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Contribution of Prostaglandins to exercise hyperaemia: workload, ethnicity and sex matter!

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Abstract

The contribution of prostaglandins (PGs) to exercise hyperaemia is controversial. In this review, we argue this is partly explained by differences between studies in exercise intensity. The effects of cyclooxygenase (COX) inhibition and PG assays, PGs contribute more at moderate to heavy, than 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 women in 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 3 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.

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-heavy load 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 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% 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 4-5s 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 2-3 fold increases PGE_2 and 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 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 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 contain 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 $PGF_{2\alpha}$ 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 Figure 1).

In vitro, increased intraluminal shear rate, or graded fall in PO_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 PO_2 could augment interstitial PGE_2 accumulation (Figure 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.

Turning to PO_2, arteriolar dilatation during muscle contractions was attenuated when tissue PO_2 was maintained by raising superfusate PO_2 (Gorczynski & Duling, 1978). Further, when young men breathed 40%O_2 during isometric contraction at 60% MVC to limit the fall in tissue PO_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 contraction ceased 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 PO_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 released 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); Junejo, Ray & Marshall, unpublished observation). Thus, it

seems reasonable to propose that the release of PGI_2 and PGE_2 are largely dependent on the fall in tissue PO_2 during both rhythmic and isometric contractions.

Peri-arteriolar PO_2 falls transiently during muscle contraction, whereas PO_2 shows a sustained fall around capillaries and post-capillary venules (Lash & Bohlen, 1987). PO_2 close to skeletal muscle fibres falls gradually with increasing exercise intensity, to ~3mmHg 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 (Figure 1). The increase in arterial PO_2 achieved with 40%O_2 must steepen the PO_2 gradients along the vascular tree and to the muscle fibres, raising PO_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; Figure 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 PO_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 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 Figure 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 Figure 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 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; Nyberg *et al.*, 2013).

These results indicate the contributions of PGs to exercise hyperemia 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 (Figure 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 2nd 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 - 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 PO_2 arising from attenuated exercise hyperaemia leads to compensatory increases in the release of ATP and adenosine, (see Mortensen *et al.* (2007); Marshall and 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 2nd messengers by which they act, ie 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 potassium (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 (Figure 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, EETs (epoxyeicosatrienoic acids) 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 hyperpolarisation of endothelial and/or vascular smooth muscle cells blunt dilatation that might otherwise be induced by PGs and/or NO.

Accordingly, superfusion of hamster cremaster muscle with K^+ at 10mM, 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 PO_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 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-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 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: 10/8, male/ 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 hours; none were taking medication. Experiments were performed in a temperature-controlled room at 21-23^{o}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/ 1s relaxation). An audio signal and visual display of the output of the dynamanometer 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 Figure 2). For each recording of FBF, the slope of the increase in forearm circumference was computed over the first 1-2 heart beats following venous occlusion at 50mmHg to optimize the accuracy of the FBF measurement (Junejo et al, 2018). 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 (Figure 2A). 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 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 is the important factor, the findings of Kelly *et al.* (2004) are consistent with this idea. For, 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 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 men during 10 and 20% MVC (Casey *et al.*, 2014), but *larger* in women than 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 WE men. The trend for post-exercise FVC to be smaller in BA women than WE women did not reach statistical significance (Figure 2B). Secondly, within both ethnicities, women showed *smaller* post-exercise increases in FVC than men (Figure 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 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 WE women, attenuating the peak FVC by ~ 30% in all 3 groups (Figure 3). By contrast, COX inhibition had no effect in BA women (Figure 3). Thus, even though post-exercise vasodilatation is smaller in BA than WE men, and even though BAs have smaller proportions of oxidative fibres (Ceaser & Hunter, 2015), the fall in tissue PO_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 (Figure 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.

Competing interests

Neither of the authors has any conflicts of interest.

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	Male WE (n=10)	Female WE (n=8)	Male BA (n=10)	Female BA (n=8)
Age (yrs)	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**

Table 1: Characteristics of Male and female white Europeans (WE) and Black Africans (BA). Values are shown as mean ± SEM Baseline values for gender groups of White Europeans (WE) and Black Africans (BA). ** , *: $P<0.01$, $P<0.05$ respectively, male vs female within ethnicity

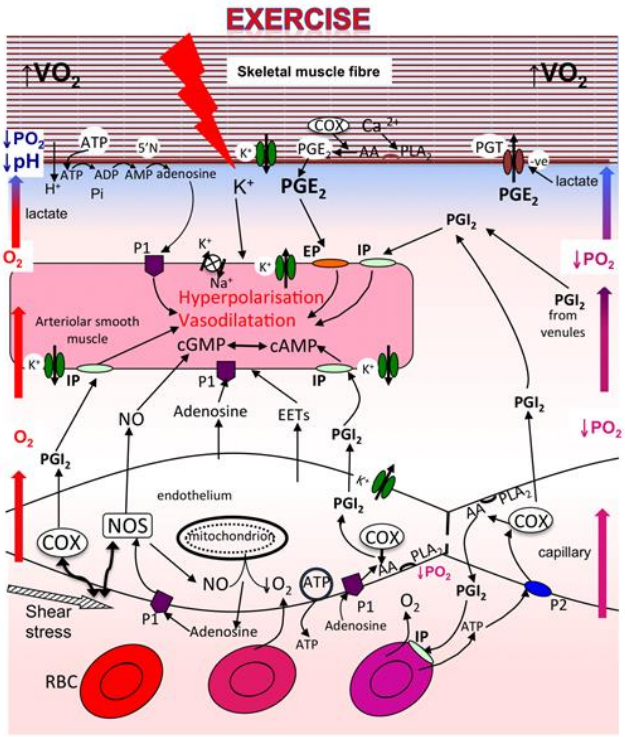

Figure 1. Schematic diagram showing mechanisms by which prostaglandins (PGE_2 and PGI_2) are released during exercise and mechanisms by which PGs induce dilatation.

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 metabolised to adenosine by ectonuceotidases, and adenosine is

released by endothelial cells as a consequence of fall in PO_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. Abbreviations: 5'N: 5'nucleotidase, cGMP; cyclic GMP, COX; cyclooxygenase, NOS; NO synthase, PLA_2; phospholipase A_2. For further details see text. Adapted from Marshall & Ray (2012).

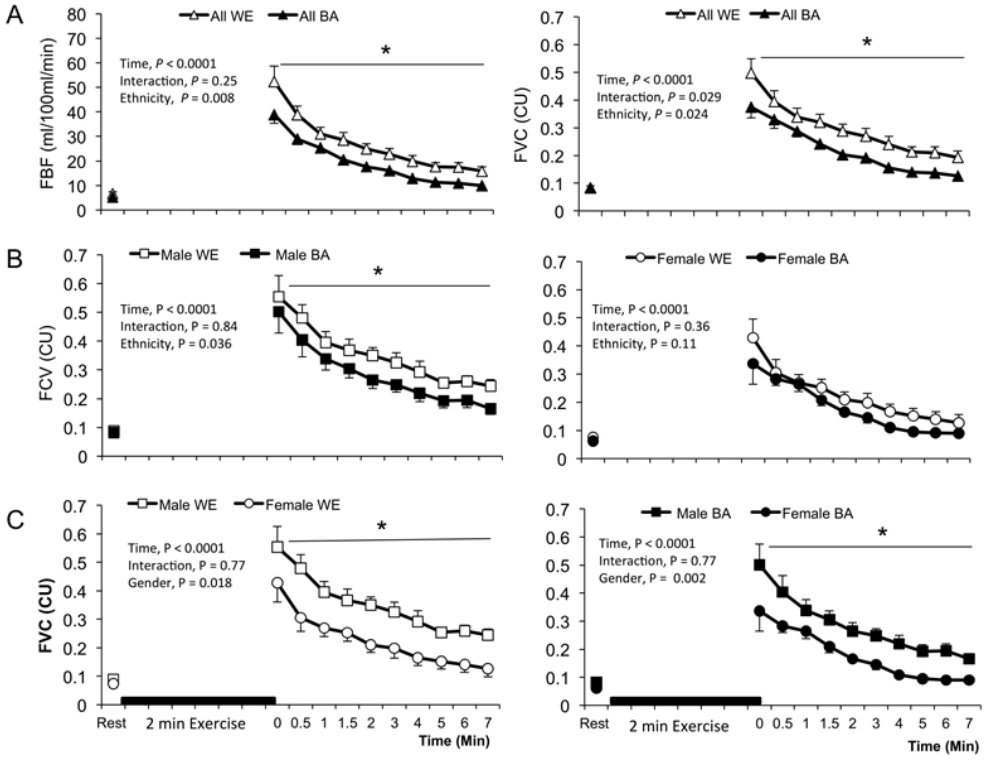

Figure 2: Effects of rhythmic contractions at 60% MVC for 2 min on forearm vasculature of young WE and BA men and women. A: Comparisons between all WEs and all BAs for post-exercise forearm blood flow (FBF; left) and forearm vascular conductance (FVC; right). B: Comparisons between WE and BA men (left) 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. *, ** $p < 0.05$, $p<0.01$ respectively from immediately contractions ceased (time 0) until 7 min.

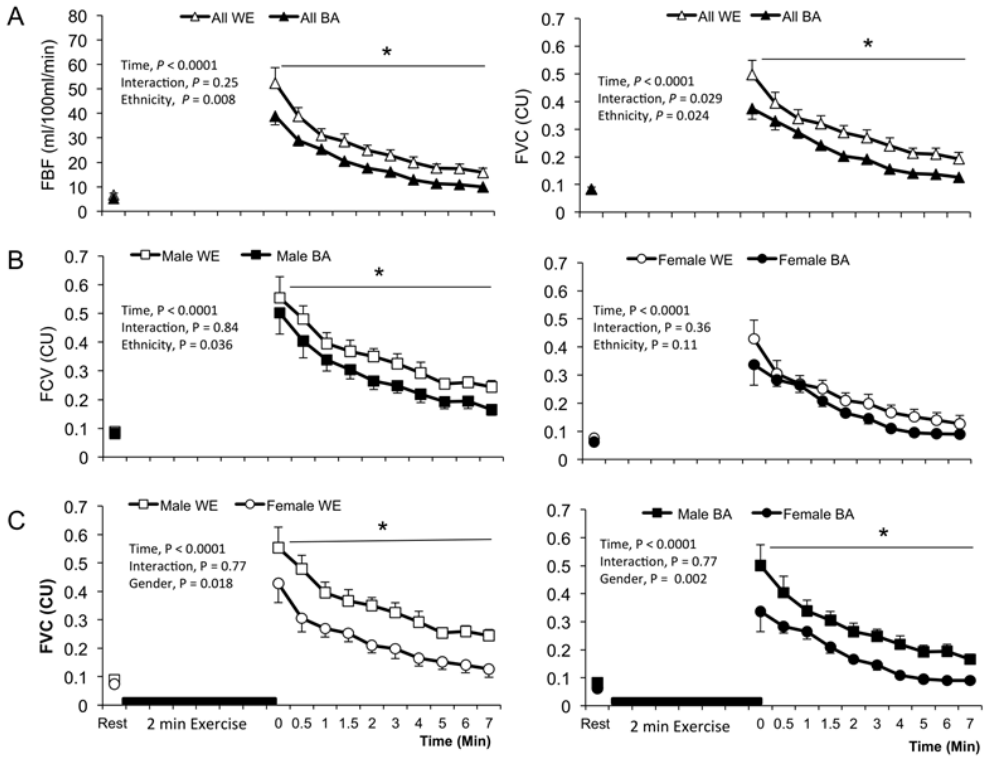

Figure 3: Effect of COX inhibition with aspirin on peak change in Forearm vascular conductance (FVC) following rhythmic contractions at 60% MVC for 2 min in WE and BA men (above) and WE and BA women (below). Values are shown mean ± SEM. §, §§: $p<0.05$, $p<0.01$ respectively before vs after aspirin.

Abstract Figure. 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 young women, both in those of White European (WE) and Black African (BA) ethnicity. However, the dilator effects of PGs are deficient in BA women implying endothelial dysfunction.