FORM 2 – FULL RESEARCH PROPOSAL

Principal Investigator[1]

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Collaborators [2]

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Research title[3]

Sleep disturbances, tissue oxygenation and acclimatization at high altitude

Lay summary[4]

Central sleep apnea is almost universally observed at high altitude but its impact on tissues oxygenation and its relationship with altitude acclimatization still remain to clarify. The brain appears especially sensitive to hypoxia and may show the largest oxygenation changes during sleep apnea at altitude. Also, whether sleep apnea at high altitude should be consider as a positive or deleterious mechanisms for acclimatization remains unclear. This study aims to assess changes in cerebral and muscle oxygenation during sleep during a prolonged sojourn at high altitude and to determine the relationships between sleep perturbations, tissue oxygenation and acclimatization.

Scientific proposal, background[5]

Background.

Altitude sojourners to high altitude frequently experience sleep disturbances,

often reporting restless and sleepless nights. Others describe a feeling of suffocation on awakening from sleep. A characteristic waxing and waning breathing pattern known as periodic breathing accompanies sleep at high altitude; typically consisting of 2 to 4 breaths, separated by an apnea from the next burst of 2 to 4 breaths (1). Changes in the ventilatory responses to hypoxia and CO_2 may be responsible for a narrowing of the difference between the eupneic arterial CO_2 partial pressure and the apneic threshold. Arterial hypocapnia due to altitude-induced hyperventilation would subsequently trigger central sleep apnea. Hence, while periodic breathing and central events during sleep clearly increase with altitude, whether this higher incidence of central events alters altitude tolerance is still debated. A recent study from our laboratory suggested that good responders to high altitude (i.e. with few symptoms of acute mountain sickness, AMS) have significantly more central events in hypoxia but also a higher average arterial oxygen saturation during sleep compared to AMS susceptible subjects (2). These results are against the concept that central sleep apnea is deleterious for acclimatization at high altitude but remain to be confirmed on the field. We recently showed by using near infrared spectroscopy (NIRS) that the brain shows larger deoxygenation compared to the muscle during normobaric hypoxic exposure at rest (6), suggesting that beyond the changes in arterial oxygen saturation, tissue and potentially individual specificities exist regarding changes on oxygenation induced by reduced inspiratory oxygen pressure. The impact of sleep apnea and associated cyclic changes in arterial oxygenation on cerebral and muscle oxygenation as assessed by NIRS at high altitude has never been investigated. It may provide additional insights on the impact of sleep disturbances and acclimatization at high altitude.

Scientific proposal, aims and hypotheses[6]

This study aims to assess changes in cerebral and muscle oxygenation during wakefulness and sleep during a prolonged sojourn at high altitude and to determine the relationship between sleep apnea, changes in cerebral and muscle oxygenation and symptoms of AMS. We hypothesize that i) cerebral oxygenation will be significantly reduced and will show cyclic variations during sleep apnea and ii) acclimatization is associated with more central events and higher average arterial and tissue oxygenation during sleep.

Scientific proposal, methods[7]

Subjects. Subjects will be investigated at sea level and at high altitude (at Manaslu base camp -5000 m-, after a 9-day trek to this altitude), with various degrees of AMS. Subjects will be pre-screened at base camp by using the Lake Louise Questionnaire. The objective will be to investigate 12 subjects with Lake Louise score >3 and 12 subjects with Lake Louise score <1. Based on previous experience of the MEDEX group in similar conditions (3), the 2 groups with the requested Lake Louise scores should be easily obtained with the total amount of volunteers planned during the MEDEX MANASLU 2015 experiment (>50 subjects).

Study design. Subjects will be investigated during the night immediately after arrival at Manaslu Base camp and after 3-4 days at the same altitude. Subjects will be investigated during the whole night with polygraphy (naso-bucal flux, ECG, respiratory movement, finger pulse oximetry; ApneaLink Air RESMED, Germany) and quadriceps and cerebral NIRS (PortaLite, ARTINIS, The Netherlands). Subjects will be characterized with the Lake Louise questionnaire for symptoms of AMS and with standard questionnaires for sleep quality.

In a subset of volunteers climbing the Manaslu, we will perform the first NIRS recordings during wakefulness and sleep at very high altitude (>6000 m) by using new portable NIRS devices.

Near infrared spectroscopy. A portable NIRS device (PortaLite, ARTINIS, The Netherlands) will be used to monitor relative concentrations in pre-frontal oxygenated-haemoglobin (ΔO_2Hb), deoxygenated-Hb (ΔHHb) and total-Hb ($tHb = O_2Hb + HHb$). Theoretical and performance details of NIRS have been previously described (4). NIR-determined hemodynamic reflects the dynamic balance between O_2 demand and O_2 supply in the tissue microcirculation and can be used at high altitude to assess cerebral oxygenation and perfusion changes (5).

Polygraphy. Standard polygraphy will be performed to detect periodic breathing and apnea-induced changes in oxygen saturation. A light polygraph (ApneaLink Air RESMED, Germany) will be used to record nasal flow, snoring, respiratory effort, heart rate and oxygen saturation.

Scientific proposal, expected results[8]

While sleep disturbances are among the most remarkable physiological

consequences of high altitude exposure, their roles and consequences regarding acclimatisation to high altitude and altitude diseases remain unclear. Also, while intermittent hypoxia induced by sleep apnoea is commonly described based on arterial oxygenation, there is increasing interest about the effect of intermittent hypoxemia on tissue oxygenation per se. It is suspected that large differences may exist between tissues in terms of average values and cyclic variations in oxygenation levels. In a recent study (unpublished data) we showed for instance the large difference existing in cerebral and muscle oxygenation during intermittent hypoxemia induced in healthy subjects (see figure 1). Therefore, this study should clarify first the link between symptoms and acclimatisation at high altitude and periodic breathing and determine whether periodic breathing is a marker of acclimatization. Second, this study by using the model of periodic breathing at high altitude should clarify the consequences of changes in arterial oxygenation on tissue oxygenation during sleep. This should help understanding the relative vulnerability of some organs compared to others in condition of hypoxia.

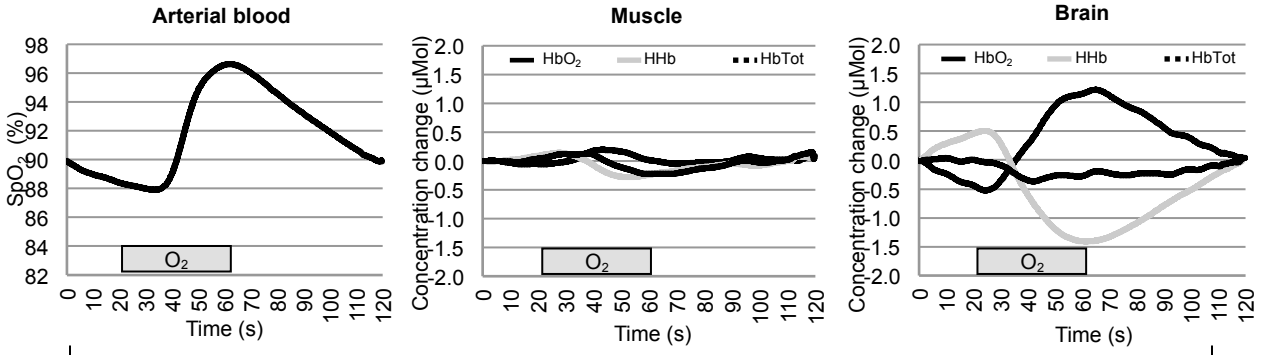

Figure 1. Average arterial, muscle and brain mean oxygenation changes measured in 13 healthy subjects over 2-min intermittent hypoxia cycles.

Dissemination plan, target journal(s)[9]

Sleep, European Respiratory Journal, Sleep Medicine, High Altitude Medicine and Biology

Dissemination plan, timeline[10]

Data analysis. NIRS and polygraphy data, expedition + 5 months.

Results discussion and article preparation. Article 1 (Sleep apnea at high altitude in symptomatic and asymptomatic subjects), expedition + 8 months. Article 2 (Effect of periodic breathing at high altitude on tissue oxygenation), expedition + 9 months

Conference presentation and article submission. Expedition + 6-10 months

Research requirements, participants[11]

Total time spent by one volunteer for this study: 45 min preparation before sleeping, one night recording, 15 min at waking-up for sensor removal.

Research requirements, personnel[12] 3-4 researchers are required to run this experiment in several subjects simultaneously each night. Fully trained operators for polygraphy and NIRS recordings are needed. The entire group of experimenters should be able to evaluate 4 volunteers per night.
Research requirements, equipment[13] For near infrared resonance spectroscopy: PortaLite (ARTINIS, The Netherlands; Power recruitment: 100-240 VAC; Weight: 5 kg) For polygraphy: ApneaLink Air (RESMED, Germany; Power recruitment: 100-240 VAC; Weight: 5 kg)
Research requirements, consumables[14] The only consumables are ECG electrodes, cannulas, and cleaning kits for sensors.
Research requirements, logistics[15] Equipment total weight: ~20 Kg Laboratory requirements: Sensors installation and recording will be performed in subjects' tents.
Research requirements, research cost[16] All research devices are available within our laboratory or will be lent by companies (as in previous projects from our team) and will be budgeted for 8 000 €. This research cost will be met by industrial and local public institution sponsorship.

[Print full name of principle investigator and collaborators here and all sign in next column][17]	**VERGES**  **RUPP**  **BOUZAT**  **WALTHER**  **ESTEVE** 	**08-07-14**

[1]Title, full name, current post, department, institution, contact postal address, email address, telephone (including country and area code)
[2]Title, full name, department, institution, email address
[3]Max 20 words

[4]Project summary in simple English. Max 200 word
[5]Provide rationale for study
[6]Concise; specific and directional hypotheses
[7]Participants; research design; study schematic; procedures; statistical analyses; identification of main outcome measure; justification of sample size
[8] Graphs as likely to be presented in manuscript depicting theoretical relationships but correct units and physiologically plausible absolute values; explanatory text to justify relationships (based on previous literature)
[9]Target journal(s)
[10]Timeline from research proposal to submission of, manuscript to target journal (including conference presentations and 1st draft of introduction/methods/results/discussion sections)
[11]Total time participants will spend on study; 12Risk to participants and how risks will be mitigated
[12]Staff required to run project successfully
[13]Make, model, where equipment will be sourced from, rough estimate of power requirements
[14]Plastics, paper, disposable accessories for equipment, etc
[15]Rough estimates of: sample transport (if required); equipment total weights; laboratory requirements (space, environmental conditions, services (water, electric, light, waste disposal)
[16]Direct expenditure related to project and explanation of how these costs will be met. Do not include expedition fees or logistics, or indirect salaries
[17]Principal Investigator and Collaborators must provide consent to submit proposal. This can be done with either physical or electronic signatures on the research proposal, or alternatively each researcher may email j.h.macdonald@bangor.ac.uk the following text: "I [INSERT NAME] approve the full research proposal entitled [INSERT TITLE]"

- Formatting
  - Please type information into table above and expand table as necessary
  - Min 12 point, min 1.5 line spacing, 2cm margins, times new roman, reference format as per Journal of Applied Physiology guidelines, include page numbers and principal investigator surname in a footer on every page; scientific proposal section should not exceed six pages of A4 plus references; research requirements should not exceed four pages of A4
- Submission
  - Email one pdf file to j.h.macdonald@bangor.ac.uk
  - Closing date: 24.12.13, 1200, Greenwich Mean Time
  - Please also ensure all researchers have read, completed and submitted form 3: researcher application form
  - Please also ensure the principle investigator has read, completed and submitted form 4: principal investigator contract.
  - Suggest at least four reviewers
    - Must have no known conflict of interest
    - Provide title, full name, position, department, institution, email address and phone number (including country and area code)
  - You will receive confirmation of submission within five working days
- Queries
  - Contact MEDEX Manaslu 2015 Research Lead
    - Jamie Macdonald PhD, Extremes Research Group, Bangor University
    - Email: j.h.macdonald@bangor.ac.uk
    - Tel: +44 1248 383272

References.

1. **Ainslie PN, Lucas SJ, and Burgess KR**. Breathing and sleep at high altitude. *Respir Physiol Neurobiol* 2013.
2. **Nespoulet H, Wuyam B, Tamisier R, Saunier C, Monneret D, Remy J, Chabre O, Pepin JL, and Levy P**. Altitude illness is related to low hypoxic chemoresponse and low oxygenation during sleep. *Eur Respir J* 40: 673-680, 2012.
3. **Oliver SJ, Sanders SJ, Williams CJ, Smith ZA, Lloyd-Davies E, Roberts R, Arthur C, Hardy L, and Macdonald JH**. Physiological and psychological illness symptoms at high altitude and their relationship with acute mountain sickness: a prospective cohort study. *J Travel Med* 19: 210-219, 2012.
4. **Rolfe P**. In vivo near-infrared spectroscopy. *Annu Rev Biomed Eng* 2: 715-754, 2000.
5. **Rupp T, Esteve F, Bouzat P, Lundby C, S. P, Levy P, Robach P, and Verges S**. Cerebral hemodynamic and ventilatory responses to hypoxia, hypercapnia and hypocapnia during 5 days at 4,350 m *J Cereb Blood Flow Metab* in press: 2013.
6. **Rupp T, Leti T, Jubeau M, Millet GY, Bricout VA, Levy P, Wuyam B, Perrey S, and Verges S**. Tissue deoxygenation kinetics induced by prolonged hypoxic exposure in healthy humans at rest. *Journal of biomedical optics* 18: 095002, 2013.