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Title: The impact of hypoxaemia on vascular function in lowlanders and high altitude indigenous populations

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Abstract

Exposure to hypoxia elicits widespread physiological responses that are critical for successful acclimatization; however, these responses may induce apparent maladaptive consequences. For example, recent studies conducted in both the laboratory and field (e.g. high-altitude) have demonstrated that endothelial function is reduced in hypoxia. Herein, we review the several proposed mechanism(s) pertaining to the observed reduction in endothelial function in hypoxia including: a) changes in blood flow patterns (i.e. shear stress), b) increased inflammation and production of reactive oxygen species (i.e. oxidative stress), c) heightened sympathetic nervous activity, and d) increased red blood cell concentration and mass leading to elevated nitric-oxide scavenging. Although some of these mechanism(s) have been examined in lowlanders, less in known about endothelial function in indigenous populations who have chronically adapted to environmental hypoxia for millennia (e.g. the Peruvian, Tibetan, and Ethiopian highlanders). There is some evidence indicating that healthy Tibetan and Peruvian (i.e. Andean) highlanders have preserved endothelial function at high-altitude, but less is known about the Ethiopian highlanders.

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However, Andean highlanders suffering from chronic mountain sickness, which is characterized by an excessive production of red blood cells, have markedly reduced endothelial function. This review will provide a framework and mechanistic model for vascular endothelial adaptation to hypoxia in lowlanders and highlanders. Elucidating the pathways responsible for vascular adaption/maladaptation to hypoxia has potential clinical implications for disease featuring low oxygen delivery (e.g. heart failure, pulmonary disease). In addition, a greater understanding of vascular function at high-altitude will clinically benefit the globally estimated 85 million high-altitude residents.

Key words: Endothelial function, high altitude, hypoxia, indigenous highlanders, acclimatization

List of Abbreviations:

EE, excessive erythrocytosis

F_IO_2, fraction inspired of oxygen

FMD, flow-mediated dilatation

GTN, glyceryl trinitrate, or nitroglycerin

Hb, haemoglobin

LBNP, lower-body negative pressure

NO, nitric oxide

OSS, oscillatory shear stress

ROS, reactive oxygen species

SaO_2, arterial haemoglobin saturation of oxygen

SNA, sympathetic nervous activity

Introduction:

The vast majority of humans dwell at or slightly above sea level (i.e. <1500m) where the availability of oxygen is abundant. When ascending to high altitude, oxygen concentration stays constant (i.e. ~21%), but atmospheric pressure drops, resulting in a decreased partial pressure of inspired oxygen and saturation of arterial haemoglobin (SaO_2). The lowered oxygen availability at high altitude, termed hypobaric hypoxia, poses a physiological challenge to organisms reliant upon aerobic metabolism. Once restricted to mountaineers and explorers, there is a growing number of sea level residents sojourning to high altitude (>2500m). Due to a combination of the expansive growth of the

internet and affordable air travel, the once considered exotic regions are now well documented and accessible. This can be exemplified by the tragic overcrowding at Sagarmatha (i.e. Mount Everest), where there have already been 11 deaths in 2019 after the Nepalese government issued a record 381 permits (Government of Nepal, 2019). Globally, it has been estimated that >100 million individuals travel to high altitude locations each year, largely for tourism and religious pilgrimages (Burtscher, 1999; Basnyat, 2014).

In contrast to lowland born residents, select populations have lived at high altitude for millennia. These populations, Peruvian (i.e. Andean), Tibetan, and Ethiopian highlanders, seem to have acquired distinctive physiological phenotypes while living in their respective hypoxic environments. Although there is considerable debate regarding specific durations, the general consensus is that the Old World plateaux (Ethiopian and Tibetan) have been settled for longer than the Altiplano in the New World (Andeans; Aldenderfer, 2006; Beall, 2006, 2007; Alkorta-Aranburu *et al.*, 2012; Zhang *et al.*, 2018), as outlined in *figure 1*. High altitude physiology research offers valuable insight into the mechanism(s) underpinning adaptation to hypoxaemia, which holds clinical relevance to diseases characterized by low levels of oxygen (e.g. heart failure, stroke, chronic obstructive pulmonary disease; Berger & Grocott, 2017). Moreover, understanding the fundamental aspects of chronic high altitude acclimatization in indigenous populations may permit a unique perspective on the long-term consequences of these vascular adaptations. Indeed, characterizing the physiological traits in these indigenous populations may inform local health care and clinical practice for the global estimated 85 million high altitude residents (Beall, 2014).

The acute and chronic physiological adaptations that occur with exposure to high altitude (>2500m) aim to acclimatize the human body to increase oxygen delivery. Some of these processes are noticeable, such as an increase in ventilation, while others are imperceptible, such as elevations in haemoglobin concentration ([Hb]) and mass, and reductions in blood bicarbonate levels. More recently, it has been suggested that some of these adaptations may compromise vascular function,

and more specifically, the endothelium. The objective of this review is to provide an overview and inform our current understanding of how hypoxaemia influences endothelium-*dependent* vasodilation, from acute and chronic perspectives. Herein, we highlight the laboratory- and field-based literature in lowlanders and in the Andean, Tibetan, and Ethiopian highlanders.

The initial sections of this review is dedicated to describing the impact of hypoxaemia on endothelial function in lowlanders and discusses what are currently considered the primary mechanism(s) responsible for these changes, including: 1) pro-atherogenic blood flow patterns, 2) oxidative-inflammatory-nitrosative stress, 3) elevated sympathetic nervous activity (SNA), and 4) increased haemoglobin concentration and mass leading to increased nitric-oxide (NO) scavenging. The latter portion of the review details the impact of life-long exposure to hypoxaemia in the distinct high altitude indigenous populations, and we conclude the review by briefly discussing the implications of these collective findings.

Hypoxia and endothelial function in lowlanders

There are several different methods used to measure the many aspects of endothelial function in humans. Given the scope of this review, we focus primarily on endothelial-*dependent* vasodilation as a marker of endothelial function. Below, we highlight the studies that employed arguably the most popular methods used to assess endothelial function in humans: 1) non-invasive conduit artery flow-mediated dilation (i.e. FMD) in response to a shear stress stimulus (i.e. endothelial-*dependent* vasodilation), sometimes combined with subsequent administration of a donor (e.g. glyceryl trinitrate; GTN) to assay smooth muscle sensitivity to exogenous NO (i.e. endothelial-*independent* vasodilation); and 2) invasive intra-arterial infusion of vasoactive substances such as acetylcholine and sodium nitroprusside to assess endothelial-*dependent* and endothelial-*independent* smooth muscle NO-mediated vasodilation, respectively. Comprehensive reviews on these techniques have been published elsewhere (Tousoulis *et al.*, 2005; Thijssen *et al.*, 2011).

There are advantages and disadvantages associated with laboratory- and field-based hypoxia research. The laboratory offers a controlled environment with equipment, supplies, and personnel readily available; however, its ecological validity remains in question since there are many variables encountered during high altitude field research that are not encountered in the laboratory, such as reduced atmospheric pressure (with the exception of hypobaric chambers), cold and arid climates, increased ultraviolet exposure, differences in diet, and exercise (i.e. trekking). To add further complexity, variability in these encountered stressors at high altitude makes between study comparisons difficult in certain instances (e.g. trekking vs automobile ascent; differences in length and severity of altitude exposure). Nevertheless, in order to gain a broad understanding on how hypoxaemia alters human physiological function both variations of experimentation are required (i.e. laboratory- and field-based). Below, we discuss the impact of hypoxaemia on endothelial function and the proposed mechanism(s) that govern these changes.

Studies in acute hypoxia (laboratory)

Administering hypoxia in laboratory-based studies is usually conducted using modified inspired gases or hypobaric chamber. A study by Frøbert et al. (2008) assessed the ratio of brachial artery FMD (60 seconds post-occlusion) to endothelial-*independent* vasodilation (i.e. GTN) five-minutes after steady-state breathing of 12.5% O_2 (~4000m equivalent). The authors found that endothelial-*dependent* function was reduced in middle-aged men with and without cardiovascular health risk factors (Frobert *et al.*, 2008). Similarly, in more recent studies using a slightly more severe degree of hypoxia (F_IO_2 = 0.11; equivalent to ~5000m), it was found that endothelial function decreased following 20-minutes (Tremblay *et al.*, 2018b), and after 1, 3.5, and 5.5 hours of exposure (Lewis *et al.*, 2014). Following a 30-

minute exposure to moderate (end-tidal partial pressure of oxygen = 75mmHg [equivalent to ~2000m]) or severe (end-tidal partial pressure of oxygen = 50mmHg [equivalent to ~4600m]) isocapnic hypoxia, endothelial function was reduced even after accounting for reduced shear stress stimulus (Lewis *et al.*, 2017). Although the mechanism(s) responsible for the observed decline in shear stress during acute hypoxia remain unknown, these authors attributed it to heightened sympathetic vascular constraint (Weisbrod *et al.*, 2001). In contrast, 15-minutes of severe isocapnic hypoxia (end-tidal partial pressure of O_2 = 45 mmHg; equivalent to ~5000m) did not reduce endothelial function in healthy adults (Rieger *et al.*, 2017). The latter study suggests that 15-minutes of hypoxia exposure may not be enough to elicit a reduction in endothelial function. Although these studies utilized highly sophisticated methods that allow for breath-by-breath end-tidal gas control (i.e. end-tidal forcing), is should be noted that these studies are likely less applicable to high altitude investigations, since the partial pressure of end-tidal CO_2 is inherently reduced at high altitude due to increases in ventilation. These aforementioned studies are also subject to the confounds of elevated end-tidal CO_2, which may induce alterations in blood flow patterns and increase SNA (Somers *et al.*, 1989; Vantanajal *et al.*, 2007; Steinback *et al.*, 2009), which is known to reduce endothelial function via mechanisms related to vascular constraint (*described in more detail below*).

Another study found no change in endothelial function after 4 hours in a hypobaric chamber simulating ~4000m; however, the FMD test incorporated a proximal, rather than distal, cuff occlusion compared to all other studies conducted in hypoxia (Iglesias *et al.*, 2015). Consistency in the methodology used to conduct an FMD test is of critical importance since cuff placement has been shown to influence the degree of endothelial function

observed (Agewall *et al.*, 2001). An alternative method to assess vascular function, albeit typically in the resistance (i.e. microvascular) blood vessel bed, is to utilize forearm intra-arterial infusions. There has only been one investigation that examined the effect of hypoxaemia on endothelial function at rest in the laboratory using this approach (Berger *et al.*, 2005), and this technique has yet to be completed at high altitude likely due to its invasive nature and need for adequate highly trained medical support. The study by Berger and colleagues (2005) found that endothelial-*dependent* vasodilation in response to intra-arterial (brachial) infusion of acetylcholine was impaired in high altitude pulmonary oedema susceptible patients (exaggerated hypoxic pulmonary vasoconstriction), but preserved in control participants following 4 hours of 12% O_2 (~4500m equivalent; Berger *et al.*, 2005). These data indicate that systemic hypoxic endothelial dysfunction contributes to the exaggerated increase in pulmonary artery pressure, which may increase susceptibility to high altitude pulmonary oedema (see *figure 2*).

Studies at high altitude

Upon ascent to high altitude between 3800-5050m, a decrease in endothelial function has been observed in most (Lewis *et al.*, 2014; Bakker *et al.*, 2015; Tymko *et al.*, 2017b; Tremblay *et al.*, 2018a; Tremblay *et al.*, 2018b), but not all studies (Bruno *et al.*, 2016; Tremblay *et al.*, 2017; Tymko *et al.*, 2017a). The disparity between the literatures might be due to different methodological approaches between investigations. For example, the three studies that reported reductions in FMD involved ascent to high-altitude over 7-10 days of trekking for several hours each day at altitudes >3700m in the Himalaya (Lewis *et al.*, 2014; Bakker *et al.*, 2015; Tymko *et al.*, 2017b; Tremblay *et al.*, 2018a; Tremblay *et al.*, 2018b). The study by Lewis *et al.* (2014) observed reduced FMD and endothelium-

independent vasodilation after 3 (shear stress stimulus was unchanged), and 12-14 days (shear stress stimulus was reduced), at 5050m following an 8-day trek. Importantly, the reductions in endothelial function were accompanied by impairments in smooth muscle function (i.e. reduced vasodilation in response to GTN) – indicating that the reduced vasodilatory response may be due to impaired smooth muscle vasodilation rather than endothelial function (Lewis *et al.*, 2014).

As part of a separate expedition in the same region, Bakker *et al*. (2015) tested the hypothesis that dietary nitrate supplementation, to increase NO bioavailability, would prevent high altitude trekking associated decreases in endothelial function. After 5 days above 2500m, participants were tested at 4200m, and endothelial function was decreased after placebo administration, but preserved with dietary nitrate supplementation compared to pre-trek baseline levels (acquired at 1370m). Following 4 weeks of trekking, endothelial function remained reduced one day after returning to 1370m. Thus, high altitude trekking expeditions are associated with reductions in endothelial function. This may be due to reductions in NO bioavailability [as suggested by Bakker et al. (2015)], decreased vascular smooth muscle function (Lewis *et al.*, 2014), or potentially, a reduced FMD shear stress stimulus.

In contrast, the studies that reported no change in endothelial function involved participants without AMS ascending rapidly to high altitude via cable car (Bruno *et al.*, 2016), and healthy participants traveling to high altitude via automobile [*studies part of the same research expedition* (Tymko et al., 2017a; Tremblay et al., 2018b)]. The methodological difference between these studies raises the possibility of a moderating impact of trekking exercise at altitude, in addition to the severity of altitude exposure. To expand on the latter point, the collective findings from these field research studies suggest that a high altitude threshold exists for reduced endothelial function in-between 3500 and 4000m. This threshold is likely variable between participants, but should be a consideration for future research. The length of hypoxia exposure is also an important consideration; however, it seems that endothelial function is reduced early on during the acclimatization process

(1-3 days; Lewis *et al.*, 2014; Tremblay *et al.*, 2018a), but no studies have investigated the impact of long-term acclimatization (i.e. several months) in otherwise healthy lowlanders. In addition, smooth muscle function (i.e. GTN or SNP administration) should be considered in future studies to dissociate endothelial-*dependent* and endothelial-*independent* vascular function. The primary mechanism(s) that govern these hypoxaemia-related changes in endothelial function are discussed in the following several sections.

Shear stress

The direction and magnitude of shear stress acutely and chronically regulates endothelial function (reviewed in Green *et al.*, 2017; see *abstract figure*), and it is calculated as the product of shear rate (4 x peak envelope blood velocity / arterial diameter), and if collected, adjusted to whole blood viscosity (Gnasso *et al.*, 2001). Arterial segments that are chronically exposed to low and/or oscillatory shear stress [OSS; nearly equal parts of forward shear stress (antegrade; towards periphery) and backward shear stress (retrograde; towards heart)], due to vessel geometry, such as arterial branch points and curvatures, preferentially develop atherosclerotic lesions (Caro *et al.*, 1969). Low mean shear stress may facilitate early atheroma by reducing mass transport from the arterial wall, which is one of the primary reasons exercise, an intermittent episodic high shear stress stimulus, is vasoprotective in nature (Caro *et al.*, 1971). Furthermore, OSS is enhanced with aging (Casey *et al.*, 2012), pre-eclampsia (Scholten *et al.*, 2014), menopause (Somani *et al.*, 2019), and anabolic steroid use (de Souza *et al.*, 2019). To examine the causality of OSS it can be experimentally-induced in otherwise healthy upstream arteries by inflating a pneumatic cuff on the forearm to a moderate pressure (~50-75 mmHg; Thijssen *et al.*, 2009; Tremblay *et al.*, 2019a). Exposure to 20-30 minutes of low and/or OSS has been shown to reduce brachial artery endothelial function in healthy humans (Thijssen *et al.*, 2009; Tremblay *et al.*, 2019a). However, populations with

pre-existing endothelial dysfunction (i.e. aging, chronic obstructive pulmonary disease, cardiovascular diseases) may be less susceptible to OSS induced reductions in endothelial function (Schreuder *et al.*, 2015; Thijssen *et al.*, 2015; Barak *et al.*, 2017), indicating a potential floor effect as opposed to resistance to OSS *per se*. In contrast, episodic increases in antegrade shear stress (e.g. exercise or heating) improves endothelial function (Carter *et al.*, 2014; Green *et al.*, 2017), and induces an athero-resistant endothelial phenotype (reviewed in: Green *et al.*, 2017).

Reductions in endothelial function have shown to be mediated, at least in part, due to alterations in shear stress (i.e. increases in retrograde shear stress; Thijssen *et al.*, 2009). Acute and sustained hypoxic exposure has been shown to increase retrograde shear stress (Iwamoto *et al.*, 2015; Katayama *et al.*, 2016; Lewis *et al.*, 2017; Tremblay *et al.*, 2018a; Tremblay *et al.*, 2018b), and decrease mean shear stress (Lewis *et al.*, 2017; Tremblay *et al.*, 2018a; Tremblay *et al.*, 2018b). These pro-atherogenic alterations in shear stress have a well-established deleterious impact on endothelial function (Padilla *et al.*, 2009; Thijssen *et al.*, 2009; Tremblay *et al.*, 2019a), and thus, alterations in baseline shear stress magnitude and pattern may contribute to the observed reductions in endothelial function. To further investigate the effects of shear stress on endothelial function, two studies during the 2016 UBC-Nepal Expedition (Willie *et al.*, 2018) examined endothelial function and performed measures of blood viscosity to calculate the shear stress stimulus during a 9-day trekking ascent to 5050m, and following 5-7 days at 5050m (Tremblay *et al.*, 2018a; Tremblay *et al.*, 2018b). Brachial artery endothelial function was decreased at 4371m (day 7 of trekking) and remained reduced upon arrival, and after 5-7 days at 5050m while the shear stress stimulus remained unchanged (Tremblay *et al.*, 2018a; refer to *figure 3*). Blood viscosity increased during ascent and remained elevated after 5-7 days at 5050m, and as such, played a crucial role in determining the shear stress stimulus for the FMD test; had blood viscosity not been taken into account, the shear stress stimulus, would have been underestimated by ~20-30% (Tremblay *et al.*,

2018a; Tremblay *et al.*, 2018b). This highlights the importance of blood viscosity measures in interpreting shear stress and endothelial function with high altitude exposure.

However, the deleterious impact of atherogenic shear stress on endothelial function during hypoxia may be specific to the upper limb. Although similar shear stress changes occur in the superficial femoral artery on ascent to 5050m, endothelial function is preserved (Tremblay *et al.*, 2018a; see *figure 3*). This may be due to a localized benefit of trekking on the exercised limb; it has previously been shown that increased antegrade shear, even in the presence of some increase in retrograde diastolic shear, enhances endothelial function, whereas unopposed increases in retrograde shear are detrimental to function (Green *et al.*, 2017). However, this explanation remains speculative in the context of hypoxia, and little is known regarding changes in lower limb endothelial function in hypoxia. In contrast, when baseline shear stress is unchanged and endothelial function is preserved at high altitude, the endothelium appears to be more vulnerable to OSS associated dysfunction (Tremblay *et al.*, 2017). In addition to shear stress, changes in baseline conduit artery diameter can impact the degree of observed FMD (Atkinson & Batterham, 2013). Indeed, hypoxia-associated increases in OXINOS stress and SNA facilitate a haemodynamic milieu that is often seen with aging and disease at sea level that promotes pro-atherogenic shear stress patterns in the upstream conduit arteries.

Oxidative-inflammatory-nitrosative (OXINOS) stress

Another proposed mechanism that impacts vascular endothelial health relates to the molecular disruption of redox control, derived as the balance between antioxidants and pro-oxidants (see *abstract figure*). A “tipping of the balance” favoring the latter results in oxidative-inflammatory stress, characterized by an excessive formation of free radicals, reactive oxygen species (ROS), lipid peroxidants and inflammation. This has the potential to limit $NO^{\bullet}$ bioavailability subsequent to its

diffusion-controlled reaction with superoxide radicals ($O_2^{\bullet-}$) that ultimately forms peroxynitrite ($ONOO^-$), giving rise to nitrosative stress (Gryglewski *et al.*, 1986).

$$NO^{\bullet} + O_2^{\bullet-} \xrightarrow{k \approx 1 \times 10^{10}\ M^{-1}.s^{-1}} ONOO^-$$

These reaction pathways, known collectively "OXINOS" (oxidative-inflammatory-nitrosative) stress, can directly impact the vasculature and contribute towards endothelial dysfunction (Bailey *et al.*, 2013; Bailey, 2019b). Elevated oxidative stress has been associated with structural damage to the vasculature and exists in several different cardiovascular disease states (Schachinger *et al.*, 2000; Gokce *et al.*, 2002). Interestingly, vitamin C, a ROS scavenger, has little effect on endothelial function in healthy humans (Chambers *et al.*, 2001), but improves endothelial function in populations with elevated OXINOS stress (Taddei *et al.*, 1998; Chambers *et al.*, 2001). Administration of vitamin C prevents reductions in endothelial function incurred by exposure to low and OSS (Johnson *et al.*, 2013), implicating that OXINOS stress as a contributing mechanism responsible for low and OSS induced reductions in endothelial function.

To what extent acute hypoxia impacts free radical formation and the corresponding link to endothelial function has been complicated by a traditional reliance on indirect, non-specific biological "footprints" of oxidative damage that exhibit markedly different thermodynamic and kinetic properties (Bailey *et al.*, 2004). Only few studies have employed electron paramagnetic resonance spectroscopy, which represents the most direct, specific, and sensitive analytical technique for the molecular detection and characterisation of free radicals, *sine qua non* (Bailey *et al.*, 2018). In the studies that have, acute and chronic exposure to normobaric/hypobaric hypoxia has consistently been shown to promote the systemic (Bailey *et al.*, 2009a; Woodside *et al.*, 2014), skeletal (Bailey *et al.*, 2004), pulmonary (Bailey *et al.*, 2010), and cerebral (Bailey *et al.*, 2009b; Bailey *et al.*, 2017; Bailey *et al.*, 2018) formation of a variety of free radical species including lipid-derived alkoxyl radicals ($LO^{\cdot}$) that have the thermodynamic capacity to inactivate $NO^{\cdot}$ and impair endothelial

function and associated metrics of arterial function (Bailey *et al.*, 2009b; Bailey *et al.*, 2013; see *figure 4*). In support, both the systemic and regional bioavailability of bioactive NO metabolites has consistently been shown to be reduced by hypoxia with a corresponding elevation in 3-nitrotyrosine (3-NT; Bailey *et al.*, 2009b; Bailey *et al.*, 2010), a surrogate biomarker of $ONOO^-$ formation, indirectly confirming oxidative annihilation of $NO^{\cdot}$ that proceeds at diffusion-controlled rates (Bailey *et al.*, 2011):

$$(O_2^{\bullet -}/LO^{\cdot} + NO \xrightarrow{k = 16-20 \ x \ 10^9 \ M.s^{-1}} ONOO^- \xrightarrow{tyrosine \ nitration} 3\text{-NT})$$

Several competing theories have been proposed to explain the source and underlying mechanism(s) that serve to promote oxidation in hypoxia, which remains controversial given the fundamental importance of molecular O_2 for peroxyl radical formation and subsequent propagation of the lipid peroxidation chain. Accumulating evidence in vitro has identified increased mitochondrial $O_2^{\bullet -}$ release by complex III of the electron transport chain possibly by increasing ubisemiquinone lifetime with ROS release to the intermembrane space notwithstanding separate contributions from extra-mitochondrial sources including NADPH oxidase/xanthine oxidase/phospholipase A2 activation, haeme auto-oxidation and liberation of catalytic iron with the capacity to promote Fenton and Haber–Weiss–mediated formation of the hydroxyl radical (Bailey *et al.*, 2018). However, it is important to emphasize that while historically considered as toxic, mutagenic "accidents" of in-vivo chemistry limited to cellular oxidative damage and vascular pathophysiology, recent evidence has identified that given the emerging role of reactive oxygen-nitrogen species as important signal transductions, a physiological level of (elevated) OXINOS appears hormetically beneficial during lifelong adaptation to the hypoxia of high altitude in order to preserve cellular O_2 homeostasis (Bailey, 2019b). In support, systemic OXINOS has been shown to be permanently elevated in healthy well-adapted highlanders compared to lowlander controls, whereas the OXINOS response is more exaggerated (i.e. marking the transition from physiological to pathological) in maladapted patients

suffering from high altitude illness and vascular endothelial dysfunction (Bailey *et al.*, 2013; Bailey *et al.*, 2019a).

Sympathetic nervous activity

The organization of the peripheral sympathetic nervous system is important for the control of blood flow to various tissues and organs in order to meet metabolic demand on a moment-by-moment basis. Sympathetic neural fibers release noradrenaline, which binds to α- and β-adrenergic receptors located on the surface of smooth muscle cells, which usually signal for vasoconstriction and vasodilation, respectively (see *abstract figure*). A classic methodology utilized to elevate SNA is through lower-body negative pressure (LBNP), which reduces central blood volume and signals for increased SNA based on afferent feedback from cardiopulmonary and arterial baroreflexes. Lower-body negative pressure has been shown to reduce endothelial function in some (Hijmering *et al.*, 2002; Thijssen *et al.*, 2014), but not all investigations (Dyson *et al.*, 2006; Tymko *et al.*, 2017b), without altering smooth muscle function or the FMD shear stress stimulus (Hijmering *et al.*, 2002).

The discrepancy between these investigations may be due to differences in the magnitude of LBNP (i.e. suction level) or duration of exposure. Intra-arterial infusion of phentolamine, a non-selective α-adrenergic receptor inhibitor, prevents the reduction in endothelial function during LBNP, suggesting that heightened SNA contributes to the LBNP-induced endothelial impairment (Hijmering *et al.*, 2002). However, LBNP also induces pro-atherogenic shear stress in the brachial artery (Padilla *et al.*, 2010; Thijssen *et al.*, 2014), which could, independent of sympathetic vasoconstrictor tone, provoke reductions in endothelial function (*see shear stress section*). Thijssen *et al.* (2014) sought to delineate these mechanisms (i.e. SNA vs atherogenic shear stress), by employing a similar magnitude of LBNP with 1) forearm heating to reduce retrograde shear patterns observed during moderate LBNP (i.e. ~35 mmHg) and 2) forearm cuff inflation to exaggerate

increases in atherogenic shear stress. Lower-body negative pressure reduced endothelial function in the non-heated limb, but this reduction was abolished in the heated arm, which led the authors to conclude that SNA *per se* was not directly responsible for reduced endothelial function during LBNP, but SNA reduces endothelial function indirectly via increases in retrograde shear stress (Thijssen *et al.*, 2014). In support of this theory it was recently demonstrated that mild levels of LBNP (e.g. -10 mmHg) elicited a significant increase in SNA, but did not alter retrograde shear, and consequently, did not alter endothelial function (Tymko *et al.*, 2017b). In addition, more recent studies have shown that post-exercise reductions in endothelial function are prevented with α-adrenergic blockade (Atkinson *et al.*, 2015; Tymko *et al.*, 2017a), providing more evidence of the strong influence of SNA on endothelial function.

Acute hypoxia (Saito *et al.*, 1988; Leuenberger *et al.*, 1991; Duplain *et al.*, 1999; Fisher *et al.*, 2018), and chronic high altitude exposure (Duplain *et al.*, 1999; Hansen & Sander, 2003; Fisher *et al.*, 2018; Simpson *et al.*, 2019), has been associated with heighted SNA (see *figure 5*). The mechanism(s) underpinning the increase in sympathetic neural outflow during hypoxia exposure remains unclear; however, there is evidence that elevated peripheral chemoreflex drive (Somers *et al.*, 1989), red blood cell production (Oshima *et al.*, 2018), intracranial pressure (Schmidt *et al.*, 2018), and pulmonary artery pressure (Moore *et al.*, 2011), may be responsible. Worth noting, the mechanism(s) responsible for sympathetic nervous “hyper”-activity in hypoxia seem to be influenced by the length of exposure; for example, the peripheral chemoreflex has been shown to mediate increases in SNA during acute (Somers *et al.*, 1989), but not chronic exposure (Hansen & Sander, 2003; Fisher *et al.*, 2018; Simpson *et al.*, 2019).

Heightened SNA has been shown to reduce endothelial function in a number of studies at sea level (Hijmering *et al.*, 2002; Thijssen *et al.*, 2014; Atkinson *et al.*, 2015; Tymko *et al.*, 2017a), but only few have attempted to test the direct link between hypobaric hypoxia associated elevated SNA and reductions in endothelial function. A sea level hypoxic chamber study conducted by Lewis *et al*.

(2014) discovered that following 4 hours of hypoxic exposure (F_IO_2 = 0.11; equivalent altitude ~5000m), administration of α_1-adrenergic receptor blockade reversed the ~28-36% observed reduction in endothelial function compared to normoxia, suggesting that elevated α_1-adrenergic SNA contributes to the impairment in endothelial function in acute hypoxia. Recently, two high altitude studies have explored the impact of SNA on endothelial function immediately after moderate-intensity exercise at 3800m (Tymko *et al.*, 2017a), and mild lower-body differential pressure at 5050m (Tymko *et al.*, 2017b); however, these studies showed that altering SNA at high altitude had no effect on endothelial function on acclimatized lowlanders. Sympathetic nervous activity may also alter conduit artery diameter, which directly influences the degree of observed FMD (Atkinson & Batterham, 2013). Although there are inconsistencies with available data showing that brachial artery baseline diameter does not change (Lewis *et al.*, 2014; Bakker *et al.*, 2015), or slightly decreases (Tremblay *et al.*, 2017; Tymko *et al.*, 2017a; Tymko *et al.*, 2017b; Tremblay *et al.*, 2018a), with high altitude exposure; allometrically correcting for baseline diameter is an important consideration when investigating the impact of hypoxaemia on endothelial function (Atkinson & Batterham, 2013). A summary of all laboratory and field studies that investigated the impact of hypoxia on endothelial function in lowlanders is provided in *table 1*.

Impact of hypoxia on endothelial function in highlanders

Humans are the only mammals to have colonized all of Earth's most extreme environments, and the mechanism(s) of human adaptation in the different domains have naturally attracted the attention of physiologists for centuries. Permanent settlements and sojourners at high altitude are subject to potent environment stressors, specifically to hypoxaemia, as well as cold, arid climates, increased ultraviolet radiation, and undergo various physiological adaptations and acclimatization. Hence, populations that have thrived at high altitude for millennia have evolved distinct physiological

phenotypes. Physiological comparisons between these populations are difficult due to differences in residing altitudes, physical activity, and diet. The following section is dedicated to summarizing our current understanding on how, and if, these high altitude populations have adapted from a vascular function perspective.

Andean highlanders with and without Chronic Mountain Sickness

There is good evidence that the Andean plateau has been populated for at least 7,000 years (Aldenderfer, 2008; Haas *et al.*, 2017). The Quechua and Aymara populations of this region present with what can be considered an exaggerated form of typical lowlander high altitude adaptation with increased [Hb] levels and haematocrit, together increasing the oxygen content in arterial blood, so that it actually exceeds that of lowlanders at sea level (Beall, 2007). As a result, blood viscosity increases, eliciting opposing increases in shear stress and vascular resistance, the balance of which determines oxygen delivery and influences endothelial function. Healthy male and female Andean highlanders display similar endothelial function compared to lowlanders at sea level (Kametas *et al.*, 2002; Rimoldi *et al.*, 2012; Bailey *et al.*, 2013), despite higher haematocrit and blood viscosity and elevated OXINOS (Bailey *et al.*, 2013), suggesting preserved endothelial function. In addition, oxygen administration (to normalize the hypoxaemia) does not improve endothelial function in this population (Rimoldi *et al.*, 2012). Pregnant Andean highlanders present with lower blood viscosity and haematocrit compared to non-pregnant female Andeans (Kametas *et al.*, 2002; Kametas *et al.*, 2004), and do not present impairments in endothelial function during pregnancy. These data indicate that in healthy Andean highlanders, endothelial function does not appear compromised by hypobaric hypoxia; however, more work is required to determine this since the authors of these studies did not control or correct for between-group differences in shear stress stimuli.

The haematological response to hypoxia is characterized by erythropoiesis, which leads to increased [Hb] and thereby increases the oxygen carrying capacity of the blood (Pugh, 1964; Faura *et al.*, 1969; Ge *et al.*, 2002). Although this response is generally beneficial, when exaggerated or ineffective at improving oxygenation, erythrocytosis can become pathogenic. Chronic mountain sickness is characterized by excessive erythrocytosis (EE), defined as [Hb] ≥21 g dl^{-1} in men and ≥19 g dl^{-1} in women (Leon-Velarde *et al.*, 2005), and the presence of signs and symptoms, defined by the Qinghai CMS score. By comparison, the Canadian Blood Services classifies normal [Hb] levels as between 14-18 g dl^{-1} in men and 12-16 g dl^{-1} in women. Chronic mountain sickness is diagnosed based on the presence of EE combined with three or more of the following symptoms: breathlessness, palpitations, sleep disturbance, cyanosis, dilation of veins, paresthesia, headache, or tinnitus (Leon-Velarde *et al.*, 2005). An estimated 5-10% of all high altitude dwellers are at risk of developing EE (Leon-Velarde *et al.*, 2005; Villafuerte & Corante, 2016); however, this varies depending on the population studied. Excessive erythrocytosis is extremely rare in Tibetan high altitude natives (Pei *et al.*, 1989), but highly prevalent in some Andean highlander communities (Monge *et al.*, 1989; Monge *et al.*, 1992).

In Cerro de Pasco, EE, independent of hypoxaemia, is associated with increased cardiovascular risk, calculated using the Framingham General Cardiovascular Risk Score (Corante *et al.*, 2018). Rimoldi *et al.* (2012) reported increased arterial stiffness (carotid-femoral pulse-wave velocity and augmentation index), carotid intima-media thickness, and decreased endothelial function in individuals with CMS compared to age-matched healthy Andeans. These patients did not present traditional cardiovascular risk factors, thus the vascular dysfunction is likely specific to CMS rather than pre-existing cardiovascular

complications, and this provides important initial evidence of endothelial dysfunction (endothelium-*independent* vasodilation was similar between healthy and unhealthy groups) in this population. Andeans with EE ([Hb] > 21 g/dL) present with lower endothelial function compared to healthy Andeans (Rimoldi *et al.*, 2012; Bailey *et al.*, 2013; Tremblay *et al.*, 2019b); which improves with oxygen administration (Rimoldi *et al.*, 2012), and haemodilution (Tremblay *et al.*, 2019b), suggesting that hypoxaemia, hyperviscosity and/or high [Hb] contribute to this apparent impairment (see *figure 6*). Participants with CMS also presented exaggerated markers of OXINOS stress compared to Andeans without CMS, providing a potential mechanism for the vascular dysfunction (Bailey *et al.*, 2013).

In high-altitude EE, haemodilution and bloodletting to reduce [Hb] have been noted to improve the clinical symptoms associated with CMS (Leon-Velarde *et al.*, 2005), pulmonary function (Cruz *et al.*, 1979), oxygen transport (Winslow *et al.*, 1985), and cardiac output (Manier *et al.*, 1988). The attendant reductions in iron stores (Zheng *et al.*, 2006), [Hb], blood viscosity may contribute to observed acute increases in endothelial function. Elevated blood viscosity increases vascular resistance, thereby reducing blood flow for a given perfusion pressure (Richardson & Guyton, 1959). Simultaneously, high blood viscosity increases shear stress at a given shear rate, leading to endothelium-*dependent* vasodilator production, decreasing vascular resistance. Thus, a balance between the resistance and shear stress mediated dilatation effects of elevated viscosity influences blood flow (Intaglietta, 2009; Salazar Vazquez *et al.*, 2010). Further adding to the complexity, [Hb] is a potent NO scavenger (Azarov *et al.*, 2005; refer to *abstract figure*), and can reduce endothelial-derived NO to nitrite-nitrate. Additionally, the diffusive conductance from endothelial cells to red blood cells increases as the plasma layer separating the circulating

red blood cells and endothelium narrows, which occurs with increases in haematocrit (Salazar Vazquez *et al.*, 2008). Thus, increases in [Hb] leads to higher blood viscosity, which causes opposing increases in vascular resistance and shear stress-associated vasodilation, and increases NO scavenging.

Tibetan highlanders

Human habitation of the Tibetan plateau has been ~4x longer compared to the Andean plateau (i.e. 30-40,000 years; Zhang *et al.*, 2018). Tibetans present a lower than expected [Hb] accompanied by lower arterial oxygen content; a counterintuitive strategy given the low levels of available oxygen where they reside (>3000m). Consequently, blood viscosity is markedly *lower* in Sherpa (direct descendants of the Tibetans) relative to the Andeans. This alternative adaptation may be inherited from Denisovan-based ancestors (Huerta-Sanchez *et al.*, 2014), an archaic hominid group that may have occupied the Tibetan Plateau at least ~160 thousand years ago (Chen *et al.*, 2019). Tibetans possess a high peripheral blood flow phenotype, characterized by an elevated forearm blood flow, decreased vascular resistance, and increased exhaled and circulating NO metabolites (Erzurum *et al.*, 2007). However, this may not be as pronounced or evident in the Sherpa (Schneider *et al.*, 2001; Bruno *et al.*, 2014; Tremblay *et al.*, 2018a). A high-flow phenotype, as observed in Tibetan highlanders, may decrease the likelihood of developing adverse shear stress patterns at altitude, and thus, serve as a protective mechanism to preserve conduit artery endothelial function. Sherpa display similar brachial artery endothelial function to acclimatized lowlanders (Lewis *et al.*, 2014), but slightly lower compared with lowlanders at sea level (Bruno *et al.*, 2014; Lewis *et al.*, 2014). Despite their hypoxaemic phenotype, Sherpa do not demonstrate any change in endothelial function with oxygen administration (Bruno *et al.*, 2014); this contrasts Andeans, as those with SpO2 <90% present an increase in endothelial function with oxygen administration (Rimoldi *et al.*, 2012). In Lhasa, Tibet, Tibetans present lower endothelial function and baseline arterial diameter compared

to Han Chinese (Yang *et al.*, 2016). In Kathmandu (1400m), partially de-acclimatized Sherpa who live and work at high altitude present similar endothelial function compared to lowlanders (Tremblay *et al.*, 2018a). On ascent to 5050m, although both Sherpa and lowlanders demonstrate changes in shear stress, only lowlanders experience reductions in endothelial function (Tremblay *et al.*, 2018a). Thus, Sherpa may be resistant to hypoxia- and/or shear stress-associated reductions in endothelial function.

Ethiopian highlanders

Evidence for the occupancy of the Ethiopian highlands is elusive; however, artifacts, presumably from *Homo erectus/ergaster*, have been found at altitudes of 2,300-2,400m along the rim of the Rift Valley dating back to 1.5 million years ago (Clark & Kurashina, 1979; Williams *et al.*, 1979; de la Torre, 2011). More recent estimates suggest that humans have populated the Ethiopian highlands anywhere from 500-70,000 years ago (Aldenderfer, 2003; Pleurdeau, 2005). Despite the possibility that humans or hominids have ventured to high altitudes in East Africa much earlier than Tibetans or Andeans, very little is known regarding their physiological phenotype of Amhara people residing in and around the Simien Mountains. Intriguingly, Amhara highlanders present a phenotype distinct from Tibetans and Andeans, by appearing to have a low [Hb] and higher than expected SaO_2, potentially due to an increased affinity of [Hb] for oxygen (Beall *et al.*, 2002; Beall, 2006). Conceivably, these three phenotypes (healthy Tibetan, Andean, and Ethiopian highlanders) represent distinct evolutionarily-driven strategies which permit survival and encourage oxygen delivery at high altitude (Beall, 2006). One study design employed to investigate Amhara highlanders compared individuals living in the Amhara region (Simien Mountains) to Oromo highlanders, who have resided at high altitude in the Bale mountains for only ~500 years (Lewis, 1966). The Oromo present lower SaO_2 and higher [Hb] than Amhara highlanders (Lundgrin *et al.*, 2013; Cheong *et al.*, 2017), suggesting the Oromo present a similar response to high altitude as lowlanders. An adaptive

vascular phenotype has been suggested in Amhara highlanders, based upon urinary levels of nitrate and cyclic guanosine monophosphate (Cheong *et al.*, 2017); however, these are indirect and non-specific measures and no studies of the systemic vasculature have been conducted in this population.

Conclusions

The objective of this review was to summarize the current understanding of how hypoxia (normobaric and hypobaric) affects endothelial function in lowlanders and permanent highlander populations. The primary mechanism(s) that alter endothelial function pertain to shear stress and blood flow patterns, oxidative and inflammatory stress, sympathetic nervous activity, and haemoglobin and blood viscosity. Importantly, each of these mechanism(s) are altered during chronic hypoxia exposure, and most likely, all contribute to reduced endothelial-*dependent* function observed in lowlanders at altitudes >3500-4000m. Due to limited data sets, the impact of chronic hypoxia exposure from an evolutionary perspective is less clear. There is mounting evidence indicating that Sherpa and healthy Andean highlanders have similar endothelial function compared to lowlanders at sea level; however, endothelial function in Ethiopian highlanders remains unknown. Additionally, Andeans suffering from chronic mountain sickness have markedly reduced endothelial function. The clinical implications and physiological consequences of “impaired” endothelial function at high altitude in lowlanders remains in question. A major limitation to the current available literature is that the principal method utilized to quantify the effect of hypoxia on endothelial function has been restricted to the forearm (i.e. brachial artery flow-mediated dilation), likely due to this technique being easily accessible and non-invasive. Despite the logistical hurdles encountered with high altitude field research, future studies should consider employing methodologies that focus

on more clinically relevant vasculature (e.g. coronary arteries), and test multiple vascular beds (e.g. microvasculature, separate limbs.

Although hypoxaemia is generally considered to yield negative health consequences in both acute and chronic settings, but the human body is resilient, and through several different avenues, we can acclimatize to impressive altitudes. In fact, hypoxia may be protective to some extent, as indicated by studies demonstrating reduced atherosclerosis (in mice; Kang *et al.*, 2016), lower mortality rates from coronary disease (Faeh *et al.*, 2009; reviewed in Burtscher, 2014), and lower incidences of certain types of cancer (reviewed in Thiersch & Swenson, 2018), even after correcting for important confounders such as age, sex, education, and urbanization. In light of these studies it is possible that reduced NO-mediated vascular function at high altitude is not pathogenic, but perhaps a necessary, physiological consequence for acclimatization; however, more work is required to elucidate the full clinical implications of reduced endothelial function at altitude.

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Tables

Table 1. Summary of studies that have performed flow-mediated dilation in hypoxic conditions in lowlanders.

Study	Population	Level of Hypoxia	Duration	Impact on FMD	Study Comments
Acute – laboratory					
Frobert *et al.* (2008)	10 men increased CV risk; 10 healthy men	12.5%	5 minutes	↓ FMD (3%)	Diameter measured at 60-seconds post-cuff release GTN performed in normoxia
Rieger *et al.* (2017)	16 young, healthy	Isocapnic $P_{ET}O_2$=50	15 minutes	↔ FMD	Repeated 1h, 24h, and 48h after RIPC or sham

		mmHg			
Lewis *et al.* (2017)	12 young, healthy	Isocapnic $P_{ET}O_2$=75 mmHg, and $P_{ET}O_2$=50 mmHg	30 minutes	↓ FMD (17%, and 45%)	GTN performed after 60 minutes of isocapnic hypoxia. ↓ SR_{AUC}, corrected-RH-FMD GTN, FMD/GTN. Greater reduction in 50 vs 74. ↑ retrograde shear, ↓ mean, ↓ antegrade
(Lewis *et al.*, 2014)	11 young, healthy	11%	60 minutes 210 minutes 330 minutes	↓ FMD (28%, 33%, and 36%)	α_1-adrenergic receptor blockade prior to FMD abolished the hypoxia-related reductions in FMD
Iglesias *et al.* (2015)	10 healthy male sportsmen or mountaineers	Hypobaric chamber, 4000m	4h	↔ FMD	Proximal FMD cuff placement
(Tremblay *et al.*, 2018b)	15 young, healthy men	11%	20 minutes	↓ FMD (29%)	Imposed oscillatory shear did not further reduce FMD
Acute – Field, passive ascent					
Bruno *et al.* (2016)	22 AMS-, healthy	3842m	4h	↔ FMD	↑ SR_{AUC} (AMS-)
	12 AMS+, healthy	3842m	4h	↓ FMD (43%)	↔ SR_{AUC} (AMS-)
Frick *et al.* (2006)	18 men with metabolic syndrome	1700m	Day of arrival	↔ FMD	No change in GTN, proximal cuff placement
Sustained – Passive					
Tremblay *et al.*	12 young, healthy	3800m	Day 2/3	↔ FMD	↑ SR_{AUC}, ↓ diameter. ↓ RH-FMD post-oscillatory shear

(2017)					stress.
Tymko *et al.* (2017a)	9 young, healthy	3800m	Day 3-7	↔ FMD	Decreased diameter, ↔ SR_{AUC}. α_1-adrenergic receptor blockade did not impact baseline RH-FMD. α_1-adrenergic receptor blockade resulted in larger post-exercise RH-FMD.
Rieger *et al.* (2017)	12 young, healthy	3800m	Day 8-12	↔ FMD	Repeated 1h, 24h, and 48h post RIPC/sham
Sustained – trekking					
(Lewis *et al.*, 2014)	12 healthy	5050m	Day 3 and 12-14 after 8-day trek	↓ FMD (14%)	Acetazolamide administered during ascent. ↓ GTN, ↔ FMD/GTN, ↔ SR_{AUC} day 3, ↓ SR_{AUC} day 12-14
Bakker *et al.* (2015)	11 young, healthy	Trekking above 2500m Measures at 3700m, 4200m	3700m: 3 days trekking, one day residing at 3700m 4200m: 5 days above 2500m	↓ FMD (42%)	FMD stimulus calculated as peak blood flow / peak diameter. No change in FMD with beetroot juice supplementation at 3700m. Baseline measures made at 1370m.
Frick *et al.* (2006)	18 men with metabolic syndrome	Trekking at 1700m	3 weeks	↓ FMD (49%)	Decreased diameter. FMD remained reduced 6 weeks after return to lower altitude
(Tremblay *et al.*, 2018b)	15 young, healthy men	5050m	Days 5-7 after 9-day trek	↓ FMD (25%)	Imposed oscillatory shear did not further reduce FMD

(Tremblay *et al.*, 2018a)	22 young, healthy (2 female)	3440m 4371m 5050m	Day 4 Day 7 Day 10 of trek	↔ FMD ↓ FMD (36%) ↓ FMD (46%)	Reduced brachial artery FMD at 4371m and 5050m, but no changes in superficial femoral FMD
(Tymko *et al.*, 2017b)	14 young, healthy	5050m	Days 11-14 after 9-day trek	↓ FMD (18%)	Mild changes in SNA did not alter FMD. SNA not assessed at high altitude.

Definition of abbreviations: GTN, glyceryl trinitrate; FMD, reactive hyperemia flow-mediated dilation; RIPC, remote ischemic precondition; SNA, sympathetic nerve activity; SR_{AUC}, shear rate area under the curve.

Figure Legends

Abstract Figure: Overview of the conventional mechanism(s) that govern endothelial function in normoxic and hypoxic humans. This figure depicts the primary mechanism(s) governing changes in endothelial-*dependent* vascular smooth muscle vasodilation. Chronic, hypoxia (normobaric and hypobaric) results in 1) an increase in pro-atherogenic shear stress, 2) heightened sympathetic nervous activity, 3) elevations in oxidative-nitrosative-inflammatory (OXINOS) stress defined by a free radical/inflammation-mediated reduction in vascular nitric oxide bioavailability, and 4) during chronic hypoxia exposure leading to increased red blood cell concentration – increased red blood cell mediated nitric oxide scavenging. Arterial segments that are chronically exposed to low, and oscillatory shear stress preferentially develop atherosclerotic lesions (Caro *et al.*, 1969; Ku *et al.*, 1985). Increased pro-atherogenic shear stress during hypoxia may be related to increased sympathetic nervous system outflow, which results in increased norepinephrine (i.e. NE) release sympathetic ganglia (T1-T5, upper body vasculature; T6-L2, lower body vasculature) onto α and β adrenergic receptors, which signal for smooth muscle vasoconstriction and vasodilation, respectively. A stimulus (e.g. shear stress) results in a release of endothelial endogenous calcium, which binds to calmodulin, and activates endothelial nitric oxide synthase (eNOS). The production of eNOS converts L-arginine into L-citrulline, and while doing so, releases nitric oxide. The newly produced nitric oxide is able to diffuse across the endothelial cell membrane and into the nearby smooth muscle cells. Within the smooth muscle cells, nitric oxide activates guanylate cyclase, and guanylate cyclase converts guanosine 5'-triphosphate (GTP) into cyclic guanosine 3',5'-monophosphate (cGMP). The cGMP upregulates cGMP dependent protein kinase G (PKG), which acts to inhibit myosin light chain kinase resulting in smooth muscle cell relaxation (Adelstein *et al.*, 1978; Kerrick & Hoar, 1981). Upregulated cGMP also phosphorylates voltage-gated potassium channels resulting in potassium cellular extrusion, contributing to smooth muscle relaxation (Irvine

et al., 2003). In addition, there is some evidence that suggests that NO may independently activate voltage-gated potassium channels (Bolotina *et al.*, 1994).

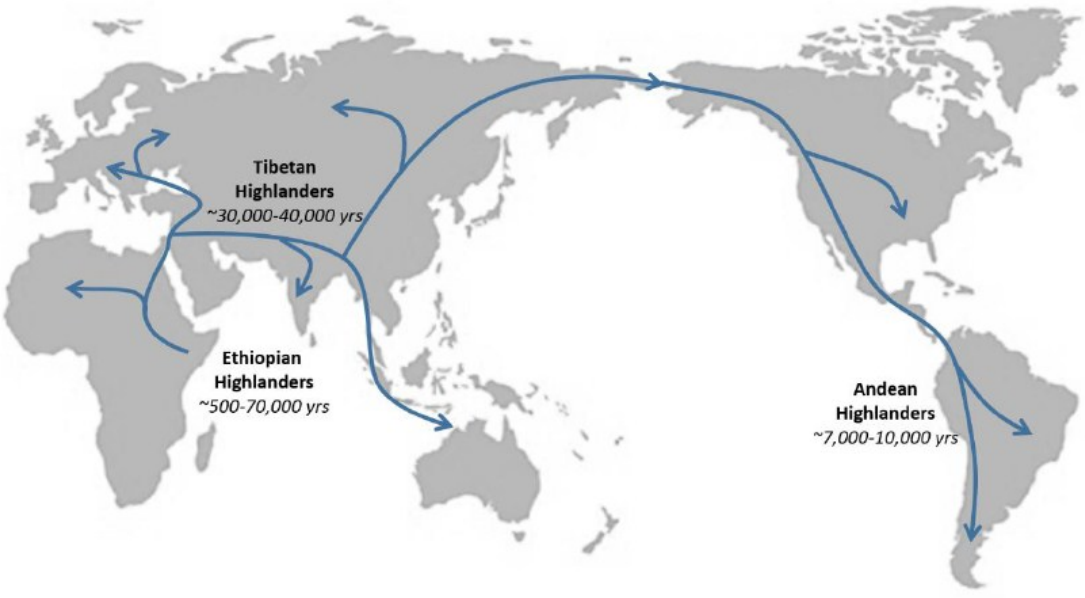

Figure 1: Timeline summary of the proposed Ethiopian, Tibetan, and Andean highlander settlements. Historical records indicate that the Oromo tribe of Ethiopia has only been settled in the highlands for ~500 years (Lewis, 1966); however, the Amhara tribe has been living at altitude potentially up to 70,000 years based on archaeological evidence (Lewis, 1966; Aldenderfer, 2003; Pleurdeau, 2005). Humans are thought to have occupied the Tibetan plateau for ~30-40,000 years (Zhang *et al.*, 2018), and eventually crossed a land bridge that once connected present day Russia and Alaska, which facilitated North and South American settlements and eventually migrating to the Andes ~7-10,000 years ago (Aldenderfer, 2008; Haas *et al.*, 2017).

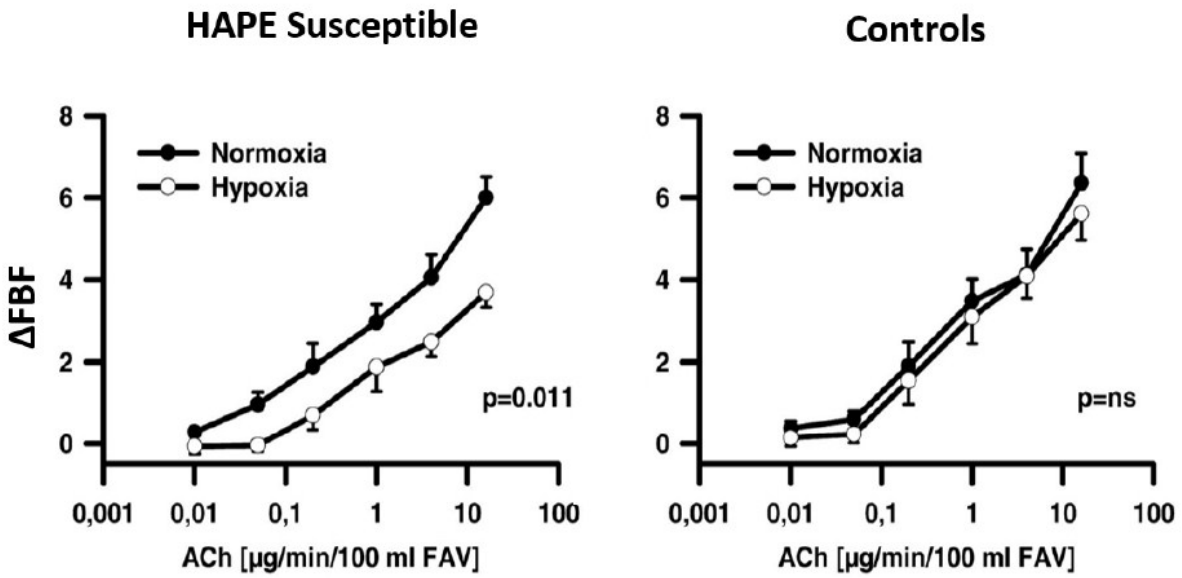

Figure 2: The effect of normobaric hypoxia on endothelial function in participants prone to developing high altitude pulmonary oedema and healthy controls. Forearm blood flow (FBF) response to acetylcholine after exposure to normoxia and hypoxia in nine mountaineers susceptible to high-altitude pulmonary oedema and nine healthy mountaineers (control). Figure adapted from (Berger *et al.*, 2005)

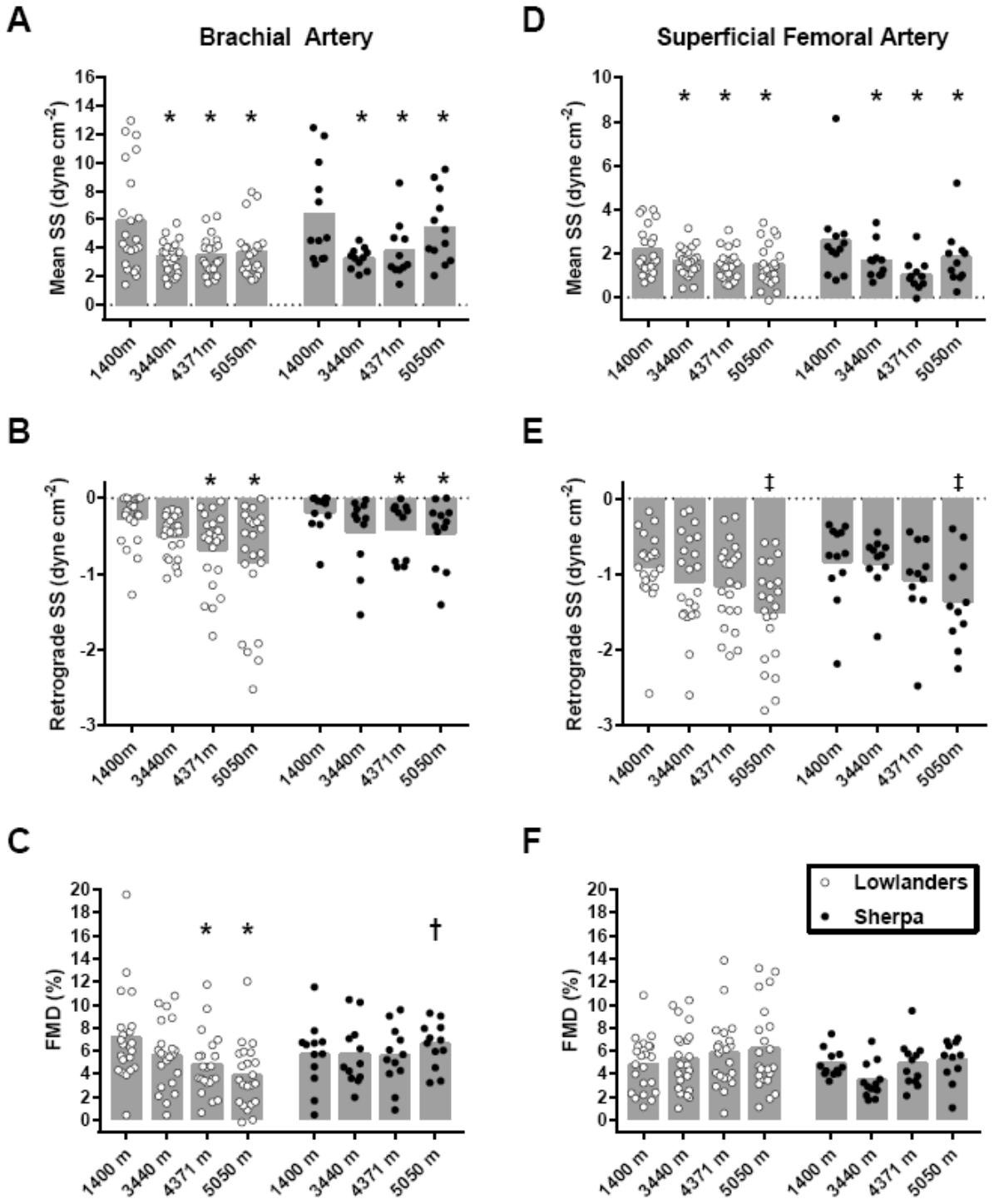

Figure 3: Effect of high altitude on shear stress and endothelial function in lowlanders and Sherpa. Upon ascent to high altitude, mean shear stress is reduced, but retrograde shear stress is elevated at high altitude in both lowlanders and Sherpa highlanders. In lowlanders, brachial artery endothelial function is reduced at 4371m and 5050m, whereas Sherpa have preserved endothelial function. In contrast, no changes in endothelial function was observed in the superficial femoral artery in both lowlanders and Sherpa. Figure adapted from (Tremblay *et al.*, 2018a).

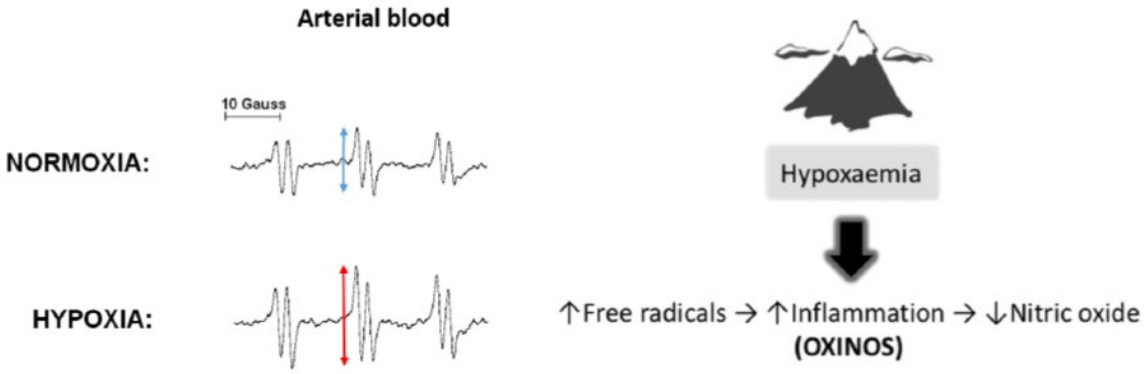

Figure 4: Free radical production with hypoxia exposure. Representative figure displaying electron paramagnetic resonance spectroscopic detection of α-phenyl-*tert*-butyl-nitrone in normoxia (21% O_2) and after 9-h passive exposure to hypoxia (12.9% O_2). Figure adapted from (Bailey *et al.*, 2009b)

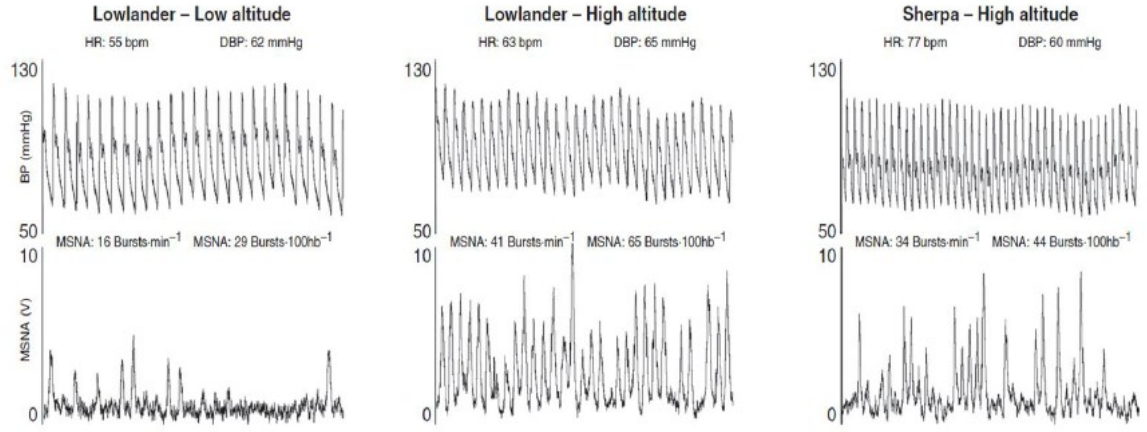

Figure 5: The effect of high altitude on resting muscle sympathetic nervous activity in lowlanders and Sherpa. Ascent to high altitude increases basal muscle sympathetic nervous activity in lowlanders, and to a lesser extent in Sherpa. Heightened sympathetic nervous activity is proposed as a potential mechanism responsible for reduced endothelial function at high altitude; however, the direct mechanism link between sympathetic nervous activity and endothelial function at high altitude has not been assessed. Figure adapted from (Simpson *et al.*, 2019), and units for muscle sympathetic nervous activity are expressed in burst frequency (number of bursts per minute) and burst incidence (the number of bursts per 100 heart beats).

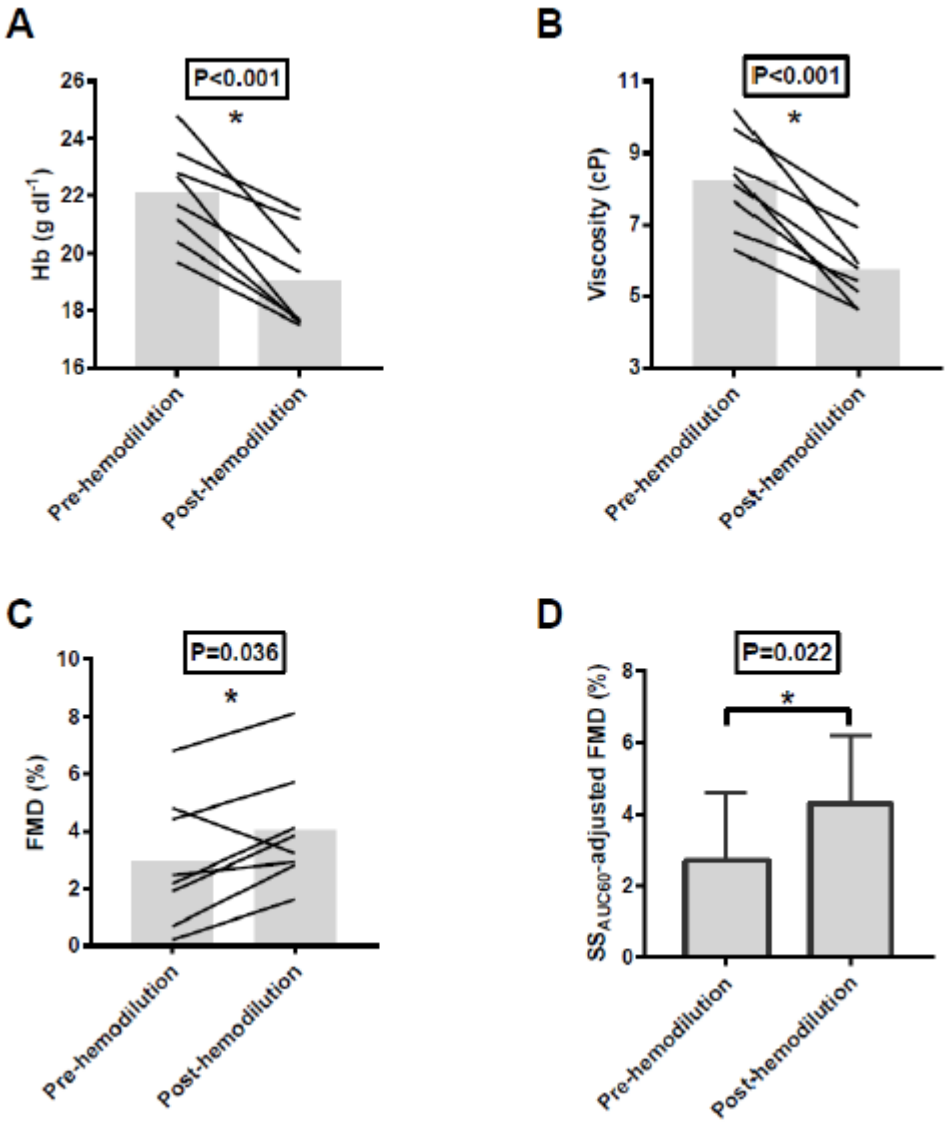

Figure 6: The effects of haemodilution on brachial artery endothelial function in Andeans with excessive erythrocytosis at 4300m. Haemodilution resulted in a significant reduction in haemoglobin concentration and blood viscosity, and an increase in endothelial function assessed by brachial artery flow-mediated dilation. Figure adapted from (Tremblay *et al.*, 2019b).