Meeting Report

Coping with hypoxia at high altitude:
How lung, blood and brain respond and crosstalk
5th International Atacama-Leh Symposium in San Pedro de Atacama, March 4-9, 2018, Chile

1,2 Edith M. Schneider Gasser, 2 Nuria Fabregas Bregolat, and 2,3 Markus Thiersch

1 Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstrasse
190, 8057 Zurich, Switzerland
2 Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zurich,
Winterthurerstrasse 260, 8057 Zurich, Switzerland
3 Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich,
Winterthurerstrasse 160, 8057 Zurich, Switzerland

The 5th International Atacama-Leh Symposium took place on March 4-9, 2018 in one
of the driest and certainly most beautiful places in the world – the Atacama Desert in
Chile. San Pedro de Atacama is located on a plateau at 2'408 meters altitude
offering a spectacular view to the surrounding volcanoes including Licancabur at the
border between Chile and Bolivia. This year's meeting was the 5th meeting since the
first Leh symposium in 2010 in Leh, Ladakh, India. The conference was very well
organized and chaired by Drs. Max Gassmann, Norbert Weissmann and Emilio
Herrera and assisted by Nuria Fabregas Bregolat. The organizers arranged an
excellent and balanced scientific program as well as unique social events including
the visit of ALMA – the currently largest radio telescope observatory in the world at
the Chajnantor Plateau in the Atacama Desert.
This year’s conference theme was “Coping with hypoxia at high altitude: How lung,
blood and brain respond and crosstalk” offering a broad insight into different high
altitude research fields, e.g. pulmonary hypertension and chronic as well as acute
mountain sickness. 50 researchers that represented 13 different countries from
South- and North America as well as Europe attended the conference. We had the
opportunity to listing to 32 exciting talks and to discuss 15 excellent posters. Further,
three posters were chosen for a short oral presentation. In this report, we summarize
the science presented in its various form at the meeting from our personal point of
view and our memories. Of course that way it is almost impossible to give full credit
to all the excellent presentations and we apologize if some attendees work is underrepresented in our report, which is by no means a reflection of scientific quality or importance.

Sunday 4th March 2018
The conference was opened in the evening by the three organizers, Drs. Max Gassmann, Norbert Weissmann and Emilio Herrera. The welcoming remarks were followed by an excellent Keynote lecture from Lan Zhao who presented an update of the current Tibet research program focusing on the difference of developing pulmonary hypertension between Han Chinese and Tibetans at high altitude. The evening ended after a jovially welcome dinner, where everybody had the opportunity to taste the typical drink (Pisco sour) and food (Ceviche).

Monday 5th March 2018
The first session was on pulmonary vasoconstriction chaired by Erik Swenson. The first speaker, Norbert Weissmann, gave an excellent presentation about the regulation of pulmonary vasoconstriction (HPV) to redirect local blood perfusion from poorly to well-ventilated areas of the lung. He introduced two currently discussed hypotheses that either increased or decreased mitochondrial reactive oxygen species regulate HPV. His data showed that HPV is induced by mitochondrial hyperpolarization and the release of mitochondrial superoxide that ultimately depolarizes the membrane of pulmonary arterial smooth muscle cells. Lan Zhao discussed the role of the hypoxia-sensitive zinc transporter ZIP12 in remodeled pulmonary vessels of animals and patients as the major regulator in pulmonary hypertension and showed us that targeting ZIP12 induces resistance to pulmonary hypertension. The session was closed by Mauricio Henriquez who used lung slices to microscopically study the contractibility of small intrapulmonary veins in the presence of purine nucleotides, which are frequently elevated in certain pulmonary diseases. The contribution of vein contraction to pulmonary arterial hypertension (PAH) has been controversially discussed and Dr. Henriquez showed us that venous contraction increased more by ATP, UTP and UDP treatment in rats with PAH when compared to healthy rats involving the purinergic receptors P2Y4 and P2Y14. The theme of the second session was “Blood at high altitude” chaired by Joachim Fandrey. The first speaker was Josef Prchal with a talk on “Hypoxia, Erythropoiesis and Tibetan Evolutionary Adaption. He started off with an excellent overview of the cellular response to normoxia and hypoxia and subsequently introduced us to
hypoxia-induced polycythemia and the protection from it in evolutionary high altitude-adapted Tibetans. He identified several Tibetan-enriched haplotypes including EGLN1 and EPAS1 that contribute to polycythemia protection. These genotypes, however, seem to be not sufficient to explain the full polycythemia protection. Martina Muckenthaler discussed the role of excessive iron accumulation in pulmonary diseases and introduced her mouse model with a point mutation in the murine ferroportin gene. The ferroportin mutation causes hereditary hemochromatosis type 4 with increased iron levels in various tissues including alveolar macrophages, epithelial cells of conductive airways, lung parenchyma and vascular smooth muscle cells. Iron accumulation causes oxidative stress and decreases both, total lung capacity and blood oxygen saturation as well as renders lung tissue more susceptible to inflammation and immune cell invasion. Max Gassmann finished the session with a presentation on human hemoglobin (Hb) and hematocrit values at varying altitudes. Approached by the world health organization (WHO) he and his colleagues performed a meta-analyses on hemoglobin and hematocrit values at high altitude to revise the currently accepted standard hematological parameters at varying altitudes. He furthermore showed that elevation lower than 1500 m above sea level influences Hb levels in men by analyzing the blood values of about 80,000 Swiss soldiers. He could show that Hb levels significantly increase with every 300 meters of increasing altitude.

After the lunch break Martina Muckenthaler chaired the 3rd session on lung at high altitude. Julián Aragonés discussed the role of HIF2α in airway response to oxygen fluctuation. He nicely showed us that the HIF2α-mTORC1 pathway promotes tumor proliferation via SLC7A5 but also takes place in hypoxic lungs – especially in the bronchial epithelium where high levels of HIF2α induce a marked proliferation. He further emphasized the central role of HIF2α in bronchial epithelium biology by showing that HIF2α also controls the expression of RELMα and β, which were previously considered to be bronchial epithelial growth factors. Heimo Mairbäurl discussed the question whether the aggravation of pulmonary vasoconstriction is caused by impaired clearance of alveolar fluids (high altitude pulmonary edema; HAPE) and the resulting impaired oxygen diffusion. He reported that both, LPS and the alveolar fluid Na-reabsorption inhibitor increased lung water and decreased arterial oxygen saturation. Observing an associated increase in right ventricular systolic pressure he concluded that poor high altitude tolerance might be associated to impaired alveolar reabsorption and epithelial ion transport. Irwin Reiss chaired the following session “disease and therapy at high altitude” and Ricardo Amaru led off
with his presentation on the characterization of erythrocytosis in chronic mountain sickness (CMS) of Andean highlanders living in La Paz (3'600 meter above sea level) and El Alto (4'000 meter above sea level). Among other clinical characteristics, he reported that Aymara highlanders suffering from CMS display normal erythropoietin serum levels but increased erythropoietin sensitivity of BFU-E’s. Thomas Haider talked about adaptation and maladaptation at high altitude focusing on the role of aquaporin 1 (AQP1) in pulmonary hypertension. He showed that the inhibition of AQP1 by Acetazolamide and by an in vivo knock down strategy (gapmers) reduces right ventricular pressure in hypoxia-exposed mice. His work suggests that aquaporin’s might be promising therapeutic targets for hypoxia-related diseases. Martin Burtscher gave a very interesting presentation on placebo and nocebo effects in high-altitude headache as the first symptom of acute mountain sickness (AMS). He further discussed the work of Benedetti and colleagues and showed us that the cyclooxygenase pathway is modulated during acute high altitude exposure by both nocebo and placebo. Interestingly, he reported that preconditioning with oxygen or aspirin as a positive stimulus improves the “success rate” of subsequent placebo administration. Martin Burtscher’s talk was followed by the poster session that nicely closed this day.

**Tuesday 6th March 2018**

A highlight of the conference was the Tuesday’s social event. Emilio Herrera organized a fantastic visit of the Atacama Large Millimeter/submillimeter Array (ALMA) Observatory that analyzes the universe beyond the visible range of light in the millimetric and submillimetric part of the spectrum. After safety instructions and a medical check at the base station (ca. 2’900 meter above sea levels) we were brought to the currently largest radio telescope worldwide at the Chajnantor Plateau (5’000 meter above sea level). Whereas the ALMA staff and our bus driver were supplied with oxygen, the conference members were able to experience the full impact of high altitude. For a few of us it was the first time at such an altitude and the unique chance to actually feel what we are usually just studying and talking about. Needless to say that this was a wonderful and unique experience and we, the authors, would like to express our gratitude to Emilio Herrera and the ALMA Observatory for making it happen.

After our return to San Pedro de Atacama we finished the day with the short presentation of selected posters. Despite being tiered or having even a headache from our aforementioned high altitude experience, we listened to three excellent presentations. Felipe Beñaldo presented his data on cinaciguat and atrial natriuretic
protein treatment in pulmonary hypertensive lambs at high altitude and nicely showed us that both compounds could be effective drugs to treat pulmonary hypertension. Rocio San Martin and Eduardo Peña presented the poster of Karem Arriaza on Zinc supplementation in chronically hypoxia-exposed rats, which results in increased right ventricular hypertrophy. Nuria Fabregas closed the day with her poster presentation on iron deficiency and erythropoietin insensitivity in a mouse model of anemia of cancer.

**Wednesday 7th March 2018**

The first session of the morning, chaired by Emilio Herrera, was on Perinatology and Children at High Altitude. Irwin Reiss discussed the adaptive processes of the placenta during pregnancy at high altitude and the consequences to neonatal microcirculation. Using an ex-vivo placental model, he demonstrated that in preeclamptic placentas, the nitric oxide (NO)-mediated vasodilatation of the fetoplacental circulation is impaired, due in part to upregulation of phosphodiesterases (PDEs). Additionally, he showed that neonates, in whom fetal development occurred at high altitude, total vessel density was significant higher than neonates at sea level, suggesting an adaptive fetal strategy to cope with hypoxia. Anibal Llanos continued the session addressing neonatal pulmonary hypertension at high altitude and proposed an alternative treatment to NO. He proposed that soluble guanylyl cyclase (sGC) enzymatic function is reduced by hypoxia and showed in neonatal lambs born in Putre Research Station, INCAS (3'600 m asl), that the use of a drug to activate oxidized sGC can reduce pulmonary arterial pressure (mPAP) and pulmonary vascular resistance (PVR) in response to acute episodes of superimposed hypoxia. Finally, a video sent by Gabriel F. Diaz, who was awarded by Colombian Society of Cardiology and therefore unable to attend the meeting, emphasized the mistake of extrapolating studies from pulmonary hypertension at sea level to explain pulmonary hypertension caused by hypobaric hypoxia; mainly due to the fact that pulmonary hypertension in hypobaric hypoxia has another epidemiology, varying in frequency, age onset, and pulmonary vascular reactivity and remodeling in response to oxygen.

The second morning session chaired by Irwin Reiss, was devoted to metabolism at high altitude. Daniel Martin and Helen McKenna exposed a fantastic translational study in which they compared the adaptive changes occurring within the mitochondrial-microcirculatory unit in Himalayan Sherpas ascending to 5300 m asl with the changes occurring in patients at different time-points during critical illness. This comparative approach provided a new framework in which the successful
adaption in Sherpas to hypoxic stress could represent therapeutic targets in critically ill patients. Patricia Siques discussed how an altered metabolic condition, such as obesity and overweight, can impair a proper acclimatization to high altitude, leading to a higher risk of high altitude diseases such as Acute Mountain Sickness and high Pulmonary Artery hypertension.

The afternoon seminars started with a session, chaired by Paul Schumacker, dedicated to cancer and chemosensitivity at high altitude. Markus Thiersch presented his work in which he evaluated the effect of high altitude on cancer progression and mortality. He showed, in a mouse model of lung cancer that exposure to high altitude did not suppress tumor growth per se but ameliorated cancer-associated comorbidities including anemia and hepatomegaly-associated increased serum bile acid levels. Till Acker, addressed the importance of tumor microenvironments for the process of invasion and metastasis and showed us that prolyl hydroxylase domain protein 3 (PHD3) is a key sensor of environmental stress signals, such as hypoxia, controlling tumor growth and metastasis. Finally, Jorge Soliz updated his work on carotid body chemosensitivity to hypoxia and hypercapnia. He showed in ex vivo carotid body preparations, how erythropoietin can have an “hormetic” modulation of the carotid sinus nerve activity (CSNA) to hypoxia and hypercapnia either increasing the CSNA when applied at low concentrations (<0.5U/ml), or decreasing CSNA when applied in high concentrations (>2U/ml) mostly due to an increase in the capacity of endothelial cells to produce NO which acts then as an inhibitor. Understanding how Epo controls the carotid body’s chemoresponse to hypoxia and hypercapnia, give new insights to understand respiratory disorders occurring at high altitude.

The second afternoon session was chaired by Daniel Martin, with the theme “Sensing and Signaling hypoxia”. Paul Schumacker presented his outstanding work in which he addressed the important role of mitochondrial ROS signals in response to cellular hypoxic stress. With the use of sensors of thiol redox status (roGFP) to assess redox signaling; genetic deletion of the Rieske iron-sulfur protein (RISP) and pharmacological interventions to perturb mitochondrial electron transport function in pulmonary artery smooth muscle and endothelium, he was able to show how that altered mitochondrial signals trigger the development of hypoxia-induced pulmonary hypertension. Serge Adnot continued the topic with another aspect in the pathology of pulmonary hypertension: cell senescence. In animal models of pulmonary hypertension, his group showed that inducing cell senescence could prevent and reverse the pathology. However, senescence occurs also in pulmonary vessels during aging or in chronic lung diseases. Therefore, in their current work, they
Thursday 8th March 2018

The last day opened with a morning session chaired by Vincent Joseph on The Heart at High Altitude. Marco Maggiorini gave a great overview on heart-lung interactions at high altitude. He explained how changes in the pulmonary artery pressure caused by high altitude can lead to an enlargement of the heart right ventricle with a preserved function. However, when acclimatization fails, pulmonary hypertension occurs within the first days of exposure and that can lead to congestive heart failure within weeks. Emilio Herrera then gave an enriched presentation on animal models for studying cardiopulmonary effects of hypobaric hypoxia. In his talk he review from the Tibetan yak to the Andean camelids the main adaptations selected by this species to survive to their extraordinary environments. He highlighted the pro and contra in the use of each animal model and summarized the main discoveries and potential treatments achieved to attenuate high-altitude cardiopulmonary complications. Julio Brito presented data on right cardiac circuitry changes and pulmonary hypertension after long term high altitude intermittent exposure. He mainly reported that in subjects exposed to high altitude intermittently, mostly due to work, a high prevalence of mild pulmonary hypertension was observed.

The second morning session, chaired by Julio Brito, was on the stress effects of Ascending to High Altitude. The first talk was given by Edith Schneider Gasser who exposed the changes in brain microvasculature, neuronal survival and cognition caused by chronic hypoxic stress after ascent to high altitude. Ascent to high altitude in long Evan rats caused a loss of spatial and visual memory and increased neuronal apoptosis. When animals were housed in an enriched environment that allowed them to explore and exercise, angiogenesis and neurogenesis were enhanced; neuronal apoptosis was reduced and memory was recovered. When VEGF signaling was inhibited, memory recovery at high altitude was not possible, addressing the pivotal role of angiogenesis in the brain adaptation to high altitude. Erik Swenson reported that although at high altitude the cerebral blood flow increases, the consumption of oxygen can decrease and alterations in the blood brain barrier may lead to cerebral edema. Also the kidney becomes hypoxic at high altitude, having many mechanisms to maintain the renal function. Increase diuresis appears to be driven by renal blood flow and tubular function, which may be partially activated by sympathetic activation. Also peripheral chemoreceptor-mediated signaling to the brain can control the renal function via an unknown efferent pathway. Sergio Muñoz presented a project aiming
to evaluate the health of workers exposed to intermittent high altitude. He compared the health status of workers at different altitudes and showed a marked effect of high altitude on sleep disturbance, hypertension and acute mountain sickness.

The last session of the meeting after lunch, chaired by Till Acker, was on Sleep, Exercise and Inflammation at High Altitude. Vincent Joseph discussed his new work on mitochondrial dysfunction in a model of sleep apnea. He showed that in ovariectomized female rats exposed to intermittent hypoxia (a model of apnea), estradiol supplementation could protect against hypertension and reduced brain oxidative stress. Intermittent hypoxia causes a dysfunction in mitochondrial respiration that could be prevented by agonist of α and β estradiol receptors. His findings leaded to conclude that sleep apnea after menopause is linked to a mitochondrial dysfunction, which can be prevented with estradiol. Joachim Fandrey gave an extremely interesting presentation evaluating the role of HIF in myeloid and dendritic cells function and immune response during the pathogenesis of inflammatory bowel disease, in particular colitis. His group showed that HIF-1 signaling is essential for myeloid cells life span and function as well as dendritic cell activation and maturation during inflammation. In addition, the role of HIF-1α in macrophage function during acute retroviral infection was evaluated with the use of myeloid cell-specific HIF-1α knockout mice. They could show that in HIF-1α-deficient macrophages, invasion after viral infection was impaired. This work highlighted the importance of HIF-1 signaling in white blood cells in response to inflammation.

During Fandrey’s talk, the earth vibrated! At 13:41 we experienced a 5.0 magnitude earthquake with its epicenter around 80 Km west from San Pedro de Atacama. Most of us never felt an earthquake before but after some time of recovery and understanding the meaning of being in a volcanic area, we could continue to listen.

Hans Hoppeler gave the last talk of the meeting in which he explained the limitations of genetic approaches to address VO2max trainability in endurance sports. Although it is proposed that key determinant of VO2max and trainability are regulated by complexes transcriptomic networks consisting of a multitude of interacting proteins, the knowledge of physiological properties of the individual proteins are not sufficient condition for understanding sedentary VO2max and its trainability.

After the closing remarks of the meeting given by Max Gassmann, we visited the El Valle de la Luna (Valley of the Moon), located 13 kilometers west of San Pedro de Atacama, in the desert. We were amazed with the various stone and sand formations, which have been carved by wind and water and the range of color and texture that look somewhat similar to the surface of the moon. We also went to El
Mirador to see the glowing red light illuminating the snow and clouds above the volcanos at sunset. That evening we gathered for the last time at dinner. The modest restaurant offered us once again the traditional drink Pisco sour and very well prepared dishes bringing the meeting to a formal end. We all will keep this meeting in best memory.

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