Time: 1600

ADRENERGIC RECEPTOR MECHANISMS IN SYMPATHETIC NEUROVASCULAR TRANSDUCTION AT HIGH ALTITUDE.

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Objective: Previously, we demonstrated blunting of sympathetic neurovascular transduction (NVT) with high-altitude hypoxia. This may contribute to the maintenance of mean arterial pressure (MAP) near sea-level values despite an increase in muscle sympathetic nerve activity (MSNA). Since vascular adrenoreceptors mediate changes in vessel tone and may undergo desensitization with hypoxia, we aimed to elucidate the relationship between NVT and adrenergic receptor reactivity at high altitude. Methods: Participants (n=10; 6M, 4F) were tested following 3-11 days at altitude (Barcroft Field Station, White Mountain CA; 3800m). Changes in forearm blood flow (FBF; Doppler ultrasonography) and vascular conductance (FVC; FBF·MAP-1*100) with incremental intra-arterial infusions of phenylephrine (specific α1-adrenergic agonist) and norepinephrine (non-selective α- and β-adrenergic agonist) were determined and compared to the contralateral arm as a control. A linear slope between FBF and FVC responses and log transformed doses was used to assess adrenergic sensitivity. NVT slopes were calculated as changes in MAP (finger photoplethysmography) corresponding to normalized amplitudes of MSNA burst sequences (microneurography) using a custom software (MATLAB). Results: Resting MSNA burst frequency (27±6 bursts·min-1) and NVT slope (0.79±0.36a.u.) had a strong negative correlation (r=-0.67; p=0.0497), indicating lower vascular responsiveness in individuals with higher sympathetic activity. NVT also exhibited a strong positive relationship with both FBF (4.12±3.89mL·100mL FAV-1·min-1·log(μg·100mL FAV1·min-1)-1, r=0.73, p=0.0416) and FVC (3.94±3.52a.u.·log(μg·100mL FAV1·min-1)-1, r=0.70, p=0.0532) sensitivity to phenylephrine, but not norepinephrine (1.20±1.19mL·100mL FAV-1·min-1·log(ng·100mL FAV-1·min-1)-1 and 1.22±1.13a.u.·log(ng·100mL FAV1·min-1)-1, respectively; both p>0.05). Additionally, there was no relationship between α1-specific and non-specific α- and β-adrenergic reactivity (both p>0.05). Conclusion: Our data indicates that α1-adrenoreceptors contribute to NVT, and blunting of NVT at high altitude may be mediated

through decreased sensitivity of α1-adrenoreceptors. However, β-adrenergic vasodilation by endogenous norepinephrine may also offset NVT. Funding: NSER.

Time: 1615

Putatively adaptive Andean single nucleotide variant in EPAS1 preserves mitochondrial oxygen consumption. . Katie O'Brien 1, Juan Zuniga-Hertz2, Elijah Lawrence2, Wanjun Gu2, Ingrid Niesman3, Carlos Vasquez2, Esteban Moya2, Marco Bauk2, James Yu2, Alexis Komor2, Hemal Patel2, Andrew Murray1, Tatum Simonson2. 1University of Cambridge, 2University of California, San Diego, 3San Diego Sate University

Study objectiveTo investigate cellular metabolic phenotype downstream of a novel putatively adaptive single nucleotide variant (SNV) in EPAS1, encoding a subunit of hypoxia inducible factor HIF-2α, identified in Andean highlanders from Cerro de Pasco, Peru (4340m).MethodsThe EPAS1 SNV (rs570553380, A>G, p.[His194Arg]) was incorporated into an isogenic human cell line (HEK293T) using CRISPR base editing. Cells heterozygous for the putatively adaptive variant alongside wild type transfected and non- transfected (n =3 per cell line) were exposed to 24hrs of normoxia or hypoxia (1% O2). Mitochondrial oxygen consumption rates (OCR) and extracellular acidification rates were measured using the Cell Mito Stress Test and Glycolysis Stress Test, respectively (Agilent Seahorse XF), corrected to cell density (sulforhodamine b (SRB) colorimetric assay). Mitochondrial ultrastructure was visualized using Transmission Electron Microscopy. Cellular growth rate was measured in high glucose and galactose media using the SRB assay and cell viability using trypan blue staining and a Countess automated cell counter (Thermo Fisher Scientific). ResultsIn comparison to wild type, cells heterozygous for the putatively adaptive EPAS1 SNV revealed suppression of OCR in normoxia, including a 32% decrease in basal respiration, a 30% decrease in ATP linked respiration and a 50% decrease in maximal respiration ($p<0.05$), alongside changes in mitochondrial ultrastructure with inflated mitochondrial cristae. Following 24hrs of hypoxia, mitochondrial OCR was suppressed in wild type lines, including a 1.4 fold decrease in maximal respiration ($p<0.05$), and cells displayed the inflated mitochondrial cristae phenotype. In heterozygous cells, OCR and cristae phenotype remained unchanged from normoxic levels. No change was evident in cellular glycolytic capacity, growth rate or viability between cell lines.ConclusionTogether these results indicate cellular mechanisms downstream of a novel Andean variant in EPAS1 that may prime cells for hypoxic exposure by preserving mitochondrial O2 consumption with concomitant alterations in mitochondrial morphology.

Time: 1630

Expedition 5300 - The increase in blood viscosity in lowlanders exposed to high altitude is not only due to the rise of haematocrit. Emeric STAUFFER1, 2, Aurélien PICHON3, Benoit CHAMPIGNEUL4, Michaël FURIAN5, Lars KARSTNER6, 7, Ivan HANCCO4, Paul ROBACH4, 8, Julien V. BRUGNIAUX4, Philippe CONNES1, Samuel VERGES4. 1Laboratoire Interuniversitaire de Biologie de la Motricité (LIBM) EA7424, Team « Vascular Biology and Red Blood Cell », Université Claude Bernard Lyon 1, Université de Lyon, France, 2Explorations Fonctionnelles Respiratoires, Médecine du sport et de l'Activité Physique, Hospices Civils de Lyon, Hôpital Croix Rousse, Lyon, France, 3Université de Poitiers, Laboratoire MOVE, Poitiers, France , 4Univ. Grenoble Alpes, Inserm, CHU Grenoble Alpes, HP2, 38000 Grenoble, France, 5Pulmonary Division, University Hospital Zurich, 8092 Zurich, Switzerland, 6Theoretical Medicine and Biosciences, Saarland University, Homburg, Germany, 7Experimental Physics, Saarland University, Saarbrücken, Germany., 8National School for Mountain Sports, Site of the National School for Skiing and Mountaineering (ENSA), Chamonix, France

Introduction: Humans ascending to high altitude exhibit an increase in blood viscosity, which is classically attributed to the rise of hematocrit. However, blood viscosity is also dependent on the rheological properties of red blood cells (RBC). Few studies have investigated changes in RBC deformability and aggregation in lowlanders exposed to high altitude. The present study assessed the effect of acute high altitude exposure on blood viscosity and RBC rheology in lowlanders during a sojourn in the highest city in the world (La Rinconada, Peru, 5,100 m).Methods: Eleven volunteers native from lowland underwent 5 days at 3,800 m followed by 10 days at 5,100 m and another 5 days at 3,800 m. Blood was sampled 1 month before exposure to altitude (sea level pre), after 15 days at altitude and 2 weeks after return to sea level (sea level post). Blood viscosity (cone plate viscometer), RBC deformability (ektacytometry) and aggregation (syllectometry) measurements were performed on each blood fresh sample.Results: Haemoglobin concentration and haematocrit increased with altitude and returned to baseline after 2 weeks at sea level. Blood viscosity followed the same kinetic (6.9±1.5 cP at sea level pre, 16.7±3.1 cP at 5,100m and 7.3±1.3 cP at sea level post; $p<0.001$). RBC deformability decreased and RBC aggregation increased at altitude and returned to baseline after 2 weeks at sea level (elongation index at 3 Pa 0.318±0.015 UA at sea level pre, 0.322±0.048 UA at 5,100m and 0.375±0.02 UA at sea level post; $p=0.001$; aggregation index: 53.5±5.2% at sea level pre, 77.7±2% at 5,100m and 48.9±15% at sea level post; $p<0.001$).Conclusion: The increase in blood viscosity observed in lowlanders exposed to altitude is not only due to the rise in haematocrit but is also explained by a reduction of RBC deformability and an increase of RBC aggregation.

Time: 1645

CEREBROVASCULAR CO2 REACTIVITY IN ADULTS BORN PRETERM AT HIGH-ALTITUDE.

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Premature birth impairs cardiac and ventilatory responses to both hypoxia and hypercapnia, but little is known about cerebrovascular responses. We, therefore, investigated cerebrovascular CO2 reactivity at both sea-level (SL) and after 2 days at high-altitude (HA, 3375m) in young healthy preterm born adult males (n=16; gestational age, 29±1 weeks), and their age-matched term born counterparts (n=15; 40±0 weeks). Participants were exposed to two consecutive 4-minute hyperoxic hypercapnic conditions (3%CO2-97%O2; 6%CO2-94%O2), followed by two periods of voluntary hyperventilation to elicit hypocapnia, at both SL and HA. We measured middle cerebral artery blood velocity (MCAv; Transcranial Doppler), end-tidal CO2 and arterialized capillary blood gases. Hypocapnic and hypercapnic CO2 reactivity, and the sigmoidal response midpoint (X0), were analyzed using a sigmoid curve fitting. Hypocapnic CO2 reactivity increased at HA compared to SL in term born (+173±326%, P=0.026), but not in preterm (-21±107%, P=0.572, respectively), adults. HA exposure increased hypercapnic CO2 reactivity only in preterm adults (+125±144%, P<0.001). In HA, both hypocapnic and hypercapnic CO2 reactivity responses were different between preterm and term born individuals (P=0.012 and P=0.020, respectively). While X0 was similarly reduced at HA in both preterm (39.4±2.4 to 29.8±2.0 mmHg, P<0.001) and term born participants (41.1±2.9 to 31.1±1.0 mmHg, P<0.001), preterm adults exhibited a lower X0 compared to their term born peers independently of HA (P=0.025). Resting MCAv increased at HA compared to SL in term born (+24±39%, P=0.036), but not in preterm (-4±27%, P=0.278), adults. The SL-to-HA increase in MCAv was correlated with the change in PaO2/PaCO2 ratio in term born individuals only (r2=0.45, P=0.035). In conclusion, exposure to HA reveals differential cerebrovascular function in preterm compared to term born adults, as assessed by CO2 reactivity.Funding: Swiss National Science Foundation (nr. 320030L_192073) and Slovenian Research Agency (nr. N5-0152).

Time: 1700

TIME DOMAINS OF DNA METHYLATION PATTERNS DURING HIGH-ALTITUDE ACCLIMATIZATION.

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Objective: To measure changes in DNA methylation in response to acute and generational high-altitude exposure, in key hypoxia-inducible factor (HIF) pathway genes including EPAS1 (HIF-2α) and EGLN1 (PHD2). Hypothesis: Acute high-altitude exposure would produce increased methylation of the EGLN1 promoter region and decreased methylation of the EPAS1 promoter region, facilitating HIF-pathway activation. While in high-altitude natives we hypothesized that healthy individuals would have DNA methylation levels similar to acclimatized sojourners and individuals with maladaptive excessive erythrocytosis (EE) would have methylation levels comparable to sojourners on the first few days of high-altitude exposure. Methods: 31 participants of sea-level ancestry were recruited. DNA was isolated from venous

blood samples which were collected during fasting at sea level and over three days of acclimatization to 3800m elevation in Bishop, CA. In addition, 24 multigenerational Andean high-altitude residents from Cerro de Pasco Peru (4300m) were recruited and separated into control and EE groups based on hemoglobin levels. Local DNA methylation levels within EPAS1 and EGLN1 were obtained using a high-resolution melt technique. Results: Amplicons in both EPAS1 and EGLN1 showed significantly increased methylation levels in sojourners after 3 days of acclimatization as compared to their sea-level values. Additionally, EE highlanders showed higher levels of methylation in these regions than their healthy counterparts. Conclusion: These changes in DNA methylation likely play a key role in acclimatization and adaptation to chronic hypoxia and future studies will explore these mechanisms. This work has implications for understanding the role of epigenetics in diseases associated with hypoxia such as COPD, COVID-19, and sleep apnea, as well as the role epigenetics may play in long-term evolutionary adaptations. Funding: This work was funded by a UCR Regent's Faculty Fellowship and the UCR School of Medicine.

Time: 1715

The ABC of hypoxia - what is the norm?. Chris Donnelly1, 2, Sabine Schmitt1, Cristiane Cecatto1, Luiza Cardoso1, Timea Komlodi1, 3, Nicolas Place2, Bengt Kayser2, Erich Gnaiger1. 1Oroboros Instruments, Austria, 2Institute of Sports Sciences, University of Lausanne, Switzerland, 3Semmelweis University, Hungary

Hypoxia is a condition of oxygen levels below normoxia and opposite to hyperoxia. We here define the normoxic reference state by three complementary precepts: (A) ambient normoxia at sea level in the contemporary atmosphere and corresponding dissolved O2 concentrations at air saturation of aqueous environments; (B) biological compartmental O2 levels at ambient normoxia under physiological activity of healthy organisms in the absence of environmental stress; and (C) O2 levels above the respiratory oxygen control region. In the oxygen control region, the capacity for O2 consumption is compromised by hypoxic partial O2 pressure as evaluated by O2kinetics of respiration or other critical functions. The ABC of hypoxia distinguishes deviations from these reference points caused by different mechanisms: (ΔA) ambient alterations of oxygen levels; (ΔB) biological O2 demand exceeding O2 supply under pathological or experimental limitations of convective O2 transport or O2 diffusion; and (ΔC) critical oxygen pressure in oxygen kinetics shifted by pathological and toxicological effects or environmental stress. The ABC of hypoxia may be of help in the design, interpretation and communication of in vitro and in vivo experimental studies.

Time: 1730

THE IMPACT OF INFLAMMATION ON BRAIN OXYGENATION. Jeff Dunn1, Qandeel Shafqat1, Ying Wu1. 1Department of Radiology, Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary

Low levels of oxygen (hypoxia) in the brain can have a significant impact on brain function as well as quality of life measures (sleep, concentration etc). There is increasing evidence that inflammation, both local and systemic, can cause changes in the regulation of cerebral blood flow (CBF), molecular responses to hypoxia, such as HIF-1α regulation, and vascular reactivity. Near-infrared spectroscopy can be used to assess oxygenation in human brain. We show evidence from frequency domain NIRS measures of microvessel oxyhemoglobin saturation, that there is hypoxia in many people with MS and with long covid—which may relate to inflammation. We also show data from direct measurements of brain pO2 in two animal models that are measured while awake. The Experimental Autoimmune Enchephalomyelitis (EAE) model is an inflammation induced autoimmune model that results in demyelination and immune cell activation. The lipopolysaccharide injection model is one where injection of bacterial glycolipid stimulates a systemic immune response much like septic shock. We implanted fibre optic based pO2 sensors into the brain of the mice. The sensors have fibre bundles that attach to a control system but still allow for free movement. Behaviour and either cortical or hippocampal pO2 was measured. EAE mice show fluctuations in pO2 at times of peak disease—with all animals showing hypoxia at some period. The LPS mice have similar reactions, in that significant fluctuations in pO2 occur, with many periods of severe hypoxia. We show that inflammation is associated with brain hypoxia under a range of conditions in animal models and humans. This hypoxia could impact perception of sickness as well as impair recovery processes. The hypoxia could also be a target for treatment with oxygenation strategies including vasodilators, increased hemoglobin, or oxygen therapy.

Time: 1745

PUTTING HYPOXIA TO WORK: TAKING A STAB AT GENERATING INTRINSIC, RECYCLABLE MRI CONTRAST. Joseph Fisher1. 1University of Toronto

Introduction. Gadolinium (Gd), a paramagnetic molecule used for MRI contrast is invasive, risks toxicity, allergy, and environmental pollution. Deoxyhemoglobin (dOHb) is also paramagnetic, and can be produced by making the lung hypoxic. But rapid precise targeting of lung PO2 is resisted by its large functional residual capacity. Here we report generating precise [dOHb] as MRI contrast and comparing the resulting MRI images and hemodynamic measures to those obtained with a clinical standard, Gd, in patients with brain tumors. Method. We studied 9 patients (8 M; age 23-67 y) with low to high grade glioblastoma. Lung gas control was via sequential gas delivery (SGD) and prospective gas targeting breath by breath. We applied a hypoxic gas sequences twice consecutively: from normoxia, to PO2 at 40 mmHg (SaO2 of about 75%) for 60 s, then reoxygenation to normoxia within one inspiration (total 2 min). Each patient also received an injection of 5 ml Gd. Following the hypoxia sequence and the Gd injection we ran a T2* gradient echo planar sequence with TR 1750 ms voxel size 2.5 mm isotropic. We compared resting perfusion measures generated using both dOHb and Gd, using standard tracer kinetics and a new analysis of step re-oxygenation (under review).Results. Six patients completed the study (1 had excessive motion; 2 refused Gd). All patients attained

target arterial blood gases with little discomfort. Resting perfusion measures obtained using dOHb were highly comparable to those obtained with Gd.Conclusion. Hypoxia-induced dOHb provides an attractive MR contrast agent capable of obtaining contrast imaging and resting perfusion metrics highly similar to those obtained using Gd. Precise, repeatable dOHb generation is automated, brief, non-invasive, comfortable, and suited for repeated study, such as for monitoring for tumor progression.Words: 280

Time: 1800

ORGAN-SPECIFIC FUEL REWIRING IN ACUTE AND CHRONIC HYPOXIA REDISTRIBUTES GLUCOSE AND FATTY ACID METABOLISM. Ayush Midha1, Yuyin Zhou2, Bruno Queliconi2, Alec Barrios2, Cyril Fong1, Joseph Blecha1, Henry VanBrocklin1, Youngho Seo1, Isha Jain2. 1UCSF, 2UCSF/Gladstone

Objective: Oxygen deprivation can be detrimental. However, chronic hypoxia is associated with decreased incidence of metabolic syndrome and cardiovascular disease in high-altitude populations. Previously, hypoxic fuel rewiring has primarily been studied in immortalized cells. Here, we describe how systemic hypoxia rewires fuel metabolism to optimize whole-body adaptation. Acclimatization to hypoxia coincided with dramatically lower blood glucose and adiposity. Methods and Results: Using in vivo fuel uptake and flux measurements, we found that organs partitioned fuels differently during hypoxia adaption. Acutely, most organs increased glucose uptake and suppressed aerobic glucose oxidation, consistent with previous in vitro investigations. In contrast, brown adipose tissue and skeletal muscle became "glucose savers," suppressing glucose uptake by 3-5-fold. Interestingly, chronic hypoxia produced distinct patterns: the heart relied increasingly on glucose oxidation, and unexpectedly, the brain, kidney, and liver increased fatty acid uptake and oxidation. Hypoxia-induced metabolic plasticity carries therapeutic implications for chronic metabolic diseases and acute hypoxic injuries.Of note, this story is currently In Revision at Cell Metabolism.Lab website: https://ishajainlab.com/Funding: ADM was supported by the National Institute of General Medical Sciences 409 (NIGMS) Medical Scientist Training Program, Grant T32GM141323. IHJ was supported by NIH 410 DP5OD026398. IHJ, BBQ and AMB were supported by Defense Advanced Research Projects 411 Agency, Biological Technologies Office (BTO) Program: Panacea issued by DARPA/CMO under 412 Cooperative Agreement No. HR0011-19-2-0018