordingly, endothelial dysfunction is particularly pronounced in BA women. Flow-mediated dilatation, was smaller in young early-middle-aged BA than WE women (Perregaux *et al.* 2000; Bransford *et al.* 2001) and reactive hyperaemia was smaller in young BA than WE women (Aiku *et al.* 2016). Flow-mediated dilatation and reactive hyperaemia are NO-dependent, but also mediated by PGs and EDHFs (Engelke *et al.* 1996; Stoner *et al.* 2012; Crecelius *et al.* 2013; Green *et al.* 2014).

In the only study to date comparing COX inhibition on exercise hyperaemia in young men and women, infusion of COX inhibitor during light contractions at 15% MVC attenuated the vasodilatation to similar extents in men and women (Kellawan *et al.* 2015), but whereas in their earlier study on men and women (Schrage *et al.* 2004), in which COX inhibition transiently attenuated the increase in FVC, Kellawan *et al.* (2015) found COX inhibition *augmented* the increase in FVC in both sexes. There was no obvious explanation for the disparity.

The results described above from our own study on BAs and WEs were performed 30 min after a placebo drink (orange squash in water), so that the results could be compared with those obtained in comparable experiments on a different day, starting 30 min after COX inhibition with aspirin (600 mg in orange squash, see Win & Marshall, 2005). COX inhibition attenuated post-exercise vasodilatation in both WE and BA men and in WE women, attenuating the peak FVC by ∼30% in all three groups (Fig. 3). By contrast, COX inhibition had no effect in BA women (Fig. 3). Thus, even though post-exercise vasodilatation is smaller in BA than in WE men, and even though BAs have smaller proportions of oxidative fibres (Ceaser & Hunter, 2015), the fall in tissue P_{O_2} during contractions at 60% MVC is apparently sufficient to allow PGs whose release is largely O_2-dependent, to be released in BA men and make a substantial contribution to exercise hyperaemia.

Thus, our results in WE women provide no indication that oestrogen facilitates the contribution of PGs to exercise hyperaemia relative to WE men as might have been expected from effects of oestrogen on COX (Huang & Kaley, 2004). Moreover, comparison of peak increases in FVC in WE and BA women (Fig. 3) suggests that *absence* of the PG contribution played a major part in blunting post-exercise dilatation in BA women. Given endothelium-dependent dilatation is depressed in young BA, relative to WE women (Perregaux *et al.* 2000; Bransford *et al.* 2001; Aiku *et al.* 2016), we suspect the absence of PG involvement largely reflects impaired endothelial function. Indeed, our results suggest that disturbed vasodilator contributions of PGs to exercise hyperaemia in young BA women may serve as an early functional marker of their increased risk of hypertension and cardiovascular disease (Hertz *et al.* 2005; Geronimus *et al.* 2007).

Concluding remarks

Seen against a background of well over a century of experimentation on exercise hyperaemia, mostly performed on WE men, our results demonstrate pronounced differences between young people of WE and BA ethnicities and between sexes in the magnitude of exercise hyperaemia evoked by rhythmic contractions at 60% MVC. The relative contribution of O_2-dependent PGs to these responses is similar in both WE and BA men and WE women, but is absent in BA women. From an experimental viewpoint, these are good reasons to take ethnicity and sex into account in any investigation of exercise hyperaemia. From physiological and clinical perspectives, it will be important to establish whether the smaller hyperaemic responses in BAs and especially in BA women reflect different oxidative/glycolytic profiles and release of O_2-dependent and O_2-independent dilators, or early signs of cardiovascular disease.

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Additional information

Competing interests

Neither of the authors has any conflicts of interest.

Author contributions

A.O.A. and J.M.M. conceived the original studies described in this review; A.O.A. took responsibility for performing these studies and for analysing and interpreting the data. A.O.A. and J.M.M. drafted the manuscript, critically revised it and approved the final version. Both authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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