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Title: The 2018 Global Research Expedition on Altitude-related Chronic Health (REACH) to Cerro de Pasco, Peru: An Experimental Overview

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Running Title: Global REACH High Altitude Expedition to Peru

Abstract: In 2016, the international research team - Global Research Expedition on Altitude-related Chronic Health (REACH) - was established and executed a high altitude research expedition to Nepal. The team consists of ~45 students, principal investigators and physicians with the common objective of conducting experiments focused on high altitude adaptation in lowlanders, and highlanders with lifelong exposure to high altitude. In 2018, Global REACH traveled to Peru where we performed a series of experiments in the Andean highlanders. The experimental objectives, organization and characteristics, and key cohort data from Global REACH's latest research expedition are outlined herein. Herein, fifteen major studies are described that aimed to elucidate the physiological differences in high altitude acclimatization between lowlanders (n=30) and Andean born highlanders with (n=22) and without (n=45) Excessive Erythrocytosis (EE). After baseline testing in Kelowna, BC, Canada (344m), Global REACH travelled to Lima, Peru (~80 m), and then ascended by automobile to Cerro de Pasco, Peru (~4300m) where experiments were conducted over 25 days. The core studies focused on elucidating the mechanism(s) governing cerebral and peripheral vascular function, cardiopulmonary regulation, exercise performance, and autonomic control. Despite encountering serious logistical challenges, each of the proposed studies were completed at both sea level and high altitude amounting to ~780 study sessions and >3000 hrs of experimental testing. Participant demographics and data related to acid-base balance and exercise capacity are presented. The collective findings will contribute to our understanding of how lowlanders and Andean highlanders have adapted under high altitude stress.

New Findings: What is the central question of this study? Herein, a methodological overview of our research team's latest high altitude research expedition to Peru is provided. What is the main finding and its importance? The experimental objectives, expedition organization, measurements, and key cohort data are discussed. The select data presented in the current manuscript demonstrated some of the hematological differences between lowlanders and Andeans with and without excessive erythrocytosis, and that relative exercise capacity was similar between study groups at high altitude. The forthcoming findings from our research expedition will contribute to our understanding of lowlander acclimatization and highlander adaptation.

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Title: The 2018 Global Research Expedition on Altitude-related Chronic Health (REACH) to Cerro de Pasco, Peru: An Experimental Overview

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What is the central question of this study?

Herein, a methodological overview of our research team's (Global REACH) latest high altitude research expedition to Peru is provided.

What is the main finding and its importance?

The experimental objectives, expedition organization, measurements, and key cohort data are discussed. The select data presented in the current manuscript demonstrate the hematological differences between lowlanders and Andeans with and without excessive erythrocytosis, and that exercise capacity was similar between study groups at high altitude. The forthcoming findings from our research expedition will contribute to our understanding of lowlander and indigenous highlander high altitude adaptation.

Abstract

In 2016, the international research team - Global Research Expedition on Altitude-related Chronic Health (REACH) - was established and executed a high altitude research expedition to Nepal. The team consists of ~45 students, principal investigators and physicians with the common objective of conducting experiments focused on high altitude adaptation in lowlanders, and highlanders with lifelong exposure to high altitude. In 2018, Global REACH traveled to Peru where we performed a series of experiments in the Andean highlanders. The experimental objectives, organization and characteristics, and key cohort data from Global REACH's latest research expedition are outlined herein. Herein, fifteen major studies are described that aimed to elucidate the physiological differences in high altitude acclimatization between lowlanders (n=30) and Andean born highlanders with (n=22) and without (n=45) Excessive Erythrocytosis (EE). After baseline testing in Kelowna, BC, Canada (344m), Global REACH travelled to Lima, Peru (~80 m), and then ascended by automobile to Cerro de Pasco, Peru (~4300m) where experiments were conducted over 25 days. The core studies focused on elucidating the mechanism(s) governing cerebral and peripheral vascular function, cardiopulmonary regulation, exercise performance, and autonomic control. Despite encountering serious logistical challenges, each of the proposed studies were completed at both sea level and high altitude amounting to ~780 study sessions and >3000 hrs of experimental testing. Participant demographics and data related to acid-base balance and exercise capacity are presented. The collective findings will contribute to our understanding of how lowlanders and Andean highlanders have adapted under high altitude stress.

Key words: High-altitude, hypobaric hypoxia, Andean highlanders, Global REACH

Introduction

Indigenous populations of the high plateaux of Tibet (Sherpa), Peru (Quechua), and Ethiopia (Amhara and Oromo) have developed distinct physiological adaptations to thrive in their respective hypoxic environments (Beall, 2006). Although there is considerable debate on when these plateaux were occupied, the consensus is that the Old World plateaux have been inhabited longer (Ethiopian ~50,000 to ~70,000 years; Tibetan ~30,000 to ~40,000 years), compared to the Altiplano in the New World (Peruvian ~7,000 to ~10,000 years) (Aldenderfer, 2003, 2008; Alkorta-Aranburu et al., 2012; Beall, 2006, 2007a; Beall et al., 2002; Haas et al., 2017; Pleurdeau, 2005; Zhang et al., 2018). The majority of these indigenous populations show impressive function at high altitude compared to lowlanders – function that arises from fundamental changes in oxygen delivery and utilization. The Sherpa typically have lower hemoglobin concentration compared to other highlanders at similar altitudes such as the Peruvian Quechuan (Beall, 2006; Gassmann et al., 2019). Some (Gilbert-Kawai, Milledge, Grocott, & Martin, 2014; Tremblay, Hoiland, et al., 2018), but not all studies (Liu et al., 2016; Wang et al., 2018), indicate that Sherpa rely on enhanced blood flow and endothelial function to sustain convective oxygen delivery, and also on cellular metabolic adaptations to utilize oxygen more efficiently (Horscroft et al., 2017). In contrast, the Quechua highlanders exhibit increased hemoglobin concentration in response to high altitude exposure, but they are more prone to developing chronic mountain sickness, of which excessive erythrocytosis (EE) is one characteristic feature (being defined as a hemoglobin concentration of $\geq$ 19 g dl^{-1} and $\geq$ 21 g dl^{-1} in females and males, respectively). Although the pathological origins of hypoxia-induced EE are still not fully understood and may be secondary to existing disease (Villafuerte & Corante, 2016), or due to genetic drift (Bermudez et al., 2019; A. M. Cole, Petousi, Cavalleri, & Robbins,

2014; Hsieh et al., 2016; Zhou et al., 2013), EE leads to elevated blood viscosity (which increases the resistance to blood flow through the vasculature), and elevated systemic oxidative-inflammatory-nitrosative stress (Bailey et al., 2018; Bailey et al., 2013). Less is known about the adaptations (or maladaptations) of Ethiopian highlanders (Amhara and Oromo populations) from the Simien and Bale mountains, respectively. The Amhara, but not Oromo highlanders, have hemoglobin concentrations similar to lowlanders, yet higher than expected arterial oxygen saturation, potentially due to an increased affinity of hemoglobin for oxygen (Beall, 2006; Beall et al., 2002), which can be altered by temperature, pH, PCO_2, and 2,3-bisphosphoglyceric acid. Conceivably, the Sherpa, Quechua, and Amhara phenotypes represent distinct evolutionarily-driven strategies that permit survival and performance at high altitude (Beall, 2006, 2007b; Beall et al., 2002).

In 2016, our international research team, Global Research Expedition on Altitude-related Chronic Health (REACH) was formed with the research objective to execute a series of experiments on the Tibetan, Peruvian, and Ethiopian highlanders. High altitude physiology research offers valuable insight on the mechanistic adaptation to hypoxia, which is translatable to a myriad of clinical diseases characterized by hypoxemia (Levett et al., 2010). The timing of this research is urgent, as migration and modernization are rapidly changing traditional ways of life and altitude exposure of high altitude dwellers (Beall, 2013). In October 2016, our research team successfully conducted a series of experiments in lowlanders and Sherpa at the Ev-K2 Pyramid International Laboratory/Observatory near Mt. Everest basecamp (5050m) (Willie et al., 2018), and in July of 2018, Global REACH traveled to Cerro de Pasco, Peru (4300m) to conduct a similar series of experiments in the Quechua highlanders with and without EE. An estimated 5-10% of high altitude dwellers are at risk of developing EE (Leon-Velarde et al., 2005; Villafuerte

& Corante, 2016), which is a public health concern primarily in the Andes, but also in Kyrgyzstan, Northern India, and among migrants to high altitude in Tibet and the United States (Pei et al., 2012; Penaloza & Arias-Stella, 2007; Sahota & Panwar, 2013). Among the highest reported prevalence is in the mining city of Cerro de Pasco, Peru, where 15% of men aged 30-39, and 34% aged 60-69 present with EE (Monge, Leon-Velarde, & Arregui, 1989). Additionally, a recent meta-analysis demonstrated that there are differences in hemoglobin concentration between highlander populations (Gassmann et al., 2019), and in agreement with previous reports (Beall, 2006, 2007b; Beall et al., 2002), this meta-analysis indicated that Andean highlanders have higher hemoglobin concentration and hematocrit compared to other highlander populations (e.g. Sherpa and Ethiopians).

Similarly to the overview manuscript written based on our team's research expedition to Nepal (Willie et al., 2018), we provide a methodological summary of Global REACH's research expedition to Cerro de Pasco, the second installment of our team's common research objective to study and compare the Tibetan, Peruvian, and Ethiopian highlanders. In both expeditions the core studies focused on elucidating the mechanism(s) governing cerebral and peripheral vascular function, cardiopulmonary regulation, exercise performance, and autonomic control. The principal scientific themes, experimental details, and key cohort data of our Peru expedition are discussed within. The results of the specific studies outlined will be published as separate manuscripts.

Method and Materials

Ethical approval. In accordance with the *Declaration of Helsinki*, except for registration in a database, the lowlander and highlander based studies were approved by the UBC Clinical Research Ethics Board (H17-02687 and H18-01404, respectively), and local Peruvian ethics committee for the Universidad Peruana Cayetano Heredia (#101686). All lowlander and highlander study participants provided informed consent (English and Spanish consent forms were available) after each study was thoroughly explained in their native language. Prior to voluntary consent, we provided opportunity for questions directly with each study principal investigator. For each study involving highlanders in Cerro de Pasco, Peru, an official translator was present for the entire testing duration to ensure proper communication was maintained between the researchers and participant. All participants were free to withdraw without justification or penalty from all experiments at any time. Andean participants were monetarily compensated for their time based on a payment suggested by the Universidad Peruana Cayetano Heredia research ethics board.

Global REACH. Our research team, consisting of 45 students, principal investigators, and physicians commenced baseline studies in March 2018, and travelled to Cerro de Pasco between June 26-28th 2018, where the majority of the team remained until July 22nd 2018 (*refer to figure 1*). The leadership for the research expedition originated from the University of British Columbia - Okanagan (Canada), Unversity of Alberta (Canada), Duke University (United States), Loma Linda University (United States), University of Innsbruck (Austria), University of Boulder Colorado (United States), Cardiff Metropolitan University (United Kingdom), University of Cambridge (United Kingdom), University of South Wales (United Kingdom), and Bangor

University (United Kingdom). The research expedition, was aided by the help of local collaborator, Dr. Francisco Villafuerte, and three graduate students from the Universidad Peruano Cayetano Heredia, Peru.

Participants. All Global REACH members were of European descent (two generations self-reported) and were born and currently living below 1500m. All lowlander participants avoided visiting high altitude (>1500m) for at least six-months prior to the expedition. Each expedition study had different sample sizes pooled from the same cohort of participants according to prospective power calculations (*see below*). Global REACH members were included in up to 11 experiments at either sea level and/or high altitude with adequate recovery between each and close attention to non-contamination between protocols (e.g. washout following drug infusion). The Andean highlanders were only tested at high altitude. Andean highlanders were recruited through an established database compiled in-part by Dr. Villafuerte from the Universidad Peruana Cayetano Heredia, Lima, Peru. Highlanders were recruited by telephone by one of three local translators. During a familiarization visit to the high altitude laboratory, a detailed self-reported family and altitude history was collected from all Andean participants including altitudes during childhood, in adulthood, and for the 12-months preceding the studies. All recruited Andean participants (and at least two previous known generations) were born at, and permanently lived at high altitude (within the Cerro de Pasco region). After providing informed consent, each recruited Andean participant was requested to fill out a Qinghai CMS questionnaire (Leon-Velarde et al., 2005). Studies involving both lowlanders and Andean highlanders attempted to age and sex match participants (i.e. match Andean highlanders to the already enrolled lowlanders). Prior to any study at either low or high altitude, participants were

asked to refrain from exercise and caffeine for a minimum of 12-hours, and were fasted for a minimum of two-hours. We also obtained an antecubital venous blood sample for the measurement of hematocrit and hemoglobin. Due to the high number of proposed investigations that needed to be completed within a short time-frame, six laboratories in Cerro de Pasco ran simultaneously and typically operated for 12-18 hrs/daily. Study participants were included if they were between the ages of 18-60 years old without any medical history of cardiovascular, cerebrovascular, pulmonary, metabolic disease, or history of working in the local mines (e.g. Lead, Cobalt, and Sulphur mines). However, blood analyses for heavy metals such as Lead and Cobalt were not performed and are potential cofounders to our data sets. A subset of premenopausal women aged 18-30 were recruited to investigate potential sex differences within healthy (i.e. non-EE) Andeans.

Chronic Mountain Sickness. . Chronic mountain sickness was determined using the Qinghai CMS score (Leon-Velarde et al., 2005). A score of zero (i.e. absent), to three (i.e. severe), was assigned for the following signs and symptoms: breathlessness/palpitations, sleep disturbance, cyanosis, venodilation, paresthesia, headache, and tinnitus. The sum of the score for each symptom and EE defines CMS severity as absent (0-5), mild (6-10), moderate (11-14), and severe ($\geq$ 15).

Low and high altitude laboratories. Between February-April 2018, baseline studies were conducted in the Cerebrovascular Physiology Laboratory at the University of British Columbia – Okanagan (Kelowna, BC, Canada). There, the large laboratory (P_B = ~730 mmHg, humidity= ~30%, laboratory temperature = ~22^{o}C) was divided into five separate simultaneous testing

areas, and one space was dedicated to blood analysis compliant with standard research governance guidelines. Global REACH arrived in Cerro de Pasco in late June 2018. The testing facility associated with the Universidad Peruana Cayetano Heredia in Cerro de Pasco was expansive (P_B = ~450 mmHg, humidity= ~75%, laboratory temperature = ~15ºC), being able to accommodate six complex (i.e., invasive and/or pharmacological) studies running simultaneously. Electricity was consistent for the majority of expedition; however, on ~5 days we encountered long-term black-outs and power surges. The latter resulted in short-lasting electrical fires. Therefore, all equipment was equipped with surge protectors, and after the first local electrical blackout (of several), a large generator served as a back-up power source for the remainder of our expedition. Another regular issue encountered was water supply shortage. The laboratory was not accustomed to housing ~45 guests at once (excluding Andean participants). Our team consistently ran out of water for the facility's toilets and sinks, and needed to get the water reservoir filled 1-2 times per week.

Equipment Logistics. Over four metric tonnes of equipment and consumable items (e.g. needles, syringes, saline), with a value amounting to ~1.3 million dollars (USD) were packed in a combination of pelican cases, duffle bags, suitcases, and waterproof barrels. Back-up equipment for nearly every research device was packed in case of equipment failure at high altitude. This equipment travelled as checked-baggage on multiple international flights with each Global REACH team member. More fragile equipment, such as Duplex ultrasound machines and laptops, accompanied team members as carry-on luggage. Appropriate documents for legal importation of all equipment and consumable items were obtained prior to traveling to Peru. Despite these efforts, ~15% of our equipment was rejected entry into Peru upon arrival; however,

through sustained perseverance by both Global REACH members and our local Peruvian collaborators, these items were released from Peru customs within ~14 days. In advance, 43 K- and T-size compressed gas cylinders were purchased (Linde Industrial Gas, Lima, Peru) and delivered to the high altitude laboratory in Cerro de Pasco. One small compressed gas cylinder of 100% oxygen was acquired as a safety precaution in the event of an emergency evacuation of a team member (or Andean participant) related to altitude illness – thankfully, no serious adverse health outcomes were experienced. Several studies required blood and plasma samples to be flash frozen and stored in liquid nitrogen Dewars prior to transportation back to the Canada/UK/USA for batch analyses. Liquid nitrogen was sourced locally in Lima, Peru from an industrial gas supplier (Linde).

Ascent profile to Cerro de Pasco. Global REACH members traveled to Lima, Peru, in June ~2-7 days prior to departure to Cerro de Pasco. During this time, final equipment organization and preparations were completed, including the processing of several documents to release the equipment that remained in Peru customs. Research equipment, personal luggage, and liquid nitrogen Dewar's were transported by a one-tonne cube truck to Cerro de Pasco (~270 km; ~7-8 hour drive). Global REACH team members, along with local collaborators were transported by cargo van on three separate days (June 26th, 27th, and 28th). Participants refrained from taking prophylactic medication against high altitude illness (e.g. Acetazolamide). However, if participants requested medication to relieve symptoms while at high altitude, or were requested by our physicians to undergo treatment, it was made available to them and their health and symptoms were monitored closely. The majority of the Global REACH team spent 3-4 weeks in Cerro de Pasco completing all studies.

Commonly Employed Methodologies

Dynamic end tidal forcing. Several studies required the precise control of arterial blood gases for a portion, or for the entire duration of their protocol. To accomplish this, we employed a portable dynamic end-tidal forcing system (*Airforce*, UBC, Canada) to control the partial pressure of end-tidal oxygen and carbon dioxide ($P_{ET}O_2$ and $P_{ET}CO$, respectively), as a surrogate for the partial pressure of arterial oxygen and carbon dioxide (PaO_2 and $PaCO_2$, respectively) (Tymko, Ainslie, MacLeod, Willie, & Foster, 2015; Tymko et al., 2016). Our custom-built system uses independent gas solenoid valves for O_2, CO_2, and N_2 and controls the volume of each gas being delivered to an inspiratory reservoir through a mixing and humidification chamber on a breath-by-breath basis. This system has been used previously to effectively control end-tidal gases during many different physiological stressors, and its use has been validated at high altitude (Tymko et al., 2015). Although this device works extremely well in participants with background knowledge in respiratory physiology, extensive training and instruction was required from our local translators to aid the Andean participants to breathe normally on the device for specific research studies.

Respiratory and cardiovascular measurements. Nearly all respiratory and cardiovascular parameters were acquired using an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO, USA) interfaced with a personal computer. Commercially available software was used to analyze ventilatory and cardiovascular variables (LabChart V7.1, ADInstruments, Colorado Springs, CO, USA). Respired gases were sampled at the mouth and analyzed for $P_{ET}O_2$ and $P_{ET}CO_2$ (ML206; ADInstruments, Colorado Springs, CO,

USA). During the expedition, two gas analyzers failed due to pump failure and excess condensation, the latter issue was able to be resolved. Respiratory flow was also measured near the mouth using a pneumotachograph (HR 800L, HansRudolph, Shawnee, KS, USA) and a differential pressure amplifier (ML141, ADInstruments, Colorado Springs, CO, USA). One unexpected problem we encountered due to the high volume of data collection (i.e. >12 hours of continuous respiratory data collection each day) was excess condensation accumulating in the pneumotachometers resulting in respiratory "drift" and signal artifact. This issue was resolved by replacing spirometry filters more frequently (e.g. two per participant), and/or by replacing the pneumotach. Heart rate was determined from a standard lead II electrocardiogram (ML 132, ADInstruments, Colorado Springs, CO, USA), and the majority of blood pressure measurements were conducted on a beat-by-beat basis using finger photoplethysmography (Finometer pro, Finapres Medical Systems, Netherlands). At times, due to the cold environment, a hot water bottle was required to elevate and then maintain the participant's hand temperature prior to experimentation, in order to obtain a proper blood pressure waveform. Additionally, all studies recorded manual blood pressure measurements before and throughout experimentation to both confirm and calibrate the finometer blood pressure waveform.

Transcranial and duplex Doppler ultrasound. Several studies required the use of transcranial Doppler (TCD) and/or duplex Doppler ultrasound. These techniques function based on the same fundamental principles; however, Duplex ultrasound allows for the simultaneous acquisition of both blood vessel image and blood velocity (Thomas, Lewis, Hill, & Ainslie, 2015), whereas TCD only records blood velocity of an insonated blood vessel (Willie et al., 2011). The same experienced sonographers were used for all studies, and they used commercially available

ultrasound machines (TCD, Spencer Technologies, PMD150B; Duplex ultrasound, uSmart 3300, Terason; Vivid, GE, Fairfield, CT, USA). For our studies, TCD ultrasound utilized a low frequency probe (2 MHz) to assess blood velocity in the middle and posterior cerebral arteries using previously described techniques (Willie et al., 2011). Our peripheral Duplex ultrasound machines used a higher-frequency (10 MHz) linear array probe and were portable with a short battery life (~90-minutes), therefore, routine access to power supplies were needed. The peripheral Duplex ultrasound machines were used to measure blood flow through the brachial, renal, common carotid, internal carotid, external carotid, and vertebral arteries using previously established guidelines and principles (Thomas et al., 2015). Data backups were performed daily to multiple portable encrypted solid-state hard drives. In previous high altitude expeditions, our team has encountered several failures with older spinning hard drives likely due to the reduction in barometric pressure. Therefore, we ensure that we only use solid-state external and internal hard drives while at high altitude.

Transthoracic Echocardiography. For studies assessing cardiac function and/or pulmonary pressure, a portable, battery-powered, cardiac ultrasound machine was employed (Vivid Q, GE, Fairfield, CT, USA). Ultrasound images were acquired and analyzed using a range of echocardiographic techniques including two-dimensional, Doppler and speckle-tracking modalities in accordance with published guidelines (Lang et al., 2015; Rudski et al., 2010). Pulmonary artery systolic pressure (PASP) was a primary outcome variable for a number of studies, and was measured by Doppler echocardiography based upon measurement of the maximum velocity of the tricuspid regurgitation jet (Bertini et al., 2009). The peak systolic pressure gradient of the right ventricle (Δ P max) to the right atrium was calculated by the

simplified Bernoulli equation ($4*V^2$), where V is the peak systolic velocities of the tricuspid regurgitate. Pulmonary artery systolic pressure was then determined by adding the right atrial pressure. Right atrial pressure was estimated by evaluation of the inferior vena cava diameter and response to a deep inspiration (Aessopos, Farmakis, Taktikou, & Loukopoulos, 2000).

Blood sampling. Blood samples were obtained following placement of an indwelling cannula located in either a radial/brachial artery, or forearm antecubital vein as required. Blood was drawn directly into a safePico syringe (Radiometer, Copenhagen, Denmark) for immediate blood gas analysis (*outlined in detail below*) and into Vacutainers® (Becton, Dickinson and Company, Oxford, UK) containing either K_2-ethylenediaminetetraacetic acid (K_2-EDTA), serum separation gel or sodium citrate, before centrifugation at 600*g* (4 °C) for 10-minutes. Plasma, serum and red blood cell slurry were decanted into 2 mL cryogenic vials (Simport™, Fischer Scientific Ltd, UK) and immediately snap frozen and stored in liquid nitrogen (-196 °C) prior to international transportation back to the United Kingdom, USA, and Canada for specialist batch analyzes. Worth noting, due to the reduced barometric pressure in Cerro de Pasco, and volume percent of plasma in individuals with EE, ~twice as many vacutainers were required (compared to typical low altitude studies) to account for the reduced blood volume collected.

Blood gas analysis. Both venous and arterial blood samples were collected and analyzed by either a stationary commercial blood gas (ABL90 Flex, Radiometer Canada), or a portable blood analyzer (i-STAT, Abbot Point of Care, Princeton, New Jersey). Both devices (ABL90 and i-STAT) have barometric and temperature sensors within to correct for high altitude environments. The Radiometer ABL90 analyzer aspirates blood samples into a chamber containing electrodes

that are selective for the variables of interest. The ABL90 analyzer, although reliable and functional at altitude, required constant calibration, and encountered numerous blood clots likely due to a combination of the high volume of blood samples analyzed (~1000 samples) and the abnormally viscous blood encountered in the EE Andean participants. In general, throughout the expedition our research team consistently encountered higher than expected blood clotting incidents in both venous and arterial catheters, especially in the Andeans with EE. It was interesting to note that several samples obtained from the EE patients were hemolyzed (red tinge in plasma/serum), which was resolved following isovolemic hemodilution, implicating excessive polycythemia and increased red blood cell "fragility" as potential contributory factors. The portable i-STAT devices were used as a back-up in case the ABL90 required flushing or calibration, and were used for a few select studies that were measuring either venous or arterial blood (~250 samples in total). Despite the apparent electrical demise of our hot water bath early in the expedition, we successfully acquired novel measures of blood viscosity after some technical ingenuity, which involved removing, dismantling, and mending the equipment's electrical motor. Following collection into lithium-heparin vacutainers, whole blood viscosity was measured in duplicate at a shear rate of 225 s^{-1} and was body temperature corrected using a cone and plate viscometer (DV2T Viscometer, Brookfield Amtek, USA) (Baskurt et al., 2009; Gnasso et al., 2001; Tremblay, Howe, Ainslie, & Pyke, 2018).

Blood volume testing. Blood volume, plasma volume, and hemoglobin mass was determined using the modified carbon monoxide rebreathing method, as described previously (Schmidt & Prommer, 2005), and has been successfully used by our research team (Stembridge et al., 2019). Briefly, the protocol consisted of a venous blood draw for the determination of hemoglobin

concentration and the percentage of carboxyhaemoglobin (HbCO) via co-oximetry (ABL 90, Radiometer, Denmark). Subsequently, the participants began rebreathing 100% oxygen via a closed circuit (Bloodtec, GbR, Germany) whilst carbon monoxide was added to the gas mixture (1.0 ml kg^{-1}). Following two-minutes of rebreathing, a second venous blood draw was taken for the assessment of the same hematological parameters. Hematocrit was assessed via centrifugation and micro-hematocrit reader. This technique has previously used successful at high altitude (Ryan et al., 2014), and proven to be reliable against gold standard labelling techniques (Siebenmann, Keiser, Robach, & Lundby, 2017).

Arterial cannulation and pharmacological infusion. After local anesthesia (2% lidocaine) a 4.45-cm, 20 gauge catheter (Arrow, Markham, ON, Canada) was inserted under aseptic conditions into the brachial artery of the non-dominant arm for local pharmacological infusions and measurement of mean arterial pressure (MAP). Pharmacological agents were infused using a standard infusion only syringe pump (PhD Ultra Syringe Pumps, Harvard Apparatus, Holliston, MA, USA).

Cognitive function. Cognitive function was assessed using cognitive performance assessment software (Cogstate Ltd., Melbourne VIC, Australia). This standardized and automated computerized battery of neuropsychological tests (W. R. Cole et al., 2013), required participants to respond to playing cards, which assess different aspects of cognitive function (e.g. attention, psychomotor performance, working memory). Male and female Andeans who were asked to complete these neuropsychological tests were provided the Spanish version after the test was thoroughly explained by one of our local translators.

Microneurography. Muscle sympathetic nerve activity was obtained in either the radial or peroneal nerve (dependent upon the study) by inserting a Tungsten microelectrode into a muscle nerve fascicle of a sympathetic nerve bundle and a reference electrode subcutaneously 2–3 cm from the recording electrode (Mano, Iwase, & Toma, 2006). This technique was conducted by the same experienced microneurographers. Neural signals were collected using commercially available recording systems (662C-3, Bioengineering of University of Iowa, Iowa City, IA; Neuro AMP EX FE185, ADInstruments, Colorado Springs, CO, USA). Common electrical noise issues were encountered at both sea level and high altitude, and these issues were exaggerated when using the back-up generator as a power source. Linking the systems to an earth-ground (fence post in close proximity to the research laboratory) reduced electrical noise issues. One of the nerve traffic analyzers was part of the equipment locked in Peru customs upon arrival. This made running two microneurography-based studies problematic at the high altitude laboratory. It was not until the arrival of this equipment to the laboratory that both microneurography studies could run simultaneously as originally planned. The nerve signals were amplified (gain 70 000–160 000), band-pass filtered (700–2000 Hz), full-wave rectified, and integrated with a resistance-capacitance circuit (time constant 0.1 s). Criteria for adequate MSNA recording included: (1) pulse synchrony, (2) facilitation during the hypotensive phase of the Valsalva maneuver, and suppression during the hypertensive overshoot after release, (3) increases in response to breath holding, and (4) insensitivity to a gentle skin touch or a loud shout (Delius, Hagbarth, Hongell, & Wallin, 1972). Earphones (Audiotechnica ATH M40x) were used during microneurography searching to reduce distraction related to ambient noise.

Exercise testing. Maximal cardiopulmonary exercise testing was performed in the semi-recumbent position on an electronically braked cycle ergometer (Corival Paediatric, Lode B.V., Groningen, Netherlands). The bicycle frame that the participant laid on was custom made in Cerro de Pasco using wood purchased from a local store, and was assembled in the laboratory using common power tools. The protocol was explained thoroughly by a local translator prior to the exercise test and participants were instructed to maintain a cadence between 70 and 80 rpm with verbal feedback given throughout the experiment. Following an initial rest period, the first stage of exercise was two-minutes in duration at 20 W with subsequent increments of 20 W every minute until the participant reached volitional exhaustion. Peak power output was calculated as the 20 W increment divided by 60, multiplied by the time into the last stage and added to the power output from the last completed stage. Respiratory measures were assessed via breath-by-breath online gas analysis (Oxycon Mobile, Carefusion, San Diego, CA, USA), heart rate was recorded using a heart rate strap and transmitted to a Polar Electro RS4000 watch (Polar Electro, Kemple, Finland), and peripheral oxygen saturation measured via fingertip pulse oximetry (Choice Mmed, MD300C2, Beijing Choice Electric Technology Co Ltd, Beijing, China). Peak oxygen consumption ($\dot{V}O_{2peak}$) was calculated as the highest oxygen uptake ($\dot{V}O_2$) over a 30-second average. Importantly, these data are only applicable to other studies that utilized semi-recumbent cycling exercise, and may not be useful when comparing against other modes of exercise (e.g. trekking, running etc.).

Sleep Monitoring. Sleep architecture was assessed using a wrist-worn ambulatory sleep system (WatchPat Central Plus, Itamar Medical, Israel). This system incorporates arterial pulsatile volume changes (via peripheral arterial tone signal) in the finger, pulse oximetry, and actigraphy,

to algorithmically evaluate and score metrics of sleep disordered breathing (e.g. apnea-hypopnea index, oxygen desaturation index, rapid eye movements versus non-rapid eye movements stages of sleep) (Yalamanchali et al., 2013). These devices have been previously validated to differentiate between central and obstructive sleep apnea (Pillar et al., 2019), and have been used at high altitude (Carr et al., 2020; Lipman et al., 2015; Orr et al., 2018). However, these devices have not been fully validated at high altitude; therefore, the data pertaining to oxygen desaturation, sleep staging, and apnea-hypopnea index will be cautiously interpreted. Questionnaires performed before sleep and upon awakening were also conducted to quantify sleep quality.

Nitric oxide synthesis. Nitric oxide (NO) for clinical use was not available in Peru when these studies were conducted. To overcome this challenge, pure NO was produced in small quantities from the reaction of sodium nitrite (a common food additive) with hydrochloric acid (Arlin B. Blood, PhD, personal communication). This reaction produces small quantities of nitric oxide compounds (NOx) such as NO_2 and N_2O_2 in addition to pure NO gas itself. To eliminate the other NOx compounds, the gas resulting from the initial reaction with acid was mixed with a solution of sodium hydroxide (NaOH), resulting in pure NO. All the reactions were performed in an anaerobic environment with the use of 50 ml syringes, initially flushed with nitrogen. The pure NO immediately diluted with nitrogen to reduce the concentration of NO to a few hundred parts per million (ppm). Finally, this diluted NO/N_2 was mixed with oxygen immediately before it was used, to produce a mixture of ~21% oxygen, 79% N_2, and 40 ppm NO. After verification of the gas concentrations with analyzers for oxygen and NO, subjects inhaled this mixture from a Douglas bag for studies #4 and #8 outlined in Table 1.

Blood analysis and transport. Approximately 4000 samples were transported back to the laboratory in Lima for analysis and international shipment, whilst ~200 samples remained in Lima for the measurement of serum concentrations of iron (trans-ferritin, ferritin, iron, etc.) and plasma erythropoietin (MedLab, Lima, Peru). The majority of biological samples were immediately shipped on dry ice (-78.5°C; Marken Ltd; temperature verified) to the United Kingdom, Canada, and United States for subsequent analysis. These analyses included the following measurements of systemic endothelial microparticles [including endothelial activation (i.e., $CD62e^{+}$) and apoptosis (i.e., $CD31^{+}/42b^{-}$)] and oxidative-inflammatory-nitrosative-structural (OXINOS) stress that have previously been described in detail elsewhere (Bailey et al., 2018; Bailey et al., 2017).

1. *Oxidative Stress:* Samples will be analyzed for the ascorbate free radical directly using X-band electron paramagnetic resonance spectroscopy. Serum lipid hydroperoxides will be assayed spectrophotometrically as complementary biomarkers of lipid peroxidation. Plasma ascorbic acid and lipid soluble antioxidants will be assayed by fluorimetry and high-performance liquid chromatography.

2. *Inflammatory Stress:* high-sensitivity (hs) C-reactive protein and tumor necrosis factor-α will be assayed by hs enzyme-linked immunosorbent assay (ELISA).

3. *Nitrosative Stress:* Plasma and red blood cell concentrations of nitrite, *S*-nitrosothiols and *S*-nitrosoHb will be determined by ozone-based chemiluminescence. Plasma 3-

nitrotyrosine, a biomarker reflecting the oxidative inactivation of NO, will be measured by hs-ELISA.

4. *Structural stress: S*100β, neuron-specific enolase, myelin basic protein, neurofilament light, ubiquitin carboxy-terminal hydrolase-L and glial fibrillary acidic protein (biomarkers of blood-brain barrier integrity, neuronal-parenchymal damage and glial damage respectively) will be measured by hs-ELISA.

Data Analysis

Sample Size Estimates. Sample sizes were determined *a priori* based on study specific effect size estimates. Moreover, based on our research team's previous experience with high altitude expeditions, adjustments were made to account for expected participant dropout (e.g. (Foster et al., 2014; Hoiland et al., 2019; Lewis et al., 2014; Simpson et al., 2019; Smith et al., 2014; Stembridge et al., 2019; Tremblay, Thom, Yang, & Ainslie, 2017; Tymko et al., 2017; Willie et al., 2014)). Sample sizes were determined using statistical power tests that assumed a minimum statistical power of 80% (or 0.8). Statistical significance was set at an alpha value of 0.05. Depending on the variability of the primary outcome of each study, 10-58 study participants were recruited.

Statistics. Statistical analysis included in the current manuscript was performed using SigmaStat V13 (Systat, Chicago, IL, USA), and all data are reported as mean ± SD. Statistical significance was set at $P<0.05$. For statistical comparisons between lowlanders at sea level and high altitude, paired *t*-tests were used (e.g. table 3 and 4). For statistical comparisons between male lowlanders

521 at high altitude, and male Andean (EE+ and EE-), a one-way analysis of variance design was
522 used. For statistical comparison between male and female healthy (i.e., non-EE) Andeans, un-
523 paired *t*-tests were used. When significant F-ratios were detected, differences between means
524 were determined using Bonferonni-corrected independent samples *t*-tests.
525

General Results and Characterization of Cohorts

Studies conducted and participants recruited. In total, 15 separate *a priori* studies (see table 1), amounting to >780 study sessions were completed between sea level testing in Kelowna, BC, Canada (344m), and over ~25 days in Cerro de Pasco (4300m). This amounted to 829 experimental hours in lowlanders at sea level, 1522 experimental hours in lowlanders at high altitude, 500 experimental hours in non-EE Andean highlanders, and 219.75 experimental hours in Andean highlanders with EE. Table 2 highlights the participant demographics (age, height, weight and body mass index) for lowlanders (n=30), and highlanders with (n=22; all male) and without (n=45; 11 female) EE.

Arterial blood data (lowlanders and Andeans). Table 3 displays arterial blood data obtained in 13 male lowlanders at sea level (Lima, Peru) immediately prior to ascending to Cerro de Pasco, and after 14 days of high altitude exposure. In addition, table 3 illustrates baseline arterial blood data in 16 male Andean participants with and without EE at 4300m. Compared to sea-level, lowlanders after 14 days of high altitude acclimatization had higher pH, but lower $PaCO_2$, PaO_2, SaO_2 and HCO_3 (all $P<0.001$). Lowlanders after 14 days of high altitude exposure had an elevated arterial blood pH and SaO_2 compared to Andeans with EE ($P<0.01$). Lowlanders at altitude had lower $PaCO_2$ and HCO_3^- compared to EE Andean participants (both $P<0.001$). Lowlanders at high altitude had the same arterial blood pH ($P=0.316$), $PaCO_2$ ($P=1.00$), PaO_2 ($P=1.00$), HCO_3^- ($P=1.00$), and SaO_2 ($P=1.00$) compared to non-EE male Andean participants. Compared to non-EE male Andean participants, Andeans with EE had a lower arterial blood pH ($P=0.014$) and SaO_2 ($P<0.001$), but an elevated $PaCO_2$, and HCO_3^- (both $P<0.001$). There were no differences in PaO_2 observed between male Andeans with and without EE ($P=0.087$) with our

549 statistical model (Lowlanders vs Andeans); however, when a less conservative unpaired t-test
550 was performed between male Andean groups, Andeans with EE had a lower PaO_2 compared to
551 non-EEAndeans (P=0.03). In Figure 2, a proton-bicarbonate (i.e. Davenport) diagram was used
552 to demonstrate the various acid–base compensation and comparisons between groups.
553
554 ***Venous blood data (lowlanders and Andeans).*** Table 4 displays venous blood data obtained in
555 male lowlanders at sea-level (n=24; Kelowna, BC), and after 14 days of high altitude exposure
556 (n=17). In addition, table 4 illustrates baseline venous blood data in male Andean participants
557 with EE (n=21), males and female Andeans without EE (n=26 and n=11, respectively). After 14
558 days of high altitude acclimatization, lowlanders had an increase in hemoglobin and blood
559 viscosity compared to sea level values (both P<0.001). Lowlanders at altitude had lower
560 hemoglobin and blood viscosity compared to male Andean with EE (both P<0.001), and
561 Andeans without EE (both P<0.001). Male Andean EE participants had higher hemoglobin and
562 blood viscosity compared to non-EE Andeans (both P<0.001). Notably, four participants' (all
563 EE+; Hb of these participants = 24.7±1.1 g dL^{-1}) blood viscosity *exceeded* the upper limit of the
564 viscometer (>10.22 cP), thus, their blood viscosity was reported as 10.22 cP. Female Andeans
565 had lower hemoglobin and blood viscosity compared to male Andeans without EE (P=0.007 and
566 P=0.004, respectively).
567
568 ***Exercise performance (lowlanders and Andeans).*** Figure 3 displays exercise performance data
569 between lowlanders at sea level, lowlanders at high altitude, Andean male participants with and
570 without EE, and Andean female participants. At high altitude, lowlanders had a lower absolute
571 and relative VO_2 max (P=0.001), and peak power output (P<0.001), compared to sea level

values. Lowlanders at high altitude had an elevated peak power output compared to male Andeans with EE (P<0.001) and non-EE Andeans (P<0.001). Absolute VO_2 max was higher in lowlanders at high altitude compared to male Andean participants with (P=<0.001) and without EE (P<0.001). Relative VO_2 max was similar in lowlanders at high altitude compared to male Andean participants with (P=0.619) and without EE (P=0.492). No differences in absolute VO_2 max (P=0.91), and relative VO_2 max and peak wattage were observed between male Andean participants (both P=1.00). Female Andeans had a similar relative VO_2 max (P=1.00), and a lower absolute VO_2 max (P=0.001), and peak wattage compared to non-EE male Andeans (P=0.015).

Discussion

Despite some of the major obstacles encountered during the expedition outlined throughout this manuscript, our research team, Global REACH, collected ~90% of the data for the proposed projects. Below we outline some of the lessons learnt from this expedition and discuss in further detail the expected forthcoming publications, and finally, discuss the potential clinical translation and future goals of the Global REACH research team.

Major challenges. The success of large-scale field research expeditions typically depends on establishing appropriate and adequate local support. Through a historical connection, our research team was capable of organizing contact with local Peru collaborators: Dr. Francisco Villafuerte and his laboratory team consisting of graduate students and administrators. Six months prior to the research expedition, two members of Global REACH traveled to Cerro de Pasco to collect some preliminary data and construct initial planning for our core studies with our local collaborators. Without the local Peruvian team and their unfailing help and support, several aspects of the expedition would not have been possible including: 1) recruitment of Andean highlanders using their established database, 2) organizing transport to the high altitude laboratory in Cerro de Pasco, and 3) local ethical approval for all proposed studies. Our team had full-time access to multiple graduate students of Dr. Villafuerte who handled nearly all recruitment and screening of participants and offered their skills of being a translator between the study principal investigators and Andean study participants.

During our Nepal expedition in 2016, our research team collected data upon ascent to high altitude (5050m) without the confounding influence of participants taking prophylactic acetazolamide (Willie et al., 2018). In order for our data to remain consistent between our Nepal

and Peru high altitude expeditions, we aimed to refrain from taking any high altitude medications. However, if participants fell severely ill from altitude and/or non-altitude related sickness, our physicians had adequate medication available for treatment. Unlike our expedition in Nepal, which involved a gradual ascent to 5050m over a period of ~7-10 days, we were transported to altitude by automobile over a 7-8 hour rapid ascent to Cerro de Pasco. Due to this, nearly all participants reported symptoms of acute mountain sickness (via Lake Louise scoring system), with several having to be treated with Acetazolamide after arrival (n=6). No participants had to be evacuated due to altitude related illness; however, these individuals were either removed from specific studies due to illness or were rescheduled to participate once they were fully recovered and endured a complete drug washout time (i.e. at least five half-lives).

Lastly, the biggest challenge of this expedition was the logistical aspect of transportation and importation of research equipment. Unfortunately, despite a substantial effort to organize and submit importation documents for legal entry into Peru, a large portion of our equipment was initially rejected entry into Peru. Rectifying this issue required countless hours and financial assurances from the local university to release this equipment. This unforeseen complication resulted in the delay of multiple research projects; however, through equipment sharing and working excessive hours, our research team was able to cope with the temporary equipment loss until it arrived ~14 days after arrival to Cerro de Pasco. For these reasons, we recommend future expeditions to bring back-up and duplicate equipment whenever possible in case of any equipment issues related to foreign country importation.

Arterial blood gases and acid-base balance. When a lowlander travels to high altitude, hyperventilation occurs as a result from hypoxemia associated peripheral chemoreceptor

stimulation, the $PaCO_2$ falls, and the arterial blood pH rises in accordance with the Henderson–Hasselbach equation:

$$pH = pK + \log \frac{[HCO_3^-]}{0.03 PaCO_2}$$

Equation 1: Henderson-Hasselbach equation where $[HCO_3^-]$ is the bicarbonate concentration in millimoles per liter and the $PaCO_2$ is in mmHg.

However, the kidney responds by eliminating bicarbonate, which is prompted via reductions in PCO_2 in the renal tubular cells. This results in a more alkaline urine due to decreased reabsorption of bicarbonate ions. The decrease in plasma bicarbonate then moves the bicarbonate/$PaCO_2$ ratio back towards its normal equilibrium. This relationship is known as metabolic compensation for respiratory alkalosis. The compensation may be complete, in which case the arterial pH returns to ~7.40 or, more often, incomplete with a steady-state pH that exceeds ~7.40. There are two noteworthy and novel observations from the arterial blood gas data (*see Table 3*). First, two-weeks of acclimatization at 4300m in lowlanders resulted in comparable changes in SaO_2, PaO_2, $PaCO_2$, and HCO_3^- when compared to non-EE Andean highlanders who have resided at this elevation for many years. In contrast, despite the comparable changes in $PaCO_2$ (and hence likely stimulus to reduce HCO_3^-), pH remained elevated in lowlanders. Thus, despite comparable oxygenation and metabolic compensation in the Andeans – as indexed via HCO_3^- – it failed to compensate for the respiratory alkalosis (i.e., elevated pH; *refer to figure 2*). It is possible that these results could be directly due to differences in strong base ions since differences in standard base excess can alter blood pH for a given $PaCO_2$ (Morgan, 2009).

651 Unfortunately, we did not acquire standard base ion in lowlanders at this time-point since these
652 blood samples were analyzed using iSTAT cartridges that did not output these data. Also of note
653 was that the female Andeans had a lower blood Hb and viscosity compared to non-EE male
654 Andean highlanders. These results align with previous findings demonstrating that
655 premenopausal female highlanders have lower incidences of CMS and EE, which may be related
656 to elevated levels of progesterone during their menstrual cycle resulting in elevated ventilation,
657 thus SaO_2 – a primary stimulus for red blood cell production (Leon-Velarde et al., 1997; Leon-
658 Velarde, Rivera-Chira, Tapia, Huicho, & Monge, 2001).

659 Albeit over a shorter period, the time course of the change in arterial pH when normal
660 subjects ascend abruptly to high altitude has been studied by several investigators (Dempsey,
661 Forster, Chosy, Hanson, & Reddan, 1978; Lenfant, Torrance, & Reynafarje, 1971; Severinghaus,
662 Mitchell, Richardson, & Singer, 1963). In one study, lowlanders were taken within five hours
663 from sea level to an altitude of 4509m (P_B = 446 mmHg) and remained there for four days. The
664 arterial pH rose to a mean of about 7.47 within 24-hours and then gradually declined but was still
665 ~7.45 at the end of the four-day period (Lenfant et al., 1971). In another study, four normal
666 subjects were taken abruptly to 3800m for eight days. The arterial pH rapidly rose from a mean
667 of 7.424 at sea level to 7.485 after two days, and remained constant, being 7.484 at the end of
668 eight days (Severinghaus et al., 1963). In a further study, 11 lowlanders traveled to 3200m
669 altitude where they remained for 10 days (Dempsey et al., 1978). The arterial pH rose by 0.03–
670 0.04 units within two days, and then remained essentially unchanged. In all instances, the $PaCO_2$
671 continued to decline as did the plasma bicarbonate concentration. Consistent with the current
672 data, it appears that the return of the arterial pH to (or near to) its sea level value is very slow and
673 may not occur even after years of exposure to high altitude (as confirmed by the elevated pH

observed in non-EE Andeans). Some studies have collected arterialized blood indirectly (e.g. ear lobe and finger) to measure acid-base balance during short term (Samaja, Mariani, Prestini, & Cerretelli, 1997), and long-term exposure to high altitude (Porcelli et al., 2017). In one study, arterialized ear lobe blood pH was elevated at 5050m and remained elevated for three-weeks in lowlanders (Samaja et al., 1997), while in another study conducted at the equivalent of ~3800m arterialized finger capillary blood pH remained elevated for 300 days (Porcelli et al., 2017). There are some aspects to our data that make ours unique compared to previous reports: 1) we collected actual arterial blood from the radial artery; and 2) we report data from a large participant cohort and arterial blood samples were taken at the exact same time point (sea-level, day 1, and day 14), after a controlled ascent to 4300m, while refraining from altitude illness medications.

Another interesting observation consistent with previous reports is the relative hypoventilation in the EE Andeans, as reflected in the arterial hypoxemia and attenuated reductions in $PaCO_2$ compared to the non-EE Andean and lowlander groups (Beall, 2006). The mechanism(s) driving this relative hypoventilation has been explained in detail elsewhere (Villafuerte & Corante, 2016). Nevertheless, it is interesting to note that despite this greater hypoxemia and attenuated reductions in $PaCO_2$, the greater reductions in HCO_3^- resulted in full metabolic compensation for the respiratory alkalosis i.e., comparable pH in Andeans with EE to lowlanders at sea level. Worth noting, Davenport diagrams (e.g. *figure 2*) provide only basic insight into acid-base derangements at high altitude; therefore, one of our future goals is to collect additional data to employ more sophisticated acid-base models to better understand this important physiology.

Exercise performance. Similar to previous reports, our data indicate that exercise performance (both relative VO_2 max and peak wattage) is reduced with high altitude exposure in lowlanders (Cymerman et al., 1989; Smith et al., 2014; Tymko et al., 2017; Wehrlin & Hallen, 2006). Our exercise data in male Andean highlanders supports previously published data that indicate that Andeans with EE have a similar VO_2 max and peak wattage compared to non-EE male Andean participants despite substantially elevated Hb and reduced SaO_2 (Groepenhoff et al., 2012; Swenson, 2012). To ensure all lowlander and highlander participants performed a maximal effort during the exercise protocol verbal encouragement was provided by several research personnel and at least one Spanish translator. In addition, although the statistical differences detected between groups (lowlanders and highlanders) were different for relative VO_2 max and peak wattage (*refer to figure 3*), the mean trends between groups were similar.

Comparison to Nepal 2016 expedition. Although the fundamental goals of our international research team's expeditions to Nepal and Peru were similar, there are distinct differences between these two expeditions that warrant further comment. First, there were a large number of "ascent" studies conducted in both lowlanders and Sherpa over a gradual ascent to a slightly higher altitude (5050m vs 4300m). The Sherpa studied during the ascent investigations permanently lived at altitudes >3500m within the Khumbu valley, and were requested to descend to Kathmandu (1400m) for 9 ± 3 days prior to gradual ascent with the research team to the pyramid laboratory. Reasoning for this was to partially de-acclimatize the Sherpa from an autonomic, endocrine, cardiovascular, and respiratory standpoint; however, the descent and re-ascent altitude profile likely did not alter the Sherpa's hemoglobin concentration since the life cycle of red blood cells is ~three months (Berlin, Waldmann, & Weissman, 1959).

In Nepal, our research team also tested a large cohort of Sherpa at 5050m that did not descend to lower altitude in a number of studies, but these Sherpa also resided permanently at altitudes >3500m within the Khumbu valley. Another important consideration is the difference in lifestyles and environment between Nepal and Peru. Our Sherpa cohort, by nature, were likely to be more physically active compared to the Andeans, having to walk long distances on a daily basis and serving as guides in nearby mountains. Nevertheless, previous reports indicate similar rates of obesity between Sherpa and Andean highlanders (~10% vs ~8%, respectively; (Sherpa et al., 2010; Woolcott et al., 2016)). It is also worth acknowledging that our Andean highlanders had access to a greater variety of food in Cerro de Pasco compared to the Sherpa in the Khumbu valley.

Clinical Translation. Research on high altitude physiology offers complementary insight into biological adaptation to hypoxia. Ways to apply results from these expeditions are multifaceted, with implications for military deployment to high altitude (e.g. Afghanistan), for the growing numbers (>1 million) of lowlanders vacationing at high altitude destinations, and for commercial flight personnel who are hypoxic during flight (mild hypoxia). These data can have direct translational impact for patients in critical care and in other clinical situations of chronic hypoxia (e.g. lung disease, heart failure, circulatory shock). For example, we observed that several blood samples obtained from Andeans with EE were “hemolyzed”, which was resolved following isovolemic hemodilution. Importantly, these research expeditions will give us novel insight into the current physiological status of local highlanders (Tibetan, Peruvian, and Ethiopian), which may lead to related health benefits in these populations. The results from this study will be a valuable step toward effective treatments for the potential cardiopulmonary consequences of EE.

The future of Global REACH. Due to the numerous publications, the local cultural benefit (e.g. CMS and EE research), the international scientific relationships gained (e.g. Dr. Villafuerte), and the opportunity for training highly qualified personnel with each expedition (e.g. graduate and medical student training), our research team, Global REACH, intends to organize future large-scale high altitude research expeditions. We know significantly less about high altitude related adaptation in the Ethiopian Amhara and Oromo highlanders from the Simien and Bale mountains, respectively. The Amhara have hemoglobin concentrations similar to lowlanders, yet higher than expected oxygen saturation, potentially due to an increased affinity of their hemoglobin for oxygen (Beall, 2006; Beall et al., 2002). Only few sophisticated physiological data sets exist on Ethiopian highlanders. Conceivably, the Sherpa, Quechua, and Amhara phenotypes represent distinct evolution-driven strategies that permit survival and performance at high altitude. Ethiopia will be the final installment in Global REACH's trilogy of high altitude adaptation studies.

Conclusion

The 2018 Global REACH expedition to Peru was comprised of 15 independent studies on three distinct cohorts: lowlanders (n=30), non-EE Andeans (n=45), and Andeans diagnosed with EE (n=22). Studies were conducted at sea level (Kelowna, BC; 344mm) and after ascending to Cerro de Pasco (4300 m) from Lima (~80 m) over a 7-8 hour automobile ride, which focused on cardiovascular, cerebrovascular, cardiopulmonary, autonomic and neurocognitive aspects of human physiological responses to hypobaric hypoxia acclimatization. The findings from this

765 study will be reported in several forthcoming publications according to their respective *a priori*
766 hypotheses.
767

Declarations

Ethics approval and consent to participant. In accordance with the *Declaration of Helsinki*, the lowlander and highlander based studies were approved by the UBC Clinical Research Ethics Board (H17-02687 and H18-01404, respectively), and local Peruvian ethics committee for the Universidad Peruana Cayetano Heredia (#101686). All lowlander and highlander study participants signed the approved consent form (English and Spanish forms were available) after each study was thoroughly explained in their native language.

Availability of data and material. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests. We have no competing interests to declare.

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Table 1: Overview of experimental studies

Study	Study Title	Study Aim	Participants	Techniques used
1	The common carotid artery vasomotor response to the cold pressor test at sea level and high altitude in lowlanders and Andeans: the role of oxygen	To determine the effects of altitude on the common carotid artery vasomotor response to the cold pressor test at sea level and high altitude	Lowlanders (n =14); non-EE Andeans (n = 12)	Duplex ultrasound; finger photoplethysmography
2	Nitric oxide-mediated endothelium-dependent vasodilation in lowlanders at sea level and high altitude	To determine the influence of oxidative stress on endothelial dependent vasodilatory function in lowlanders with chronic exposure to high altitude.	Lowlanders (n =11)	Venous occlusion plethysmography with intra-brachial infusions of acetylcholine and sodium nitroprusside
3	Investigating the role of haemoglobin concentration, plasma volume and absolute blood volume on cardiac function and exercise capacity in high altitude natives	To explore whether hemoglobin mass or absolute blood volume is associated with exercise performance in the Andean population, and whether differences in performance are related to cardiac structure and function	Lowlanders (n =12); healthy Andeans (n = 40)	CO rebreathing; venous blood sampling; Duplex ultrasound; maximal exercise test
4	Sympathetic function in lowlanders and high altitude Andean's with and without EE	To investigate sympathetic nervous activity in Peruvian highlanders and identify a specific link between sympathetic hyperactivity and elevated pulmonary arterial pressure	Lowlanders (n =18); non-EE Andeans (n = 10); Andeans with EE (n=10)	Microneurography to assess muscle sympathetic nervous activity; Duplex ultrasound; finger photoplethysmography
5	The effects of oxidative stress on cutaneous vasodilation at sea level and high altitude	To determine the role of oxidative stress on cutaneous vascular function at sea level and high altitude	Lowlanders (n =11); non-EE Andeans (n = 11)	Microdialysis; laser-Doppler flowmetry
6	The effects of venesection on peripheral and central vascular function in Andeans	To quantify the influence of reductions in hematocrit (via blood removal) on central and peripheral vascular function	Andeans with EE (n=10)	Duplex ultrasound; arterial/venous blood sampling; finger

	with EE	in Andean highlanders suffering from EE		photoplethysmography
7	The factors effecting resting and active (exercising) skeletal muscle blood flow through the process of acclimatization, adaptation and maladaptation to high altitude	To provide a integrative assessment of the factors influencing skeletal muscle blood flow during adaptation and maladaptation to high altitude	Lowlanders (n =11); non-EE Andeans (n = 10); Andeans with EE (n = 8)	Exercise tests; Microneurography to assess muscle sympathetic nervous activity; Brachial catheterization; Duplex ultrasound; local arterial pharmacological infusions
8	Pulmonary vascular changes to acute and chronic high altitude hypoxia	To study pulmonary vascular responses at rest and during exercise, at both low and high altitude in lowlanders and in Andeans with and without EE	Lowlanders (n =11); non-EE Andeans (n = 13); Andeans with EE (n = 9)	Duplex ultrasound
9	Endothelial function and shear stress in high altitude Andeans with and without EE	To characterize resting shear stress patterns and assess endothelial function via flow-mediated dilation in response to transient and sustained elevations in shear stress	Non-EE Andeans (n = 33); Andeans with EE (n = 20)	Duplex ultrasound; handgrip exercise
10	The global cerebral blood flow and intracranial pressure response to hypobaric hypoxia in high-altitude Andeans with and without EE	To determine the effects of high altitude on cerebral blood flow and intracranial pressure in high-altitude Andeans with and without EE	Non-EE Andeans (n = 33); Andeans with EE (n = 20)	Duplex ultrasound
11	The effects of iron supplementation on vascular function between lowlanders and high altitude Andeans	To determine the effect of iron sucrose intravenous supplementation on peripheral vascular function between healthy lowlanders and Andean highlanders	Lowlanders (n = 24); non-EE Andeans (n = 24)	Duplex ultrasound; venous blood sampling;
12	The effect of a positive expiratory pressure mask with dead space on sleep, acute mountain sickness and cognitive function during	To assess the combined effect of positive expiratory pressure and dead space on sleep, acute mountain sickness and cognitive function during normobaric and hypobaric hypoxia.	Lowlanders (n = 15)	Sleep monitoring

	normobaric and hypobaric hypoxia			
13	Redox-regulation of cerebrovascular function during acute exposure to environmental hypoxia	To examine to what extent free radical-mediated alterations in nitric oxide bioavailability contribute towards systemic vascular impairment following acute exposure to environmental hypoxia and to what extent this translates to the cerebrovasculature and corresponding implications for cognition.	Lowlanders (n = 12); non-EE Andeans (n = 18)	Duplex ultrasound; Transcranial Doppler ultrasound; finger photo-plethsmography; venous blood sampling; electron paramagnetic resonance spectroscopy; ozone chemiluminescence; hs-ELISA; fluorimetry; HPLC; neurovascular coupling; cognition
14	The effect of high altitude exposure on the regulation of cerebral blood flow during heat and cold stress	To understand how high-altitude acclimatization impacts the regulation of cerebral blood flow during heat and cold challenges.	Lowlanders (n =11)	Duplex ultrasound; esophageal and rectal temperature monitoring; arterial blood sampling;
15	Renal reactivity at high altitude	To determine the effects of acute and chronic altitude exposure on kidney function in lowlanders and Andean highlanders	Lowlanders (n = 30); non-EE Andeans (n = 17); Andeans with EE (n = 11)	Duplex ultrasound; urine sampling;

Definition of abbreviations: EE, excessive erythrocytosis. Each of these investigations had distinct outcome measurements, and data were not pooled across individual studies.

Table 2: Participant demographics in Peru

	Lowlanders	**Males EE-**	**Males EE+**	**Females EE-**
n	30 (3F)	35	22	10
Age (yrs)	29.9 ± 7.8	30.2 ± 11.2	44.1 ± 12.8*	25.5 ± 3.1
Stature (cm)	175.3 ± 5.8	162.6 ± 4.4	159.8 ± 5.8	152.1 ± 5.0
Mass (Kg)	72.5 ± 7.9	63.8 ± 8.1	66.7 ± 9.9	53.6 ± 4.1
BMI (Kg/m^2)	23.5 ± 1.6	24.2 ± 3.2	26.1 ± 3.3	23.2 ± 2.1

Definition of abbreviations: yrs, years; cm, centimeter; kg, kilogram; m, meter; EE+, Andeans with excessive erythrocytosis; EE-, Andeans without excessive erythrocytosis. *P<0.05, Males EE- vs EE+.

Table 3: Arterial Blood Data

	Male LL (344m)	Male LL Day 14 (4300m)	Male Andean EE+ (4300m)	Male Andean EE- (4300m)
n	13	13	16	16
pH	7.42 ± 0.02	7.47 ± 0.03*	7.42 ± 0.02†	7.45 ± 0.03‡
$PaCO_2$ (mmHg)	40.8 ± 2.1	28.6 ± 1.5*	35.0 ± 3.2†	29.2 ± 3.6‡†
PaO_2 (mmHg)	98.7 ± 7.4	50.2 ± 5.6*	44.2 ± 3.7†	49.42 ± 7.1
HCO_3 (mmol/l)	26.2 ± 1.5	20.7 ± 1.3*	22.5 ± 1.5†	20.0 ± 2.0‡
SaO_2 (%)	97.8 ± 0.7	87.8 ± 2.6*	79.0 ± 4.7†	86.4 ± 4.0‡

Definition of abbreviations: mmHg, millimeters of mercury, mmol, millimoles; l, liters; EE+, Andeans with excessive erythrocytosis; EE-, Andeans without excessive erythrocytosis. LL, low-landers. *P<0.05, low-landers sea level vs low-landers Day 14. †P<0.05, vs low-landers Day 14. ‡EE+ vs EE-.

Table 4: **Venous Blood**

	Male LL (344m)	Male LL Day 14 (4300m)	Male Andean EE+ (4300m)	Male Andean EE- (4300m)	Female Andean (4300m)
n	24	17	21	26	11
Hb (g/dl)	14.9 ± 1.0	17.4 ± 1.7*	22.6 ± 1.7†‡	18.5 ± 1.7†	16.8 ± 1.3┼
Blood viscosity (cP)	4.3 ± 0.7	4.9 ± 0.6*	8.5 ± 1.2†‡	6.0 ± 0.8†	4.9 ± 0.7┼

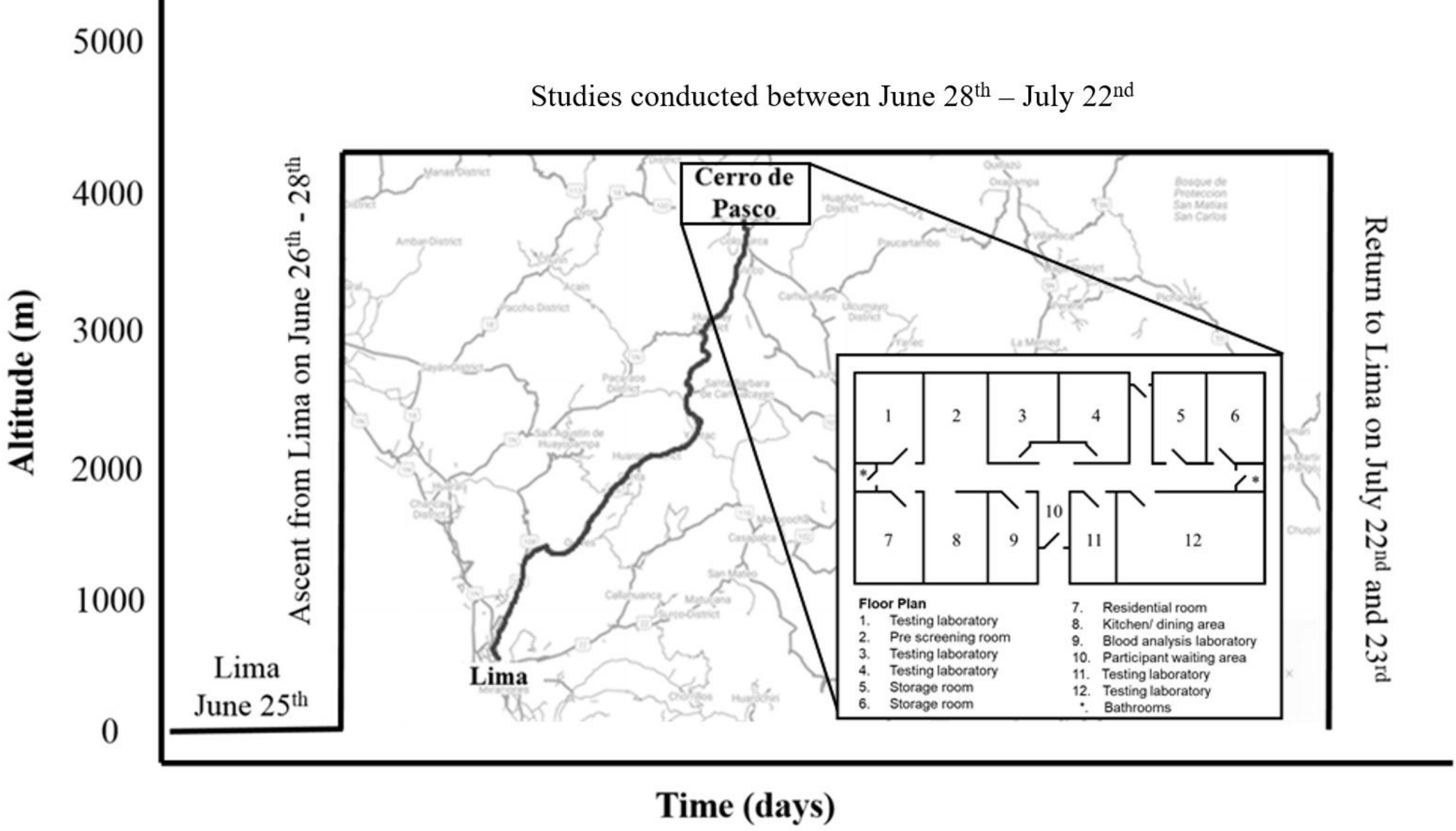
Definition of abbreviations: g, grams; l, liters; EE+, Andeans with excessive erythrocytosis; EE-, Andeans without excessive erythrocytosis. LL, low-landers. *P<0.05, low-landers sea level vs low-landers Day 14. †P<0.05, vs low-landers Day 14. ‡P<0.05, male EE+ vs male EE-. ┼P<0.05, male EE- vs female EE-.

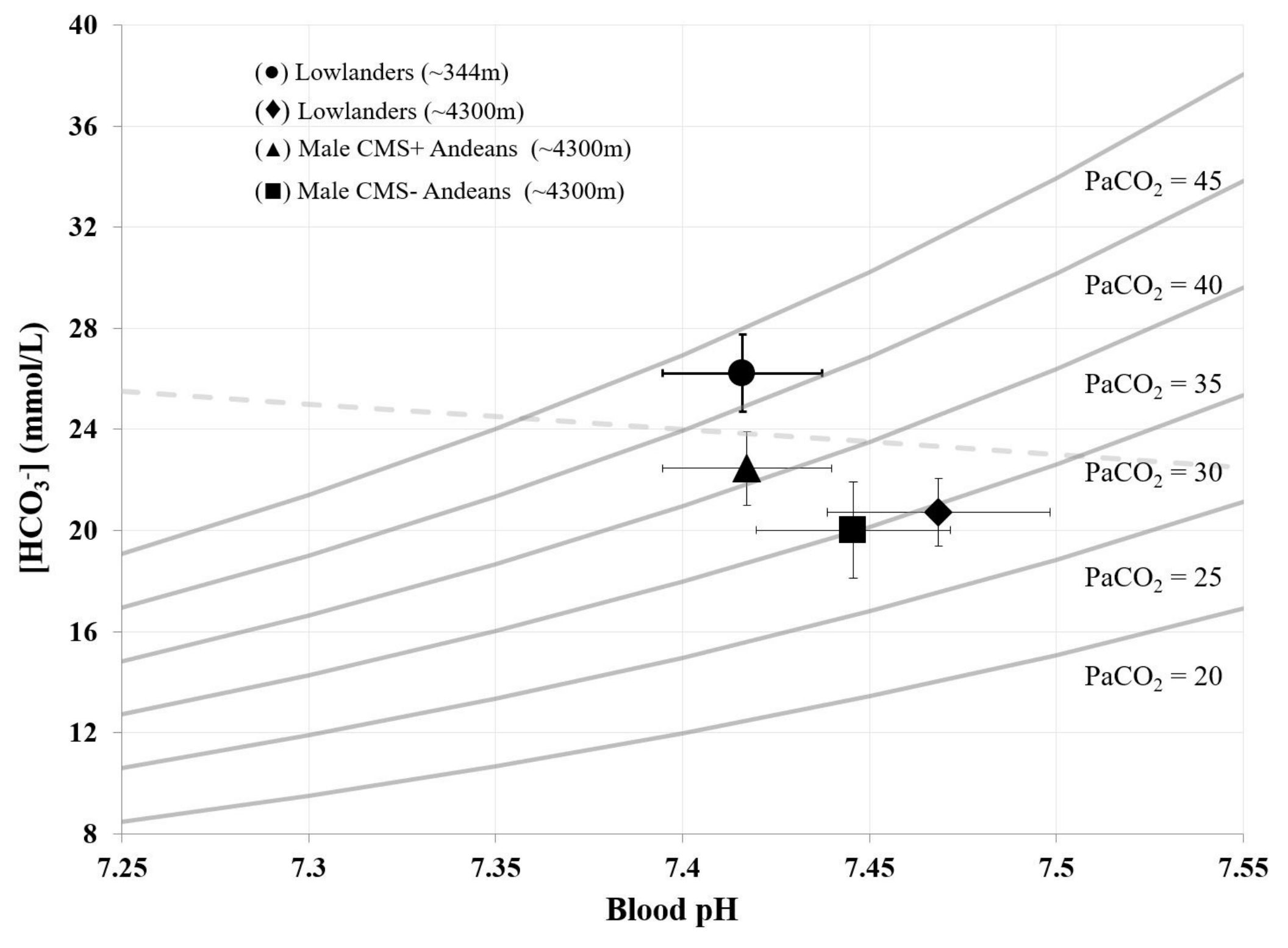
Figure Legends

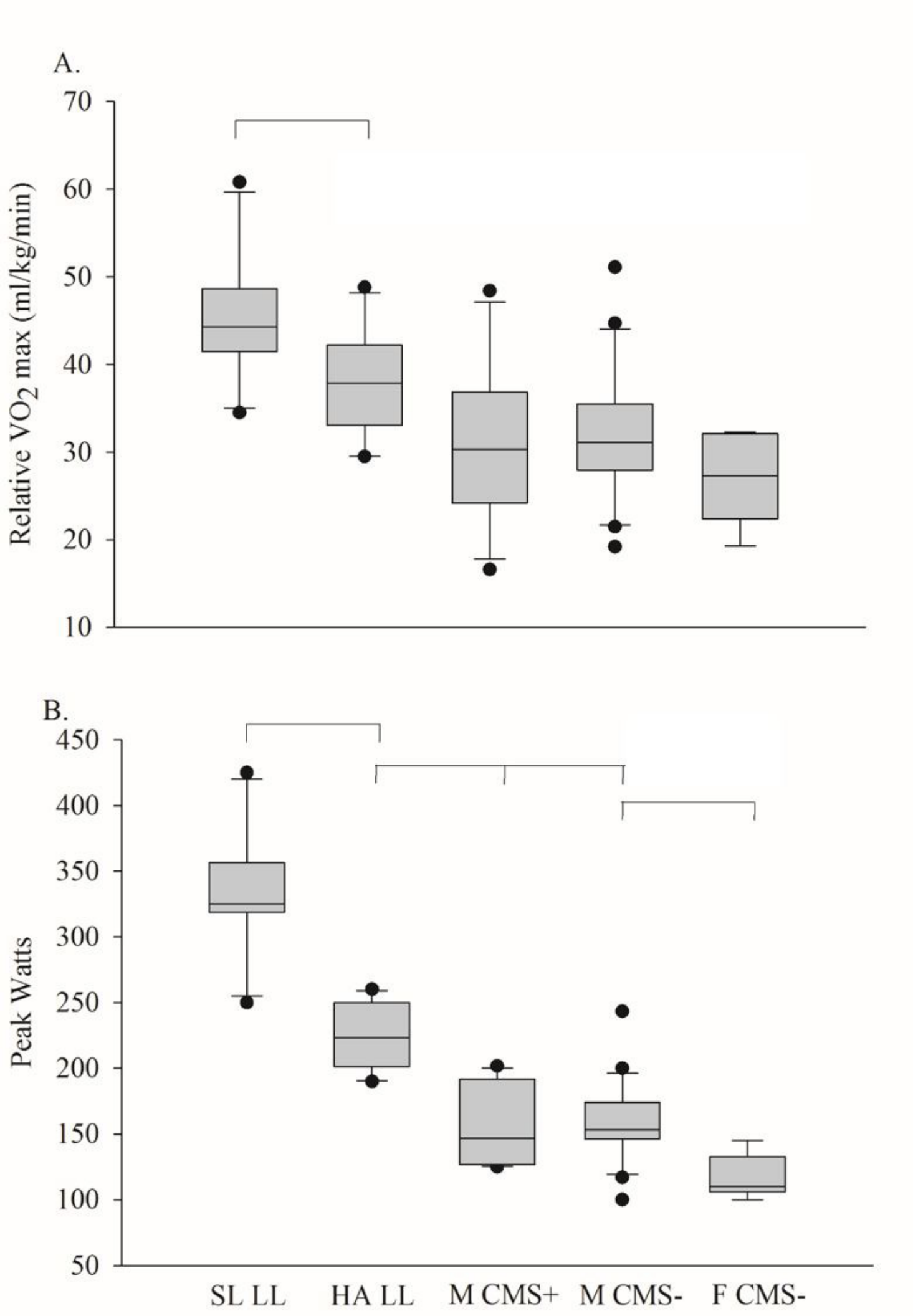
Figure 1. A timeline of Global REACH's latest research expedition to Peru, and a schematic of the laboratory facility used in Cerro de Pasco.

Figure 2. Davenport diagram illustrating acid-base balance between male lowlanders at sea level (●), male lowlanders after 14 days at 4300m (♦), Andean highlanders diagnosed with EE at 4300m (▲), Andean highlanders without EE at 4300m (■). *Definition of abbreviations:* EE, excessive erythrocytosis; HCO_3^-, bicarbonate; $PaCO_2$, partial pressure of arterial carbon dioxide.

Figure 3. Exercise performance between male lowlanders and Andean highlanders. Panel A, box and whisker plot for relative VO_2 max (ml/kg/min). Panel B, box and whisker plot for peak wattage obtained during the exercise test. *Definition of abbreviations*: EE, excessive erythrocytosis; F, female; HA, high altitude; kg, kilograms; LL, lowlanders; M, male; min, minutes; ml, milliliters; SL, sea level. Brackets represent differences between data sets ($P<0.05$).


Studies conducted between June 28th – July 22nd
Altitude (m)
5000
4000
3000
2000
1000
0
Time (days)
Ascent from Lima on June 26th - 28th
Return to Lima on July 22nd and 23rd
Lima
June 25th
Cerro de Pasco
Lima
1
2
3
4
5
6
7
8
9
10
11
12
Floor Plan
1. Testing laboratory
2. Pre screening room
3. Testing laboratory
4. Testing laboratory
5. Storage room
6. Storage room
7. Residential room
8. Kitchen/ dining area
9. Blood analysis laboratory
10. Participant waiting area
11. Testing laboratory
12. Testing laboratory
*. Bathrooms


(●) Lowlanders (~344m)
(♦) Lowlanders (~4300m)
(▲) Male CMS+ Andeans (~4300m)
(■) Male CMS- Andeans (~4300m)
PaCO2 = 45
PaCO2 = 40
PaCO2 = 35
PaCO2 = 30
PaCO2 = 25
PaCO2 = 20
[HCO3-] (mmol/L)
40
36
32
28
24
20
16
12
8
7.25
7.3
7.35
7.4
7.45
7.5
7.55
Blood pH


A.
70
60
50
40
30
20
10
Relative VO2 max (ml/kg/min)
B.
450
400
350
300
250
200
150
100
50
Peak Watts
SL LL
HA LL
M CMS+
M CMS-
F CMS-