Contribution of prostaglandins to exercise hyperaemia

Aiku, Abimbola; Marshall, Janice

DOI:
10.1113/JP278033

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

Abimbola O Aiku

&

Janice M Marshall

Institute of Clinical Sciences

College of Medical & Dental Sciences

University of Birmingham

Birmingham B15 2TT

Short title: Prostaglandins and exercise hyperaemia

Key words: prostaglandins, ethnicity, sex, hyperaemia, vasodilatation, exercise

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.

Funding

The authors gratefully acknowledge the financial support of the Tertiary Education Trust Fund (TET Fund), and the University of Ibidan, Nigeria

References

Aiku AO, Martin U & Marshall JM. (2016). 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.

Armstrong ML, Dua AK & Murrant CL. (2007). Potassium initiates vasodilatation induced by a single skeletal muscle contraction in hamster cremaster muscle. *J Physiol* **581,** 841-852.

Bagher P & Segal SS. (2011). Regulation of blood flow in the microcirculation: role of conducted vasodilation. *Acta Physiol (Oxf)* **202,** 271-284.

Barbosa TC, Kaur J, Stephens BY, Akins JD, Keller DM, Brothers RM & Fadel PJ. (2018). Attenuated forearm vascular conductance responses to rhythmic handgrip in young African-American compared with Caucasian-American men. *Am J Physiol Heart Circ Physiol* **315,** H1316-H1321.

Berna N, Arnould T, Remacle J & Michiels C. (2001). Hypoxia-induced increase in intracellular calcium concentration in endothelial cells: role of the Na(+)-glucose cotransporter. *Journal of cellular biochemistry* **84,** 115-131.

Berthiaume F & Frangos JA. (1992). Flow-induced prostacyclin production is mediated by a pertussis toxin-sensitive G protein. *FEBS Lett* **308,** 277-279.

Boushel R, Langberg H, Gemmer C, Olesen J, Crameri R, Scheede C, Sander M & Kjaer M. (2002). Combined inhibition of nitric oxide and prostaglandins reduces human skeletal muscle blood flow during exercise. *J Physiol* **543,** 691-698.

Bransford TL, St Vrain JA & Webb M. (2001). Abnormal endothelial function in young African-American females: discordance with blood flow. *J Natl Med Assoc* **93,** 113-119.

Burkholder TJ. (2007). Mechanotransduction in skeletal muscle. *Front Biosci* **12,** 174-191.

Campbell WB & Fleming I. (2010). Epoxyeicosatrienoic acids and endothelium-dependent responses. *Pflugers Arch* **459,** 881-895.

Campia U, Choucair WK, Bryant MB, Waclawiw MA, Cardillo C & Panza JA. (2002). Reduced endothelium-dependent and -independent dilation of conductance arteries in African Americans. *J Am Coll Cardiol* **40,** 754-760.

Cardillo C, Kilcoyne CM, Cannon RO, 3rd & Panza JA. (1998). Racial differences in nitric oxide-mediated vasodilator response to mental stress in the forearm circulation. *Hypertension* **31,** 1235-1239.

Cardillo C, Kilcoyne CM, Cannon RO, 3rd & Panza JA. (1999). Attenuation of cyclic nucleotide-mediated smooth muscle relaxation in blacks as a cause of racial differences in vasodilator function. *Circulation* **99,** 90-95.

Casey DP, Shepherd JR & Joyner MJ. (2014). Sex and vasodilator responses to hypoxia at rest and during exercise. *J Appl Physiol (1985)* **116,** 927-936.

Ceaser T & Hunter G. (2015). Black and White race differences in aerobic capacity, muscle fiber type, and their influence on metabolic processes. *Sports Med* **45,** 615-623.

Chan BS, Endo S, Kanai N & Schuster VL. (2002). Identification of lactate as a driving force for prostanoid transport by prostaglandin transporter PGT. *American journal of physiology Renal physiology* **282,** F1097-1102.

Cowley AJ, Stainer K, Rowley JM & Wilcox RG. (1985). Effect of aspirin and indomethacin on exercise-induced changes in blood pressure and limb blood flow in normal volunteers. *Cardiovasc Res* **19,** 177-180.

Crecelius AR, Kirby BS, Luckasen GJ, Larson DG & Dinenno FA. (2013). Mechanisms of rapid vasodilation after a brief contraction in human skeletal muscle. *Am J Physiol Heart Circ Physiol* **305,** H29-40.

Crecelius AR, Luckasen GJ, Larson DG & Dinenno FA. (2014). KIR channel activation contributes to onset and steady-state exercise hyperemia in humans. *Am J Physiol Heart Circ Physiol* **307,** H782-791.

de Wit C, von Bismarck P & Pohl U. (1994). Synergistic action of vasodilators that increase cGMP and cAMP in the hamster cremaster microcirculation. *Cardiovasc Res* **28,** 1513-1518.

Duffy SJ, New G, Tran BT, Harper RW & Meredith IT. (1999). Relative contribution of vasodilator prostanoids and NO to metabolic vasodilation in the human forearm. *Am J Physiol* **276,** H663-670.

Edmunds NJ, Moncada S & Marshall JM. (2003). Does nitric oxide allow endothelial cells to sense hypoxia and mediate hypoxic vasodilatation? In vivo and in vitro studies. *J Physiol* **546,** 521-527.

Edwards G, Feletou M & Weston AH. (2010). Endothelium-derived hyperpolarising factors and associated pathways: a synopsis. *Pflugers Arch* **459,** 863-879.

Ellsworth ML, Ellis CG & Sprague RS. (2016). Role of erythrocyte-released ATP in the regulation of microvascular oxygen supply in skeletal muscle. *Acta Physiol (Oxf)* **216,** 265-276.

Endo T, Imaizumi T, Tagawa T, Shiramoto M, Ando S & Takeshita A. (1994). Role of nitric oxide in exercise-induced vasodilation of the forearm. *Circulation* **90,** 2886-2890.

Engelke KA, Halliwill JR, Proctor DN, Dietz NM & Joyner MJ. (1996). Contribution of nitric oxide and prostaglandins to reactive hyperemia in human forearm. *J Appl Physiol (1985)* **81,** 1807-1814.

Feletou M, Huang Y & Vanhoutte PM. (2011). Endothelium-mediated control of vascular tone: COX-1 and COX-2 products. *Br J Pharmacol* **164,** 894-912.

Fordy GR & Marshall JM. (2012). Breathing 40% O(2) can attenuate postcontraction hyperaemia or muscle fatigue caused by static forearm contraction, depending on timing. *Exp Physiol* **97,** 362-374.

Frandsen U, Bangsbo J, Langberg H, Saltin B & Hellsten Y. (2000). Inhibition of nitric oxide synthesis by systemic N(G)-monomethyl-L-arginine administration in humans: effects on interstitial adenosine, prostacyclin and potassium concentrations in resting and contracting skeletal muscle. *J Vasc Res* **37,** 297-302.

Frisbee JC, Maier KG, Falck JR, Roman RJ & Lombard JH. (2002). Integration of hypoxic dilation signaling pathways for skeletal muscle resistance arteries. *Am J Physiol Regul Integr Comp Physiol* **283,** R309-319.

Geronimus AT, Bound J, Keene D & Hicken M. (2007). Black-white differences in age trajectories of hypertension prevalence among adult women and men, 1999-2002. *Ethn Dis* **17,** 40-48.

Gilligan DM, Panza JA, Kilcoyne CM, Waclawiw MA, Casino PR & Quyyumi AA. (1994). Contribution of endothelium-derived nitric oxide to exercise-induced vasodilation. *Circulation* **90,** 2853-2858.

Gonzales JU, Thompson BC, Thistlethwaite JR, Harper AJ & Scheuermann BW. (2007). Forearm blood flow follows work rate during submaximal dynamic forearm exercise independent of sex. *J Appl Physiol (1985)* **103,** 1950-1957.

Gorczynski RJ & Duling BR. (1978). Role of oxygen in arteriolar functional vasodilation in hamster striated muscle. *Am J Physiol* **235,** H505-515.

Green DJ, Dawson EA, Groenewoud HM, Jones H & Thijssen DH. (2014). Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. *Hypertension* **63,** 376-382.

Green S, Thorp R, Reeder EJ, Donnelly J & Fordy G. (2011). Venous occlusion plethysmography versus Doppler ultrasound in the assessment of leg blood flow during calf exercise. *Eur J Appl Physiol* **111,** 1889-1900.

Gupta M, Singh N & Verma S. (2006). South Asians and cardiovascular risk: what clinicians should know. *Circulation* **113,** e924-929.

Hammer LW, Ligon AL & Hester RL. (2001). ATP-mediated release of arachidonic acid metabolites from venular endothelium causes arteriolar dilation. *Am J Physiol Heart Circ Physiol* **280,** H2616-2622.

Hecker M, Mulsch A, Bassenge E & Busse R. (1993). Vasoconstriction and increased flow: two principal mechanisms of shear stress-dependent endothelial autacoid release. *Am J Physiol* **265,** H828-833.

Heffernan KS, Jae SY, Wilund KR, Woods JA & Fernhall B. (2008). Racial differences in central blood pressure and vascular function in young men. *Am J Physiol Heart Circ Physiol* **295,** H2380-2387.

Hellsten Y. (1999). The effect of muscle contraction on the regulation of adenosine formation in rat skeletal muscle cells. *J Physiol* **518 (Pt 3),** 761-768.

Hellsten Y & Frandsen U. (1997). Adenosine formation in contracting primary rat skeletal muscle cells and endothelial cells in culture. *J Physiol* **504 (Pt 3),** 695-704.

Hellsten Y, Maclean D, Radegran G, Saltin B & Bangsbo J. (1998). Adenosine concentrations in the interstitium of resting and contracting human skeletal muscle. *Circulation* **98,** 6-8.

Hertz RP, Unger AN, Cornell JA & Saunders E. (2005). Racial disparities in hypertension prevalence, awareness, and management. *Arch Intern Med* **165,** 2098-2104.

Hillig T, Krustrup P, Fleming I, Osada T, Saltin B & Hellsten Y. (2003). Cytochrome P450 2C9 plays an important role in the regulation of exercise-induced skeletal muscle blood flow and oxygen uptake in humans. *J Physiol* **546,** 307-314.

Huang A & Kaley G. (2004). Gender-specific regulation of cardiovascular function: estrogen as key player. *Microcirculation (New York, NY : 1994)* **11,** 9-38.

Hunter SK, Schletty JM, Schlachter KM, Griffith EE, Polichnowski AJ & Ng AV. (2006). Active hyperemia and vascular conductance differ between men and women for an isometric fatiguing contraction. *J Appl Physiol (1985)* **101,** 140-150.

Joyner MJ & Wilkins BW. (2007). Exercise hyperaemia: is anything obligatory but the hyperaemia? *J Physiol* **583,** 855-860.

Juel C, Pilegaard H, Nielsen JJ & Bangsbo J. (2000). Interstitial K(+) in human skeletal muscle during and after dynamic graded exercise determined by microdialysis. *Am J Physiol Regul Integr Comp Physiol* **278,** R400-406.

Junejo RT. (2017). Contribution of oxygen-dependent mechanisms to vascular responses of exercise in young and older men: the role of prostaglandins and adenosine. (PhD Thesis). In *School of Clinical and Experimental Medicine, Centre for Cardiovascular Sciences*. University of Birmingham, United Kingdom.

Junejo RT, Ray CJ, Marshall, JM (2018). Cuff inflation time significantly affects blood flow recorded with venous occlusion plethysmography. *Eur J Applied Physiol* 119 (3): 665–674.

Kagaya A & Homma S. (1997). Brachial arterial blood flow during static handgrip exercise of short duration at varying intensities studied by a Doppler ultrasound method. *Acta Physiol Scand* **160,** 257-265.

Kahn DF, Duffy SJ, Tomasian D, Holbrook M, Rescorl L, Russell J, Gokce N, Loscalzo J & Vita JA. (2002). Effects of black race on forearm resistance vessel function. *Hypertension* **40,** 195-201.

Kappus RM, Bunsawat K, Brown MD, Phillips SA, Haus JM, Baynard T & Fernhall B. (2017). Effect of oxidative stress on racial differences in vascular function at rest and during hand grip exercise. *J Hypertens* **35,** 2006-2015.

Karamouzis M, Karamouzis I, Vamvakoudis E, Ampatzidis G, Christoulas K, Angelopoulou N & Mandroukas K. (2001). The response of muscle interstitial prostaglandin E(2)(PGE(2)), prostacyclin I(2)(PGI(2)) and thromboxane A(2)(TXA(2)) levels during incremental dynamic exercise in humans determined by in vivo microdialysis. *Prostaglandins Leukot Essent Fatty Acids* **64,** 259-263.

Kellawan JM, Johansson RE, Harrell JW, Sebranek JJ, Walker BJ, Eldridge MW & Schrage WG. (2015). Exercise vasodilation is greater in women: contributions of nitric oxide synthase and cyclooxygenase. *Eur J Appl Physiol* **115,** 1735-1746.

Kelly DE, Scroop GC, Tonkin AL & Thornton AT. (2004). Cardiovascular responses to orthostatic and other stressors in men and women are independent of sex. *Clin Exp Pharmacol Physiol* **31,** 50-56.

Kent-Braun JA, Ng AV, Doyle JW & Towse TF. (2002). Human skeletal muscle responses vary with age and gender during fatigue due to incremental isometric exercise. *J Appl Physiol (1985)* **93,** 1813-1823.

Kilbom A & Wennmalm A. (1976). Endogenous prostaglandins as local regulators of blood flow in man: effect of indomethacin on reactive and functional hyperaemia. *J Physiol* **257,** 109-121.

Koller A & Kaley G. (1990). Prostaglandins mediate arteriolar dilation to increased blood flow velocity in skeletal muscle microcirculation. *Circ Res* **67,** 529-534.

Lamb IR & Murrant CL. (2015). Potassium inhibits nitric oxide and adenosine arteriolar vasodilatation via K(IR) and Na(+)/K(+) ATPase: implications for redundancy in active hyperaemia. *J Physiol* **593,** 5111-5126.

Lash JM & Bohlen HG. (1987). Perivascular and tissue PO2 in contracting rat spinotrapezius muscle. *Am J Physiol* **252,** H1192-1202.

Lim To WK, Kumar P & Marshall JM. (2015). Hypoxia is an effective stimulus for vesicular release of ATP from human umbilical vein endothelial cells. *Placenta* **36,** 759-766.

Limberg JK, Eldridge MW, Proctor LT, Sebranek JJ & Schrage WG. (2010). Alpha-adrenergic control of blood flow during exercise: effect of sex and menstrual phase. *J Appl Physiol (1985)* **109,** 1360-1368.

Marshall JM & Ray CJ. (2012). Contribution of non-endothelium-dependent substances to exercise hyperaemia: are they O(2) dependent? *J Physiol* **590,** 6307-6320.

McKay MK, Gardner AL, Boyd D & Hester RL. (1998). Influence of venular prostaglandin release on arteriolar diameter during functional hyperemia. *Hypertension* **31,** 213-217.

McLennan IS & Macdonald RE. (1991). Prostaglandin synthetase and prostacyclin synthetase in mature rat skeletal muscles: immunohistochemical localisation to arterioles, tendons and connective tissues. *J Anat* **178,** 243-253.

Melikian N, Wheatcroft SB, Ogah OS, Murphy C, Chowienczyk PJ, Wierzbicki AS, Sanders TA, Jiang B, Duncan ER, Shah AM & Kearney MT. (2007). Asymmetric dimethylarginine and reduced nitric oxide bioavailability in young Black African men. *Hypertension* **49,** 873-877.

Messina EJ, Sun D, Koller A, Wolin MS & Kaley G. (1992). Role of endothelium-derived prostaglandins in hypoxia-elicited arteriolar dilation in rat skeletal muscle. *Circ Res* **71,** 790-796.

Mo FM & Ballard HJ. (2001). The effect of systemic hypoxia on interstitial and blood adenosine, AMP, ADP and ATP in dog skeletal muscle. *J Physiol* **536,** 593-603.
Mortensen SP, Gonzalez-Alonso J, Damsgaard R, Saltin B & Hellsten Y. (2007). Inhibition of nitric oxide and prostaglandins, but not endothelial-derived hyperpolarizing factors, reduces blood flow and aerobic energy turnover in the exercising human leg. *J Physiol* **581,** 853-861.
Mortensen SP, Nyberg M, Thaning P, Saltin B & Hellsten Y. (2009). Adenosine contributes to blood flow regulation in the exercising human leg by increasing prostaglandin and nitric oxide formation. *Hypertension* **53,** 993-999.
Murrant CL, Dodd JD, Foster AJ, Inch KA, Muckle FR, Ruiz DA, Simpson JA & Scholl JH. (2014). Prostaglandins induce vasodilatation of the microvasculature during muscle contraction and induce vasodilatation independent of adenosine. *J Physiol* **592,** 1267-1281.
Murrant CL & Sarelius IH. (2015). Local control of blood flow during active hyperaemia: what kinds of integration are important? *J Physiol* **593,** 4699-4711.
Myers TO, Messina EJ, Rodrigues AM & Gerritsen ME. (1985). Altered aortic and cremaster muscle prostaglandin synthesis in diabetic rats. *Am J Physiol* **249,** E374-379.
Nowak J & Wennmalm A. (1978). Effect of exercise on human arterial and regional venous plasma concentrations of prostaglandin E. *Prostaglandins Med* **1,** 489-497.
Nyberg M, Al-Khazraji BK, Mortensen SP, Jackson DN, Ellis CG & Hellsten Y. (2013). Effect of extraluminal ATP application on vascular tone and blood flow in skeletal muscle: implications for exercise hyperemia. *Am J Physiol Regul Integr Comp Physiol* **305,** R281-290.
Nyberg M, Mortensen SP, Thaning P, Saltin B & Hellsten Y. (2010). Interstitial and plasma adenosine stimulate nitric oxide and prostacyclin formation in human skeletal muscle. *Hypertension* **56,** 1102-1108.
Opie LH & Seedat YK. (2005). Hypertension in sub-Saharan African populations. *Circulation* **112,** 3562-3568.
Ozkor MA, Rahman AM, Murrow JR, Kavtaradze N, Lin J, Manatunga A, Hayek S & Quyyumi AA. (2014). Differences in vascular nitric oxide and endothelium-derived hyperpolarizing factor bioavailability in blacks and whites. *Arterioscler Thromb Vasc Biol* **34,** 1320-1327.
Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Herr MD & Proctor DN. (2007). Sex differences in leg vasodilation during graded knee extensor exercise in young adults. *J Appl Physiol (1985)* **103,** 1583-1591.
Perregaux D, Chaudhuri A, Rao S, Airen A, Wilson M, Sung BH & Dandona P. (2000). Brachial vascular reactivity in blacks. *Hypertension* **36,** 866-871.
Radegran G & Saltin B. (1999). Nitric oxide in the regulation of vasomotor tone in human skeletal muscle. *Am J Physiol* **276,** H1951-1960.
Ray CJ, Abbas MR, Coney AM & Marshall JM. (2002). Interactions of adenosine, prostaglandins and nitric oxide in hypoxia-induced vasodilatation: in vivo and in vitro studies. *J Physiol* **544,** 195-209.
Richardson RS, Newcomer SC & Noyszewski EA. (2001). Skeletal muscle intracellular PO(2) assessed by myoglobin desaturation: response to graded exercise. *J Appl Physiol (1985)* **91,** 2679-2685.
Russ DW & Kent-Braun JA. (2003). Sex differences in human skeletal muscle fatigue are eliminated under ischemic conditions. *J Appl Physiol (1985)* **94,** 2414-2422.
Saito Y, Iemitsu M, Otsuki T, Maeda S & Ajisaka R. (2008). Gender differences in brachial blood flow during fatiguing intermittent handgrip. *Med Sci Sports Exerc* **40,** 684-690.

Salvemini D, Kim SF & Mollace V. (2013). Reciprocal regulation of the nitric oxide and cyclooxygenase pathway in pathophysiology: relevance and clinical implications. *Am J Physiol Regul Integr Comp Physiol* **304,** R473-487.

Schrage WG, Eisenach JH & Joyner MJ. (2007). Ageing reduces nitric-oxide- and prostaglandin-mediated vasodilatation in exercising humans. *J Physiol* **579,** 227-236.

Schrage WG, Joyner MJ & Dinenno FA. (2004). Local inhibition of nitric oxide and prostaglandins independently reduces forearm exercise hyperaemia in humans. *J Physiol* **557,** 599-611.

Shoemaker JK, Naylor HL, Pozeg ZI & Hughson RL. (1996). Failure of prostaglandins to modulate the time course of blood flow during dynamic forearm exercise in humans. *J Appl Physiol (1985)* **81,** 1516-1521.

Stein CM, Lang CC, Nelson R, Brown M & Wood AJ. (1997). Vasodilation in black Americans: attenuated nitric oxide-mediated responses. *Clin Pharmacol Ther* **62,** 436-443.

Stoner L, Erickson ML, Young JM, Fryer S, Sabatier MJ, Faulkner J, Lambrick DM & McCully KK. (2012). There's more to flow-mediated dilation than nitric oxide. *J Atheroscler Thromb* **19,** 589-600.

Testa M, Rocca B, Spath L, Ranelletti FO, Petrucci G, Ciabattoni G, Naro F, Schiaffino S, Volpe M & Reggiani C. (2007). Expression and activity of cyclooxygenase isoforms in skeletal muscles and myocardium of humans and rodents. *J Appl Physiol (1985)* **103,** 1412-1418.

Trappe TA & Liu SZ. (2013). Effects of prostaglandins and COX-inhibiting drugs on skeletal muscle adaptations to exercise. *J Appl Physiol (1985)* **115,** 909-919.

Tu J, Le G & Ballard HJ. (2010). Involvement of the cystic fibrosis transmembrane conductance regulator in the acidosis-induced efflux of ATP from rat skeletal muscle. *J Physiol* **588,** 4563-4578.

Vyskocil F, Hnik P, Rehfeldt H, Vejsada R & Ujec E. (1983). The measurement of K+e concentration changes in human muscles during volitional contractions. *Pflugers Arch* **399,** 235-237.

Wilson JR & Kapoor SC. (1993). Contribution of prostaglandins to exercise-induced vasodilation in humans. *Am J Physiol* **265,** H171-175.

Win TS & Marshall JM. (2005). Contribution of prostaglandins to the dilation that follows isometric forearm contraction in human subjects: effects of aspirin and hyperoxia. *J Appl Physiol (1985)* **99,** 45-52.

Zhu S, Han G & White RE. (2002). PGE2 action in human coronary artery smooth muscle: role of potassium channels and signaling cross-talk. *J Vasc Res* **39,** 477-488.

	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

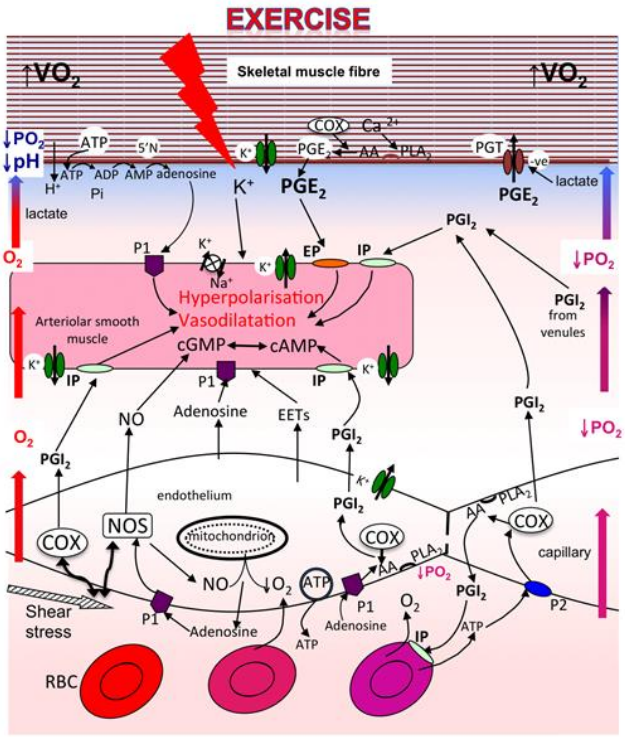

Figure 1. Schematic diagram showing mechanisms by which prostaglandins (PGE_2 and PGI_2) are released during exercise and mechanisms by which PGs induce dilatation.

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).

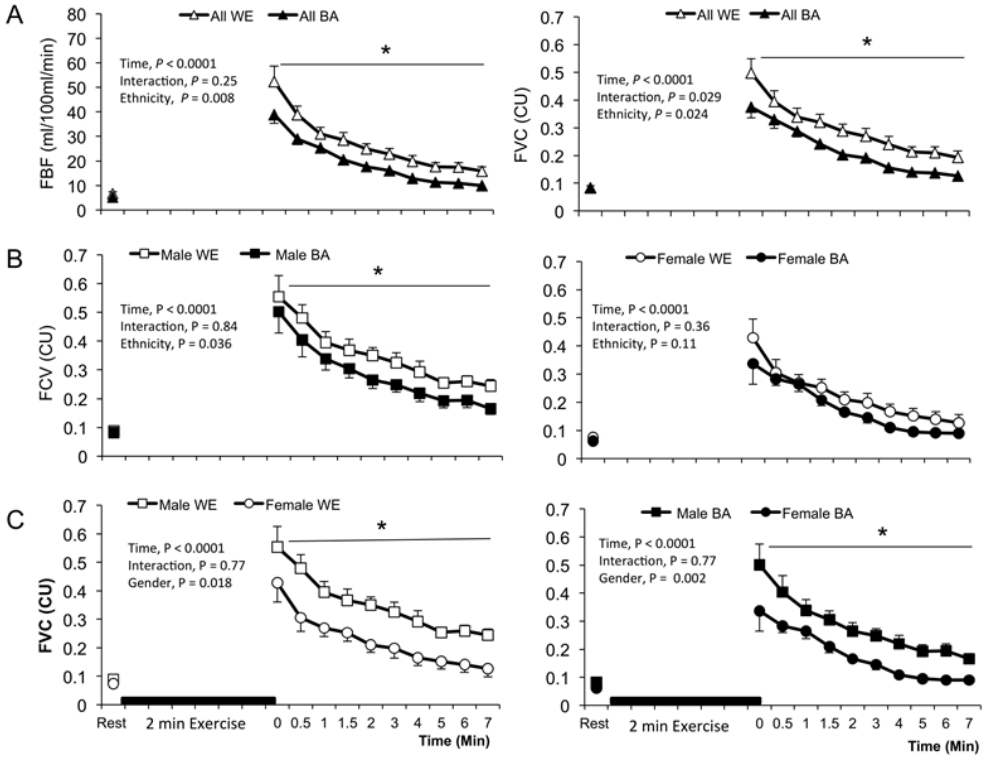

Figure 2: Effects of rhythmic contractions at 60% MVC for 2 min on forearm vasculature of young WE and BA men and women. A: Comparisons between all WEs and all BAs for post-exercise forearm blood flow (FBF; left) and forearm vascular conductance (FVC; right). B: Comparisons between WE and BA men (left) 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.

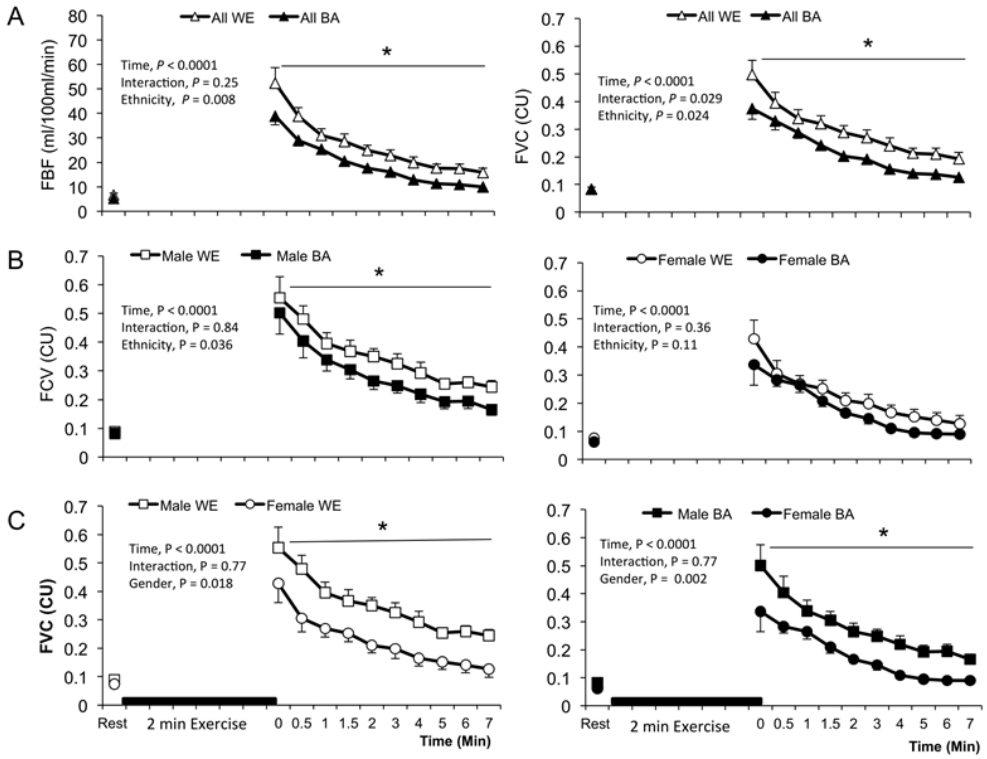

Figure 3: Effect of COX inhibition with aspirin on peak change in Forearm vascular conductance (FVC) following rhythmic contractions at 60% MVC for 2 min in WE and BA men (above) and WE and BA women (below). Values are shown mean ± SEM. §, §§: $p<0.05$, $p<0.01$ respectively before vs after aspirin.

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.