UNDERSTANDING THE VARIABLE HUMAN RESPONSE TO HYPOXIA: PHYSIOLOGICAL, GENETIC, AND EPIDEMIOLOGICAL INVESTIGATIONS OF ACUTE MOUNTAIN SICKNESS SUSCEPTIBILITY

by

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Abstract

High-altitude (and simulated high-altitude) environments can be extraordinarily stressful for low-altitude organisms because of the reduced oxygen availability (*i.e.* hypoxia). Humans, who live primarily at low altitude, can adjust physiologically (*i.e.,* acclimatise or acclimate) to hypoxic environments; however, the human acclimatisation response to hypoxia is highly variable, evident from the differential susceptibility to acute altitude illnesses, such as acute mountain sickness (AMS). For my dissertation, I attempted to identify some of the physiological, genetic, and epidemiological variables that could explain the variation in hypoxia tolerance. I conducted (i) two studies using a normobaric hypoxia chamber at the University of British Columbia; (ii) two field studies in a mountainous region of the Nepalese Himalaya; and (iii) two meta-anaylyses. The most important findings of my dissertation are that (i) oxygen saturation (S_{PO_2}) and heart rate (HR) were not strong markers of AMS susceptibility in laboratory or field settings; (ii) a low fraction of exhaled nitric oxide (F_{ENO}) was associated with increased susceptibility to AMS in the laboratory but not in the field; (iii) physiological responses (F_{ENO}, S_{PO_2}, HR, blood pressure) to hypoxia were repeatable on two normobaric hypoxia exposures; (iv) AMS severity was lower on the second of two identical normobaric hypoxia exposures (but headache severity was similar); (v) in a large Nepalese sample, age, sex, ascent rate, and preventative strategies were associated with AMS susceptibility; (vi) the severity of AMS was similar in brothers; (vii) there were biogeographical differences in AMS susceptibility in the Nepalese sample; (viii) polymorphisms of the *FAM149A* gene were associated with AMS severity; (ix) AMS history was a poor predictor of future AMS outcomes; and (x) sleep quality was weakly related to other AMS symptoms. In conclusion, this dissertation demonstates that the measured physiological variables (F_{ENO}, S_{PO_2}, HR, blood pressure) were not associated with AMS status, that a genetic basis to the variation in AMS susceptibility is likely, and that the Lake Louise Score definition of AMS should be amended. Our understanding of acute altitude tolerance in humans may be aided by the redefinition of AMS.

Preface

Portions of the introduction (Chapter 1) have been published in a series of review articles about the genetics of altitude illnesses and altitude tolerance (MacInnis et al. 2010, 2011) altitude adaptation (MacInnis and Rupert 2011) and twin studies in hypoxia (MacLeod et al. 2013). I wrote the original draft of each of the manuscripts for which I am listed as the first author, and I wrote large portions (including the portions contained in my dissertation) of the manuscript for which I am listed as the second author. Excerpts, figures, and tables from manuscripts published in *High Altitude Medicine and Biology* are reprinted with permission from HIGH ALTITUDE MEDICINE AND BIOLOGY (Vol. 13, Issue 2; Vol. 12, Issue 2; Vol. 11, Issue 4), published by Mary Ann Liebert, Inc., New Rochelle, NY.

Chapter 3 has been published in *Respiratory Physiology & Neurobiology* (MacInnis et al. 2012a). E.A. Carter and I designed the experiment through consultation with Drs. M.S. Koehle and J.L. Rupert. E.A. Carter and I collected the data in the UBC Environmental Physiology Laboratory with assistance from B. Boere and Dr. P. Wang. I performed the statistical analyses and wrote the first draft of the manuscript; E.A. Carter, Dr. M.S. Koehle, and Dr. J.L. Rupert reviewed and revised the manuscript. The UBC Clinical Research Ethics Board approved the study (ethics certificate number: H10-02323; project title: "NO chamber study"). This manuscript is reprinted with permission from Elsevier BV, Amsterdam, Netherlands.

Chapter 4 has been published in *Wilderness and Environmental Medicine* (MacInnis et al. 2014) and is reprinted with permission from the Wilderness Medical Society. I conceived of and designed this experiment in consultation with Drs. M.S. Koehle and J.L. Rupert. I collected the data with assistance from S. Koch, K.E. MacLeod, E.A. Carter, R. Jain, and N. Richard. I performed the data analysis and wrote the first draft of the manuscript. S. Koch, K.E. MacLeod, E.A. Carter, Dr. M.S. Koehle, and Dr. J.L. Rupert reviewed and revised the manuscript. S. Ko assisted with the analysis of heart rate and oxygen saturation data. The UBC Clinical Research Ethics Board approved the study (ethics certificate number: H11-02659; project title: "NO and AMS 2").

Chapter 5 was published in *PLOS ONE* (MacInnis et al. 2013a). I designed this experiment in consultation with E.A. Carter, Dr. M.S. Koehle, and Dr. J.L. Rupert, and I led the 3-week research expedition in Nepal. Data collection was completed in Langtang National Park with the assistance of many colleagues: Dr. N. Widmer, Dr. M.G. Freeman, Dr. A. Subedi, and

B. Pandit were responsible for the high-altitude camp and the data collected there, and E.A. Carter, Dr. A. Siwakoti, and U. Timalsina and I were responsible for the low-altitude camp and the data collected there. (*N.b.,* E.A. Carter and U. Timalsina also assisted with data collection at the high-altitude site on the night of *Janai Purnima*). Members of the Mountain Medicine Society of Nepal (especially Dr. G.B. Thapa, Dr. A. Lohani, Gobi Bashyal, and Bhuwan Acharya) provided important logistic support in Nepal. The kind staff at the Langtang View Hotel (Dhunche), the Hotel Lakeside (Gosainkunda), and local businesses in Dhunche assisted with data collection by providing space and food to subjects and researchers. I performed the data analyses and wrote the first draft of the manuscript; all authors reviewed and revised the manuscript. The UBC Clinical Research Ethics Board (ethics certificate number: H11-03516; project title: "Nepal II: The Genetics and Physiology of Altitude Illness") and the Nepal Health Research Council (ethics certificate number: Ref. 60) approved the study. Regional approval of the study was provided by the Rasuwa chief district, public health, and district health officers.

A portion of Chapter 6 contains data collected in Nepal in 2010. Drs. M.S. Koehle and J.L. Rupert designed the experiment, and I accompanied them to Nepal and collected saliva samples and physiological data. Members of the Himalayan Rescue Association (Bhuwan Acharya, Drs. Ashish Lohani, Sagar Koirala, Sagar Panthi, Sushil Pant, and Smith Giri) assisted with subject recruitment, translating questions, and diagnosing subjects. Dr. Buddha Basnyat and Gobi Bashyal provided logistic support. The UBC Clinical Research Ethics Board (ethics certificate number: H05-70208; project title: "The Role of Genetic Variants in the Development of Altitude-Related Pathologies") and the Nepal Health Research Council (ethics certificate number: Ref. 227) approved the study. The remainder of the data in this chapter is from the 2012 expedition to Nepal that I led. The acknowledgements for this study are the same as those for Chapter 5. In addition, Dr. I. Manokhina assisted with the preparation of DNA samples for genotyping. The genome-wide association study has not yet been published.

Chapter 7 (and Appendix G) has been published in the *British Journal of Sports Medicine* (MacInnis et al. 2013c). I conceived of the study, collected the data with assistance from J. Strong, and wrote the majority of the first draft of the manuscript. I performed the initial statistical analysis; however, Dr. K.R. Lohse chose and implemented the final statistical analysis. Dr. K.R. Lohse also contributed significantly to the writing and revising of the manuscript, including the creation of the figures. Dr. M.S. Koehle reviewed the statistical analyses and

assisted with the writing and revising of the manuscript. All authors approved the final manuscript. Reproduced from [Is previous history a reliable predictor foracute mountain sickness susceptibility? A meta-analysis of diagnostic accuracy. MacInnis MJ, Lohse KR, Strong JK, and Koehle MS. Epub ahead of print. copyright notice year: 2014] withpermission from BMJ Publishing Group Ltd.

Chapter 8 has been published in *High Altitude Medicine & Biology* (MacInnis et al. 2013b), and is reprinted with permission from HIGH ALTITUDE MEDICINE AND BIOLOGY (Vol. 14, Issue 4), published by Mary Ann Liebert, Inc., New Rochelle, NY. I designed and performed the analysis, and wrote the first draft of the manuscript. Drs. S.C. Lanting and M.S. Koehle reviewed the statistical analyses and assisted with the writing of the manuscript. Dr. Rupert approved the manuscript. Attributions for the data collection and the ethical approval are the same as those for Chapter 5.

Appendix F has not been published. I conceived of the analysis, collected the necessary data, and wrote the first draft of the manuscript. E.A. Carter and Dr. J. Donnelly reviewed the collected data, and E.A. Carter, and Drs. J. Donnelly, J.L. Rupert, and M.S. Koehle assisted with the writing and revising of the manuscript.

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List of Symbols

a: altitude (used in equation for P_B)
ºC: Degrees Celsius
C_{aO_2}: Arterial oxygen content
CO_2: Carbon dioxide
C_{vO_2}: Venous oxygen content
D_{O_2}: Oxygen delivery
D_{LO_2}: Lung diffusion capacity for oxygen
F_{ENO}: Fraction of exhaled nitric oxide
FEV_1: Forced expiratory volume in one second
F_{IO_2}: Fraction of inspired oxygen
F_{O_2}: Fraction of oxygen
FVC: Forced vital capacity
H^+: Hydrogen ion
H_2O: Water
h^2: Narrow-sense heritability
Hb: Hemoglobin
I^2: Heterogeneity
m: Meters
mmHg: Milimeters of mercury
n: Sample size
N_2: Nitrogen
NO: Nitric oxide
O_2: Oxygen
p: p-value
P_{H_2O}: Partial pressure of water vapour
P_{AO_2}: Partial pressure of alveolar oxygen
P_{aO_2}: Partial pressure of arterial oxygen
P_B: Barometric pressure
P_{cO_2}: Partial pressure of pulmonary capillary oxygen
P_{ENO}: Partial pressure of exhaled nitric oxide
P_{IO_2}: Partial pressure of inspired oxygen
P_{O_2}: Partial pressure of oxygen
ppb: Parts per billion
P_{tO_2}: Partial pressure of tissue oxygen
P_{vO_2}: Partial pressure of mixed venous oxygen
Q: Cardiac output
r: Pearson's correlation
S_{aO_2}: Arterial oxygen saturation
S_{PO_2}: Pulse oxygen saturation
t: t-test statistic

V_{CO_2}: Volume of carbon dioxide produced
V_E: Minute ventilation
V_{O_2}: Volume of oxygen consumed
$V_{O_2\ max}$: Maximum aerobic capacity
z: z-score
χ^2: Chi-squared statistic

List of Abbreviations

AIC: Akaike information criterion
AIMS: Ancestry informative markers
AMS: Acute mountain sickness AMS+: Acute mountain sickness positive
AMS-: Acute mountain sickness negative
AMS-C: Cerebral acute mountain sickness score
ANOVA: Analsis of variance
ATS: American Thoracic Society
BH: Breath hold
BIC: Bayesian information criterion
BP: Blood pressure
bp: Base pair
CADO: Combined altitude depleted oxygen
CBF: Cerebral blood flow
CFA: Confirmatory factor analysis
CFI: Comparative fit index
cGMP: Cyclic guanosine monophosphate
CGRP: Calcitonin gene-related peptide
CI: Confidence interval
CLLS: Children's Lake Louise Score
CMDS: Classical multi-dimensional scaling
Corr: Correlation
DBP: Diastolic blood pressure
dbSNP: SNP database
DL: Dizziness/ lightheadedness
DNA: Deoxyribonucleic acid
DZ: Dizygotic
EFA: Exploratory factor analysis
eNOS: Endothelial nitric oxide synthase
ERS: European respiratory society
ESQ: Environmental Symptom Questionnaire
FAD: Flavin adenine dinucleotide
FDR: False discovery rate
FMN: Flavin mononucleotide
FN: False negative
FP: False positive
FPR: False positive rate
FW: Fatigue / weakness
GI: Gastrointestinal
GLIDERS: Genome-wide linkage disequilibrium repository and search engine
GWAS: Genome-wide association study
HA: Headache
HAPE: High-altitude pulmonary edema
HACE: High-altitude cerebral edema
HH: Hypobaric hypoxia

HR: Heart rate
HS: Hackett's score
HVR: Hypoxic ventilatory response
HWE: Hardy-Weinberg Equilibrium
H1: First hypoxic exposure
H2: Second hypoxic exposure
ICC: Intraclass correlation
ICP: Intracranial pressure
iNOS: Inducible nitric oxide synthase
LLS: Lake Louise Score
LLSQ: Lake Louise Score Questionnaire
L-NMMA: L-NG-Monomethylarginine
M: Mean
MADA: Meta analysis of diagnostic accuracy
MAF: Mean allele frequency
MAP: Mean arterial pressure
MZ: Monozygotic
N (sens): Sensitivity
NA: Not applicable
NADPH: Nicotinamide adenine dinucleotide phosphate
NCBI: National Center for Biotechnology Information
NH: Normobaric hypoxia
nNOS: Neuronal nitric oxide synthase
NOS: Nitric oxide synthase
NR: Not reported
NSAID: Non-steroidal anti-inflamatory drugs
ONSD: Optic nerve sheath diameter
P (spec): Specificity
PAP: Pulmonary artery pressure
PVR: Pulmonary vascular resistance
QUADAS-2: Quality assessment of diagnostic accuracy studies
Ref.: Reference
RER: Respiratory exchange ratio
RMSEA: Root mean square square error of approximation
RNase: Ribonuclease
ROC: Receiver operator characteristic
RR: Risk ratio
SB: Single breath
SBP: Systolic blood pressure
SD: Standard deviation
SE: Standard error
SENS: Sensitivity
sGC: Soluble guanylate cyclase
SH: Sham exposure
SMD: Standardized mean difference
SNP: Single nucleotide polymorphism

SQ: Sleep quality
SSQ: Study-specific questionnaire
Stat.: Statistic
TB: Tidal breathing
TLI: Tucker Lewis index
TN: True negative
TP: True positive
UBC: University of British Columbia
WLSMV: Weighted least squares mean variance

Acknowledgements

A PhD dissertation is a lot of work, and I have a tremendous amount of gratitude for the many people who assisted me along the way. Many colleagues, friends, family members, subjects, and funders made my research possible. I would like to express my gratitude here so that readers will know how much help I had, but I owe all of these individuals and agencies much more than a written "thank you."

My interest in molecular biology was fostered during my undergraduate degree. I was lucky to spend time working under the supervision of Drs. Michael Tanchak (Cape Breton University), Illimar Altosaar (University of Ottawa), and Tim Rawlings (Cape Breton University), all of whom provided excellent mentoring and encouragement. While I chose a field different from theirs, they prepared me well for a career in research.

Several UBC undergraduate students worked with me on projects in our laboratory. Brendan Boere, Samuel Ko, Sally Lin, Jenny Strong, and Sean Nugent each conducted research projects (KIN 499 or BIOL 490) with me, and Radha Jain volunteered to help without receiving course credit. I am grateful to these students for their enthusiasm and assistance with data collection and data analysis. These students are acknowledged for their specific contributions in the preface. Sean Nugent also joined the lab group for a summer as an NSERC Undergraduate Student Research Award recipient. He led the way with some side projects (that are not a part of my thesis) for which I am very grateful.

My time at the University of British Columbia (UBC) has allowed me to connect with many great people. I received guidance and support from a number of senior PhD students, including Cynthia Thomson, Pei Wang, Meaghan MacNutt, and Luisa Giles. I also received technical support from the postdoctoral fellows in our research group, Drs. Shawnda Lanting and Irina Manokhina. Furthermore, some of the projects I dreamed up were complicated, and the statistics were initially outside of my skill set. Dr. Keith Lohse and Tavinder Ark helped me perform the correct and necessary statistics to answer the questions I posed, and their assistance was exceptional. Dr. Carolyn Brown provided helpful support with the genetic aspects of my research, and I am thankful for her help.

A number of labmates made significant contributions to my dissertation. Eric Carter was my roommate for a year and a collaborator for many of my dissertation projects. He provided irreplaceable assistance in Nepal, contributed to numerous projects in the lab, and helped me get

the most out of several scientific conferences. (*N.b.*, I still hold him responsible for stranding us on the Ice Fields Parkway on our 2011 trip to the Hypoxia Symposium in Lake Louise, AB). Normand Richard helped fuel my coffee addiction, kept me humble while cycling, and leant his hypoxia expertise to some early drafts of my thesis. Kristin MacLeod proofread my papers, helped me gain weight with her baking after I lost a few kg in Nepal, and provided moral support. Sarah Koch is the nicest person with whom I have had the chance to work. She helped keep me sane, fit, and upbeat over the years we worked together. Finally, I overlapped briefly with Sean Sinden and Maha Elashi, but they helped make our lab a fun work environment and helped me when I needed it the most.

I was lucky to conduct two research projects in the spectacular Nepalese Himalaya. Organizing and carrying out international research is difficult to say the least, and I would have come home with little data (if I came home at all) without the aid of many people. On the 2010 expedition, Dr. Michael Koehle led the trip, and I tagged along. We had a great team of Nepali medical students assisting us: Ashish Lohani, Smith Giri, Sagar Koirala, Sushil Pant, and Sagar Panthi. We also received logistical and field support from Dr. Buddha Basnyat, Bhuwan Acharya and Gobi Bashyal. I learned a lot about traveling, fieldwork, and life on this trip, and I knew I would come back. In 2012, I led my own research expedition to our field site. I brought a crack team with me (Eric Carter, Michael Freeman, and Nadia Widmer), and an exceptional team of Nepali medical students joined our team in country (Ashmita Siwakoti, Ankita Subedi, Bidur Pandit, and Utsav Timalsina). We received tremendous logistical support from Dr. Kamal Thapa, Dr. Ashish Lohani, Bhuwan Acharya, and Gobi Bashyal. Together we pulled off a huge project of which I am genuinely proud. I would not have been able to get data for my thesis without all of these individuals. Specifically, Michael Freeman, Ashmita Siwakoti, and Kamal Thapa were intrumental in obtaining ethical approval, Eric Carter risked his health to increase our sample size, and Nadia Widmer kept me alive and (reasonably) well when I was was feeling crappy. Lastly, I owe much gratitude to the Nepalese pilgrims who decided to take part in our studies and to the local government for allowing us to collect data in their region.

While the projects in the Nepal were the most demanding logistically, the project described in Chapter 4 was the most gruelling. I gave up sleeping for many nights to pull off this overnight study in our laboratory, but this study would not have been possible without Sarah Koch, Kristin MacLeod, and Eric Carter each suffering through some sleepless nights

themselves. The lab, which is reasonably warm and comfortable during the day, is surprisingly inhospitable at night: the cold air, the unexpected overnight visitors, and the lack of comfortable furniture made the hours between 10 PM and 7 AM pass extremely slowly. Each of these individuals contributed significantly to this project, and I am grateful for their help.

In 2012, I was granted a Michael Smith Foreign Study Supplement grant to spend 4 months abroad as a visiting scientist. With short notice, Dr. Kenny Baillie welcomed me into his laboratory at the Roslin Institute in the autumn of 2013, and he provided a great opportunity for me to gain hands-on genomics experience in a world-class institute. My association with Dr. Baillie also led me to collaborate with a talented group of undergraduate students who are organizing and leading Apex 4, a high-altitude research expedition to Bolivia in June 2014. Time spent with Shona Main, Sandy Jackson, Alistair Rocke, Calum Stannett, and Georgie Barber kept me busy and also provided opportunities to lend my expertise to a promising and exciting research project. I am thrilled to have had the chance to be part of their research team.

Having sufficient funding for my dissertation made my life a lot easier and allowed me to focus my time on research. The majority of my funding came from the Natural Science and Engineering Research Council of Canada (NSERC). I was the recipient of an NSERC Canada Graduate Scholarship (CGS) – Master's, an NSERC CGS – Doctoral, and an NSERC Michael Smith Foreign Study Supplement, and I am extremely grateful for the support of NSERC. I also received funding from UBC (UBC Affiliated Fellowship, UBC Four Year Fellowship, and a Graduate Entrance Scholarship), the UBC Faculty of Education (Doug and Ellen Poulter Scholarship, Dean of Education Scholarship, Mary Elizabeth Simpson Scholarship, two Graduate Student Research Grants, and a Travel Award), the UBC School of Kinesiology (Graduate Student Initiative Tutition Award and a Travel Award), and the UBC Faculty of Graduate Studies (Travel Award).

Staff in the UBC School of Kinesiology helped keep my paper work and finances in order. While the entire team in the administration office was helpful, I would specifically like to thank Kyla Hicks, Helen Luk, Kathy Manson, and Ayaz Mahmood. When I came back from Nepal with 'receipts' scribbled on scraps of paper in Nepali, they were more than understanding and incredibly helpful. Kyla Hicks cheerful emails always put a smile on my face. Furthermore, the support of the School Director, Dr. Bob Sparks, meant a lot to me.

My committee consisted of Drs. Trish Schulte, Bill Sheel, and my supervisor, Dr. Michael Koehle. They kept me on track throughout my dissertation, challenged my ideas, and taught me a lot about research. The varied specialties of my committee members allowed me to follow my nose and investigate numerous aspects of acute mountain sickness. Michael Koehle went above and beyond for a supervisor: he assisted tremendously with the planning of the Nepal expeditions, provided funding for some of my projects, imparted a tremendous amount of wisdom related to the clinical aspects of acute mountain sickness, and was always free to chat when I had an idea or a question. He provided tremendous help anytime I needed it, and I am grateful for the opportunity to join his laboratory.

My family and friends have put up with a lot during my dissertation. Not only did I leave home to pursue my PhD, I also happened to choose a university 6000 km away from home. As a result, I was not able to spend as much time as I would have liked with my parents, brother, sister, and friends from home while I was at UBC; however, they always understood, and they supported me. I appreciated their phone calls, visits, and care packages. Even if they did not understand exactly what I was doing, they recognized that it was important to me, and they helped me more than they know.

Finally, my wife, Katie, has had the greatest impact on me during my dissertation. When I left Nova Scotia, I also left Katie behind while she finished her Chartered Accounting program. Without any hesitation, she moved across the country 2 years later. Katie did not complain when I worked late, slept in the laboratory, or left the country to do my research. She never showed much duress or impatience about the uncertainties that come with an academic career, and I appreciated this a lot. Katie helped me relax after a stressful day: she was my running partner, study date, and best friend. Her work ethic and attitude helped me keep working hard when I lost motivation, and for this I owe her a lot.

For Katie,

my inspiration and motivation.

Chapter 1 Introduction

1.1 Why study human responses to altitude?

With its highest peak only just surpassing 500 m, Cape Breton, Nova Scotia is hardly an incubator for high-altitude scientists. The "mountains" in the Cape Breton Highlands have little in common with any of the world's great mountain ranges (dwarfed even by their Scottish counterparts). Ascending the major climbs of the Cabot Trail (North Mountain [445 m], French Mountain [455 m], and Smokey Mountian [366 m]) may be difficult, but cyclists do not have the excuse of altitude to explain their struggles. Local waterfalls, such as Uisge Ban Falls and North River Falls, seem high until you travel amongst the Himalaya in monsoon season, where waterfalls are rated on their potential to wash away the land beneath your feet. Yet, in spite of a youth spent near sea level, altitude has always fascinated me.

My first introduction to the science of high altitude (and exercise physiology for that matter) was through *Daniels' Running Formula* (Daniels 2005), a training manual for runners. I used this book as a guide while coaching cross-country running in Coxheath, Nova Scotia during my undergraduate degree. I still use the book as a guide for my running workouts. One sentence stands out as particularly relevant to my research now: referring to altitude training, Daniels writes, "Not all endurance runners thrive at altitude. Some benefit little, and others have significant breakthroughs." While I likely forgot this sentence shortly after reading it, this physiological variation in response to altitude came to be the theme of my PhD research (although I chose to study the variability in altitude tolerance, not altitude training).

The hypoxia of high altitude is an unrelenting stress that is unparalleled by other environmental stressors. Heat, cold, and UV radiation can significantly impact one's health and activities, but they are not usually constant (i.e. they tend to vary diurnally and seasonally), and the severity of each of these stressors can also be partially mitigated with relatively simple technology (*e.g.* clothing, sunglasses, sunblock, air conditioning) and behavioral adjustments (*e.g.* seeking shade, working at night). Aside from minor fluctuations with barometric pressure, the stress of hypoxia is essentially fixed at a given location. You cannot wait until night for the hypoxia to go away; you cannot take shelter inside to protect your brain; you cannot change your clothing to increase oxygen saturation in your blood. Bottled supplemental oxygen can reduce the stress of hypoxia, but it is generally reserved for mountaineers approaching the death zone (>8000 m) or medical emergencies. This is not to say that other environmental stressors are

minor; the opposite is certainly true. The point I wish to make is that the hypoxic stress of high altitude is different. Ascend to the summit of Mount Everest (8848 m) anytime in your life, and you will experience approximately the same degree of hypoxia that Messner and Habeler overcame in 1978 on the first summit of Mount Everest without supplemental oxygen. Also consider that, in addition to hypoxia, other environmental stressors (namely cold and UV radiation) are intrinsic to high-altitude environments, making existence at high altitude, even if only brief, supremely challenging.

High altitude is so stressful because humans need oxygen, and oxygen is less available at high altitude. Yet, humans travel to and live at high altitude. Acclimatisation and adaptation to altitude are responsible for our tolerance of altitude in the short term and long term, respectively: while the low oxygen availability of any given altitude is constant, the physiological stress it imposes on humans is mitigated by physiological changes. Humans may not function as well at altitude as they do at sea level, but with time, they function better. What makes high-altitude biology so fascinating is that humans do not acclimatise equally well, and the reasons for this differential ability to acclimatise are not clear: is it physiological, anatomical, genetic, psychological or something else? On the same ascent to high altitude, some humans adjust well and others need to descend to stay alive, but why? This question was the focal point of my PhD dissertation.

1.2 The high-altitude environment

1.2.1 Barometric pressure and altitude

Barometric pressure (P_B) is the force exerted on a surface by the weight of the atmosphere above that surface. Standard pressure is 760 mmHg, which is approximately the sea level P_B value. The P_B is the main determinant of the oxygen availability in natural settings: based on Dalton's Law, the partial pressure of oxygen (P_{O_2}) is equal to the product of P_B and the fractional concentration of oxygen (F_{O_2}), which is 20.93 % in the physiologically relevant regions of the atmosphere (*i.e.,* below 10 km); therefore, the P_{O_2} at sea level is approximately 160 mmHg (Figure 1.1). The mass of air above any surface on Earth decreases as altitude increases, which results in a lower P_B and a commensurate reduction in P_{O_2} (Figure 1.1). As an example, the P_B on the summit of Mount Everest (8848 m) was measured to be 253 mmHg, which resulted in a

P_{O_2} of approximately 53 mmHg (West 1999), a value that is approximately one-third of the sea-level P_{O_2}.

The partial pressure of inspired oxygen (P_{IO_2}) is lower than the P_{O_2} in the environment (Figure 1.1). The fraction of oxygen in inspired air (F_{IO_2}) is equal to the concentration of oxygen in the atmosphere; however, air becomes humidified upon inhalation, and the fraction of air that is humidified does not contain O_2. The humidification of inhaled air is responsible for the difference between P_{O_2} and P_{IO_2}. At body temperature (37°C), the partial pressure of water vapour (P_{H_2O}) is equal to 47 mmHg (West 2012; Figure 1.1); thus, to calculate the P_{IO_2}, the P_{H_2O} in humidified air at 37°C is subtracted from the P_B prior to multiplying by the F_{IO_2}:

$$\mathbf{P_{IO_2} = F_{IO_2} * (P_B - 47)} \quad \textbf{Equation 1.1}$$

As a result, the P_{IO_2} at sea level (assuming a P_B of 760 mmHg) would be approximately 150 mmHg (10 mmHg below the P_{O_2}; Figure 1.1).

The P_B is also determined by the latitude, season, and prevailing weather of a given location (West et al. 1983; West 1996). Air masses accumulate near the equator because of increased heating from the sun. The relatively greater air masses above the equator increase the P_B at the equator compared to the poles. For the same reason, P_B is lower in the winter than the summer in seasonal areas. Finally, meteorological events are associated with fluctuations in the P_B, with high and low pressure systems increasing and decreasing the P_B, respectively. The effects of these variables on the P_B have three main practical applications in high-altitude physiology: (i) the P_B on mountains near the equator is greater than the P_B of equivalently high mountains nearer to the poles; (ii) the P_B on mountains in seasonal areas (*i.e.,* away from the equator) is greater in the summer than the winter; and (iii) the P_B of a site will change depending on the prevailing weather, typically being lower with precipitation and higher with dry weather. As shown above, a greater P_B results in a greater P_{O_2}.

Despite the many factors affecting the P_B, at a given altitude it can be estimated with the following equation:

$$\mathbf{P_B = EXP\ (6.63268 - 0.1112\ a - 0.00149\ a^2)} \quad \textbf{Equation 1.2}$$

where a is the altitude in km (West 1996). When predictions from this equation were compared to actual field data across a range of altitudes, the differences were typically very small (less than

1% of the P_B). The equation was accurate for locations within 45° of the equator in the summer; however, in the winter, the accuracy of the equation was best for locations within 30° of the equator. Despite this limitation, the equation still covers many of the world's major peaks throughout the year, as many are located near the equator. The equation was still accurate (within 6 mmHg of the measured P_B) above 45° of latitude for altitudes below 5000 m. There are relatively few mountains above 45° latitude exceeding this altitude (*e.g.,* Denali in Alaska), so the equation is considered to be reasonable estimate of high altitude P_B throughout the world (West 1996).

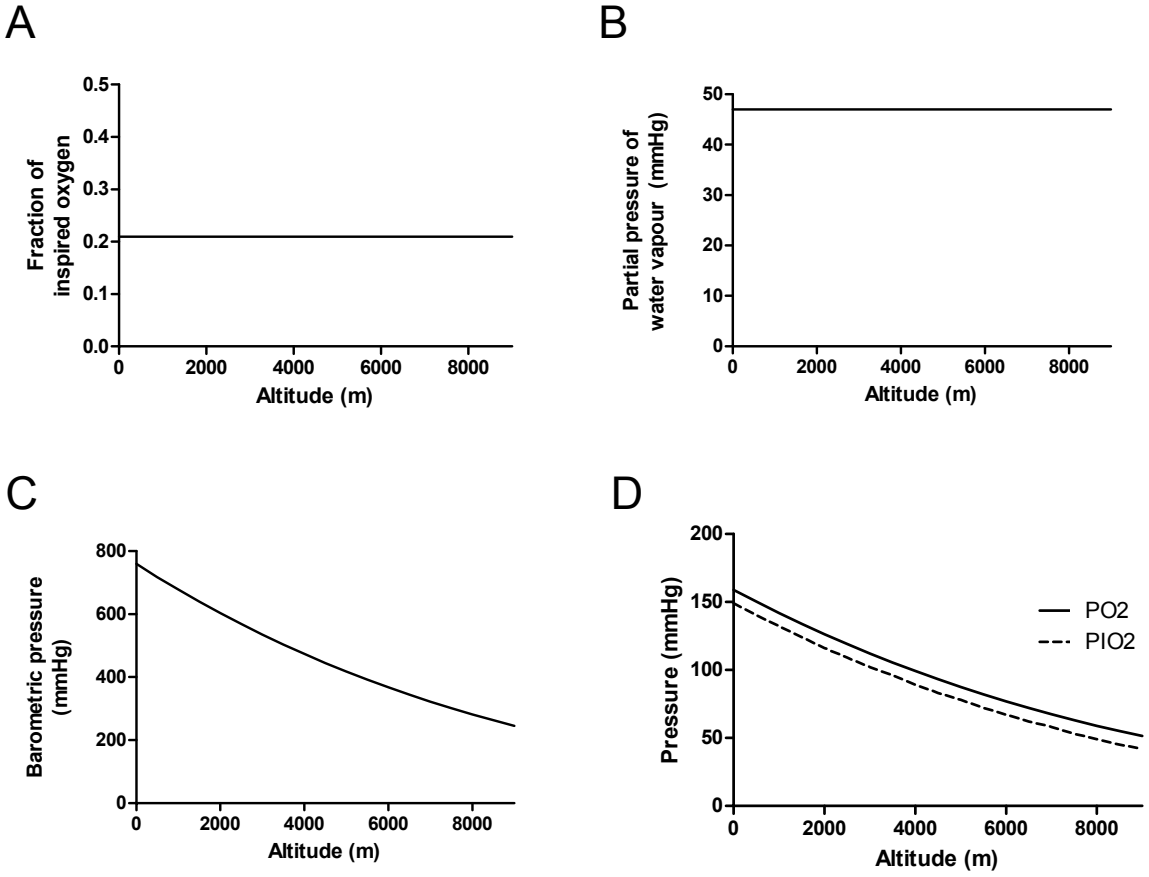

Figure 1.1. The effects of altitude on several physiologically relevant variables. Panels A-D depict the relationships between altitude and (A) the fraction of inspired oxygen (F_{IO_2}), (B) the partial pressure of water vapour (P_{H_2O}), (C) the barometric pressure (P_B), (D) a comparison of the partial pressure of oxygen (P_{O_2}) and the partial pressure of inspired oxygen (P_{IO_2}). The P_B was calculated using the formula provided by (West 1996), and P_{IO_2} was calculated with equations in Section 1.1.1.

1.2.2 Hypobaric and normobaric hypoxia

The physiological stress of high-altitude environments is due mostly to hypoxia, the decreased availability of oxygen (Martin and Windsor 2008). The term hypoxia is technically defined as any P_{IO_2} below that of sea level (*i.e.,* 150 mmHg); however, hypoxia does not necessarily elicit physiological stress, as very small decrements in the P_{IO_2} are likely to have very minor effects, if any, on humans.

There are two important modes of hypoxia: hypobaric hypoxia and normobaric hypoxia. Hypobaric hypoxia is a reduction in P_{IO_2} due to a reduction in the P_B. It is the mode of hypoxia associated with high-altitude environments, but it can also be generated artificially by partially evacuating a sealed chamber to lower the P_B of that chamber. Normobaric hypoxia is a reduction in P_{IO_2} due to a reduction in the F_{IO_2}. For normobaric hypoxia, the P_B remains ambient, which necessitates a corresponding increase in the partial pressure of another gas (usually N_2). Normobaric hypoxia can be generated by lowering the F_{IO_2} (usually through the addition of N_2 or oxygen-depleted air) in a partially sealed chamber. A comparison of the F_{IO_2} and the P_B needed to simulate a given P_{IO_2} is provided in Figure 1.2.

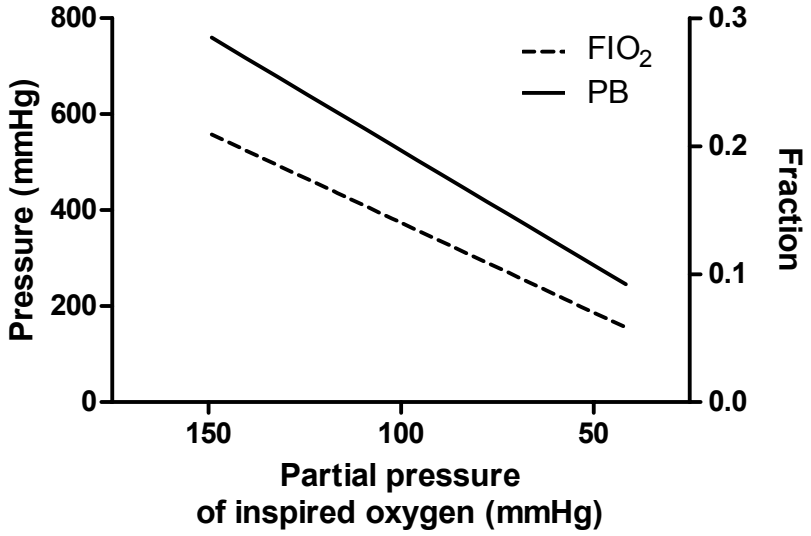

Figure 1.2 A comparison of conditions needed to generate normobaric and hypobaric hypoxia. The barometric pressures (P_B; solid line) and fractions of inspired oxygen (F_{IO_2}; broken line) required to produce a given partial pressure of inspired oxygen (P_{IO_2}) when the other variable is held constant (at an F_{IO_2} of 0.2093 and at a P_B of 760 mmHg, respectively) are shown. The solid line is indicative of hypobaric hypoxia, and the broken line is indicative of normobaric hypoxia. Note that the lines are not parallel because the partial pressure of water vapour has a greater effect on the P_{IO_2} at a lower P_B.

The two modes of hypoxia are capable of simulating the hypoxia of high-altitude environments (*i.e.,* the P_{IO_2}) equally well. Laboratory-generated hypobaric hypoxia is the most realistic substitute for high altitude, as the P_B and F_{IO_2} match the natural conditions of a high-altitude environment. Although hypobaric hypoxia is preferred from a theoretical perspective, it is not ideal in many other ways: hypobaric hypoxia chambers are very expensive, require highly-trained staff, and can harm the occupants if pressure is reduced too rapidly (Singh et al. 2010). Alternatively, simulating altitude with normobaric hypoxia is very common in research settings and commercial activities because it is more convenient (e.g. less technical equipment), safer, and less expensive than doing so with hypobaric hypoxia (Conkin and Wessel 2008; Richard and Koehle 2012). While still potentially dangerous, subject evacuation is easier because the chamber can be opened without needing to recompress to the ambient P_B, and there is no risk of injury due to rapid decompression on "ascent" to the simulated altitude (e.g., decompression sickness; Conkin et al. 2013). In some instances, the two modes of hypoxia are combined in what is known as combined altitude depleted oxygen (CADO). With CADO, the reduction in P_{IO_2} is due to simultaneous decreases in the P_B and the F_{IO_2}. Like hypobaric hypoxia, the generation of CADO requires a sealed chamber; however, CADO is safer for attaining very high altitudes because it minimizes the risks associated with rapid decompression (*e.g.,* decompression sickness and barotraumas; Singh et al. 2010).

The equivalent air altitude model states that any combination of P_B and F_{IO_2} resulting in an equivalent hypoxic P_{IO_2} will produce equivalent physiological responses (Conkin and Wessel 2008). Under this model, for example, exposure to a P_B of 447 mmHg and an F_{IO_2} of 0.2093 (P_{IO_2} = 84 mmHg) should lead to physiological responses equivalent to those observed during exposure to a P_B of 760 mmHg and an F_{IO_2} of 0.118 (P_{IO_2} = 84 mmHg). It is important to note that this model predicts equivalent physiological responses to two conditions having the same P_{IO_2}, not the same P_{O_2}. This technicality is imperative, as equivalent P_{O_2} values can result in nonequivalent P_{IO_2} values (when the P_B differs between the two conditions; Girard et al. 2012). This occurs because the P_{H_2O} represents a greater proportion of the P_B in hypobaric hypoxia than it is in normobaric hypoxia (*e.g.,* 47/447 > 47/760). In the previous example of two isohypoxic conditions, the P_{O_2} would be 94 mmHg for the former and 90 mmHg for the latter.

The equivalent air altitude model implies that the hypobaria of high altitude is irrelevant physiologically and that hypoxia is the sole determinant of physiological responses observed at altitude; however, one cannot travel to altitude without being exposed to hypobaria, and hypobaric exposure can have effects on the body independent of hypoxia (Epstein and Saruta 1972; Loeppky et al. 2005). Thus, hypobaric and normobaric hypoxia might not be completely equivalent, despite their potential to generate an equivalent P_{IO_2} (Conkin and Wessel 2008). For my dissertation, I did not explore the differences between the two modes of hypoxia; however, because I performed experiments in both hypobaric and normobaric hypoxia, their potentially different physiological effects will be discussed later in the introduction.

1.2.3 Categorizing altitudes

Altitude is often classified into discrete categories for convenience. One such classification system divides altitudes into six zones: low altitude (< 1500 m), intermediate altitude (1500 - 2500 m), high altitude (2500 - 3500 m), very high altitude (3500 - 5800 m), extreme altitude (5800 - 8000 m), and the death zone (> 8000 m; Imray et al. 2011). This categorization roughly delineates the signs and symptoms of increasing physiological stresses of altitude on humans. Only minor physiological effects are evident below 1500 m, (e.g. decrements in aerobic exercise performance becoming evident at altitudes as low as 580 m in highly trained athletes (Gore et al. 1997). Above 1500 m, maximum oxygen consumption begins decreasing at a rate of ~1% per 100 m ascended in (untrained) individuals (Fulco et al. 1998). Humans develop altitude illnesses after rapid ascent to altitudes above 2500 m, and the incidence and severity of altitude illnesses increase with ascent to very high altitudes (Maggiorini et al. 1990). Between 2500 m and 5800 m, mental and psychomotor impairments have been observed as well (reviewed in Wilson et al. (2009). The ceiling for altitude acclimatisation is thought to be just below 6000 m, as prolonged stays above this altitude are not thought to be possible (Imray et al. 2010; Imray et al. 2011). The highest permanent settlement in the world is at 5100 m (La Rinconada, Peru), although a temporary mining settlement at 5950 m existed for over 2 years at Aucanquilcha, Chile (West 1999; West 2002). Finally, any travel above 8000 m requires extensive acclimatisation at lower altitudes, and even after many weeks spent above 2500 m, the duration of time one can survive above 8000 m (without using supplementary oxygen) is severely restricted, justifying the name “death zone.”

1.2.4 Human travel to altitude

Millions of low-altitude residents travel to altitudes of 2500 m or higher each year (e.g. (Burtscher 2004; Gallagher and Hackett 2004; Richalet et al. 2012). Reasons for ascending to altitude include (i) professional obligations such as mining (Richalet et al. 2002; West 2012), military operations (West et al. 1983; West 1996; Rodway and Muza 2011), and astronomy (Forster 1984a); (ii) spiritual experiences (*e.g.,* pilgrimages to sites such as Gosainkunda (Basnyat et al. 2000) and Mount Kailash (Basnyat 2013)); and (iii) recreational activities such as skiing (Luks and Swenson 2007; Hatzenbuehler et al. 2009), hiking (Richalet et al. 2012), and mountaineering (McIntosh et al. 2008).

Humans are facultative aerobes, requiring continuous supplies of oxygen to survive. Low-altitude residents are accustomed to a normoxic or nearly normoxic environment (*i.e.,* a P_{IO_2} that is ~150 mmHg) and, upon exposure to environmental hypoxia, their demands for oxygen remain (or in some cases increase to support the changes described below; Westerterp 2001); therefore, physiological accommodations are necessary to provide sufficient oxygen to body tissues if humans are to function well in hypoxic conditions. To appreciate the physiological changes that occur in humans upon acute exposure to altitude/hypoxia, it is important to first understand the basic principles of oxygen delivery under normoxic conditions. The following section will discuss the transport of oxygen from the environment to the cells in humans.

1.3 Oxygen delivery in humans in normoxic conditions

1.3.1 Convection and diffusion

A complex series of convective and diffusive steps, involving several organs and systems, is needed to bring oxygen from the environment to the cells (Figure 1.3). The convection of oxygen is an active process, requiring energy to generate flow; the diffusion of oxygen is a passive process, requiring a concentration gradient for oxygen to flow (from areas of high concentration to areas of low concentration; Leach and Treacher 1998). Although diffusive transport may not require energy directly, the establishment of the gradient (*i.e.,* between the alveolar space and blood) may have some energetic cost. Oxygen delivery can be subdivided based on the systems in which the steps primarily occur: (i) convection and diffusion in the respiratory system and (ii) convection and diffusion in the cardiovascular system; however,

interactions between the two systems cannot be ignored when describing the physiology of oxygen uptake and delivery.

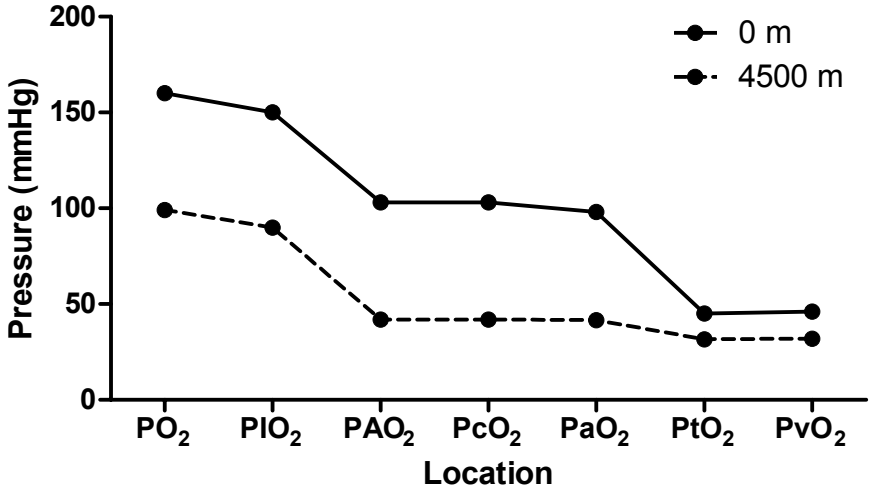

Figure 1.3 A schematic of the oxygen cascade in a human at 0 m and 4500 m. The partial pressure of oxygen is given for the atmosphere (P_{O_2}), inspired gas (P_{IO_2}), alveolar gas (P_{AO_2}), pulmonary capillary blood (P_{cO_2}), arterial blood (P_{aO_2}), tissue (P_{tO_2}), and mixed venous blood (P_{vO_2}). The values at each location were calculated from resting data using an online tool provided by http://www.prognosis.org/physiology/.

1.3.2 Oxygen transport in the respiratory system

Oxygen delivery begins in the lungs, which act as an interface between the body and the environment. Expansion of the chest lowers the pressure in the lungs relative to the external environment and, through convective transport, air from the environment is inspired into the lungs. The partial pressure of oxygen in the alveolar space (P_{AO_2}) is approximated by the equation:

$$P_{AO_2} = P_{IO_2} - P_{ACO_2} / RER \quad \text{Equation 1.3}$$

where P_{ACO_2} is the partial pressure of CO_2 in the alveoli, and RER is the respiratory exchange ratio, which can be defined as:

$$RER = V_{CO_2} / V_{O_2} \quad \text{Equation 1.4}$$

where V_{CO_2} is the volume of carbon dioxide exhaled and V_{O_2} is the volume of oxygen inhaled. Because the P_{AO_2} is greater than the P_{O_2} in the pulmonary arterial blood (P_{cO_2}), oxygen diffuses from the alveolar space, across the alveolar capillary membrane, into the blood.

Successful diffusion of oxygen from the alveolar space to the blood is dependent on sufficient oxygen driving pressure from the alveolar space, a large and thin membrane-capillary

surface area, the strong oxygen affinity of hemoglobin, and a sufficient red blood cell transit time (*i.e.,* adequate time for oxygenation to occur; Sheel et al. 2010). The rate of oxygen diffusion in the lungs (D_{LO_2}) is defined by the equation:

$$\mathbf{D_{LO_2} = V_{O_2} / (P_{AO_2} - P_{vO_2})} \quad \textbf{Equation 1.5}$$

where P_{vO_2} is the P_{O_2} in venous blood.

Once across the blood-gas interface of the alveolar wall, oxygen dissolves in the blood, and most oxygen molecules bind to hemoglobin (some oxygen [~0.3 mL/dL at sea level] is transported dissolved in solution). The content of oxygen in the arterial blood (C_{aO_2}) is determined by the concentration of hemoglobin (Hb), the oxygen saturation of the blood (S_{aO_2}), and the partial pressure of oxygen in the arterial blood (P_{aO_2}), as described by the following equation:

$$\mathbf{C_{aO_2} = 1.36 * [Hb] * S_{aO_2} + 0.003\ P_{aO_2}} \quad \textbf{Equation 1.6}$$

The first term of this equation represents the content of blood bound to hemoglobin, and the second term, which is a very small fraction of the C_{aO_2}, represents the content of oxygen dissolved in the blood. The S_{aO_2} is dependent on the P_{aO_2}, as shown by the oxygen dissociation curve in Figure 1.4.

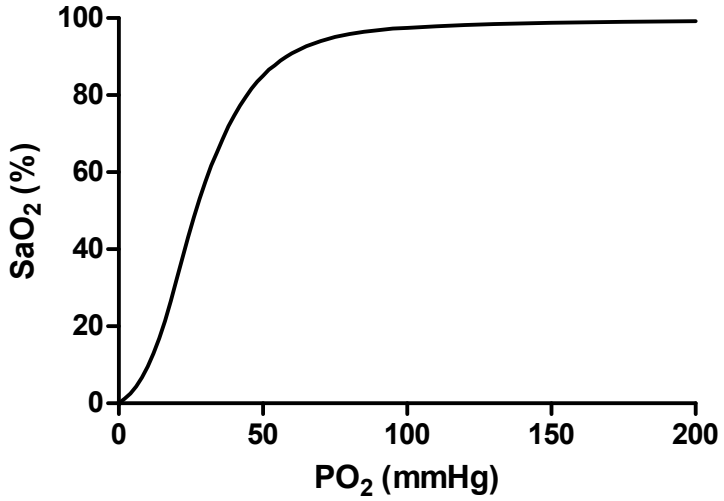

Figure 1.4 A schmatic of the oxygen-dissociation curve. The figure depicts the relationship between the oxygen saturation of blood and the partial pressure of oxygen in the blood. Data shown is from Severinghaus (1979), with a pH of 7.4 and at a temperature of 37ºC.

1.3.3 Oxygen transport in the cardiovascular system

The heart pumps oxygen-depleted blood returning from the systemic circulation to the lungs before pumping the oxygenated blood throughout the body to the tissues requiring oxygen. The pumping of blood is a form of convective transport. The amount of oxygen pumped (or the oxygen delivery, D_{O_2}) through the circulatory system depends directly on the cardiac output (Q; the amount of blood that is pumped out of the left side of the heart) and the C_{aO_2}, as shown in this equation:

$$\mathbf{D_{O_2} = Q * C_{aO_2}} \quad \textbf{Equation 1.7}$$

As blood travels from arteries through capillaries, oxygen diffuses from the blood to the tissues because of a lower P_{O_2} in the tissues than in the blood. The rate of diffusion is dependent on the concentration gradient and the diffusion distance (Leach and Treacher 1998). Oxygen eventually reaches the mitochondria of the cells, where it is used as an electron donor in the generation of adenosine triphosphate (ATP) during aerobic cellular respiration. The volume of oxygen extracted from the blood (*i.e.,* V_{O_2}), is equal to the difference between the C_{aO_2} and the venous oxygen content (C_{vO_2}) multiplied by the cardiac output:

$$\mathbf{V_{O_2} = Q * (C_{aO_2} - C_{vO_2})} \quad \textbf{Equation 1.8}$$

The delivery of oxygen is enhanced by decreasing the affinity of Hb for oxygen: increased temperatures, increased concentrations of 2,3-diphosphoglycerate (2,3-DPG), and decreased pH (*i.e.,* more acidic) shift the oxygen-dissociation curve to the left (Leach and Treacher 1998). See Figure 1.4. Similarly, the affinity of Hb for oxygen is increased (and oxygen uptake at the lungs is enhanced) when any of these parameters moves in the opposite direction (*i.e.,* decreased temperature, decreased 2,3-DPG, and increased pH each shift the oxygen dissociation curve to the right).

1.3.4 Interrelationships between the respiratory and cardiovascular systems

The respiratory and cardiovascular systems are highly interconnected. Efficient gas exchange in the lungs is dependent on the matching of alveolar ventilation (V_A) and perfusion (Q) throughout the lungs. A V_A / Q mismatch (*e.g.,* relatively low ventilation in an area of relatively high perfusion or vice versa) would impair gas exchange and lead to hypoxemia (low blood oxygen content; Treacher and Leach 1998). Similarly, pulmonary diffusion limitation

could lead to hypoxemia. The difference in P_{AO_2} and P_{aO_2} (the alveolar-arterial gradient, A-aP_{O_2}) due to diffusion limitation can be calculated from the equation:

$$\mathbf{A\text{-}aP_{O_2} = (P_{AO_2} - P_{vO_2}) * EXP\ (-D_{LO_2}/\ ß\ *Q)\ Equation\ 1.9}$$

where, ß is the average slope of the O_2-disassociation curve between systemic arterial and mixed venous values (*i.e.* $[C_{aO_2} - C_{vO_2}]/[P_{aO_2} - P_{vO_2}]$), and Q is pulmonary blood flow (which is equal to cardiac output; Wagner 2010). From this equation, it is evident that the A-aP_{O_2} increases (*i.e.*, gas exchange is impaired) when P_{AO_2} decreases, P_{vO_2} decreases, ß increases, Q increases, or D_{LO_2} decreases. Pulmonary diffusion does not limit oxygen transport in resting healthy humans at sea level, as capillary blood is fully oxygenated at one-third of the transit distance through the lung (Treacher and Leach 1998); however, it can be potentially limited under certain circumstances such as during exercise or ascent to altitude.

1.4 Acclimatisation to hypoxia

1.4.1 Why is acclimatisation to hypoxia necessary?

Without physiological adjustments, decreases in the P_{IO_2}, whether due to hypobaric or normobaric hypoxia, would lead to decreases in the P_{aO_2}, impairing the diffusion of oxygen to the tissues of the body and restricting oxygen availability. Furthermore, without any physiological adjustments, the maintenance of sea level physiological processes dependent on O_2 would not be possible in hypoxia. In turn, the maximum altitudes to which humans could ascend (without supplemental O_2) would be greatly reduced, restricting both sojourns to, and the settlement of, high-altitude regions.

1.4.2 The process of acclimatisation

With respect to high-altitude biology, acclimatisation refers to any transient and beneficial physiological adjustment to hypoxic stress. Acclimatisation to hypoxia occurs through a suite of acute and chronic molecular, cellular, tissue, and systemic responses that increase oxygen supply in the body (Sarkar et al. 2003). Of these adjustments, the systemic responses are the best understood and the most apparent, although, these systemic responses are ultimately manifestations of multiple and interacting molecular, cellular, and tissue responses. Acclimatisation increases the availability of oxygen to the cells of the body relative to that of the

initial arrival.

The time required for acclimatisation depends on the physiological variable in question, making a definitive conclusion on the timeline of acclimatisation difficult (Imray et al. 2011). The timeline for acclimatisation to hypoxia also depends on the altitude to which an individual is exposed and the unknown individual characteristics. More time is required to acclimatise to higher altitudes, but the time to acclimatise to a given altitude can be reduced by first acclimatising to a lower altitude (generally >1500 m; Muza et al. 2010; Staab et al. 2013). On induction to a higher altitude, some individuals will still acclimatise quicker than other individuals with the same extent of pre-acclimatisation (Muza et al. 2010).

Acclimatisation cannot completely compensate for the restriction in oxygen availability regardless of the duration of the exposure. Thus, acclimatisation only dampens the effects of hypoxia; it does not abolish the effects of hypoxia. The following section highlights some of the physiological responses to hypoxia, the majority of which are involved in the process of acclimatisation, as they function to increase oxygen availability to the tissues of the body.

1.5 Physiological responses to hypoxia

1.5.1 Humans in hypoxia

As my dissertation was entirely focused on humans, and in the interest of keeping the introduction suitably brief, only data from human studies will be discussed in this section. Furthermore, because acute exposures to altitudes above 5000 m are very difficult to tolerate (*i.e.,* humans are likely to become unconscious with rapid ascent to altitudes above 5500 m; Wilson et al. 2009) the following sections will focus on the physiological responses to acute altitude exposures below 5000 m (or the equivalent of 5000 m for simulated altitude). Similar responses (but of a greater magnitude) are likely to occur at higher altitudes.

1.5.2 Respiratory responses to hypoxia

Immediately upon exposure to hypoxia, the decrement in P_{IO_2} leads to a decrease in the P_{AO_2}. The decrease in P_{AO_2} leads to an almost immediate decrease in the P_{aO_2} and the S_{aO_2} (*i.e.,* hypoxemia develops). Peripheral chemoreceptors in the carotid bodies sense the hypoxemia (reviewed in Weir et al. 2005) and signal the respiratory centres in the medulla to increase minute ventilation (V_E). The increase in V_E due to hypoxia is known as the hypoxic ventilatory

response (HVR), and it is a measure of the carotid body chemoreflex sensitivity (Duffin 1990). With an increased V_E, the P_{ACO_2} decreases and the P_{AO_2} is elevated (see Equation 1.3).

Increased V_E raises P_{aO_2} and S_{aO_2}, but the hypocapnia associated with increased V_E leads to alkalosis in the blood, which limits the extent to which V_E will increase acutely (reviewed in Martin et al. 2010). Even in isocapnic conditions, where CO_2 is held constant, V_E still decreases after the initial rise in response to hypoxia, meaning that the decrease in V_E is not caused exclusively by the hypocapnia; this decrease is termed the hypoxic ventilator decline (Powell 1998). The alkalosis that develops as a result of increased V_E shifts the oxygen-dissociation curve to the left, raising the S_{aO_2} for a given P_{aO_2} (Sheel et al. 2010). The decrease in [H+] (*i.e.,* increase in pH) also triggers renal bicarbonate excretion to compensate for the alkalosis (Goldfarb-Rumyantzev and Alper 2014). With continued exposure to hypoxia, one's sensitivity to CO_2 increases (*i.e.,* V_E is greater for a given P_{aCO_2}), allowing V_E to increase again despite the lowered P_{aCO_2} (Martin et al. 2010). During continued exposure to hypoxia, one's V_E gradually increases over several days or weeks until finally reaching a plateau (Powell 1998).

In response to the alveolar hypoxia, pulmonary vessels constrict (Hambraeus-Jonzon et al. 1997; Imray et al. 2011) leading to increases in pulmonary vascular resistance (PVR) and consequently increases in pulmonary artery pressure (PAP). While potentially beneficial in disease states and following trauma, hypoxic pulmonary vasoconstriction may be maladaptive at altitude because of the generalized hypoxia (Grover et al. 1986; Bärtsch and Gibbs 2007). Specifically, the increase in PAP likely impairs oxygen diffusion by shunting blood from the alveolar capillaries (Lovering et al. 2008) and by causing leakage of fluid into the lung that increases the diffusion distance for oxygen to travel (Maggiorini et al. 2001). The PAP increases in proportion to the altitude (Groves et al. 1987), and increased PAP persists for generations at altitude (Bärtsch and Gibbs 2007). In the systemic vasculature, hypoxia has the opposite effect, triggering the vasodilation of arterioles, which increases blood supply in proportion to metabolic demands.

1.5.3 Cardiovascular responses to hypoxia

Upon exposure to hypoxia, oxygen delivery is impaired for a given cardiac output due to the reduction in C_{aO_2}. Acutely, at rest in hypoxia, heart rate is elevated due to increased

sympathetic activity and vagal withdrawal (Koller et al. 1988), leading to immediate increases in cardiac output because stroke volume is unchanged (Naeije et al. 1982). While the immediate increase in heart rate (within minutes) increases the cardiac output, there is a gradual decrease in stroke volume upon exposure to altitude (due to plasma volume reduction) that returns cardiac output to baseline values after approximately 3 days (Klausen 1966). The reduction in plasma volume is partially due to diuresis (see below) as well as decreased water intake and increased water loss from ventilation and perspiration (reviewed in Naeije 2010); however, preventing dehydration does not prevent the plasma volume reduction (Sawka et al. 1996). Over the first few weeks at 3000 m, heart rate remains elevated and stroke volume remains depressed, resulting in a slightly lower cardiac output relative to sea level (Klausen 1966), although some studies report that cardiac output was similar at high altitude and sea level at rest (Banchero et al. 1966; Vogel et al. 1974).

The increased sympathetic activity associated with hypoxia should, in theory, also trigger an increase in systemic blood pressure (Wolfel et al. 1994); however, this vasoconstriction is antagonized by the direct vasodilatory effects of hypoxemia. As a result, systemic blood pressure may be slightly increased or unaffected upon acute exposure to hypoxia (reviewed by Luks 2009; Naeije 2010).

1.5.4 Hematological responses to hypoxia

Hemoglobin concentration is increased at altitude, acutely due to reductions in plasma volume and chronically (more than 3 weeks at altitudes below 4000 m; Sawka et al. 2000) due to increased red blood cell production (*i.e.,* erythropoiesis). In the first few hours of altitude/hypoxia exposure, the general hypoxic diuretic response decreases total body water (Jain et al. 1980) and plasma volume (Stäubli et al. 1986). For example, an increase of 0.5-1.0 g of hemoglobin per 100 mL of blood was observed in the first two days at moderate altitude (1500-2000 m), which was accounted for by a 200-300 mL decrease in the overall plasma volume (Bärtsch and Saltin 2008). Diuresis is due partly to increased sodium and water excretion, and urine volume and sodium output in hypoxia positively correlated with the hypoxic ventilatory response (Swenson et al. 1995). Whether or not this response is completely beneficial is debatable: diuresis increases hemoglobin concentration and reduces the volume load on the brain and the lungs, but it may lead to hyperviscosity of the blood and potentially to thrombosis

(Martin et al. 2009; Goldfarb-Rumyantzev and Alper 2014). With greater durations of hypoxic exposure (*i.e.,* weeks), erythrocyte volume increases (Sawka et al. 2000; Jacobs et al. 2012), leading to further increases in hemoglobin mass and hemoglobin concentration (Reynafarje et al. 1959).

Regardless of the mechanism, the greater concentration of hemoglobin in the blood raises the C_{aO_2} of the blood. The C_{aO_2} can return to and even surpass (with extended stays) its sea level value; however, it is important to note that oxygen delivery is the volume of oxygen that is pumped through the system, and simply transporting a volume of oxygen throughout the body is not sufficient to ensure the arrival of oxygen at the mitochondria of cells: impaired diffusion due to the relatively low P_{aO_2} developing at high altitude limits oxygen from reaching the mitochondria. This fact is emphasized by those studies (e.g. Young et al. 1996) showing that exogenous increases in erythrocyte volume do not improve exercise capacity at high altitude despite increasing C_{aO_2}.

1.6 Altitude tolerance

1.6.1 Acute mountain sickness

At the organismal level, the purpose of the physiological responses to hypoxia is to allow humans to survive and function well in hypoxic conditions. While humans can acclimatise to altitudes of almost 6000 m, complete acclimatisation requires days or weeks depending on the system. If acclimatisation is insufficient (*i.e.,* due to a rapid ascent or exposure to an extreme altitude), hypoxia will have detrimental effects on the health of humans.

Just as full acclimatisation is difficult to define because of the number of systems affected by hypoxia, the measurement of hypoxia tolerance is very difficult. In addition to the individual systems affected by hypoxia, insufficient hypoxia acclimatisation leads to numerous global (*i.e.,* functional) effects. The study of hypoxia/altitude tolerance necessitates the quantification of subjects' tolerances of hypoxia/altitude. Many variables could plausibly be used as a marker of hypoxia tolerance, both physiological variables (*e.g.,* P_{aO_2}, oxygen carrying capacity) and functional variables (*e.g.,* cognitive performance, exercise capacity, athletic performance, etc.). Researchers often use acute altitude illnesses as measures of hypoxia/altitude tolerance in humans, likely because these illnesses also reflect an individual's wellbeing and safety.

Inadequate acclimatisation to hypoxia often manifests as one of three (potentially

overlapping) conditions, termed 'acute altitude illnesses': acute mountain sickness (AMS), high-altitude pulmonary edema (HAPE), and high-altitude cerebral edema (HACE; Hackett and Roach 2001). Of the three manifestations of inadequate hypoxia acclimatisation, AMS is the most common but also the most benign, and HAPE and HACE, which are relatively rare, can be lethal without immediate medical intervention. For my dissertation, I chose AMS susceptibility as a measure of altitude/hypoxia tolerance. A brief discussion of HAPE and HACE is warranted given their potential relationships with AMS; however, for brevity, information about HAPE and HACE is provided in Appendix A

Acute mountain sickness is a primarily cerebral condition that manifests in response to normobaric or hypobaric hypoxia exposure. Because AMS generally resolves over time as the individual acclimatises, AMS is generally believed to be the result of insufficient acclimatisation. In 1993, the Lake Louise Consensus Group defined AMS as the presence of a headache upon recent ascent to an altitude of 2500 m or higher in addition to one or more of the following symptoms: nausea (or vomiting), fatigue, lightheadedness/dizziness, or difficulty sleeping. The symptoms defining AMS are similar to those associated with a variety of conditions, including carbon monoxide poisoning, alcohol hangover, migraine, and viral illnesses (Hackett and Roach 2001; Imray et al. 2010). In addition to the altitude requirement, for a malaise to be considered AMS, the symptoms should resolve within 8 hours of return to low altitude or with the administration of hyperbaric or supplemental oxygen treatment. This last criterion is often not tested because of logistical difficulties. There are no clinical signs of AMS.

Usually, AMS onset is 6-12 hours after arrival to a new altitude (> 2500 m), but AMS can develop as early as the first hour depending on the individual, the altitude, and the ascent rate (Hackett and Roach 2001). Symptoms of AMS typically resolve within 24-48 hours if no further ascent is performed (Imray et al. 2010), but because AMS is a sign of insufficient acclimatisation, it can precede more serious altitude illnesses, such as HAPE and HACE (Gallagher and Hackett 2004). This does not imply that AMS develops into either HAPE or HACE; rather, AMS often occurs before, or simultaneously with, HAPE and HACE. Given sufficient time, many humans can acclimatise to altitudes as high as ~ 5800 m (Barry and Pollard 2003; Imray et al. 2011), and once acclimatised, can remain in these high-altitude environments without risk of developing AMS again.

1.6.2 Acute mountain sickness pathophysiology

The pathophysiological mechanism underlying the symptoms of AMS is unknown (Imray et al. 2010); however, many potential mechanisms have been postulated. It is generally accepted that AMS is a result of cerebral perturbations in response to a hypoxic environment. The following is a quote from pioneering physiologist Sir Joseph Barcroft (1924), discussing the cause of AMS:

> "Taking it, therefore, as settled that mountain sickness is due to oxygen want, the question arises, "oxygen want of what?" And the answer is, "of the brain." Such evidence as is at our disposal goes to show that the brain wants but little oxygen; that little, however, it wants very badly indeed."

The regulation of cerebral vasculature is an extremely complex task, as the brain responds to changes in perfusion pressure, metabolic requirements, autonomic neural activity, and humoral factors (Ainslie and Smith 2011). With some degree of input from these components, cerebral blood flow (CBF) increases upon ascent to high altitude and returns to sea-level values after acclimatisation (~ 1 week; Severinghaus et al. 1966). Multiple hypotheses posit that the development of AMS is related to the sequelae arising from the increased CBF and increased intracranial pressure (ICP) that occur in response to hypoxia. Hansen and Evans proposed that AMS developed when the brain was compressed due to increased cerebral venous volume, increased cerebral edema, or decreased cerebral spinal fluid reabsorption (Imray et al. 2010). Similarly, Ross (1985) suggested that cerebral hypoxic intracellular (cytotoxic) edema and increased cerebral vasodilation were the causes of AMS. To explain the "random nature of cerebral mountain sickness," Ross (1985) suggested that those individuals with smaller intracranial and intraspinal capacities would have a lower compliance to buffer brain swelling and a greater susceptibility to AMS. This hypothesis became known as the "tight-fit" brain hypothesis. The postulations of Hansen and Evans and of Ross are based on hypoxic exposures exhausting the buffering capacity of the cranium, which would elevate the ICP to cause headache through some mechanism (Imray et al. 2010).

In a thorough review, (Bailey et al. 2009a) described what he referred to as the "traditional model" of AMS pathophysiology as follows: in hypoxia, hemodynamic and molecular forces increase cerebral capillary hydrostatic pressure, which disrupts the blood-brain barrier, causing extracellular (vasogenic) edema, increased intracranial volume (ICV), increased ICP, and brain swelling. In this model, headache is suggested to result from the stretching of pain-sensitive

fibers in the trigeminovascular system (Sanchez del Rio and Moskowitz 1999). As the trigeminal nerve is associated with other types of headache (Goadsby et al. 2002), this is an appealing hypothesis.

A role for elevated ICP in the pathophysiology of AMS is uncertain. In a recent study, cerebral venous distension was apparent in response to hypoxia, which would be consistent with increased ICP in hypoxia (Wilson et al. 2013). While their sample size was small, they reported that a restriction in cerebral venous outflow contributed to high-altitude headache. Studies of optic nerve sheath diameter (ONSD), which is a surrogate for ICP, are inconclusive: all studies performed to date report elevated ONSD at altitude (Sutherland et al. 2008; Fagenholz et al. 2009; Lawley et al. 2012; Keyes et al. 2013), but only two studies reported a relationship between ONSD and AMS (Sutherland et al. 2008; Fagenholz et al. 2009). Similarly, elevated intraocular pressure, another surrogate for ICP, was not related to AMS (Cushing et al. 2013). Finally, a recent study reported increased intracellular swelling without vasogenic edema (*i.e.,* a shift of fluid but no volumetric enlargement) in response to 12% O_2 (Lawley et al. 2013).

Bailey and colleagues (Bailey et al. 2009a) argued that the "traditional model" of AMS pathophysiology was insufficient to explain AMS. First, the review argued that individuals with and without AMS were comparable in terms of brain swelling, vasogenic edema, blood-brain barrier permeability, and lumbar pressure (Bailey et al. 2005). Furthermore, in studies by Kallenberg et al. (2007) and Schoonman et al. (2008), vasogenic edema was present in subjects with and without AMS. Collectively, these studies demonstrated that vasogenic edema could not explain the pathophysiology of AMS (Bailey et al. 2009a). Interestingly, intracellular (cytotoxic) edema only developed in subjects with AMS (Kallenberg et al. 2007; Schoonman et al. 2008). Based on the perceived unimportance of extracellular edema and the perceived importance of intracellular edema, Bailey et al. put forth an alternative model for AMS (Bailey et al. 2009a). First, they suggested that hypoxia increases free radical production, which leads to endothelial dysfunction and depressed hypoxic ventilatory control (Hildebrandt et al. 2002) that further potentiates the increase in free radical concentrations. The authors proposed that free radicals would reduce sodium potassium ATPase activity, causing extracellular fluid uptake, leading to astrocyte swelling (intracellular edema) and increased nitric oxide production. In contrast to the stretching mechanism of the previous model, Bailey et al. suggested that nitric oxide (and other free radicals) might activate the trigeminovascular system to produce the characteristic high-

altitude headache of AMS.

Although several models have been put forward, the pathophysiology of AMS is not very well understood, and there is certainly no consensus among researchers as to the etiology or even the precise definition of the condition. This limited understanding may be partly due to the difficulty in assessing AMS in humans.

1.6.3 Measuring acute mountain sickness

The study of hypoxia tolerance necessitates the quantification of individuals' tolerances of hypoxia. Often, researchers use the severity of AMS as a measure of hypoxia tolerance, although other variables could be used (*e.g.,* cognitive performance, exercise capacity, athletic performance, etc.). A self-reported questionnaire is used to diagnose AMS and quantify AMS severity because it is a subjective condition. The most commonly used questionnaire is the Lake Louise Score (LLS) Questionnaire, which asks individuals to rate each of the five symptoms discussed earlier on a scale of 0-3 (Roach et al. 1993), with 0 indicating absence of the symptom and 3 indicating that the symptom is severe (see Appendix D). If the individual has recently ascended to altitude, a total LLS ≥ 3 (with a headache score ≥ 1 and the presence of at least one other symptoms) is considered a positive AMS diagnosis. An alternative self-report questionnaire, the Environmental Symptom Questionnaire III (ESQ III) (Sampson et al. 1983), can also be used to diagnose AMS (see Appendix D). The ESQ III has 68 questions, of which 11 relate to AMS (more specifically the 11 questions relate to "cerebral AMS," as the questionnaire also measures "respiratory AMS"). Answers are given on a 0-5 Likert-type scale, with 0 indicating the symptom is absent and 5 indicating that the symptom is severe (see Appendix D). The calculation of the final score (*i.e.,* the cerebral AMS score [AMS-C]) is somewhat complicated: the response from each of the 11 questions is multiplied by a different constant, the 11 products are added, and the resulting sum is multiplied by another constant. An AMS-C score ≥ 0.7 is considered diagnostic of AMS. Note, the ESQ does not require a headache for the diagnosis of AMS, and there is some debate as to whether headache should be required in the LLS definition of AMS (Roach et al. 2011). The full ESQ III is difficult to administer in the field, but a shortened electronic version that is AMS-specific has been validated (Beidleman et al. 2007); however, ESQ is still used less frequently than the LLS, probably partly because of the added labour needed to compute the score.

Using the LLS or ESQ, one can quantify individuals' hypoxia tolerances. Acute mountain sickness can be considered a continuous variable, since its severity can be rated from 0-15 (on the LLS Questionnaire); however, AMS is often treated as an ordinal variable when individuals are divided into several groups (*e.g.,* absent, moderate, and severe AMS – *e.g.,* 0-2, 3-5, 6-15 respectively) or as a dichotomous variable when individuals are divided into two groups (*e.g.,* resistant [<3] or susceptible [≥3]). Individuals' hypoxia tolerances can also be rated based on one or more exposures to hypoxia. In some studies, a single measurement of AMS severity on one exposure to altitude is used to determine a person's AMS susceptibility; in other studies, multiple measurements of AMS severity on one or more exposures to hypoxia are used. The field of high-altitude biology does not have a consensus for assessing individuals' hypoxia tolerances.

The concept of categorizing subjects into groups based on their susceptibilities to a specific altitude illness is predicated on the idea that altitude illnesses are repeatable. If an individual always develops AMS at a given altitude and ascent rate, then one exposure would be sufficient to categorize the individual's innate hypoxia tolerance. Alternatively, if altitude illnesses are not repeatable, categorizing individuals based on susceptibility would be difficult, as variable factors (*e.g.*, diet and psychology) may affect the susceptibility of individuals. It is also possible that in a population ascending to a specific altitude at a specific rate, some individuals will always develop AMS, some individuals will never develop AMS, and other individuals will vary across exposures – sometimes developing AMS and sometimes not.

1.6.4 Physical factors affecting the incidence and severity of acute mountain sickness

The incidence of AMS varies widely depending on the altitude attained, the rate of ascent, the latitude of the mountain, and the prevailing weather (reviewed in Schoene (2007)), all factors related to the degree of hypoxia at a given point and the rate of change in the availability of oxygen during the ascent. The incidence of AMS increased linearly with altitude in a study conducted in the Swiss Alps: the incidences were 9%, 13%, 34%, and 53% at altitudes of 2850 m, 3050 m, 3650 m, and 4559 m, respectively (Maggiorini et al. 1990). Although Maggiorini *et al.* did not describe the ascent rates of their participants, other studies have demonstrated a drastic increase in the incidence of AMS when comparing a quick ascent rate to moderate altitude relative to a moderate ascent rate to the same altitude. For example, after a direct flight from 1300 m to 3740 m (a flight of <1 hour in duration) in the Nepal Himalayas, an AMS

incidence of 84% was recorded (Murdoch 1995). A similarly high incidence of AMS (80%) has been reported upon rapid (~ 3 hours) ascent of Mauna Kea (4200 m) in Hawaii (Forster 1985). Both studies had AMS rates nearly twice that reported by Maggiorini et al. (1990) at comparable altitudes reached at presumably slower ascent rates (actual rates were not provided, but subjects were described as "climbers" and presumably ascended by foot; Maggiorini et al. 1990).

The actual altitude of a location is a proxy for the P_B, and a number of factors other than altitude can affect the incidence of AMS. For example, an influence of latitude on AMS incidence can be expected because of the effect of latitude on the prevailing P_B (and subsequently the P_{IO_2} (Reeves et al. 1994). Similarly, weather patterns and temperature can affect P_B and influence the incidence of AMS (West 1996; Moore and Semple 2006). Comparisons between ascents on different mountains/routes are also confounded by several factors that do not affect the P_B (*e.g.,* terrain, mode of ascent, etc.).

Whether hypobaria has an effect independent of hypoxia on the incidence and severity of AMS is unclear. Many studies have investigated whether or not respiratory and cardiovascular responses are equivalent to the two modes of hypoxia (NH and HH), but the results of these comparisons have been mixed (reviewed in Richard and Koehle 2012). From the few studies comparing responses to short NH and HH exposures, it seems that ventilation is slightly greater in NH, but oxygen saturation is not different (reviewed in Richard and Koehle 2012). It is worth noting that the level of evidence assigned to many of the studies included in this particular review was low due to methodological challenges (*e.g.,* differences in the duration of exposure, the control conditions, and the blinding of subjects). In a recent Point: Counterpoint article, Mounier and Brugniaux (Millet et al. 2012a) argued for the equivalency of the two modes (or at least that differences between the two modes are too small to be clinically relevant). These authors suggested that oxygen sensing was not affected by hypobaria and that the molecular mechanisms responsible for acclimatisation occur in either mode. The alternative argument from Millet and colleagues (Millet et al. 2012b), for a difference between the two modes, concluded that "the clinical evidence regarding the differences between HH and NH is still lacking in the field of medicine and sport performance."

In several studies, the incidence and severity of AMS were greater in hypobaric hypoxia relative to an equivalent normobaric hypoxia (Roach et al. 1996; Loeppky et al. 2005), implying that there is an interaction between hypobaria and hypoxia in the development of AMS;

however, other studies (Singh et al. 2010; Self et al. 2011; Richard et al. 2014) have shown the severity and incidence of AMS to be similar between the two modes of hypoxia. Comparisons across studies of AMS in different conditions are difficult because of variability in the durations of hypoxic exposure, subject-blinding procedures, washout lengths between exposures, and randomization strategies. That sample sizes were very small (*e.g.,* a range of 6-11 subjects for studies lasting hours) complicate matters further. Imray et al. (2010) suggested that with sufficient time for AMS to develop, normobaric and hypobaric hypoxia likely elicit similar AMS incidences and severities. It may be that the two modes differ to some degree, but the differences are not clinically relevant in the exposures commonly experienced (Küpper et al. 2011). Compared to normobaric normoxia, hypobaric normoxia did not have any detectable effects on AMS symptoms or the investigated cardiorespiratory variables, suggesting that the hypobaria of 4550 m (*i.e.,* oxygen was added to provide normoxic conditions) did not have a significant effect (Richard et al. 2014).

1.6.5 Physiological and epidemiological factors affecting acute mountain sickness

Determining who is and who is not susceptible to AMS prior to ascent is a very difficult task, and developing methods to do so is one of the major research areas of high-altitude biology. The unclear pathophysiology of AMS makes predictions difficult. Hypoxia causes AMS, and susceptibility to AMS is presumably related to variations in the ability of individuals to successfully acclimatise to hypoxia. The basis of the variation in hypoxia acclimatisation has not been determined, but it may stem from variations in physiological processes that (i) regulate the body's ability to deliver oxygen to tissues in an environment with a lower P_{O_2}, (ii) allow the body to use oxygen more efficiently in an environment with a lower P_{O_2}, (iii) combat molecular and/or systemic changes brought on by exposure to an environment with a lower P_{O_2}, or (iv) some combination of these processes.

Examples of potential physiological risk factors include, but are not limited to those listed in Table 1.1. Despite the number of variables investigated, none has proven to be reliable enough to be useful in predicting AMS outcomes prior to ascent.

Table 1.1 Examples of physiological variables that have been tested for associations with acute mountain sickness.

Variable	Authors
Hypoxic ventilatory response	Nespoulet et al. 2012
Oxygen saturation	Karinen et al. 2010
Oxygen saturation during hypoxic exercise	Richalet et al. 2012
Cardiac response to hypoxic exercise	Richalet et al. 2012
Decrease in power output during hypoxic exercise	Richalet et al. 2012
Fluid balance	Loeppky et al. 2005
Exhaled nitric oxide	You et al. 2012
Exhaled carbon monoxide	You et al. 2012
Lung diffusion capacity	Nespoulet et al. 2012
Central sleep apnea events	Nespoulet et al. 2012
Cerebral blood flow	Baumgartner et al. 1994
Heart rate	Loeppky et al. 2003
Heart rate variability	Loeppky et al. 2003
Physical exertion	Roach et al. 2000
Body mass index	Karinen et al. 2010
Aerobic capacity	Karinen et al. 2010

Several epidemiological studies of AMS have investigated whether differences in age and sex affect AMS susceptibility. Most studies report that AMS occurs less frequently in older individuals (Honigman et al. 1993; Gaillard et al. 2004; Richalet et al. 2012). Some have suggested that the decreased risk of AMS with increased age is due to the natural decrease in brain size that occurs with aging, which would provide greater compliance for swelling under the "tight fit brain" hypothesis (Ross 1985). With respect to sex, the results of studies are less congruent. Several studies (Kayser 1991; Honigman et al. 1993; Basnyat et al. 2000; Richalet et al. 2012) reported that females were more susceptible to AMS; however, other studies reported similar incidences between sexes (Hackett et al. 1976; Maggiorini et al. 1990; Schneider et al. 2002; Gaillard et al. 2004). Because AMS is self-reported, it is very difficult to rule out the possibility that cultural differences between males and females could contribute to the differences between sexes.

1.6.6 Previous history of acute mountain sickness

Many review papers suggest that the best predictor of AMS occurrence is a previous history of AMS (Hackett and Roach 2001; Schoene 2007; Imray et al. 2011). Imray et al. (2011) stated that "An individual's past performance at altitude is the main predictor of their [sic] future performance." This idea is echoed by West (2012), who stated that "Individuals who develop

AMS on one ascent are more likely to develop the disease [condition] on subsequent ascents." In actuality, there is a dearth of evidence to demonstrate the degree to which AMS is repeatable. This evidence may be weak due to the retrospective history used by most studies and the lack of a gold standard for the diagnosis of AMS (which lowers the quality of a history of AMS).

Multiple field studies that measured AMS history retrospectively reported that a history of AMS is a risk factor for future incidence of AMS. For example, Schneider et al. (2002) and Pesce et al. (2005) reported that the incidence of AMS increased with the 'AMS history score' (essentially a retrospective rating of AMS symptom severity on previous ascents to >3000 m) of individuals who ascended to 4559 m and attempted to ascend to 6992 m, respectively. Similarly, Richalet et al. (2012) showed that subjects with a history of severe high-altitude illness (mostly severe AMS but also HAPE or HACE) were more likely to develop AMS than subjects who had also been to altitude but did not develop a high-altitude illness.

Three longitudinal studies (*i.e.,* studies in which AMS history was measured prospectively) have attempted to determine the repeatability of AMS. The earliest study (Robinson et al. 1971) is difficult to interpret because subjects were given vasopressin in one of the exposures and placebo in the other (Aoki and Robinson 1971). The authors suggested that the 11 subjects responded similarly to the two exposures, but statistical support for this claim was not provided. Forster (1984b) had a larger sample size and supported his claim of repeatability with statistics; however, it was likely that the subjects were at least partially acclimatised to the altitude because the washout was only 5 days (after spending 5 days at altitude). Rexhaj et al. (2011) avoided many of the flaws in the previous studies. Twenty-seven children (age range: 8-16 years) and 29 adults were exposed to 3450 m on two separate occasions 9-12 months apart, allowing sufficient time for a washout. Sixty-two percent and 22% of adults and children developed AMS on the first exposure, respectively; Forty-eight and 15% of adults and children developed AMS on the second exposure, respectively. The positive and negative predictive values (PPV and NPV) were 78% and 100%, respectively, for adults and 0% and 48%, respectively, for children. The incidence of AMS was 11% lower on the second exposure (43% *vs.* 32%), suggesting that the first exposure may have improved subjects' altitude tolerances. Note that children under 12 years of age were assessed using the children's LLS (Yaron et al. 1998), whereas adults were assessed with the LLS. The low incidence of AMS in adults may indicate that AMS at relatively low altitudes is somewhat repeatable.

Whether AMS history was retrospective or prospective, none of the studies described above convincingly demonstrated that AMS was repeatable for a number of reasons. Firstly, the majority of the studies lacked a control condition to limit subject bias and to demonstrate that subjective scores were due to hypoxia (not to the settings of the experiment). Second, only three studies (*i.e.,* the longitudinal studies) ensured that the conditions of the two exposures were identical. Finally, even if subjects with a history of AMS were more likely to develop AMS than subjects with a negative history of AMS (*i.e.,* a statistically significant odds ratio), many studies had numerous false positives and false negatives, questioning the strength of AMS history as a diagnostic tool for predicting future AMS events.

Table 1.2 Descriptions of studies that reported sufficient data to calculate the recurrence rate of AMS.

Author	Year	Altitude (m)	Total (n)	AMS susceptible (n)	Recurrence Rate	Diagnosis
Alizadeh et al.	2012	5671	349	143	55%	LLS >5
Forster	1984	4200	1	1	100%	SSQ
Forster	1984	4200	18	NR	NR	SSQ
Honigman et al.	1993	1920–2956	2711	1492	34%	SSQ
Mairer et al.	2009	2200–3500	431	115	21%	LLS ≥4
Mairer et al. (EA)	2010	3454	79	25	36%	LLS ≥4
Mairer et al. (WA)	2010	3817	83	29	45%	LLS ≥4
Moore et al.	1986	4800	12	8	100%	Hackett's Score
Nilles et al.	2009	4267	51	19	42%	LLS ≥ 3
Pesce et al.	2005	6962	919	NR	NR	LLS >4
Rexhaj et al.	2011	3450	56	24	58%	LLS (French version) ≥3
Richalet et al.	1988	6119- 8848	128	55	58%	Diary of symptoms
Richalet et al.	2012	4000	729	235	56%	Hacketts Score (>6)
Roach et al.	1995	2500	97	17	41%	LLS ≥ 3 with a headache score ≥1
Robinson et al.	1971	4267	11	NR	NR	Headache & somatic discomfort score
Roscoe et al.	2008	4300	317	NR	NR	LLS ≥3
Schneider et al.	2002	4559	706	300	42%	AMS-C (ESQ) >0.70
Wagner et al.	2006	4419	359	142	NR	LLS ≥3
Wagner et al.	2008	4419	884	308	47%	LLS ≥3
Wagner et al.	2012	4260	56	23	74%	LLS ≥3 with a headache score ≥1
Wang et al.	2010	3952	1066	174	47%	LLS ≥4
Wiseman et al.	2006	5350	38	NR	NR	Y/N
Wu et al.	2009	4292–4779	585	114-126	NR	LLS ≥ 3
Ziaee et al.	2003	4200	449	85	72%	LLS (headache +1 other symptom)

NR, not reported; LLS, Lake Louise Score; SSQ, study symptom questionnaire; AMS-C, cerebral acute mountain sickness score; ESQ, Environmental Symptom Questionnaire.

1.6.7 Acute mountain sickness prevention

The best method to avoid AMS while ascending to altitude is a sufficiently slow ascent rate to allow time for acclimatisation. The Wilderness Medical Society Consensus states that

ascending to altitudes below 2800 m represents a low risk of developing AMS and ascent rate is not a concern; however, for travel above 3000 m, the guidelines state that sleeping altitudes should not be increased by more than 500 m in a single day (Luks et al. 2010). Furthermore, the guidelines also recommend a non-ascent day (*i.e.,* no increases in elevation) every 3 or 4 days of ascent to allow additional time for acclimatisation. Using a randomized control trial with a 15- or a 19-day ascent of the Muztagh Ata (7456 m), Bloch et al. (2009) demonstrated that the slow ascent protocol improved the chances that climbers reached Camp III (6865 m) and effectively reduced the severity and incidence of AMS relative to the quick ascent profile. Additional support for the benefits of slow ascent are retrospective studies that found lower incidences and severities of AMS in groups that ascended slower (Hackett et al. 1976; Schneider et al. 2002).

Although a slow ascent can prevent or ameliorate AMS, it is not a panacea. Caution is not always possible because of time constraints (*e.g.,* military deployment, medical rescue, travel by high-altitude train), and even if a slow ascent rate is followed, some individuals will still develop AMS (Bartsch et al. 2004). Several pharmaceuticals and dietary strategies have also been suggested for preventing AMS. Paralleling their ascent-rate recommendations, the Wilderness Medical Society Guidelines recommend against using pharmaceutical prophylaxis in low-risk situations in which AMS is unlikely (or, if occurring, likely to be very minor); however, when the risk of AMS is higher, the guidelines recommend acetazolamide, a carbonic anhydrase inhibitor, as the preferred pharmaceutical for prophylaxis. Leaf and Goldfarb (2007) have suggested multiple physiological effects of this drug that would theoretically reduce AMS severity and incidence: decreased carotid body activity leading to improved sleep quality; acidosis leading to increased central chemoreceptor activity; increased ventilation and P_{aO_2}; and increased diuresis. Multiple studies have validated the effectiveness of acetazolamide in the prevention of AMS (Forwand et al. 1968; Basnyat et al. 2006; van Patot et al. 2008; Gertsch et al. 2010). Alternatively, dexamethasone, a steroid, has also been shown to reduce AMS symptom severity in prospective trials (Ellsworth et al. 1987). The mechanism through which dexamethasone reduces AMS severity is not well understood. While acetazolamide and dexamethasone can reduce the incidence of AMS, they do not improve work performance at altitude (Muza et al. 2010). Furthermore, high doses of acetazolamide can reduce endurance capacity at altitude (Garske et al. 2003).

Diet may have an effect on AMS symptoms. Increased carbohydrate ingestion improves oxygen saturation and ventilation in response to hypoxia (Golja et al. 2008). While Consolazio et al. (1969) reported a positive effect of carbohydrate supplementation in reducing AMS symptoms, Swenson et al. (1997) did not. Coca leaves are a traditional AMS preventative in the Andes, and garlic is a traditional AMS preventative in the Himalaya, but the effectiveness of each has not been proven in randomized control trials, and there is not much evidence in support of, or contradicting, the effectiveness of either preventative supplement.

Because AMS develops when an individual is insufficiently acclimatised to a given hypoxic stress, acclimatisation to hypoxia through recent exposures can also decrease AMS severity. In a retrospective study, 5 or more days of exposure to an altitude of 3000 m or higher in the preceding 2 months was associated with a lower prevalence of AMS (Schneider et al. 2002). Similarly, residence at moderate altitude (~2000 m) was associated with a higher S_{PO_2}, smaller reduction in plasma volume, and decreased severity of AMS upon exposure to 4300 m relative to a control group living at 50 m (Staab et al. 2013). Normobaric hypoxia exposure might be less effective than hypobaric hypoxia exposure: 7 nights of normobaric hypoxia (progressively decreasing F_{IO_2} from 16.2% to 14.4%) had little effect on daytime AMS-C scores relative to a sham procedure (Fulco et al. 2011). Intermittent hypobaric hypoxia exposures may reduce the likelihood of developing AMS upon exposure to high altitude (Reviewed in Muza et al. 2010). For example, 15 days of intermittent altitude exposure (4 hours; hypobaric hypoxia equivalent to 4300 m) reduced AMS severity during a 30-hour exposure to 4300 m (Beidleman et al. 2004).

1.6.8 Acute mountain sickness treatment

If someone develops AMS, the initial treatment is to rest without ascending until the condition resolves (usually 24-48 hrs). If there is no improvement, a moderate descent (*e.g.,* 500 to 1000 m) is often sufficient to alleviate AMS symptoms (Hackett and Roach 2001). If descent is not possible, 'simulated' descent using supplemental oxygen or hyperbaric therapy (*e.g.,* a Gamow bag; King and Greenlee 1990) can be used (Hackett and Roach 2001). If symptoms worsen, descent becomes imperative because AMS can advance to HACE, a potentially fatal condition (see Appendix A; Barry and Pollard 2003). Several pharmaceutical options are available to treat AMS. Acetazolamide, although better for prevention than treatment, can be

used to treat mild AMS; however dexamethasone is more effective in treating AMS of any severity (but especially for moderate to severe AMS; Luks et al. 2010). If AMS is treated with acetazolamide and symptoms dissipate, symptoms will not return when treatment is withdrawn (without further ascent); however, because dexamethasone does not induce acclimatisation (*i.e.,* dexamethasone alleviates symptoms instead of causing physiological changes that improve acclimatisation to alleviate symptoms), AMS symptoms might return when treatment is withdrawn (personal communication, M. Koehle). As with its prophylactic effects, the mechanisms through which dexamethasone improves AMS symptoms are not understood.

1.6.9 The impact of acute mountain sickness

Each year, ~35 million people travel into the mountains and onto high plains, and the prevalence of AMS can be extremely high (see Section 1.2.4). Considering that preventing the condition is relatively simple, AMS may be so common because public awareness is often lacking. For example, only 55% of respondents at an American high-altitude ski resort had knowledge of AMS (Hatzenbuehler et al. 2009). Even if people are aware of the risks, the nature of the ascent to altitude often determines the time available for acclimatisation: tourists on a weeks-long holiday may have many days to acclimatise while trekking, whereas, trekkers with less time might not prioritize acclimatisation, potentially putting themselves at a higher risk of developing AMS (or HAPE/HACE). In a military setting, soldiers may be rushed to high-altitude for combat or disaster relief. Furthermore, in a group setting, some individuals may acclimatise quicker than others, and those who feel ill may not wish to slow the group down (and will continue to ascend when they should rest). Anecdotally, there seems to be a belief that poor fitness contributes to AMS, and individuals may continue to ascend to appear fit.

Acute mountain sickness can have significant impacts on the health and productivity of humans at high altitude. The negative health impacts of AMS have already been described, but the seriousness of AMS is magnified by the extreme and often remote locations in which it manifests. Travel in and out of these regions is often difficult and costly – if not impossible at times – and medical treatment may be limited, unavailable, or expensive. Acute mountain sickness may have negative economic impacts as well. Tourists ascending to high-altitude regions (*e.g.,* the Himalaya of Nepal, and the Machu Picchu area of Peru) may be unable to complete their treks and may be discouraged from returning in the future. Hackett estimates that

Colorado ski resorts lose $20-40 million annually because of altitude illness (Partie Lange 2001). Furthermore, productivity in high-altitude engineering projects, such as the Qinghai-Tibetan Railway (Wu et al. 2010) and in high-altitude mines, was and is severely hindered by AMS, as workers are unable to perform tasks efficiently until properly acclimatised. The Sino-Indian conflict provides an example of the costly effects of AMS at high altitude: in this conflict, the Indian army suffered more deaths from altitude illness than enemy action (Rodway and Muza 2011). Similarly, during the 2002 Operation Anaconda in Afghanistan, approximately 15% of combat-related casualties treated were cases of severe AMS (Peoples et al. 2005).

1.7 Altitude and nitric oxide

1.7.1 Nitric oxide

In recent years, nitric oxide (NO) has garnered substantial interest for its possible relationship with altitude tolerance (Janocha et al. 2011; Beall et al. 2012) and with athletic performance in hypoxia (Cermak et al. 2012; Muggeridge et al. 2014). In 1992, the journal *Science* proclaimed NO to be the "molecule of the year" after scientists discovered its role as a gaseous chemical messenger in the human body, the first gas known to have this function (Koshland 1992). Nitric oxide, or nitrogen monoxide, is a small, uncharged molecule with an unpaired electron (*i.e.,* NO is a free radical). Because NO is small and uncharged, it can easily diffuse through cell membranes, allowing NO to act as an autocrine and paracrine signaling molecule. The NO molecule is highly reactive (Beckman and Koppenol 1996), but this is not an issue for signaling; it is the concentration of NO that is biologically relevant, and a high turnover rate allows efficient and accurate communication between cells (Beckman and Koppenol 1996). Nitric oxide is part of many physiological pathways, but the following sections focus mostly on the role of NO in the cardiovascular and respiratory systems.

1.7.2 Production of NO

There are three NOS enzymes, responsible for the majority of endogenous NO production: neuronal NOS (type I; nNOS; NOS1), inducible NOS (type II; iNOS; NOS2), and endothelial NOS (type III; eNOS; NOS3; reviewed in (Ozkan and Dweik 2001). Both NOS1 and NOS3 are termed constitutive NOS, whereas NOS2 is termed inducible NOS; however, these names are misnomers, as NOS2 also produces NO constitutively and NOS1 and NOS3 can be

induced. The NOS2 enzyme produces magnitudes more NO compared to NOS1 and NOS3 (Guo et al. 1995; Dweik et al. 1998). The NOS enzymes produce NO via the catabolism of L-arginine to L-citrulline (Figure 1.5).

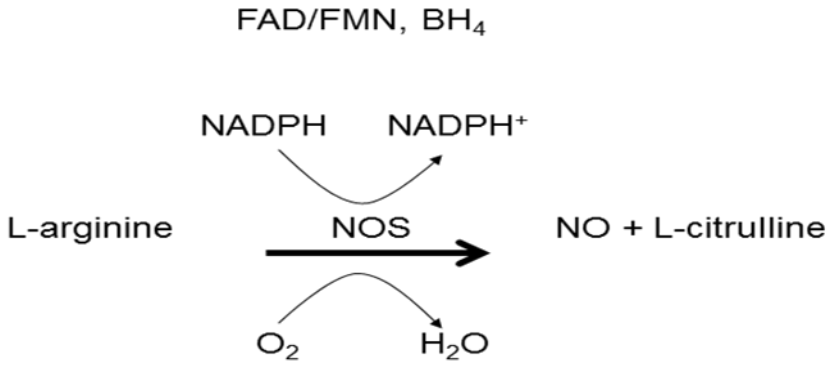

Figure 1.5 The nitric oxide synthase pathway. L-arginine is oxidized to nitric oxide (NO) and L-citrulline by a nitric oxide synthase enzyme (NOS). In this reaction, oxygen (O_2) and nicotinamide adenine dinucleotide phosphate (NADPH) are co-substrates, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and tetrahydrobiopterin (BH_4) are cofactors.

Alternatively, NO can be produced from dietary pathways via the reduction of ingested nitrate and nitrite (Lundberg et al. 2008). In this pathway, oral cavity bacteria reduce nitrate to nitrite, which is absorbed by the intestine into the blood. Nitrate from the blood is concentrated in the salivary glands and will re-enter the pathway upon swallowing. Both nitrate and nitrite can be converted to NO as they circulate throughout the body. The ingestion of a nitrate-rich meal (*e.g.,* lettuce, spinach, beet root, arugala, etc.) increased plasma nitrate and exhaled nitric oxide (Olin et al. 2001). The production of NO from food is greater than that from the NOS enzymes (Lundberg et al. 2008). The use of mouthwash, which would kill oral bacteria and interrupt the pathway, attenuated the increase in plasma nitrite after a nitrate-rich meal (Govoni et al. 2008).

1.7.3 Nitric oxide and physiology

Nitric oxide plays a key role in the regulation of basal systemic and pulmonary blood pressure under normoxic and hypoxic conditions. Nitric oxide diffuses into smooth muscle cells and activates soluble guanylate cyclase (sGC) to produce cGMP and elicit relaxation of the smooth muscle. Blood pressure increased in a dose-dependent manner when L-NMMA (N^G-monomethyl-L-arginine), an inhibitor of eNOS, was perfused into rabbit aortic tissue (Rees et al. 1989). Subsequent perfusion of L-arginine reversed the effects of L-NMMA. Similarly, in

humans, the infusion of L-NMMA lowered plasma NO concentrations and increased blood pressure, systemic vascular resistance, and pulmonary vascular resistance (Stamler et al. 1994). Again, L-arginine reversed the effects of L-NMMA. In hypoxic conditions, the PAP and PVR of humans increased, and this response was augmented when L-NMMA was infused intravenously to decrease NO production (Blitzer et al. 1996). Once more, the infusion of L-arginine restored NO production and reversed the effects of L-NMMA. Furthermore, the inhalation of exogenous gaseous NO caused pulmonary vasodilation (but not systemic vasodilation) under normoxic conditions (Frostell et al. 1991) and hypoxic conditions (Pison et al. 1993). The above studies demonstrate that manipulating endogenous NO production or providing exogenous NO affects blood pressure and vascular resistance in normoxia and hypoxia.

Nitric oxide may have a role in ventilation-perfusion matching also. Firstly, studies of anesthetized humans revealed that exhaled NO concentrations correlated with resting P_{aO_2} and the resting $AaDO_2$, implying that the NO produced in the lungs might be related to the amount of O_2 diffusing into the blood (Tsuchiya et al. 2000). In addition, the inhalation of small quantities of NO (*i.e.,* ppm concentrations) decreased PAP and increased P_{aO_2} in patients with acute respiratory distress syndrome (Gerlach et al. 1993; Puybasset et al. 1994). Even the auto-inhalation of small concentrations of nasal NO (*i.e.,* ppb concentrations) improved ventilation-perfusion matching, as blood was redistributed ventrally and cranially in the lungs to areas that were relatively under-perfused during oral breathing (Sánchez Crespo et al. 2010). These studies hint at the possibility that an innate low exhaled NO could be a marker for poor gas exchange in the lungs, which could impair acclimatisation to hypoxia.

1.7.4 Exhaled nitric oxide

Measuring NO *in situ* in the respiratory system would be invasive and difficult; however, NO produced in the respiratory system can be measured in exhaled breath. Gustafsson et al. (1991) were the first to detect NO in the exhaled breath of mammals (rabbits, guinea pigs, and humans). Exhaled NO was decreased following intravenous injection of NOS inhibitors, but the injection of air into the vasculature to prevent blood flow to the lungs did not affect exhaled NO, indicating that exhaled NO was produced in the lungs and or airways, not the blood.

The NO exhaled by humans is formed in the upper and lower respiratory airways (Dweik et al. 1998). Of these two sites, significantly more NO is produced in the upper airways (*e.g.,*

nasopharynx, oropharynx, etc.) than in the lower airways (*e.g.,* trachea, bronchi, etc.; Lundberg et al. 1994). For example, tracheotimized subjects exhaled more NO nasally than they did orally, and they also exhaled more NO orally than they did through their tracheostomies (Lundberg et al. 1994). Concentrations of NO in the nasal cavity are high due to the large amount of NO produced in the paranasal sinuses (Lundberg et al. 1995). The NO produced in the lower respiratory tract is largely produced in the epithelium and mucosa of the lungs, from where it diffuses into the lumen until it is taken up by erythrocytes or exhaled (Lundberg et al. 1996; Le Cras and McMurtry 2001).

The fraction of exhaled nitric oxide (F_{ENO}) is a measure of the concentration of NO in exhaled breath. A variety of techniques can be used to measure F_{ENO}, and the American Thoracic Society and European Respiratory Society have collectively published guidelines for the proper measurement of F_{ENO} (American Thoracic Society and European Respiratory Society 2005). The gold standard for measuring F_{ENO} is a single-breath technique performed at a constant flow rate against pressure. Briefly, the subject inhales to total lung capacity through the mouth over approximately 3 seconds and then exhales immediately. The recommended flow rate is 50 mL/s and the recommended pressure to exhale against is between 5 and 20 cm H_2O (Högman et al. 1997). At a low flow rate (*e.g.,* 50 mL/s), the F_{ENO} is indicative of the NO produced in the airways, not the alveolar space (George 2008). The single breath measure of F_{ENO} is easy to perform and highly reproducible (Lim and Mottram 2008).

1.7.5 Nitric oxide and altitude/hypoxia

Decreased NO production at altitude has been suggested to reduce gas exchange and impair pulmonary hemodynamics; however, there is little evidence to support the claim that hypoxia decreases F_{ENO} (Brown et al. 2006) or that F_{ENO} concentrations at altitude are causally related to PAP (Duplain et al. 2000). Studies of F_{ENO} in normobaric hypoxia often demonstrate stable F_{ENO} (Hemmingsson and Linnarsson 2009; Donnelly et al. 2011), although some showed a decrease in F_{ENO} (Dweik et al. 1998) and studies of F_{ENO} in hypobaric hypoxia typically report a decreased F_{ENO} (Brown et al. 2006). Therefore, this decrease in F_{ENO} in hypobaric hypoxia may be the result of a decrease in P_B, which would lower gas density in the lungs and increases NO diffusion from the conducting airways to the alveolar region, where it reacts with hemoglobin

(Hemmingsson and Linnarsson 2009). In addition, although F_{ENO} was speculated to correlate with PAP at altitude (Duplain et al. 2000), this relationship was only observed in studies of hypobaric hypoxia: in normobaric hypoxia, pulmonary artery pressure increased, but F_{ENO} was stable (Donnelly et al. 2011). Thus, decreased F_{ENO} at altitude may be a result of hypobaria while the increase in PAP is due to hypoxia. See Appendix F for more on this topic.

1.7.6 Nitric oxide and altitude illness

Because NO regulates many of the physiological responses to hypoxia, variation in the production of NO and the response to NO could explain variation in susceptibility to AMS. The F_{ENO} was lower in subjects susceptible to HAPE who were exposed to hypobaric (Duplain et al. 2000) and normobaric hypoxia (Busch et al. 2001) than in HAPE-resistant subjects. Furthermore, in patients with HAPE, the administration of exogenous NO decreased PAP, redistributed blood flow to non-edematous regions of the lungs, and improved oxygen saturation (Scherrer et al. 1996). With respect to AMS, a study by Brown et al. (2006) reported that there was no relationship between AMS susceptibility and F_{ENO}; however, this study had several limitations that may explain the lack of an association, namely a short duration of exposure (3 hours at 4200 m), a low incidence of AMS (19%), and few controls for factors affecting F_{ENO} (*e.g.,* diet). A recent field study by (You et al. 2012) has demonstrated an association between F_{ENO} and AMS status in a large group of young males who ascended to 4300 m. From the few published studies, it seems that F_{ENO} is related to altitude tolerance, although more work is needed before this relationship could be useful for predicting AMS susceptibility.

Manipulating NO production at altitude with L-arginine supplementation did not greatly affect AMS incidence or severity. Schneider *et al.* (2001) infused L-arginine intravenously in subjects at 4350 m and measured acute changes in AMS symptoms and physiological variables. The mean LLS was lowest at the end of the infusion (30 minutes), but it returned to baseline values 15 minutes post-infusion. The infusion of L-arginine did not affect heart rate, blood pressure, or oxygen saturation in this study. It is important to note that this study only looked for immediate responses to L-arginine (*i.e.,* measurements were made minutes after the infusion). In a separate study, subjects ingested L-arginine (or placebo) orally and were monitored for changes in AMS symptoms and physiological variables over a 24-hour period at 4342 m (Mansoor et al. 2005). Ingestion of L-arginine increased serum L-arginine concentrations, but there was no

difference in oxygen saturation or AMS severity between groups. Since the catabolism of L-arginine is dependent on O_2, the lack of effectiveness of this strategy in reducing AMS symptoms is not surprising, and supplementing with nitrate has been postulated to be more effective (Dauncey 2012).

Sildenafil is a phosphodiesterase type-5 inhibitor. Although it does not directly increase NO production, it does block the degradation of cGMP, thereby potentiating the effects of NO. Sildenafil prevented altitude-induced hypoxemia and pulmonary hypertension in a small randomized control trial, but it did not affect the LLS of subjects at 4350 m (Richalet et al. 2005). In the same study, the altitude-related drop in $V_{O_{2max}}$ was attenuated by sildenafil; however, another study reported no effect of sildenafil on time trial performance in normobaric hypoxia (equivalent to 3900 m; Jacobs et al. 2011).

1.8 The genetics of acute mountain sickness

1.8.1 Basics of genetics

The classic path of discovery for the genetic basis of a trait (reviewed in Ziegler et al. 2010) has not been followed for AMS (MacInnis et al. 2011). Historically, the first step is to determine whether the trait aggregates in families. If the trait is genetic, then relatives (who are genetically similar) should be more phenotypically similar than unrelated individuals for traits with genetic etiologies. Also, as humans are not 'random breeders' for sociocultural reasons, members of the same biogeographical group (even outside extended families) tend to be more genetically similar to each other than to members of other biogeographical groups. Familial aggregation and differential susceptibility across biogeographical groups support the genetic etiology of a trait, but are not sufficient to 'prove' that a trait is genetic, as families and ethnic groups can be similar for non-genetic reasons (*e.g.,* shared environments and practices). If variation in is genetic, it will be heritable (*i.e.,* the variance in the phenotype must be due – at least partially – to genetic variance). Positive findings based on the above criteria were often followed by segregation analysis to identify the mode of inheritance (*i.e.,* dominant, recessive, etc.) and linkage analysis to identify the region in which the causal variants reside. With the advent of modern genotyping techniques, these two steps are often bypassed, and high-throughput genotyping is performed to test whether specific variants contribute to variation in the trait. When this genotyping is genome-wide, it can avoid *a priori* biases associated with

candidate-gene association studies, which rely on the selection of genes (and polymorphisms) of interest based on prior knowledge of their functions (Hirschhorn and Daly 2005). Positive associations are followed by estimations of effect size to ascertain the clinical significance and by functional analysis to discover the actual role of the variant in the trait.

1.8.2 Individual patterns of susceptibility

The inter-individual variation in AMS susceptibility is likely due to inter-individual variation in the ability to acclimatise. Ross (1985) summed up the problem well: "… if a group of comparable climbers is taken to the same altitude at the same rate, one cannot predict who will have minor symptoms and who will be stuporous on the fourth day." The unpredictable development of AMS (or high altitude headache) in some, but not all individuals, has been called "random" (Ross 1985), "striking" (Imray et al. 2010), and "idiosyncratic" (Wilson et al. 2013). Despite much investigation into potential physiological, epidemiological, and psychological predispositions to AMS, the causes of these individual differences in altitude tolerance are not very well understood.

If the factors predisposing some individuals to AMS are stable, then susceptible individuals should always develop altitude illnesses on multiple exposures to a given altitude and non-susceptible individuals should never develop altitude illnesses on multiple exposures to a given altitude. While AMS is considered repeatable in the literature (Luks et al. 2010), indisputable evidence to support the repeatability of AMS is lacking (see Section 1.5.5).

1.8.3 Familial aggregation of AMS

Data on patterns of AMS within families are scarce (Table 1.3). Studies of familial aggregation and heritability of AMS would require many families to ascend to high altitude together on a single mountain with a similar rate of ascent. Such situations are rare for an opportunistic experiment, and would be challenging and expensive to arrange. In addition, if one member of a family were to get sick, the family would likely descend as a unit, before the susceptibility of other members could be ascertained. Working with families in hypoxic chamber studies could overcome these problems, but would be very time consuming, as most chambers can only accommodate a small number of individuals at one time.

Table 1.3 Studies or reports of familial groupings of acute mountain sickness (AMS) and high-altitude pulmonary edema (HAPE).

Condition	Description	Reference
HAPE	Susceptible siblings in two Peruvian families (three sisters and two brothers respectively) following ascent to 3760 m.	Hultgren et al. 1961
HAPE	A susceptible man (affected twice at between 2600 and 3125 m) whose father died with dyspnea while hiking at 3400 m.	Fred et al. 1962
HAPE	Description of a mother-daughter pair.	Scoggin et al. 1977
AMS	"Family history" was not associated with AMS.	Ziaee et al. 2003
HAPE	Description of a family with a high incidence (three out of four affected).	Norboo et al. 2004
AMS	17 infant twin pairs, seven children developed AMS including 2 monozygous pairs, one dizygous pair and one dizygous singlet.	Yaron et al. 2002; Rupert et al. 2006
HAPE	Small three generation pedigree with six affected individuals consistent with an autosomal dominant trait with incomplete penetrance. Haplotype analysis excluded linkage with following genes: *EDN1, EGLN1, EGLN2, EGLN3, EPO, EPOR HAT1, HIF1A, HIF1B*$^{++}$, *HIF2A*$^{+,++}$, *HIF3A, HPH3*$^{++}$, *HSP90*$^{++}$, *NOS2A, NOS3, NOTCH1, NOTCH2, NOTCH3, NOTCH4, OSM9*$^{++}$, *PDHA1, PDHX, PDK1, PDK2, PDK3, PDK4, PDP2, PDPR, SSAT1, VEGFA, VEGFB, VEGFC, VEGFR1*$^{++}$, *VHL*.	Lorenzo et al. 2009

[+]See erratum in (Lorenzo et al. 2010)

Only one study has assessed the role of family history in AMS, and family history was a small component of the analysis. Ziaee et al. (2003) assessed AMS (with the LLS) in trekkers (n=459) ascending Mount Damavand (5671 m), Iran. Few data were presented related to family history, except for a short statement reporting that family history was not related to AMS susceptibility. It is not clear how family history was established (whether retrospective or prospective) or how many subjects could report an informative family history (*i.e.,* the number of subjects who had families members ascend to altitude and knew their AMS status was not reported). A study of AMS in infant twins (and one set of triplets) was performed, but without any analysis of family history (Yaron et al. 1998; see Section 1.7.4)

Detecting familial aggregation of AMS may be made difficult by the effect of age on the incidence and severity of AMS. Moraga et al. (2008) suggested that children had more severe AMS than their parents at 3500 m; however, an AMS questionnaire developed specifically for

children (the Children Lake Louise Score; CLLS) was used for the children, and the LLS was used for adults, making comparisons between adults and children difficult. In a separate study, children had greater sympathetic responses to altitude than their parents, demonstrating higher PAP, systolic blood pressures, and heart rates, but the incidence of AMS was similar in children and adults (Kriemler et al. 2008). The authors note that father-child PAP were correlated, but they did not investigate the relationship between parents and their children for LLS or AMS-C scores. Perhaps investigating parents and their adult children with the same scale would be a more informative and useful approach to determining the degree to which AMS aggregates in families. In addition to age, other confounding factors could impair the utility of family studies, such as sex (see Section 1.5.4).

1.8.4 Twins and altitude/hypoxia

The classic twin study, which is an extension of family studies, compares the variance within dizygotic (DZ) twins to the variance within monozygotic (MZ) twins with the purpose of determining the nature of phenotypic variation (*i.e.*, genetic *vs.* environmental sources of variance). Because DZ twins share, on average, 50% of their genetic material with each other, and MZ twins share 100% of their genetic material with each other, a greater similarity among MZ twins relative to DZ twins can be ascribed to the greater degree of genetic similarity in MZ twins relative to DZ twins (assuming that environments are shared to the same degree for MZ and DZ twins). From these comparisons, it is possible to estimate narrow-sense heritability (h^2), the proportion of phenotypic variance due to additive genetic variance. The typical formula used is

$$\mathbf{h^2 = 2*(r_{MZ} - r_{DZ})} \quad \textbf{Equation 1.10}$$

where r_{MZ} is the correlation between monozygotic twins and r_{DZ} is the correlation between dizygotic twins. While h^2 is often cited as a measure of 'genetic-ness,' it does not demonstrate whether or not a phenotype is genetic *per se*; rather, narrow-sense heritability demonstrates whether *variation* in a population is due to *variation* in genetics (and only additive variation is included). That is, h^2 explains why individuals in a particular population differ from one another (or why related individuals are similar to each other). With respect to studies of altitude/hypoxia responses, most studies reviewed by MacLeod et al. (2013) did not actually estimate narrow-sense heritability. Instead, these studies simply tested whether DZ variance was greater than MZ variance, which answers the question 'Is the phenotype heritable?' without providing a point

estimate of heritability.

Multiple studies have investigated specific physiological responses to hypoxia in twins (reviewed in MacLeod et al. (2013)). Data from these studies suggest that variation in the hypoxic ventilatory response is likely due to genetic variation, as the variance within DZ twins was greater than the variance within MZ twins for infants (Thomas et al. 1993), adolescents (Collins et al. 1978; Kawakami et al. 1982), and adults (Kawakami et al. 1984; Akiyama et al. 1991). In contrast, the ventilatory response to hypercapnia was only heritable when the experimental procedure led to some degree of hypoxia (Kawakami et al. 1982; Kawakami et al. 1984; Kobayashi et al. 1993). In these experiments, part of the overall ventilatory response could be attributed to the hypoxic ventilatory response; therefore, variation in the ventilatory response to CO_2 does not appear to have a genetic basis. Many other studies investigated the nature of variation in heart rate, blood pressure, blood gases, and acid-base buffering, but very small sample sizes and inappropriate estimates of heritability (*e.g.,* Holzinger's equation) limit interpretations of these results (reviewed in MacLeod et al. 2013).

Only a single study has examined AMS in twins. Yaron et al. used the Children's LLS (Yaron et al. 1998) to assess the symptoms of AMS in preverbal twins (and one set of triplets) during a 6-day stay at 3109 m (Yaron et al. 2002). Seven of the 37 children developed AMS, including two pairs of MZ twins, one pair of DZ twins, and one discordant sibling from a DZ pair; therefore, 16 of 17 (94%) twin pairs (and one set of triplets) were concordant, and the only discordant pair were DZ. While a greater concordance for MZ twins relative to DZ twins supports a genetic basis to AMS, the slight difference is not compelling enough to discount the effect of a shared environment on the similar responses to altitude. Furthermore, assessing AMS in preverbal children is highly subjective, and infants may not be ideal for investigating the genetic basis of AMS. Overall, data to determine the heritability of AMS is very limited.

1.8.5 Biogeographical difference in AMS susceptibility

Part of the adaptive response to hypoxia may have been selection against genetic variants that contributed to altitude illness susceptibility, either amongst the initial migrants or in their offspring; therefore, high-altitude populations may be less susceptible to altitude illness even if resistance to AMS is not considered adaptive *per se*. Tibetans, who are considered to be high-altitude adapted (Beall 2003; Beall 2011), have been reported to be less susceptible to AMS. Wu

et al. (2005) reported that Japanese and Han Chinese reported a higher frequency and severity of AMS relative to Tibetans on an ascent of Mount Anymaqin (6282 m), that Tibetans sleep better at altitude relative to Han Chinese, and that Tibetans were less likely to develop retinal hemorrhages at altitude than American climbers (presumably of European ancestry). Resistance to AMS among Tibetans was also noted in a study of workers on the high-altitude sections of the Qinghai-Tibet railroad (Wu et al. 2009).

One issue with both of these reports is that the Tibetans were often born and living at high altitude; therefore, their improved performance at altitude may be a result of developmental changes or acclimatisation, and not necessarily genetic attributes. Li et al. (2011) examined the military medical records for 3727 young men who resided at low altitude and ascended to the Tibetan plateau between 2006 and 2009. The group was split into odd and even birth years, and Tibetans had a significantly (and strikingly) lower incidence of AMS than Han Chinese in both cohorts: adjusted odds ratios of 0.05 (95% CI: 0.01, 0.34) and 0.10 (95% CI: 0.02, 0.41). These Tibetan individuals were born and raised in low-altitude regions of China, but the authors suggested that the reduced AMS incidence is evidence that the Tibetans were genetically adapted to 'survive and thrive in a low oxygen environment' (Simonson et al. 2010). AMS susceptibility data from other high-altitude populations is unavailable. Whether or not a difference in incidence exists between different low-altitude populations is also unknown.

1.8.6 Candidate gene association studies

Candidate gene association studies test the association between one or more polymorphisms of a gene and a phenotype. For medical conditions, the frequencies of an allele (or genotype) of a polymorphism are compared between cases and controls (*i.e.,* those with and those without the condition). A gene is said to be associated with a phenotype if a variant of one of its polymorphisms is over-represented in the cases.

Candidate-gene association studies are commonly used to investigate AMS etiology. The genes selected usually encode proteins that are involved in physiological pathways thought to be associated with acclimatisation to altitude. Eighteen genes from a variety of pathways have been tested for association with AMS, and variants in nine of these genes were associated with AMS susceptibility (Table 1.4; MacInnis et al. 2010). The function of each of these genes is provided in Table 1.5 and discussed in greater depth in (MacInnis et al. 2011), but generally, many of the

genes are related to blood flow, blood pressure, pulmonary function, cardiovascular function, and oxidative stress. Overall, the data may suggest that genotype contributes to an individual's capacity to rapidly and efficiently acclimatise to altitude; however, all of these studies were small candidate-gene association studies, and replications of many of the positive associations were not attempted or failed to produce a positive result. To date, there is no strong evidence to suggest that any of the tested variants is clinically significant. Increased sample sizes and non-biased genome interrogation methods are necessary to determine which variants contribute to AMS susceptibility.

In addition to AMS, candidate gene association studies have been used to investigate the genes involved in other areas of hypoxic physiology. Several studies have examined genes (*ACE, SDHB, SDHC, SDHD, HIF1A*) for a role in determining the HVR (Patel et al. 2003; Bigham et al. 2008; Richalet et al. 2009; Hennis et al. 2010). Similarly, several studies have examined genes (*ACE, AGT, HIF1A*) for a role in determining oxygen saturation at altitude (Woods et al. 2002; Buroker et al. 2010; Hennis et al. 2010). The evidence for associations between these genes and phenotypes is inconclusive.

There are two primary weaknesses for candidate gene association studies (Daly and Day 2001). Firstly, population stratification, the unequal distribution of alleles across different populations due to heterogeneous ancestry, can lead to spurious findings if, by chance, the allele of interest is differentially represented in case and control cohorts because of differential ancestry. Ancestry Informative Markers (AIMS) can be used to control for this problem, but additional genotyping is required. Secondly, candidate gene association studies require *a priori* hypotheses and each study interrogates a very small region of the genome, leaving much of the genetic variation unexamined. One solution to avoid *a priori* hypotheses is the use of genome-wide association studies, which interrogate many variants distributed throughout the gnome.

Table 1.4 A summary of candidate gene association studies in acute mountain sickness (AMS).

Gene	Polymorphism[a]	Population (C:P)[b]	Results	Reference
ACE	*Alu* insertion/deletion; intron 16 (rs4340)	European (104:47)	no association	Dehnert et al. 2002
ACE	Alu insertion/deletion; intron 16 (rs4340)	Caucasian (244:40)	I/D genotype (day 1 only; no association day 2)	Tsianos et al. 2005
ACE	*Alu* insertion/deletion; intron 16 (rs4340) A(-240)T; promoter (rs4291) A(2350)G; silent; exon 17(rs4343)	Nepalese (59:44)	no associations	Koehle et al. 2006
ACE	*Alu* insertion/deletion; intron 16 (rs4340)	Caucasian (varies over time points)	no association	Kalson et al. 2008
ACE	*Alu* insertion/deletion; intron 16 (rs4340)	Han Chinese (60:98)	D allele	Buroker et al. 2010[f]
ADRB2	A/G; 5' UTR (rs2400707) A/G; 5; UTR (rs2053044) G/A; 5' UTR (rs12654778) G/C; 5' UTR (rs11168070) A(285)G; arg16gly; exonic (rs1042713) C(523)A; silent; exonic (rs1042718) G(1053)C; silent; exonic (rs1042719)	Nepalese (59:44)	no associations	Wang et al. 2007
AGT	T(704)C; met235thr, exon 2 (rs699)	Han Chinese (60:98)	T (met) allele	Buroker et al. 2010
AGTR1	A(1166)C; 3' UTR (rs5186)	Nepalese (59:44)	no association	Koehle et al. 2006
AGTR1	A(1166)C; 3' UTR (rs5186)	Han Chinese (60:98)	no association	Buroker et al. 2010[f]
APOB	A/G; silent; exon 2	Han Chinese (60:98)	no association	Buroker et al. 2010[f]
BDKRB2	+/- 9 bp; exon 1 (rs72348790) C(-58)T; promoter (rs1799722)	Nepalese (99:90)	no associations	Wang et al. 2010b
GNB3	A(-350)G; 5' UTR (rs2071057)	Han Chinese (60:98)	A allele	Buroker et al. 2010[f]
GSTM1	+/-	Chinese (80:43)	Negative (-/-) genotype	Jiang et al. 2005

Gene	Polymorphism[a]	Population (C:P)[b]	Results	Reference
GSTT1	+/-	Chinese (80:43)	Positive (+/+, +/-) genotype	Jiang et al. 2005
HIF1A	C(1744T;) pro582ser; exon 12 (rs11549465)	Sherpa (59:45)	no association	Droma et al. 2008
HIF1A	A/G; exon (rs11549467)	Chinese (64:64)	no association	Ding et al. 2011
	A/G; exon (rs17099141)		no association	
	A/G; 5'UTR (rs10129270)		no association	
	C/T; 5'UTR (rs41362550)		no association	
	T/C; exon (rs4902080)		no association	
	A/C; 5' UTR (rs2301113)		no association	
HSPA1A	C(+190)G; 5' UTR (rs1043618)	Chinese (173:56)	no association	Li et al. 2004
HSPA1A	G(+190)C; 5' UTR (rs1043618)	Han Chinese (100:56)	no association	Zhou et al. 2005
HSPA1B	A(1267)G; silent; exon 1 (rs1061581)	Chinese (173:56)	G/G genotype[c]	Li et al. 2004
HSPA1B	A(1267)G; silent; exon 1 (rs1061581)	Han Chinese (100:56)	G/G genotype[c]	Zhou et al. 2005
HSPA1L	G(2437)C[d]; met493thr; exon 2 (rs2227956)	Han Chinese (100:56)	B/B genotype[e]	Zhou et al. 2005
HSPA4	C(1143)T; val(288)ile; exon (rs35853823)	Chinese (64:64)	no association	Ding et al. 2011
	A/G; exon(rs2075800)		no association	
	A/G; exon (rs1061581)		no association	
	T/C; exon (rs2227956)		no association	
	A/C; exon (rs2227955)		no association	
	C/G; exon (rs9469057)		no association	
NOS3	G/A; intron 3 (rs1800781)	Nepalese (70:22)	no association	Wang et al. 2009
	G(894)T; glu298asp; exon 7 (rs1799983)		T (asp) allele	
	A(1083)T; intron 14 (rs3918186)		no association	
	A/C; intron 14 (rs3918188)		no association	
	A/G; intron 21 (rs743507)		no association	
	T/G; intron 23 (rs1808593)		no association	
	C/A; intron 24 (rs7830)		no association	
NOS3	A/G; exon (rs743507)	Chinese (64:64)	no association	Ding et al. 2011
	A/G; exon (rs1800779)		no associations	

Gene	Polymorphism[a]	Population (C:P)[b]	Results	Reference
VEGFA	C/G; exon (rs2010963)	Chinese (64:64)	no association	Ding et al. 2011
	T/C; intron (rs3025000)		no association	
	C/G; exon (rs12435848)		no association	
	C/G; exon (rs3025030)		no association	
	C/T; exon (rs3025035)		no association	
	A/C; exon (rs3778515)		no association	
	A/G; exon (rs10434)		no association	
	C/T; intron (rs1413711)		no association	
	T/C; 5' UTR (rs25648)		no association	
	A/G; exon (rs833070)		no association	
	A/G; exon (rs2075799)		no association	
	T/C; exon (rs3025039)		Association[e]	
	C/A; 5' UTR (rs699947)		no association	
	T/C; 5' UTR (rs833061)		no association	
VEGFA	C(936)T; 3′-UTR (rs3025039)	Chinese (200:200)	C/C genotype; C allele	Ding et al. 2012
	G/C; (rs3025030)		G/G genotype; G allele	
	(rs3025000)		no association	
	(rs833070)		no association	
	(rs1413711)		no association	
	(rs45533131)		no association	
	(rs302053)		no association	
	(rs3025040)		no association	
	(rs10434)		no association	
	(rs2010963)		no association	
	(rs25648)		no association	
	(rs699947)		no association	
	(rs833061)		no association	

Gene	Polymorphism[a]	Population (C:P)[b]	Results	Reference
VHL	C(589)T; exon 3 (rs28940298) A/G; 5' UTR (rs779805) T/C; intron 1 (rs779808) A/C; intron 2 (rs1678607) A(1149)G; 3' UTR	Sherpa (59:45)	no associations	Droma et al. 2008

[a] SNPs are shown as base (position) base; protein changes are shown as amino acid, position, amino acid; UTR, untranslated region; dbSNP (rs).
[b] Sample size: Control (resistant); Patients (susceptible); if n varied between polymorphisms tested in a study, the smallest sample size for which an association was reported is given.
[c] The G/G genotype is designated B/B in the paper.
[d] Listed as T(2437)C elsewhere (*e.g.* (Qi *et al.*, 2009), and dbSNP).
[e] Allele designation was unclear.
[f] An unconventional definition of AMS was used in this paper.

Table 1.5 A description of the genes investigated for a role in susceptibility to acute mountain sickness.

Gene	Name	Chromosomal Location	Rationale for candidacy gene status in altitude illness studies[a]
ABO	ABO blood group	9q34.1-q34.2	Part of demographic survey.
ACE	Angiotensin I converting enzyme 1	17q23.3	Regulation of pulmonary vascular tone; association with elite mountaineers; [ACE] is associated with acclimatisation.
ADRB2	Adrenergic, beta-2 receptor	5q31-q32	Receptor desensitization associated with right ventricular hypertrophy and secondary pulmonary hypertension.
AGT	Angiotensinogen	1q42-q43	Component of RAAS; precursor of angiotensin I.
AGTR1	Angiotensin II receptor, type 1	3q21-q25	Component of RAAS; receptor for angiotensin II.
APOB	Apolipoprotein B	2p24-p23	Role in blood pressure and pulmonary vascular tone
BDKRB2	Bradykinin receptor B2	14q32.1-q32.2	Component of RAAS; increased B2AR tone linked to AMS.
GNB3	Guanine nucleotide binding protein (G protein), beta polypeptide 3	12p13	Involved in blood pressure homeostasis
GSTM	Glutathione S-transferase mu 1	1p13.3	Protection from ROS; decreased activity of plasma glutathione S-transferases associated with AMS
GSTT1	Glutathione S-transferase theta 1	22q11.23	
HIF1A	Hypoxia-inducible factor 1, alpha subunit	14q21-q24	O_2-regulated subunit of HIF-1, which induces transcription of hypoxia responsive genes.
HSPA1A	Heat shock 70kDa protein 1A	6p21.3	Involved in stress tolerance and protein folding.
HSPA1B	Heat shock 70kDa protein 1B	6p21.3	
HSPA1L	Heat shock 70kDA protein 1-like	6p21.3	
HSPA4	Heat shock 70kDA protein 4	5q31.1	
NOS3	Nitric oxide synthase 3, endothelial	7q36	NO regulates vascular tone; polymorphisms associated with expression and activity of eNOS, hypertension and pulmonary vasoconstriction, high altitude natives, and NO concentration.
VEGFA	Vascular endothelial growth factor A	6p12	Upregulated by hypoxia in human lungs; may affect permeability.
VHL	Von Hippel-Lindau tumour suppressor	3p26-p25	Mediates pathway that degrades the HIF-1α subunit; associated with polycythemia.

[a] This is an extremely brief summary of the rationale behind choosing these genes.

1.9 Genome wide association studies and AMS

A GWAS involves the simultaneous interrogation of many polymorphisms distributed throughout the genome for the purpose of identifying those polymoprhisms that are associated with a particular phenotype (Hirschhorn and Daly 2005). GWAS are very comprehensive, as they involve the simultaneous interrogation of typically >100,000 polymorphisms. Unlike candidate gene association studies, which rely on the researcher to choose genes that are possibly involved in the phenotype, GWAS are hypothesis-free. That a biological hypothesis is not needed is the major advantage of GWAS over the candidate gene association study, especially for complex traits for which multiple genes contribute to the variation in the phenotype. In the past decade, GWAS have identified a number of genetic variants associated with numerous clinical conditions and other traits (Manolio and Collins 2009; Manolio 2010).

While a single GWAS is orders of magnitude larger than a candidate gene association study, it has some limitations. The most significant factors restricting the implementation of GWAS are the cost of the experiment and the rigorous statistical requirements necessitating large sample sizes. The cost per single nucleotide polymorphism (SNP) is significantly reduced with a GWAS, but the vast number of SNPs that are genotyped results in a cost that is substantially greater than the typical candidate-gene association study. In addition, when testing many SNPs (*e.g.,* ~10^7 SNPs) in one population, the investigator must reduce the threshold at which a statistical result is considered significant to reduce the chance of false positives (Balding 2006). One method typically used is Bonferonni correction. For 10^7 hypotheses, the threshold for significance is $0.05/10^7$, an extremely small threshold, which requires a large population for the expected small-moderate effect sizes of individual variants (Bouchard et al. 2011). False discovery rate (FDR) methods can also be applied, but again, the application of FDR decreases statistical power. In the context of AMS, GWAS also share a few limitations with candidate gene association studies. Firstly, associations depend on the accuracy of phenotypic assessments, and diagnosing AMS is difficult because non-specific and self-reported symptoms are used to make the diagnosis. Also, because the occurrence of AMS depends on so many factors, replicating studies will be difficult: researchers must try to match the altitude attained, ascent rates, subject health, subject age, weather, and latitude. Secondly, many studies of AMS are opportunistic and have small sample sizes that limit statistical power. Because of the limited power, a lack of an association may reflect a modest effect size and not a true negative.

One method to implement GWAS that reduces the cost of the experiment and the number of subjects required is the two-stage GWAS design (Shi et al. 2010). For this design, a GWAS is first performed in one population or a subset of one population. Those SNPs that meet a certain statistical criterion, which is often much relaxed relative to standard GWAS experimental designs, are then re-screened in the remainder of the original population or in additional populations to confirm the true positives and reject the false positives. Collapsing the GWAS data and the targeted genotype data for further analysis (*i.e.,* joint analysis) is a more powerful design than treating the remaining subjects from the original population as a replication of the initial GWAS (Skol et al. 2006). Many false positives are present among the "positive associations" from the first screen, but the thresholds for statistical significance become more rigorous in the second stage, which should eliminate many false positives. Ultimately, this design is a compromise: data for all SNPs will not be available for every subject in the original population, but the reduced genotyping at futile loci in subsequent populations greatly reduces the total number of loci to screen, lowering the total cost significantly. Furthermore, statistical significance becomes less of an issue as the "positive" SNPs are re-screened in additional populations for confirmation: instead of relying on one statistical test to make the claim of a positive association, replicating the associations in different populations allows for relaxed statistical significance in any one population and increases the overall validity of the results when a large sample size (*e.g.,* 1000s of subjects) is not attainable (Hirschhorn and Daly 2005).

No large candidate gene association studies or GWAS have been performed for AMS; however, a very small GWAS (using ~400 microsatellite markers and 120 subjects) was performed to investigate whether genetic variants contributed to HAPE susceptibility (Kobayashi et al. 2013). While the resolution was extremely small (i.e. 1 marker every 10.8 cM on average), the authors identified Tissue Inhibitor of Metalloproteases-3 (*TIMP3*) as a putatively causal gene for the HAPE susceptibility phenotype. An independent validation is needed to confirm this finding.

1.10 High-altitude adaptation

Some groups of humans have lived at moderate-high altitude for many generations. Of note, Tibetans have occupied the Tibetan plateau (3200-4300 m) for the past 3000-10,000 years (Aldenderfer 2011), or possibly for more than 20,000 years (Moore 2001). As described earlier,

hypoxia is an unrelenting and unavoidable stress (without modern technology that allows for the inhalation of supplemental oxygen). Thus, this long-term habitation in an environment with low oxygen availability could provide a selection pressure great enough to alter the genome of high-altitude Tibetans in comparison to their nearest low-altitude relatives. Whether or not Tibetans have adapted to their high-altitude environments has been a popular area of research in the past few years (MacInnis and Rupert 2011). Adaptation is not the same as acclimatisation, so a full review of this literature is outside of the scope of this dissertation; however, as there may be some crossover in 'strategies' for adjusting to the stress of hypoxia between acute and chronic (in this case, generations) exposures (MacInnis et al. 2011), a brief review is warranted.

Originally, two studies, published simultaneously in *Science*, reported that there were differences in the allelic frequencies of multiple genes across Tibetan and Han Chinese populations (Simonson et al. 2010; Yi et al. 2010). Interestingly, both studies identified *EPAS1* as having different frequencies in the two populations, which they attributed to this gene being under selective pressure for life at high altitude. This finding was supported by the publication of additional papers with the same general finding (Beall et al. 2010; Bigham et al. 2010; Peng et al. 2011; Xu et al. 2011). Although genes associated with high-altitude adaptation are not necessarily the same as the genes involved in high-altitude acclimatisation, it is not unreasonable to expect some crossover, given that both adaptation and acclimatisation to high altitude requires physiological adjustments to low-oxygen environments. There is similar interest in the genetic adaptations of high-altitude residents of the Andes. Of note, (Bigham et al. 2010) reported that *EPAS1* was not a candidate gene in the adaptation of Andeans to high altitude; however, *EGLN1*, a gene in the hypoxia-inducible pathway (HIF) pathway, which was also identified in some Tibetan studies, was reported to be associated with high-altitude adaptation in Andeans. Recent work in a Daghestani population living at moderate altitude (~2000 m) also revealed signs of natural selection in *EGLN1* and *HIF1A* (Pagani et al. 2012).

Chapter 2 Methods of hypoxic exposures and general hypotheses

2.1 Introduction

For my dissertation research, two different modes of hypoxia were employed to measure the hypoxia tolerance of subjects: hypobaric hypoxia and normobaric hypoxia. As described in Chapter 1, hypobaric hypoxia occurs naturally at high altitude, where the P_B is decreased relative to sea level (or artificially in a hypobaric chamber) while normobaric hypoxia can be generated using an oxygen depletion system to lower the F_{IO_2}.

2.2 University of British Columbia normobaric hypoxia chamber

Our laboratory at the University of British Columbia (UBC) houses a normobaric hypoxia chamber (Colorado Altitude Training; Louisville, CO) that can simulate altitudes as high as 4550 m (see Figure 2.1). The chamber is a cube with an approximate volume of 15.6 m^3. The walls and ceilings are composed of transparent plastic attached to an interlocking metal frame. Four oxygen concentrators operating in reverse (*i.e.*, to deplete air of oxygen) are used to generate hypoxic gas that is subsequently pumped into the chamber.

A unit inside the chamber monitors the P_B and the F_{IO_2}, adjusting the volume of hypoxic gas that needs to be added in order to control the chamber P_{IO_2}. Using West's equation (West 1996), the unit matches the P_{IO_2} of the chamber with the predicted P_{IO_2} of a given altitude. The reduction in the F_{IO_2} is compensated for by an increase in the fraction of nitrogen. An exhaust fan vents the chamber to limit CO_2 accumulation when necessary.

The chamber has a functional capacity of up to four persons depending on the experiment: it is large enough for two subjects to sleep on separate mattresses for overnight exposures or for four subjects to sit in chairs with one or more small tables in the room for daytime exposures. The main room of the chamber is attached to an entry vestibule (2.5 m^3), which (i) allows subjects to enter the chamber without substantially disturbing the F_{IO_2}, (ii) allows objects to be passed in and out of the chamber without disturbing the F_{IO_2}, and (iii) when covered, provides privacy for a bathroom. An air conditioner allows the subjects to adjust the temperature inside the chamber. The simulated altitude of the chamber is constantly displayed on the controller

panel that is located outside of the chamber. This panel can be concealed to blind the subjects to the condition, if necessary.

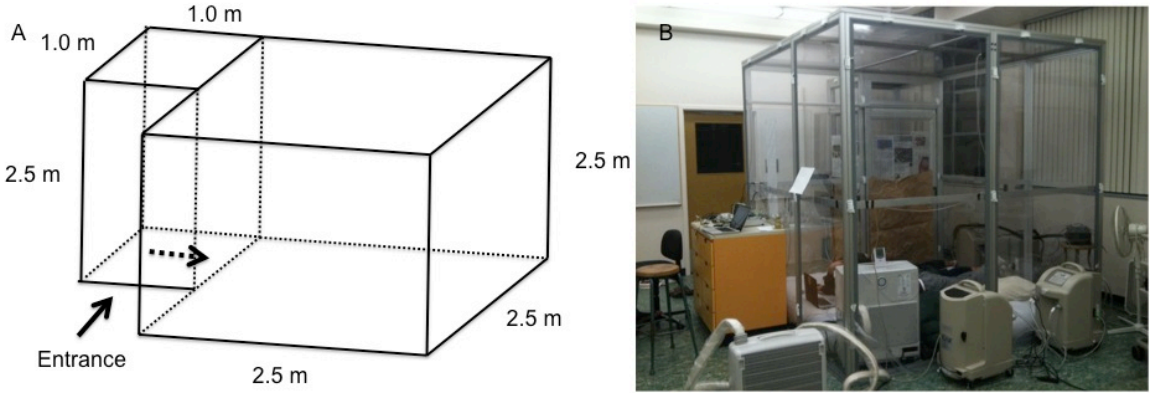

Figure 2.1 A schematic of the layout of the University of British Columbia normobaric hypoxia chamber. The main room of the chamber has an approximate volume of 15.6 m^3 and the entry lock has an approximate volume of 2.5 m^3. A hinged door is located at the entrance to the entry lock, and a sliding door connects the entry lock to the main room of the chamber. The schematic is not to scale. B. A photo of the normobaric hypoxia chamber in the Environmental Physiology Laboratory at the University of British Columbia.

2.3 The Himalaya

The world's largest and highest mountain range, the Himalaya, divides the Tibetan Plateau from the Indian sub-continent (see Figure 2.2). The Himalaya, which begins in northern Pakistan and traverses southeast to northern Bhutan, is approximately 2400 km long, varies between 150 and 400 km in width, and passes through India, China (Tibet) and Nepal. This mountain range contains 10 mountains with peaks above 8000 m, hundreds of mountains with peaks above 7000 m, and countless lower peaks. Because of the concentration of extremely high mountains (including the world's tallest, Mount Everest [*Sagarmāthā* in Nepali; *Chomolungma* in Tibetan/Sherpa]), Nepal is a premier location for high-altitude mountaineering, attracting thousands of climbers annually. Furthermore, Nepal attracts many non-mountaineering tourists, who come to trek in the more accessible but lower altitude regions of the Himalaya (e.g., 3000-6000 m).

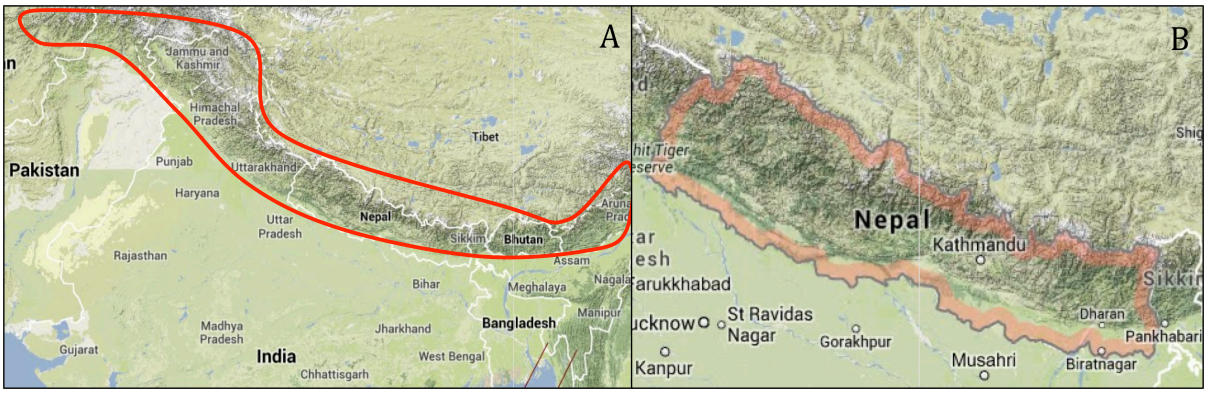

Figure 2.2 Maps of Nepal. Panel A is a map displaying the location of the Himalaya mountain range (roughly outlined in red), which stretches from Pakistan in the northwest to Bhutan in the southeast. Note that a large portion of Nepal is mountainous. Panel B is a topographical map of Nepal (delineated in pink), showing the location of the capital city, Kathmandu. Both panels were taken from Google Maps.

Although Nepal is renowned for mountaineering, the Himalaya has a significant religious meaning to many of the local people. While a full discussion of the religious importance of the Himalaya is outside the scope of this dissertation, some background is needed to appreciate the festival during which I collected large amounts of my data. The following description is summarized from a description provided by Basnyat (2010).

In Hinduism, the Himalaya represents Himavat, who is the god of snow and the father of Parvati. Shiva (who is known as both the destroyer and the transformer) is married to Parvati, and he is one of the most influential Hindu deities. According to Basnyat, in ancient times, Hindu demons and gods decided to collaborate while searching for the elixir of spiritual immortality (known as "Amrit"). The gods and demons decided to churn the ocean, using Mount Mandara as a stick, Vishnu (in the form of a tortoise) to support the mountain, and Vasuki (king of the serpents) as the rope wound around the stick. Tugging back and forth on Vasuki, the demons and gods churned the ocean for 1000 years. While not participating initially, Shiva came forward when a deadly poison (known as "Kalakut") was produced from the churning. To allow the churning process (and the quest for the elixir) to continue, Shiva drank the poison; however, the heat of the poison burned his throat. Needing water to quench the intense burning, Shiva stabbed his trident into the mountains corresponding to a location in present day Langtang National Park, creating three lakes: Gosainkunda, Bhairabkunda, and Saraswatikunda. As a tribute to Shiva's altruistic act, many pilgrims take a holy dip in Gosainkunda during the full moon of Shrawan. Other pilgrims choose to forego the trek to Gosainkunda and instead travel to the Kumbheshwar (in Lalitpur, Nepal), the location of a 5-storey pagoda temple with a pond that

is believed to connect to the lake at Gosainkunda. The festival associated with this practice is known as *Janai Purnima*. In addition to the holy dip, the festival includes the changing of the *janai* (a thread worn around the body by upper caste Hindus), and it marks the beginning of the wearing of the *raksha bandhan*, a thread bracelet continuously worn for the next 3 months (until *Gai Puja* of *Diwali*).

2.4 Gosainkunda, Nepal

Gosainkunda, Nepal (4380 m) is a small isolated site located in the Rasuwa district of Langtang National Park with few year-round residents and few permanent structures. The main access to Goainkunda is by a trail that begins in the town of Dhunche (1950 m), which is approximately 120 km due north of Kathmandu (altitude of ~1300 m; see Figure 2.3). Most pilgrims traveling to the sacred lake come from the Kathmandu valley and travel to Dhunche via bus or motorcycle. The *Janai Purnima* festival occurs in the monsoon season, meaning that travel to the lake can be dangerous: each year, the heavy rains make large sections of the road impassable in the monsoon season. Often, vehicles are forced to stop near Ramche (a very small community *en route* to Dhunche), and pilgrims continue on foot through active landslides, overflowing rivers, and piles of debris. Road quality improves approximately 10-20 km from Ramche, and buses and trucks are available for the remaining 10 km of travel to Dhunche. Some pilgrims will begin the ascent upon arrival to Dhunche; others will stay the night in Dhunche and begin the ascent the following morning.

Figure 2.3 A map displaying the route from Kathmandu, Nepal to Dhunche, Nepal. The route is ~ 120 km. The road is shown in Yellow. The image was taken from Google Earth.

The trail from Dhunche to Gosainkunda is a ~25-km single-track path that passes through multiple small communities, such as Chandanbhari (3450 m) and Laurabina Yak (3900 m; see Figure 2.4). The trail continues beyond Gosainkunda to the Laurabina Pass (~4600 m), before descending through Phedi (3740 m) to Sundarijal (~2000 m). It is possible to ascend via this secondary path, but ascending from Dhunche is the preferred option for most pilgrims coming from the Kathmandu Valley, as it is a much shorter trek and transport back to Kathmandu is readily available in Dunche. The heavy rains in combination with the steepness of the ascent (an average grade of 12%; see Figure 2.5) make the trail slick and challenging. Most pilgrims will reach Gosainkunda within 2 days of leaving Dhunche; however, some pilgrims will make the ascent in a single day and others take more than 2 days.

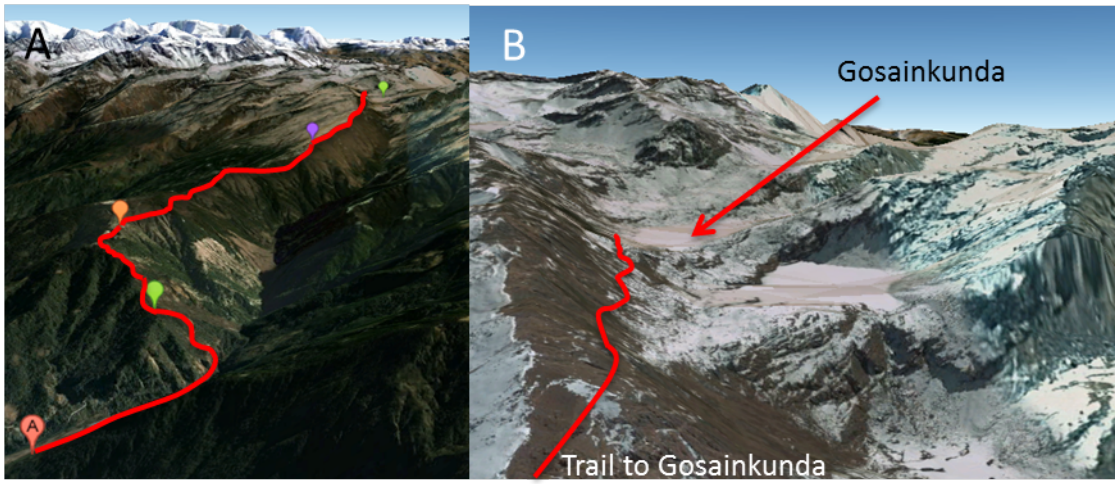

Figure 2.4 Maps of the trail from Dhunche to Gosainkunda. Panel A shows a map of the approximate route from Dhunche (red), through Deurali (green), Chandanbari (orange), Laurabinya (purple), to Gosainkunda (green). Panel B is an enlarged map of the Gosainkunda area showing the final portion of the train trail and the lake. Note that the snow and ice shown in panel B is not typical for the festival. Both images were taken from Google Earth.

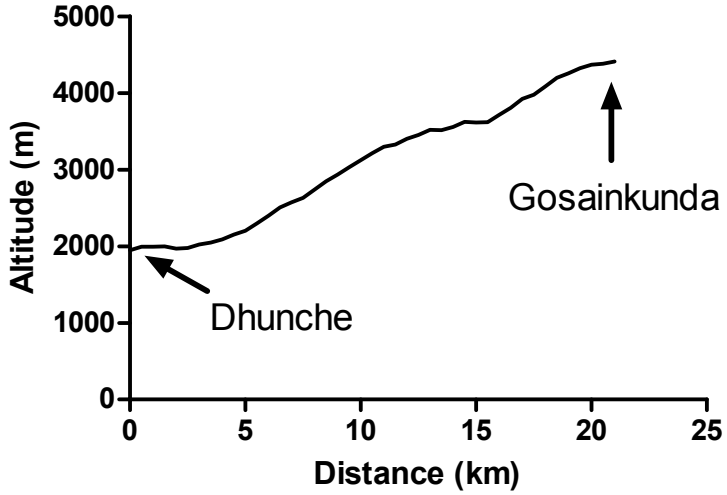

Figure 2.5 The ascent profile from Dhunche (1950 m) to Gosainkunda (4380 m). The average grade of the ascent was approximately 12%.

2.5 Gosainkunda expeditions

My dissertation involved two field studies in Gosainkunda, Nepal. The first expedition took place in 2010. During this expedition, I collected samples for DNA preparation, performed a balance assessment test, measured HR and S_{PO_2}, and attempted to measure F_{ENO}. While data were collected over 5 days, only 99 subjects were recruited due to logistical difficulties. Furthermore, due to technical difficulties, I was unable to collect any F_{ENO} data. Data for the HR and S_{PO_2} are provided in Appendix B. Ultimately, the 2010 expedition served mostly as a pilot study, although some DNA samples were analyzed as part of the GWAS in Chapter 6. I used the information gained from the 2010 expedition to plan the 2012 expedition. While the 2010 expedition had used a cross-sectional design, I decided that a longitudinal study would be needed to increase the sample size. Significantly more data were collected during the 2012 expedition, and these data are presented in Chapters 5, 6, and 8.

2.6 Questions and hypotheses

Despite decades of investigations, a definitive explanation for the variation in the human hypoxia response remains elusive. Based on the assumption that AMS is a marker of poor hypoxia acclimatisation (and conversely, the absence of AMS is a marker of adequate hypoxia acclimatisation), the two guiding questions of my thesis were:

1. Is there a genetic basis to the inter-individual variation in AMS susceptibility?
2. Can AMS susceptible individuals be identified prior to a hypoxic exposure?

These overarching questions led to the creation of multiple related questions that were constructed to guide the creation of research projects and to provide testable hypotheses. With respect to the first guiding question, the specific questions that I attempted to answer through the course of my dissertation were:

A. Is the inter-individual variation in AMS susceptibility repeatable across identical hypoxic exposures? (*n.b.,* Whether or not a history of AMS was a suitable indicator of the occurrence of future AMS episodes [on any two ascents] was also investigated).
B. Does susceptibility to AMS aggregate in families?
C. Does susceptibility to AMS aggregate in biogeographical groups (*i.e*, is there a differential susceptibility to AMS across biogeographical groups)?
D. Are there specific genetic variants that contribute to the variation in AMS susceptibility?

The second guiding question relates more to physiological and epidemiological markers of AMS susceptibility. The sub-questions were:

A. Is the F_{ENO} associated with AMS susceptibility? (*n.b.,* It was also necessary to determine if exposure to hypoxia decreased the F_{ENO} of humans).
B. Is the S_{PO_2} (during wakefulness or during sleep) associated with AMS susceptibility?
C. Are demographic (*e.g.,* age, sex) variables associated with AMS in a group of Nepalese pilgrims? (*n.b.,* this question could apply to other samples in my dissertation research; however, only the Nepal sample was large and heterogeneous enough to investigate the influence of these variables).
D. Does the consumption of Nepalese traditional AMS preventatives (*e.g.,* garlic) make individuals less susceptible to AMS?

Finally, in an attempt to validate the primary questionnaire used in investigations of altitude tolerance, I investigated the factor structure and internal consistency of the Lake Louise Score Questionnaire.

There were numerous hypotheses in my thesis. I hypothesized that there was a genetic basis to the variability in human acclimatisation to hypoxia (*i.e.,* AMS susceptibility). Given this general hypothesis, I also hypothesized that:

i. AMS susceptibility will be repeatable on multiple hypoxic exposures;
ii. AMS susceptibility will aggregate in families;
iii. AMS susceptibility will differ across biogeographical groups; and
iv. genetic variants will be associated with AMS susceptibility.

Furthermore, I hypothesized that subjects who are susceptible to AMS would be identifiable prior to and during a hypoxic exposure based on some variables but not others. Specifically, I hypothesized that subjects susceptible to AMS would:

i. have a lower F_{ENO} than resistant subjects prior to hypoxia exposure;
ii. have a lower S_{PO_2} than resistant subjects during hypoxia exposure;
iii. have a higher HR than resistant subjects during hypoxia exposure;
iv. not be identifiable based on demographic variables; and
v. not be protected from AMS by traditional Nepalese preventatives.

Finally, I hypothesized that the LLS Questionnaire would have a single-factor structure and a high internal consistency. As some of the studies described in this thesis tested multiple hypotheses, it was not possible to completely separate data chapters based on the primary questions. See Table 2.1 for a layout of this dissertation.

Table 2.1 A layout of the chapters contained in this dissertation.

Guiding question	Hypothesis	Chapter(s)
Is there a genetic basis to the inter-individual variation in AMS susceptibility?	AMS susceptibility will be repeatable on multiple hypoxic exposures	4, 7
	AMS susceptibility will aggregate in families	5
	AMS susceptibility will differ across biogeographical groups	5, 6
	Genetic variants will be associated with AMS susceptibility.	6
Can AMS susceptible individuals be identified prior to a hypoxic exposure?	AMS susceptible individuals will have a lower F_{ENO} than resistant subjects prior to hypoxia exposure	3, 4, 5
	AMS susceptible individuals will have a lower S_{PO_2} than resistant subjects during hypoxia exposure	3, 4, 5
	AMS susceptible individuals will have a higher HR than resistant subjects during hypoxia exposure	3, 4, 5
	AMS susceptible individuals will not be identifiable based on demographic variables	5
	AMS susceptible individuals not be protected from AMS by traditional Nepalese preventative	5
Is the Lake Louise Score a suitable questionnaire for assessing AMS?	The Lake Louise Score Questionnaire will have a single-factor structure and high internal consistency	8

AMS, acute mountain sickness; F_{ENO}, fraction of exhaled nitric oxide; $S_{PO_2,}$ oxygen saturation; HR, heart rate.

Chapter 3 Exhaled nitric oxide is associated with acute mountain sickness susceptibility during exposure to normobaric hypoxia

3.1 Summary

Nitric oxide (NO) is a gaseous signaling molecule that participates in a large variety of physiological functions and may have a role in the pathology of altitude illnesses, such as acute mountain sickness (AMS). The effect of normobaric hypoxia on the fraction of exhaled NO ($F_{E_{NO}}$) is a controversial area of high altitude physiology, with the effect varying widely across studies. We exposed 19 male subjects to normobaric hypoxia for 6 hours and measured $F_{E_{NO}}$ and AMS (via Lake Louise Score, LLS) each hour. For data analysis, subjects were divided into AMS-positive and AMS-negative groups based on their LLS during exposure. Eighteen subjects completed the study, and the incidence of AMS was 50%. Mean $F_{E_{NO}}$ was unchanged at hour 1 but was significantly elevated above baseline for the remainder of the normobaric hypoxia exposure ($p < 0.001$). Subjects who developed AMS had a significantly lower mean $F_{E_{NO}}$ at baseline compared to resistant subjects ($p = 0.013$). Further investigations are warranted to confirm our results and to understand the physiological basis of this association.

3.2 Rationale for the experiment

The first experiment of my dissertation was conducted in the fall of 2010, shortly after I returned from my first trip to Gosainkunda, Nepal. This experiment was designed to test whether or not simple physiological variables could serve as markers for an individual's hypoxia tolerance (*i.e.,* AMS susceptibility) under quiet resting conditions. Three non-invasive physiological measures were chosen for this experiment: fraction of exhaled nitric oxide ($F_{E_{NO}}$), blood oxygen saturation (S_{PO_2}), and heart rate (HR). The first variable, $F_{E_{NO}}$, was chosen because of two prior candidate gene associations, one demonstrating an association between a nitric oxide synthase 3 gene (*NOS3*) polymorphism and AMS (Wang et al. 2009) and the other demonstrating an association between $F_{E_{NO}}$ and the same gene (Storm van's Gravesande et al. 2003). The experiment also allowed me to test the effect of normobaric hypoxia exposure on the $F_{E_{NO}}$. I attempted to measure $F_{E_{NO}}$ in Nepalese pilgrims at Gosainkunda in 2010; however, the breathing maneuver was too challenging to explain via a translator and too difficult to perform in

a crowded clinic. The S_{PO_2} and HR were measured because both variables were associated with AMS in our 2010 Gosainkunda sample (see Appendix B) and in previous studies (Loeppky et al. 2003; Karinen et al. 2010; Koehle et al. 2010).

3.3 Introduction

Nitric oxide (NO) is a gaseous signaling molecule with a large variety of physiological functions, including airway and vascular smooth muscle relaxation and the regulation of ventilation-perfusion matching (reviewed in Ozkan and Dweik 2001). Three nitric oxide synthase (NOS) enzymes catalyze the production of NO through the oxidation of L-arginine to L-citrulline. As oxygen is a substrate in this reaction (Kwon et al. 1990), hypoxia, which would lower substrate availability, might be expected to decrease NO production in the lungs, negatively impacting physiological functions regulated by NO.

Nitric oxide produced in the lungs can be measured in the exhaled breath of humans (Gustafsson et al. 1991); however, data from studies on the effect of hypoxia on exhaled NO are inconsistent. Several groups have reported decreased exhaled NO in response to hypoxia, suggesting that the decreased availability of the oxygen substrate does decrease NO production (Brown et al. 2006; Vinnikov et al. 2011), while others have shown hypoxia to have no effect on exhaled NO (Hemmingsson and Linnarsson 2009). These conflicts might be a result of differences in procedures: studies that used hypobaric hypoxia (HH) typically reported decreased exhaled NO whereas, most studies that used normobaric hypoxia (NH) reported no effect. While the equivalent air altitude model claims that HH and NH elicit identical physiological responses for the same partial pressure of inspired oxygen, differences in physiological responses to the two conditions have been reported, leading to criticisms of this model (Conkin and Wessel 2008). The effects of NH and HH on exhaled NO are a recently acknowledged example of a discrepant physiological response to the two modes of hypoxia (Kayser, 2009). As well as differences in barometric conditions, studies of exhaled NO and hypoxia differ in terms of NO analyzer, duration of exposure, and method of measurement.

Humans must cope with hypoxia as they ascend to altitude, and for those who travel rapidly to altitudes greater than 2500 m there is a significant risk of developing AMS. The rate of ascent and the altitude attained strongly influence the probability of developing AMS, but a history of the condition may be the best predictor of susceptibility for an individual (Schneider et

al. 2002). The underlying pathology of AMS is not completely understood; however, environmental hypoxia plays a causal role. Hypoxia acclimatisation involves a suite of coordinated responses that attempt to maintain adequate delivery of oxygen to the tissues. Impaired, inadequate, or delayed acclimatisation is likely the cause of AMS, while a robust acclimatisation response may render individuals relatively resistant. As NO plays a role in the regulation of pulmonary gas exchange and blood flow, NO production will affect oxygen delivery, raising the possibility that innate differences in NO production might contribute to variation in AMS susceptibility.

Individual and population differences in exhaled NO have been associated with the ability of humans to acclimatise (Busch et al. 2001) and adapt (Beall et al. 2001; Hoit et al. 2005) to altitude, respectively. Consistent with a relationship between exhaled NO and altitude acclimatisation, the T-allele of the G894T *NOS3* polymorphism, which had previously been associated with a lower $F_{E_{NO}}$ in asthmatics at sea level (Storm van's Gravesande et al. 2003), was shown to be more common in the AMS-cohort compared to the AMS-negative cohort in a study of Nepalese pilgrims to a festival held at 4380 m in the Himalayas (Wang et al. 2009). While (Brown et al. 2006) did not find an association between the $F_{E_{NO}}$ and AMS susceptibility in HH, the unexpected low incidence of AMS upon ascent to 4200 m restricted statistical power for comparisons of $F_{E_{NO}}$ between groups.

The work described in this paper was designed to determine the effect of NH exposure on exhaled NO and to evaluate the relationship between exhaled NO and AMS susceptibility. We hypothesised that exposure to NH would not decrease exhaled NO, but individuals who exhaled less NO would be more susceptible to AMS, as defined by developing AMS within six hours of extremely rapid ascent from sea-level to a simulated altitude of 4550 m. The $F_{E_{NO}}$ was measured and AMS symptoms were evaluated repeatedly in 19 subjects who were exposed to 6-hour of NH exposure (12% O_2; 4550m equivalent).

3.4 Methods

3.4.1 Subjects

Nineteen male subjects, free of any pulmonary, cardiovascular, and neurological conditions were recruited for this study. On day 1, subjects performed spirometry to confirm

normal lung function. At this time, anthropometric measurements were taken, and the subjects were familiarised with the testing procedures. The University of British Columbia clinical research ethics board approved all procedures, and each subject provided informed consent prior to participating.

3.4.2 Normobaric hypoxia protocol

Subjects refrained from food high in nitrates (such as green leafy vegetables and cured meats) for 48 hours, exhaustive exercise for 24 hours, alcohol and caffeine for 12 hours, and food or drink for 2 hours prior to the beginning of testing. On the test day, subjects arrived at the laboratory in the morning and rested while sitting for approximately 20 minutes in normobaric normoxic conditions (altitude of ~100 m) before providing baseline measures for $F_{E_{NO}}$, HR, and S_{PO_2}. Subjects were exposed to a fraction of inspired oxygen (FI_{O_2}) of 0.12 (4550 m equivalent) upon entering the NH chamber (Colorado Altitude Training, Boulder, CO). The exposure lasted for 6 hours, and $F_{E_{NO}}$, HR, S_{PO_2}, and Lake Louise Score (LLS, see below) were measured every hour until the subjects exited the chamber. The chamber volume was approximately 15.6 m^3 (2.5 m x 2.5 m x 2.5 m) and generally, the subjects remained seated although there was space to stand and move around if they wished. Subjects watched the same nature documentary and listened to the same music. Water was provided as requested, and a standard meal of fruit and granola bars was offered after 4 hours of exposure. Two or three subjects were exposed together during each session.

3.4.3 Exhaled nitric oxide

Exhaled NO was measured using a NObreath NO analyzer (Bedfont Scientific, Kent, England) following the ATS recommended guidelines for measuring NO (American Thoracic SocietyEuropean Respiratory Society 2005) and the manufacturer's testing protocol. This device measures $F_{E_{NO}}$ with an electrochemical sensor. Briefly, subjects inhaled orally to a comfortable volume while sitting and then immediately exhaled at a rate of 50 mL/s for approximately 10 s against a pressure of 10 cm H_2O. Nose clips were not worn. The test was performed twice at each time point and the mean of these two measurements was used for analysis. Values were reported as $F_{E_{NO}}$ in parts per billion (ppb).

3.4.4 Heart rate and oxygen saturation

Heart rate and S_{PO_2} were measured using a Nonin 9600 Pulse Oximeter (Nonin Instruments, Plymouth, MN, USA) with a finger probe while subjects were seated.

3.4.5 AMS diagnosis

AMS severity was assessed each hour using the Lake Louise Score (LLS) Questionnaire (Roach et al. 1993). Subjects were divided into two groups: The AMS positive group ("AMS+") included all subjects who had a headache score of at least 1 and an LLS of 3 or greater at one or more time points during the 6-hour exposure. The remaining subjects, who had a LLS of less than three at all time points, were put in the AMS negative group ("AMS-"). Subjects were also asked not to discuss any of their symptoms with other subjects while in the chamber. The LLS questionnaire was modified to remove the question about sleeping, as subjects were required to remain awake during the experiment.

3.4.6 Statistical analysis

All values are reported as means and standard deviations (SD). Subject characteristics at baseline were compared between AMS+ and AMS- groups using independent t-tests. The effects of time exposed to NH and AMS status were assessed using two-way repeated measures ANOVA. Subjects with one or more missing data points were excluded from two-way repeated measures ANOVA. Dunnett's multiple comparison tests were used to compare mean $F_{E_{NO}}$, HR, and S_{PO_2} during NH exposure to baseline. For all statistical tests, alpha was set to 0.05.

3.5 Results

3.5.1 Subject characteristics

Seventeen of the 19 subjects completed the 6 hours of exposure to NH. One subject exited after 4 hours because he was feeling unwell (LLS of 7 before exiting chamber), and one subject exited after 4 hours because of a personal commitment (no evidence of AMS at time of departure). The subject who left with AMS symptoms was included in the analysis when possible, but the subject who was free of AMS symptoms was excluded from all analysis

because he did not complete the full 6-hour exposure and could have developed AMS in the final two hours. Data at hour 6 were not available from one subject due to technical difficulties, and the $F_{E_{NO}}$ data from one subject who had extremely high $F_{E_{NO}}$ values (>60 ppb; he did not develop AMS) were excluded from the analysis of $F_{E_{NO}}$.

Half of subjects were diagnosed with AMS. AMS+ and AMS- subjects were similar with respect to height, weight, forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and age (Table 3.1). The AMS+ group had a significantly greater LLS compared to the AMS- group (Table 3.1).

Table 3.1 Subject characteristics stratified by AMS status (AMS- or AMS+).

Measurement[a]	AMS+	AMS-	p-value
Count	9	9	NA
Height (m)	182 (7.7)	178 (4.6)	0.22
Weight (kg)	76.1 (11.1)	72.4 (6.4)	0.39
FEV_1 (L)	4.8 (0.64)	4.4 (0.3)	0.18
FVC (L)	6.3 (1.4)	5.6 (0.6)	0.23
F_{ENO} (ppb)	8.6 (6.1)	17.6 (7.1)[b]	0.013*
Age (years)	25.1 (4.4)	29.3 (6.1)	0.11
Maximum LLS	4.7 (1.3)	1.6 (0.9)	<0.001*

[a] All measurements presented here were taken at baseline, except for maximum LLS, which was measured during exposure to normobaric hypoxia.
[b] One subject was excluded because his baseline F_{ENO} value was an outlier (>60 ppb); therefore, n = 8 for this group's mean F_{ENO}.
* $p < 0.05$
FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; HR, heart rate; S_{PO_2}, pulse oxygen saturation; LLS, Lake Louise Score; NA, not applicable.

3.5.2 Heart rate and oxygen saturation

Exposure to NH had no effect on HR measured at hours 1-5, but HR was significantly elevated at hour 6 compared to baseline (Table 3.2). Mean S_{PO_2} was significantly reduced at all time points during NH exposure compared to baseline (Table 3.2). During the 6-hour exposure to NH, there was no significant difference in HR or S_{PO_2} between the AMS+ and AMS- groups (Table 3.2).

Table 3.2 Fraction of exhaled nitric oxide (F_{ENO}), heart rate (HR), and oxygen saturation (S_{PO_2}) responses to 6 hours of normobaric hypoxia exposure (F_{IO_2} of 0.12), stratified by AMS susceptibility (AMS- or AMS+).

Measure	Group	n	Time (hours)							p-value[a]
			0	1	2	3	4	5	6	
F_{ENO} (ppb)	Total	15[b]	12.3 (7.9)	13.1 (9.7)	15.6 (9.3)*	15.6 (9.1)*	16.1 (9.7)*	14.9 (8.8)*	16.3 (10.1)*	< 0.001*,
	AMS-	7	16.7 (7.2)	18.6 (10.5)	20.1 (10.10)	20.2 (9.2)	21.4 (9.6)	20.2 (8.7)	22.1 (9.7)	0.035*,
	AMS+	8	8.4 (6.5)	8.3 (6.0)	11.8 (6.9)	11.5 (7.2)	11.4 (7.6)	10.3 (6.3)	11.3 (7.8)	0.74
HR (bpm)	Total	16[c]	73.5 (10.7)	70.3 (13.2)	73.1 (12.9)	69.8 (11.0)	72.4 (11.2)	73.3 (10.9)	80.8 (12.3)*	< 0.001*,
	AMS-	8	71.8 (11.3)	66.8 (13.7)	68.9 (13.6)	66.9 (11.4)	69.8 (12.2)	71.3 (13.0)	78.3 (12.9)	0.30,
	AMS+	8	75.3 (10.5)	73.8 (12.5)	77.3 (11.5)	72.8 (10.6)	75.1 (10.2)	75.4 (8.6)	83.3 (12.1)	0.94
S_{PO_2} (%)	Total	16[c]	97.4 (1.0)	82.2 (6.4)*	82.1 (5.5)*	83.5 (4.7)*	84.2 (7.6)*	86.3 (4.8)*	86.2 (5.0)*	< 0.001*,
	AMS-	8	97.4 (0.9)	84.3 (6.8)	83.0 (6.7)	85.8 (4.6)	86.1 (6.9)	88.1 (4.1)	87.0 (4.4)	0.18,
	AMS+	8	97.5 (1.2)	80.1 (5.7)	81.1 (4.4)	81.3 (3.8)	82.3 (8.3)	84.4 (5.0)	85.4 (5.8)	0.54

[a] p-values for the main effects of time and AMS status and the interaction effect of time and AMS status, respectively, assessed using two-way repeated measures ANOVA.
[b] Two subjects with incomplete data and one outlier (> 60 ppb) were removed from analysis.
[c] Two subjects with incomplete data were removed from analysis.
* p-value is significant ($p < 0.05$) for comparisons with time 0.
† Mean value is statistically different from mean baseline value, $p < 0.05$.
F_{ENO}, fraction of exhaled nitric oxide; HR, heart rate; S_{PO_2}, pulse oxygen saturation

3.5.3 Exhaled nitric oxide

The overall mean $F_{E_{NO}}$ values at hours 2-6 were significantly elevated compared to the mean $F_{E_{NO}}$ value measured before entering the chamber (Table 3.2; Figure 3.1). There was a statistically significant effect of AMS status on the $F_{E_{NO}}$: AMS- subjects had a significantly greater mean $F_{E_{NO}}$ compared to AMS+ subjects (Table 3.2). Before entering the chamber (Time 0), the AMS+ group had a lower $F_{E_{NO}}$ than the AMS- group (Table 3.1; Figure 3.2). The $F_{E_{NO}}$ at time 0 had a specificity of 79% and a sensitivity of 85% for AMS development during the 6-hour exposure: only one subject with a $F_{E_{NO}}$ greater than 13 ppb developed AMS, and only two subjects with $F_{E_{NO}}$ values below 13 ppb did not develop AMS.

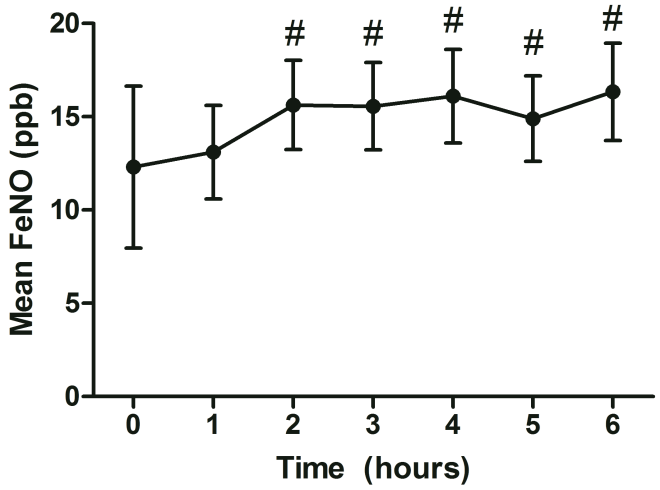

Figure 3.1 The mean fraction of exhaled nitric oxide ($F_{E_{NO}}$, ppb) before and during 6 hoours of normobaric hypoxia exposure. $F_{E_{NO}}$ was measured at baseline (Time 0) and at each hour of the exposure. The F_{IO_2} for the 6-hour exposure was 0.12. n = 15, p<0.001 for the main effect of time. Error bars represent the 95% confidence interval of the mean.

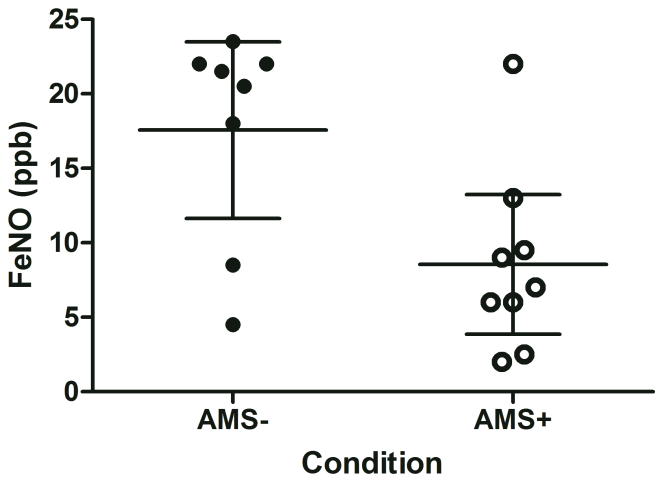

Figure 3.2 Mean fraction of exhaled nitric oxide ($F_{E_{NO}}$, ppb) measured at baseline (normobaric normoxic conditions) and stratified by AMS susceptibility (AMS- and AMS+). Individual data are shown as closed (AMS-) and open (AMS+) circles. AMS susceptibility was determined with the Lake Louise Score during 6 hours of exposure to normobaric hypoxia ($F_{I_{O_2}}$ of 0.12). n = 17, p = 0.013. Error bars represent the 95% confidence interval of the mean.

3.6 Discussion

In a group of healthy non-asthmatic men, mean exhaled NO increased from baseline during 6 hours of exposure to NH and resting exhaled NO measured before NH exposure accurately predicted AMS occurrence during a 6-hour exposure to NH. Our initial hypotheses were that NH would not affect $F_{E_{NO}}$ and that low $F_{E_{NO}}$ values at baseline and during NH exposure would be associated with greater AMS susceptibility; therefore, our data supported the latter hypothesis but not the former.

After one hour of exposure to NH, there was no change in mean exhaled NO, but exhaled NO measured at hours 2-6 was greater than exhaled NO at baseline. The increase in $F_{E_{NO}}$ could be a result of hypoxia-induced inflammation in the lungs. Measurements of $F_{E_{NO}}$ are often used to assess and manage asthma, an inflammatory airway disease in which patients tend to have elevated $F_{E_{NO}}$ compared to non-asthmatic controls (Alving et al. 1993). Rats exposed to 8 hours of NH ($F_{I_{O_2}}$ = 0.10) had increased macrophage recruitment, albumin leakage, and inflammatory mediator expression (Madjdpour et al. 2003). As macrophages produce NO through inducible

and constitutive pathways (reviewed in Ozkan and Dweik 2001), an increased macrophage recruitment during hypoxic exposure could explain the increased $F_{E_{NO}}$ reported here.

Studies of the $F_{E_{NO}}$ in NH have produced mixed results. Dweik et al. (1998) reported that the $F_{E_{NO}}$ decreased in NH (FI_{O_2} of 0.15, 0.10, and 0.05) and that the decrease was proportional to the decrease in FI_{O_2}. The difference between our results and those of Dweik et al. might be due to differences in the experimental protocols of the two studies. First, our earliest measure of $F_{E_{NO}}$ during NH exposure was at one hour, which was not different from $F_{E_{NO}}$ at baseline, indicating that the rise in $F_{E_{NO}}$ that we observed was not immediate. Similarly, two other studies (Hemmingsson and Linnarsson 2009; Donnelly et al. 2011) reported that $F_{E_{NO}}$ did not differ from baseline after 25 and 10 minutes of NH exposure, respectively. Dweik et al. allowed subjects to acclimate to hypoxia for 15 s and then measured $F_{E_{NO}}$ during a 1-min hypoxic exposure. Although our results cannot rule out the possibility that $F_{E_{NO}}$ decreases instantaneously upon exposure to NH, the short duration of exposure combined with the method of measuring $F_{E_{NO}}$ in the Dweik et al. study is a more plausible explanation for the discrepant results. Our study and the studies by Donnelly et al. (2011) and Hemmingsson and Linnarsson (2009) used a single-breath method to measure $F_{E_{NO}}$, and were not confounded by hyperventilation, as exhalation rates were held constant (50 mL/s). Because Dweik et al. (1998) measured $F_{E_{NO}}$ during tidal breathing, their results were possibly confounded by hyperventilation during assessment: $F_{E_{NO}}$ depends on expiratory flow rate (Iwamoto et al., 1994), and acute exposure to hypoxia would increase the expiratory flow rate due to hyperventilation. Similar to Dweik et al. (1998), Busch et al. (2001) reported a (non-significant) decrease in $F_{E_{NO}}$ in response to 6 hours of NH exposure (FI_{O_2} 0.12), but $F_{E_{NO}}$ was also measured during tidal breathing; accordingly, hyperventilation relative to baseline could be responsible for this observed trend as well. Additional studies reported no change in $F_{E_{NO}}$ in response to NH, but each was brief (Tsujino et al. 1996; Verges et al. 2005). Overall, the relationship between $F_{E_{NO}}$ and FI_{O_2} reported by Dweik et al. (1998) might reflect the increasing degree of hyperventilation caused by decreases in FI_{O_2}, as opposed to changes in NO production due to hypoxia (see Appendix F).

Our results suggest that lower baseline $F_{E_{NO}}$ hinders proper acclimatisation to altitude. We reported a strong relationship between $F_{E_{NO}}$ measured at baseline and the development of AMS upon subsequent exposure to NH, with AMS+ subjects having lower $F_{E_{NO}}$ at baseline compared to AMS- subjects.

Physiological evidence to support a relationship between $F_{E_{NO}}$ and AMS susceptibility has previously been reported. In anesthetised subjects, the concentration of exhaled NO correlated positively with the resting partial pressure of arterial oxygen (PaO_2) and the resting alveolar-arterial oxygen difference ($Aa\text{-}DO_2$), implying that NO measured orally might be a marker of gas exchange efficiency in the lungs (Tsuchiya et al. 2000). As acclimatisation minimizes the hypoxia-induced decrease in Pa_{O_2}, a higher Pa_{O_2} in normoxic conditions might allow for enhanced altitude acclimatisation (*i.e.* a higher Pa_{O_2} at a given altitude). The importance of NO production in the lungs might be amplified in hypoxia: inhaled NO did not affect pulmonary gas exchange or hemodynamics in healthy sheep under normoxic conditions, but sheep that inhaled NO in hypoxic conditions had decreased pulmonary artery pressure, decreased pulmonary vascular resistance, and improved ventilation-perfusion matching compared to sheep that were exposed to hypoxia without inhaling NO (Pison et al. 1993). Similarly, in patients with high altitude pulmonary edema (HAPE), a rare and pernicious form of altitude illness, the administration of exogenous NO lowered pulmonary artery pressure, redistributed blood flow to non-edematous regions of the lungs, and improved oxygen saturation (Scherrer et al. 1996). Collectively, these studies support the possibility that innately low exhaled NO could predispose one to poor gas exchange in hypoxia, which could impair acclimatisation to hypoxia.

Our results are the first to suggest an association between baseline $F_{E_{NO}}$ and AMS susceptibility. Brown et al. (2006) reported no relationship between $F_{E_{NO}}$ and self-reported AMS on ascent to 4200 m; however, the incidence of AMS in our study (~53%) was much greater than that of Brown et al. (~19%), resulting in increased statistical power for our comparison. Furthermore, the sampling method used by Brown et al. (offline; 350mL/s) was different than ours (online; 50mL/s), resulting in vastly different mean values for $F_{E_{NO}}$; however, the difference in exhalation flow rate might not explain the discrepancy between the results of the studies, as an offline 350 mL/s and an online 50 mL/s FeNO test were equally accurate diagnostic tests for

asthma (Deykin et al. 2002). In two separate studies, subjects susceptible to HAPE had a lower mean $F_{E_{NO}}$ upon exposure to NH (Busch et al. 2001) and HH (Duplain et al. 2000) compared to subjects who were resistant to HAPE. Although the pathology of HAPE is different from AMS (Hackett and Roach 2001), poor gas exchange in the lungs could contribute to both illnesses. In addition, the high incidence of AMS in patients with HAPE could explain the similar results obtained in our experiment and the experiment of Duplain et al. (2000).

Although not addressed in this study, genetic variation could contribute to variation in NO production. HAPE susceptibility and AMS susceptibility have been linked to variants of the *NOS3* gene (reviewed in MacInnis et al. (2010)), which is the predominant NOS enzyme in the endothelium of the lungs. Some of the variants of the *NOS3* gene that were associated with AMS and HAPE were also associated with lower $F_{E_{NO}}$ in a small population of asthmatics (Storm van's Gravesande et al. 2003), although this finding was not reproduced (Salam et al. 2010). The sample size in our study was too small to power a candidate gene association study.

3.7 Conclusions

In conclusion, our study demonstrated that $F_{E_{NO}}$ increased during a 6-hour exposure to NH and that baseline exhaled NO was related to AMS susceptibility during NH exposure. The increased $F_{E_{NO}}$ could be a result of hypoxia-induced inflammation in the lungs; however, no independent measures of inflammation were performed in this study. Although our association between $F_{E_{NO}}$ and AMS susceptibility is correlational, it raises the possibility of a causal relationship between the two variables. One limitation of our study design was the duration of exposure: we cannot be certain that AMS- subjects would not develop AMS during a longer exposure (although, the near instantaneous exposure to hypoxia experienced when entering the chamber likely increases the stress, suggesting that the AMS- subjects were hypoxia tolerant). Therefore, further investigations are warranted, as confirming and elucidating such a relationship would help explain the underlying pathology of AMS (and possibly other altitude illnesses such as HAPE and high altitude cerebral edema) as well as provide a tool with which to evaluate a person's acute tolerance to altitude. As millions of individuals travel to altitude each year for recreational or professional reasons, a convenient and easily administered test for predicting susceptibility to AMS would be of value to clinicians who work with visitors to altitude.

Chapter 4 Acute mountain sickness is not repeatable across two 12-hour normobaric hypoxia exposures

4.1 Summary

The purposes of this experiment were to determine the repeatability of acute mountain sickness (AMS), AMS symptoms, and physiological responses across two identical hypoxic exposures. Subjects (n = 25) spent three nights at simulated altitude in a normobaric hypoxia chamber: twice at a $P_{I_{O_2}}$ of 90 mmHg (4000 m equivalent; 'hypoxia') and once at a $P_{I_{O_2}}$ of 132 mmHg (1000 m equivalent; 'sham') with 14 or more days between exposures. The following variables were measured at hours 0 and 12 of each exposure: AMS severity (*i.e.*, Lake Louise Score; LLS), heart rate, oxygen saturation, blood pressure, and the fraction of exhaled nitric oxide. Oxygen saturation and heart rate were also measured while subjects slept. The incidence of AMS was not statistically different between the two exposures (84% vs. 56%, $p > 0.05$), but the severity of AMS (*i.e.,* LLS) was significantly lower on the second hypoxic exposure (M (SD): 3.1 (1.8)) relative to the first hypoxic exposure (4.8 (2.3); $p < 0.001$). Headache was the only symptom to have a significantly greater severity on both hypoxic exposures (relative to the sham exposure; $p < 0.05$). Physiological variables were moderately to strongly repeatable (intraclass correlation (ICC) range = 0.39, 0.86) but were not associated with AMS susceptibility ($p > 0.05$). The LLS was not repeatable across two identical hypoxic exposures. Increased familiarity with the environment (not acclimation) could explain the reduced AMS severity on the second hypoxic exposure.

4.2 Rationale for this experiment

This experiment was designed in part as a follow-up for the experiment presented in Chapter 3. I wanted to replicate the association between the fraction of exhaled nitric oxide ($F_{E_{NO}}$) and acute mountain sickness (AMS) and to determine whether heart rate (HR) and oxygen saturation ($S_{P_{O_2}}$) were associated with AMS during a longer exposure to hypoxia. To increase the external validity of the results, I increased the length of the exposure from 6 hours to 12 hours, and I had subjects sleep in hypoxia (simulating one night of high-altitude exposure). In previous studies, symptoms of AMS were reported to be worse the morning after arriving at altitude. Although replicating these previous findings was a goal of this study, the primary purpose of this

study was to determine whether AMS was repeatable. From a genetic perspective, in order to group subjects into categories of hypoxia tolerance, subjects should be sorted into the same group each time they are exposed to hypoxia (*i.e.,* the phenotype should be consistent over time). Therefore, a second hypoxic exposure was included in the design. Finally, because the symptoms are subjective, I decided it was necessary to include a sham condition in the experiment. Previous studies that reported AMS to be repeatable had not included a sham to rule out the effects of exposure to a novel environment.

4.3 Introduction

Acute mountain sickness is a relatively common form of altitude illness that can occur following rapid ascents to altitudes above 2500 m or during exposures to hypoxia (normobaric [NH] or hypobaric [HH]) in a laboratory (Hackett and Roach 2001). Humans vary significantly in their abilities to acclimatise to hypoxia, and researchers often use AMS as a marker of inadequate acclimatisation or acclimation (Hackett and Roach 2001). Despite much research, the etiology of hypoxia intolerance is not well understood (MacInnis et al. 2011), and identifying individuals who are susceptible to AMS before hypoxia exposure is difficult (*e.g.*, Barry and Pollard 2003).

Repeatability is an assessment of consistency within individuals over a series of measurements (Nakagawa and Schielzeth 2010). Although a previous history of AMS is frequently stated to be a strong risk factor for the recurrence AMS (Barry and Pollard 2003; Imray et al. 2011), evidence for the repeatability of AMS is not conclusive. Multiple studies reported associations between AMS history and AMS recurrence (Honigman et al. 1993; Schneider et al. 2002; Richalet et al. 2012); however, these studies also reported moderate numbers of false positives (positive AMS history, negative AMS diagnosis) and false negatives (negative AMS history, positive AMS diagnosis) questioning the extent to which AMS is repeatable. Three prospective studies reported that AMS was repeatable (Robinson et al. 1971; Forster 1984b; Rexhaj et al. 2011; Richalet et al. 2012), but hypoxic exposures were not necessarily comparable in two of the studies because of vasopressin use on one exposure (Robinson et al. 1971) and a high likelihood of acclimatisation on one exposure (Forster 1984b). Furthermore, the three studies lacked sham conditions to blind subjects to the conditions.

The physiological processes responsible for individual differences in AMS susceptibility

have yet to be determined, and a reliable physiological predictor of AMS remains elusive (West 2012). Currently, results are inconsistent for associations between AMS and physiological variables such as $S_{P_{O_2}}$(Karinen et al. 2010; Chen et al. 2012), HR (Loeppky et al. 2003), BP (Loeppky et al. 2003; MacInnis et al. 2012b), and the $F_{E_{NO}}$ (Brown et al. 2006; MacInnis et al. 2012a). Establishing the repeatability of AMS in conjunction with the repeatability of these physiological variables should clarify which variables are associated with AMS and which are not.

This experiment was designed to determine the repeatability of AMS, AMS symptoms, and objective physiological variables across two identical normobaric hypoxia exposures. To prevent bias in self-reported AMS symptoms, a sham exposure was included in the experimental design and subjects were blinded to the experimental conditions. We hypothesized that individual physiological responses to hypoxia would be repeatable across the two identical hypoxic exposures and that each of the physiological variables would be associated with AMS.

4.4 Materials and methods

4.4.1 Subjects

Twenty-six healthy non-smoking subjects (17 male; 9 female) were recruited, all of who resided at low altitude (*i.e.,* < 200 m above sea level) and had not ascended above 2500 m (excluding commercial flights in pressurized airliners) in the 2 months preceding each exposure. On their first visits to the laboratory, subjects were familiarized with the procedures and the testing environment. The clinical research ethics board of the University of British Columbia approved this study, and each subject provided written informed consent prior to participating.

4.4.2 Experimental design

This experiment utilized a single blind, sham-controlled design. Subjects slept three nights in a NH chamber (Colorado Altitude Training; Louisville, CO) located ~100 m above sea level at the University of British Columbia's Vancouver campus. The chamber (approximate volume of 15.6 m^3) was a transparent box housed in a large room with natural lighting. The temperature was controlled at 22°C ± 3°C, but humidity was not controlled. Subjects were exposed to NH on two occasions (H1 and H2; partial pressure of oxygen ($P_{I_{O_2}}$) = 90 mmHg;

4000 m equivalent; West 1996) and to a sham condition on one occasion (SH; $P_{I_{O_2}}$ = 132 mmHg; 1000 m equivalent; West 1996), with a minimum of 14 days between each exposure. An exhaust fan vented the chamber to limit CO_2 accumulation.

Subjects entered the chamber in the evening and remained in the chamber for 12 hours before exiting the next morning. Two subjects occupied the chamber simultaneously for most exposures, but a single subject occupied the chamber on six exposures. Subjects were randomly divided into three groups, with each group experiencing SH on the first, second, or third exposure. Making the chamber slightly hypoxic for the SH exposure was necessary to mimic the sound of the hypoxic exposures, but the SH $P_{I_{O_2}}$ did not lower the subjects' $S_{P_{O_2}}$ values relative to baseline values. Subjects were blinded to the conditions, but the researchers were not because $S_{P_{O_2}}$ values needed to be monitored as a safety precaution.

To limit confounding effects on various measurements, subjects were asked to refrain from the intake of food and drink for 2 hours, caffeine for 12 hours, alcohol for 24 hours, and food rich in nitrates for 48 hours prior to entering the chamber (Olin et al. 2001). Subjects ingested water *ad libitum* in the chamber and were offered a standard meal after 1 hour.

4.4.3 Physiological measurements

All variables were measured in room air before subjects entered the chamber (hour 0) and inside the chamber before subjects exited (hour 12). Subjects were awoken 30 minutes prior to exiting the chamber to allow for data collection.

Hypoxia tolerance was assessed using the Lake Louise Score (LLS) Questionnaire (Roach et al. 1993), which required subjects to rate five symptoms of AMS (headache, gastrointestinal symptoms, fatigue, dizziness, and sleep difficulty) on a scale of 0 (not present) to 3 (severe). A LLS $\geq$ 3 with a headache score $\geq$ 1 was considered a positive diagnosis (AMS+), and a LLS not meeting these criteria was considered a negative diagnosis (AMS-; Roach et al. 1993).

While subjects were supine, HR and $S_{P_{O_2}}$ were measured from the ear lobe with a tabletop pulse oximeter (Avant 9600, Nonin Instruments, Plymouth, MN). Both variables were recorded continuously for 5 minutes, and the mean of each interval was used for data analysis. With the subjects still supine, systolic and diastolic blood pressure (SBP and DBP, respectively)

were measured with a BPM-200 Automated Blood Pressure Monitor (BpTRU; Coquitlam, BC, Canada). The average of three measurements was analyzed. Mean arterial pressure (MAP) was estimated as 2/3 DBP + 1/3 SBP. The $F_{E_{NO}}$ was measured using a NIOX MINO handheld electrochemical analyzer (Aerocrine AB; Solna, Sweden) according to the manufacturer's recommendations and the established guidelines (American Thoracic Society and European Respiratory Society 2005). The $F_{E_{NO}}$ was measured once at hour 0 and once at hour 12. Normobaric hypoxia does not affect the accuracy of this device (Hemmingsson and Linnarsson 2009).

Nocturnal HR and $S_{P_{O_2}}$ were measured continuously while subjects slept using pulse oximetry (as described above). Six-hour blocks of data (corresponding to hours 5-11) were analyzed for each subject during each exposure. From these data, the 6-hour means for HR and $S_{P_{O_2}}$ were calculated and the proportion of time elapsed below $S_{P_{O_2}}$ thresholds (*i.e.,* time below 70% $S_{P_{O_2}}$, time below 75% $S_{P_{O_2}}$, etc.) were calculated.

4.4.4 Data analysis

Data analysis was performed using SPSS version 21.0 (IBM, Armonk, NY, USA), and alpha was set to 0.05 for all statistical tests.

The effect of hypoxia on the severity of AMS at hour 12 was determined with a one-way analysis of variance (ANOVA), and the effect of hypoxia on each individual AMS symptom at hour 12 was determined with a Friedman test. *Post-hoc* analysis was performed with Bonferroni-corrected paired samples t-tests and Wilcoxon signed-rank tests, respectively. The exact McNemar's test was used to compare the incidence of AMS and AMS symptoms across exposures. The percent agreement (Kundel and Polansky 2003) was calculated as a measure of repeatability. To determine if familiarity with the chamber affected the LLS, trend tests were used to compare LLS from the first, second, and third SH exposures.

The effect of the condition on each continuous physiological variable was determined using a two-way repeated-measures ANOVA (time [0 and 12 hours] vs. condition [H1, H2, SH]). *Post-hoc* analysis was performed with Bonferroni-corrected paired-samples t-tests within each exposure and across exposures at hour 12. The effect of condition on mean nocturnal HR and $S_{P_{O_2}}$ was determined with one-way repeated measures ANOVA and a similar *post-hoc* analysis.

The repeatability of each physiological variable was measured with an intraclass correlation (ICC), and the association between physiological variables and AMS were tested with independent t-tests.

4.5 Results

4.5.1 Subject characteristics

Twenty-five subjects (16 male and 9 female) completed this experiment, and one male subject withdrew from the study after completing two exposures. The mean age, height, and weight of subjects were 24.6 (6.2) years, 175 (8.4) cm, and 72 (12) kg, respectively. One subject's $F_{E_{NO}}$ data were excluded from analysis because she exhaled > 100 ppb (approximately 5 times larger than the mean), and high $F_{E_{NO}}$ can indicate the presence of asthma (Taylor et al. 2006), which was an exclusion criterion for the $F_{E_{NO}}$ component of the study. After eight subjects had begun the experiment, BP measurements were added as outcome variables (*i.e.*, n = 17 for these variables). Consecutive exposures were separated by a median of 21 days (range: 14, 108 days), and H1 and H2 were separated by a median of 28 days (range: 14, 138 days).

4.5.2 Normobaric chamber conditions

A mean $P_{I_{O_2}}$ of 90 mmHg was maintained for all hypoxic exposures (range: 87-93 mmHg) and a mean $P_{I_{O_2}}$ of $P_{I_{O_2}}$ mmHg (range: 129-135 mmHg) was maintained for sham exposures. The partial pressure of carbon dioxide (P_{CO_2}) was maintained below 2.6 mmHg.

4.5.3 Repeatability of AMS and its symptoms

The incidence and severity of AMS were much greater in both hypoxic exposures (H1 and H2) compared to the SH exposure (Table 4.1; Figure 4.1). The severity of AMS was significantly lower (-35%) in H2 relative to H1 ($p < 0.001$; Table 4.1), but the difference in the incidence of AMS from H1 to H2 (coincidentally also -35%) was not statistically significant ($p = 0.065$). Agreements for the incidence and severity of AMS on H1 and H2 were low (Table 4.1).

The incidence and severity of all five individual symptoms comprising the LLS were significantly greater in H1 relative to SH, but only the incidence and severity of headache symptoms were significantly greater in H2 relative to SH (Table 4.1). Agreements for the

incidence and severity of AMS symptoms were variable (Table 4.1). The incidence and severity of headache were not statistically different between H1 and H2 (Table 4.1).

The decrease in LLS across SH exposures was not statistically significant ($p = 0.066$), but the linear trend for a decrease in sleep difficulty scores was statistically significant ($p = 0.025$; Figure 4.2).

Table 4.1 The incidence and severity of acute mountain sickness and its individual symptoms in one sham (SH) and two normobaric hypoxia exposures (H1, H2).

Symptom	Incidence				Severity			
	SH[a]	H1[a]	H2[a]	Agreement[b]	SH[c]	H1[c]	H2[c]	Agreement[b]
Headache	3 (12)	21 (84)*	19 (76)*	16 (68)	0 (0,1)	2 (0,3)*	1 (0,2)*	8 (32)
Gastrointestinal	0 (0)	6 (24)*	1 (4)†	20 (80)	0 (0)	0 (0,2)*	0 (0,1)†	20 (80)
Fatigue	4 (16)	14 (56)*	5 (20)†	16 (64)	0 (0,1)	1 (0,2)*	0 (0,2)†	14 (56)
Dizziness	2 (8)	9 (36)*	6 (24)	18 (72)	0 (0,1)	0 (0,2)*	0 (0,2)	17 (68)
Sleep difficulty	18 (72)	24 (96)*	22 (88)	23 (92)	1 (0,2)	2 (0,3)*	1 (0,3)	12 (48)
AMS	1 (4)	21 (84)*	14 (56)*	14 (56)	1.2 (1.0)	4.8 (2.3)*	3.1 (1.8)*†	4 (16)

[a]The number (percentage) of subjects who developed each symptom (and AMS).
[b]The number (percentage) of subjects who had the same response on H1 and H2.
[c]For individual symptoms, the median (range) is reported, but for AMS, the mean (standard deviation) is reported.
The (*) indicates a significant difference from the sham exposure.
The (†) indicates a significant difference between the two hypoxic conditions.

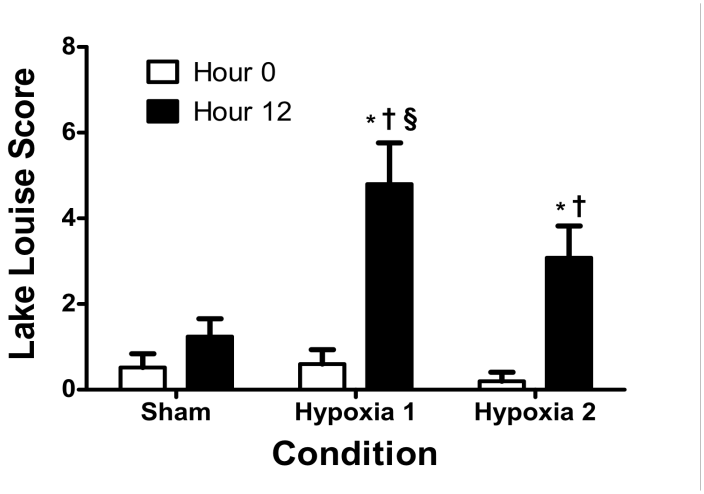

Figure 4.1 The Lake Louise Scores (LLS) at hours 0 and 12 of the sham, first hypoxic, and second hypoxic exposures. The $P_{I_{O_2}}$ of the three exposures were 132 mmHg, 90 mmHg, and 90 mmHg, respectively. The (*) denotes a 12-hour mean that was significantly greater than the 0-hour mean of the same condition ($p < 0.05$); the (†) denote a 12-hour means that was significantly greater than the 12-hour sham mean ($p < 0.05$); and the (§) denotes a 12-hour mean that was significantly greater than the 12-hour mean of the second hypoxia exposure ($p < 0.05$). Error bars represent 1 standard deviation of the mean, and n = 25.

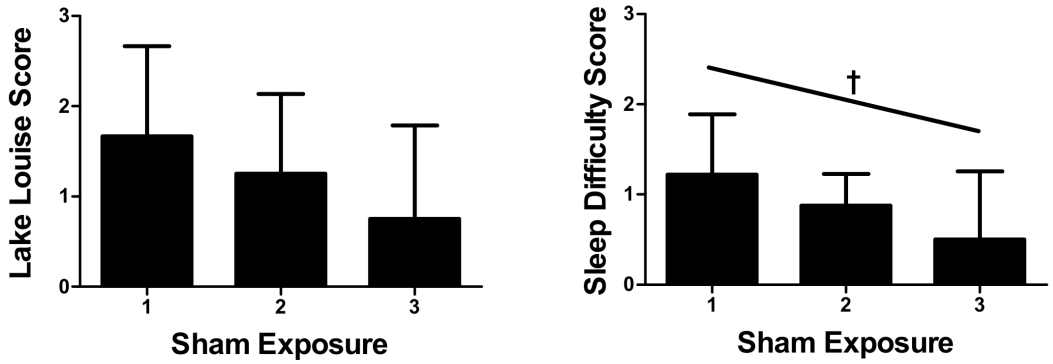

Figure 4.2 The mean Lake Louise Score (LLS) and sleep difficulty item score from hour 12 of sham conditions. Sham conditions randomly occurred on the first (n = 8), second (n = 8), or third (n=9) exposure of the study. Error bars represent 1 standard deviation of the mean. The (†) denotes a statistically significant linear trend for decreasing sleep difficulty scores ($p = 0.025$). The linear trend for LLS was not statistically significant ($p = 0.066$).

4.5.4 Repeatability of physiological responses to hypoxia

As shown in Table 4.2, $S_{P_{O_2}}$ was significantly decreased by hypoxia, HR, DBP and MAP were significantly increased by hypoxia, and $F_{E_{NO}}$ and SBP were unaffected by hypoxia. At hour 12, the means of each physiological variable were not statistically different in H1 and H2 (Table

4.2). The ICC of physiological variables at hour 12 ranged from 0.39 to 0.84, indicating moderate to strong repeatability in normobaric hypoxia (Table 4.2).

Table 4.2 Physiological responses to one 12-hour sham exposure (SH) and two 12-hour hypoxia exposures (H1 and H2).

Variable	Condition	n	Time (hours)[a]		Change (%)[b]	ICC[c]
			0	12		
HR	SH	25	62.4 (10.3)	55.2 (11.3)†	-11.5	
(bpm)	H1	25	62.3 (9.5)	64.5 (12.5)*	+3.5	
	H2	25	63.3 (9.9)	62.0 (13.5)*	+2.1	0.84‡
$S_{P_{O_2}}$	SH	25	97.9 (1.2)	98.0 (1.0)	+0.0	
(%)	H1	25	98.3 (1.3)	84.7 (4.7)*†	-13.9	
	H2	25	98.1 (1.2)	84.8 (5.2)*†	-13.6	0.39‡
$F_{E_{NO}}$	SH	24	21.6 (8.2)	21.8 (7.7)	+0.9	
(ppb)	H1	24	21.6 (10.8)	22.0 (8.7)	+1.9	
	H2	24	22.1 (9.0)	23.8 (9.2)	+7.7	0.78‡
SBP	SH	17	109.2 (11.1)	106.7 (10.6)	-2.3	
(mmHg)	H1	17	108.1 (9.1)	109.1 (7.9)	+0.9	
	H2	17	105.2 (8.1)	107.8 (8.7)	+2.5	0.65‡
DBP	SH	17	64.7 (5.4)	64.5 (7.3)	-0.3	
(mmHg)	H1	17	63.9 (5.3)	69.0 (4.8)*†	+8.0	
	H2	17	62.3 (4.4)	67.0 (7.3)†	+7.5	0.57‡
MAP	SH	17	79.6 (6.5)	78.4 (7.8)	-1.5	
(mmHg)	H1	17	78.8 (5.5)	82.2 (5.3)*†	+4.3	
	H2	17	76.6 (4.7)	80.6 (6.5)†	+5.2	0.66‡

[a] Data are presented as the mean (standard deviation).
[b] The percent change is calculated for hour 12 relative to hour 0 of the same exposure.
[c] Intraclass correlations (ICC) are calculated for mean values measured at hour 12 of H1 and H2.
*Statistically different from hour 12 of sham exposure after Bonferroni correction ($p < 0.008$).
†Statistically different from hour 0 in the same exposure after Bonferroni correction ($p < 0.008$).
‡Statistically significant intraclass correlation at hour 12 of H1 and H2 ($p < 0.05$)
HR, heart rate; $S_{P_{O_2}}$, pulse oxygen saturation; $F_{E_{NO}}$, fraction of exhaled nitric oxide; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

Nocturnal HR was greater on H1 (63.2 (10.6) bpm) and H2 (60.1 (11.5) bpm) relative to SH (52.5 (9.1) bpm; $p < 0.001$ for both comparisons), and nocturnal HR was greater on H1 compared to H2 ($p = 0.005$). Relative to SH (97.5 (1.5) %), nocturnal $S_{P_{O_2}}$ was lower on H1 (79.4 (4.6) %; $p < 0.001$) and H2 (80.2 (4.7) %; $p < 0.001$); however, nocturnal $S_{P_{O_2}}$ was similar on H1 and H2 ($p = 0.14$). The ICC of nocturnal HR and nocturnal $S_{P_{O_2}}$ were 0.86 and 0.68, respectively ($p < 0.05$ for both).

4.5.5 Predicting and diagnosing AMS

Twelve subjects were AMS+/AMS+, two subjects were AMS-/AMS-, nine subjects were AMS+/AMS-, and two subjects were AMS-/AMS+ on H1 and H2, respectively; therefore, the AMS diagnosis on H1 had a sensitivity of 86%, a specificity of 18%, a positive predictive value of 56%, and a negative predictive value of 50% for the AMS status on H2.

The AMS+ and AMS- subjects' data were only compared on H2 because the distribution of subjects was unbalanced on H1 (*i.e.*, 21 AMS+: 4 AMS- on H1). None of the physiological variables was significantly associated with AMS (Table 4.3). The mean proportion of time elapsed below each $S_{P_{O_2}}$ threshold was similar for AMS+ and AMS- subjects, and nocturnal $S_{P_{O_2}}$ was not associated with LLS (Figure 4.3).

Table 4.3 Physiological responses to the second 12-hour normobaric hypoxia exposure (H2) in subjects with (AMS+) and without (AMS-) acute mountain sickness.

Variable	n	Time (hour)	AMS- [a]	AMS+ [a]	t-statistic; p-value
HR (bpm)	25	0	60.9 (9.1)	65.1 (10.4)	-1.05; 0.31
HR (bpm)	25	5-11	57.0 (10.1)	62.5 (12.3)	-1.19; 0.25
HR (bpm)	25	12	58.4 (12.0)	64.9 (14.4)	-1.20; 0.24
$S_{P_{O_2}}$ (%)	25	0	98.2 (1.1)	98.0 (1.3)	0.46; 0.65
$S_{P_{O_2}}$ (%)	25	5-11	79.8 (4.0)	80.6 (5.3)	-0.40; 0.69
$S_{P_{O_2}}$ (%)	25	12	85.5 (2.8)	84.2 (6.5)	0.65; 0.52
$F_{E_{NO}}$ (ppb)	24	0	24.8 (10.4)	19.8 (7.4)	1.37; 0.19
$F_{E_{NO}}$ (ppb)	24	12	25.7 (9.9)	22.1 (8.5)	0.97; 0.34
SBP (mmHg)	17	0	105.0 (7.5)	105.3 (8.8)	-0.06; 0.95
SBP (mmHg)	17	12	106.8 (8.7)	108.4 (9.1)	-0.34; 0.74
DBP (mmHg)	17	0	60.7 (4.6)	63.2 (4.2)	-1.14; 0.27
DBP (mmHg)	17	12	65.0 (6.7)	68.1 (7.7)	-0.82; 0.42
MAP (mmHg)	17	0	75.4 (5.5)	77.2 (4.3)	-0.73; 0.48
MAP (mmHg)	17	12	79.2 (5.3)	81.5 (7.1)	-0.68; 0.50

[a] Data are presented as the mean (standard deviation).
HR, heart rate; $S_{P_{O_2}}$, pulse oxygen saturation; $F_{E_{NO}}$, fraction of exhaled nitric oxide; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

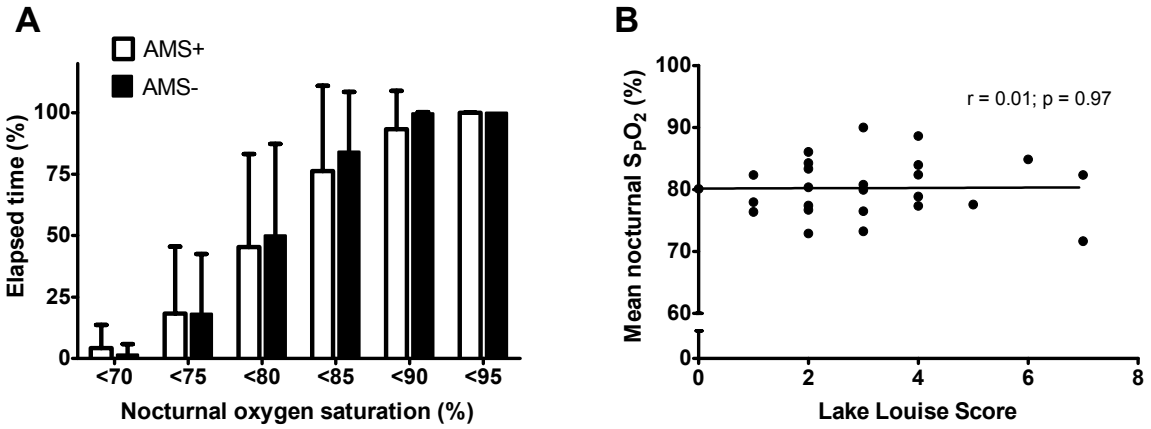

Figure 4.3 The nocturnal oxygen saturation ($S_{P_{O_2}}$) of subjects during the second hypoxic exposure (H2). Panel A displays the percentage of time elapsed below thresholds of $S_{P_{O_2}}$ for subjects with and without acute mountain sickness (AMS+ and AMS-, respectively). Panel B displays the relationship between the mean $S_{P_{O_2}}$ and the Lake Louise Score on H2. Error bars represent 1 standard deviation of the mean, and n = 25.

4.6 Discussion

This is the first experiment to assess AMS repeatability with a single blind, sham-controlled design. Contrary to our hypotheses, the severity of AMS (measured with the LLS) was lower on the second hypoxic exposure relative to the first, and none of the physiological variables was associated with AMS; however, as hypothesized, physiological variables were moderately to strongly repeatable across H1 and H2. We suggest that familiarization with the environment may be responsible for the decrease in AMS symptom severity across hypoxic exposures.

The difference in the AMS incidence between H1 and H2 was not statistically significant; however, that the severity of AMS was significantly lower in H2 still suggests that AMS was not repeatable in this experiment. The severity of AMS is more sensitive to changes than the incidence of AMS, making it a better index for repeatability: for any decrease in LLS, whether or not a change in AMS incidence would occur is dependent on (i) the threshold LLS for a positive AMS diagnosis and (ii) the mean severity of AMS induced by the experimental conditions. The lack of repeatability suggests that it may be inappropriate to categorize subjects as AMS susceptible or AMS resistant after one hypoxia exposure.

Headache, the cardinal symptom of AMS (Roach et al. 2011), was the only symptom with a significantly greater incidence and severity in H1 and H2 relative to SH. The incidence

and severity of headache were not statistically different in H1 and H2, suggesting that headache symptoms may be influenced less by familiarization than other AMS symptoms. Sleep difficulty was the only other symptom that occurred commonly on H2, but the elevated sleep-difficulty scores in SH suggest that the chamber (independent of hypoxia) was responsible for much of the difficulty sleeping.

The decrease in LLS from H1 to H2 was not likely due to hypoxia acclimation. While 5 days above 3000 m in the 2 months preceding an ascent to 4559 m decreased the risk of AMS (Schneider et al. 2002), to our knowledge, sustained acclimation multiple weeks after a single 12-hour exposure to hypoxia has not been demonstrated (in awake or sleeping subjects). A washout period similar to the one employed in this study (*i.e.*, 12-14 days) was previously used to prevent carry-over effects between two longer (22-hour) hypoxic exposures (barometric pressure 446 mmHg; 4300 m equivalent; Muza et al. 2004). That objective physiological responses were similar to H1 and H2 also suggests subjects were not acclimated to hypoxia on the second hypoxic exposure.

Our data suggest that familiarization to the environment affects the LLS. An unfamiliar sleeping environment and the anticipation of unpleasant symptoms (the potential symptoms of AMS were a required element of the consent forms) may have been responsible for the greater LLS on H1. MacNutt et al. (2012) suggested that a greater "psychological tolerance" of altitude (acquired from previous ascents) could explain the reduced AMS symptoms observed in subjects re-ascending to altitude. While our subjects did not ascend to altitude, they may have been anxious about the experiment. In support of this suggestion, sleep quality improved significantly with the number of previous exposures to the chamber, and mean HR, SBP, DBP, and MAP were slightly lower in H2 compared to H1 (at hour 12), possibly indicating reduced anxiety. Although familiarization with the environment has been postulated to be a potential confounding variable in studies of this sort (Muza et al. 2004), this study is the first to demonstrate that familiarization with the testing environment affects self-reported LLS.

That AMS severity was not repeatable is in disagreement with previous studies (Robinson et al. 1971; Forster 1984b; Rexhaj et al. 2011), but inter-study comparisons are difficult for a number of reasons. Firstly, only one previous study (Rexhaj et al. 2011) used the LLS Questionnaire, and methods of assessing AMS (*e.g.,* Hackett's score; Hackett et al. 1976) used in the other studies will likely have different psychometric properties compared to the LLS

that will affect repeatability. Secondly, the methods used to calculate or confirm AMS repeatability differed substantially across studies, preventing a direct comparison of quantitative repeatability statistics. Thirdly, methodological differences make direct comparisons problematic because two studies occurred at high altitude (Forster 1984b; Rexhaj et al. 2011), one study utilized a hypobaric chamber (Robinson et al. 1971), and our study was conducted in a normobaric chamber. Finally, we included the SH exposure to reduce subject bias, which was not done in any of the previous studies.

Pulse oximetry is frequently used in field studies of AMS, but pulse oximetry has not been conclusively demonstrated to be a reliable diagnostic tool for AMS (Windsor 2012). Mean HR (wakeful) was highly repeatable in this study, but similar to previous studies (e.g. Wagner et al. 2012a), HR was not associated with AMS. The mean $S_{P_{O_2}}$ (wakeful) had the lowest repeatability, and it was also not associated with AMS. Our $S_{P_{O_2}}$ results are in agreement with some studies (Chen et al. 2012; Wagner et al. 2012a), but not with others that showed AMS+ subjects to have lower $S_{P_{O_2}}$ values than AMS- subjects (Karinen et al. 2010; Koehle et al. 2010). The low repeatability of wakeful $S_{P_{O_2}}$ may explain mixed results in the literature. As HR is strongly affected by physical exertion and $S_{P_{O_2}}$ is strongly influenced by voluntary breathing patterns (Bilo et al. 2012), Windsor (2012) proposed that pulse oximetry data collected during sleep may be more informative than data collected during wakefulness; however, our results demonstrated that, although nocturnal HR and $S_{P_{O_2}}$ were repeatable, neither variable was associated with AMS. This finding conflicts with previous studies that reported a lower mean $S_{P_{O_2}}$ in AMS+ subjects relative to AMS- subjects (Erba et al. 2004; Nespoulet et al. 2012).

In addition to pulse oximtery, we tested BP and $F_{E_{NO}}$ for associations with AMS. Blood pressure was not associated with AMS, but our sample size was relatively small (n = 17) for these comparisons. The ICCs of SBP, DBP, and MAP were lower than those measured in normoxia (Stanforth et al. 2000), possibly due to the small range of each variable in our data (Bland and Altman 1990). The $F_{E_{NO}}$ was unaffected by normobaric hypoxia, which supports previous studies (Hemmingsson and Linnarsson 2009; Donnelly et al. 2011; MacInnis et al. 2012a), and the high repeatability of $F_{E_{NO}}$ agrees with data collected in normoxia (Kharitonov et al. 2003). The AMS+ subjects had a lower mean $F_{E_{NO}}$ than the AMS- subjects, which, although

not statistically significant ($p = 0.065$), is consistent with previous studies (MacInnis et al. 2012a; You et al. 2012).

It is important to note that exposure to normobaric hypoxia requires subjects to be confined to a small space that is unfamiliar and potentially uncomfortable, making this mode of hypoxia different from a true altitude exposure (Girard et al. 2012). For this reason (and because of the normobaric hypoxia), it is unclear whether or not our results can be generalized to high-altitude settings. We also cannot rule out the possibility that a consistent history of AMS symptoms across multiple (>2) independent exposures to hypoxia would be useful for predicting AMS on future exposures.

4.7 Conclusions

We demonstrated that AMS severity (measured with the LLS) was not repeatable in response to two identical 12-hour normobaric hypoxia exposures, and that the AMS status on H1 was not a reliable predictor of the AMS status on H2. The measured physiological responses to normobaric hypoxia were moderately-strongly repeatable, but none was associated with AMS status. Finally, a greater focus on headache may be warranted in future AMS studies: headache was repeatable across hypoxic conditions, and headache was the only AMS symptom with an elevated severity (relative to sham) on the second hypoxic exposure.

Chapter 5 A prospective epidemiological study of acute mountain sickness in Nepalese pilgrims ascending to high altitude (4380 m)

5.1 Summary

Each year, thousands of pilgrims travel to the Janai Purnima festival in Gosainkunda, Nepal (4380 m), ascending rapidly and often without the aid of pharmaceutical prophylaxis. During the 2012 Janai Purnima festival, 538 subjects were recruited in Dhunche (1950 m) before ascending to Gosainkunda. Through interviews, subjects provided demographic information, ratings of AMS symptoms (Lake Louise Scores; LLS), ascent profiles, and strategies for prophylaxis. In the 491 subjects (91% follow-up rate) who were assessed upon arrival at Gosainkunda, the incidence of AMS was 34.0%. AMS was more common in females than in males (RR = 1.57; 95% CI = 1.23, 2.00), and the AMS incidence was greater in subjects > 35 years compared to subjects ≤ 35 years (RR = 1.63; 95% CI = 1.36, 1.95). There was a greater incidence of AMS in subjects who chose to use garlic as a prophylactic compared to those who did not (RR = 1.69; 95% CI = 1.26, 2.28). Although the LLS of brothers had a moderate correlation (intraclass correlation = 0.40, p = 0.023), overall sibling AMS status was a weak predictor of AMS. The incidence of AMS upon reaching 4380 m was 34% in a large population of Nepalese pilgrims. Sex, age, and ascent rate were significant factors in the development of AMS, and traditional Nepalese remedies were ineffective in the prevention of AMS.

5.2 Rationale for this experiment

After an initial reconnaissance trip to Gosainkunda in 2010, I returned to this field site in 2012 with a better understanding of the trek, the pilgrims, and the experiments that could be performed at this location. For me, the main purpose of the 2012 field season was to collect DNA samples for a large candidate gene association study or genome-wide association study of acute mountain sickness (AMS) susceptibility. I decided that a longitudinal study, in which subjects were recruited at low altitude and followed-up at high altitude, would be the best method to recruit the large numbers needed for such a study. Based on data collected from the 2010 field season (Appendix B) and the data presented in Chapter 3, I also chose to measure the fraction of exhaled nitric oxide ($F_{E_{NO}}$), oxygen saturation ($S_{P_{O_2}}$), and heart rate (HR). In addition to these physiological variables, our research team also measured the fraction of exhaled carbon

monoxide and the optic nerve sheath diameter in smaller cohorts of our overall sample (not part of my dissertation). In addition to the physiological and genetic data collected, a large amount of questionnaire data were also collected (*e.g.,* age, sex, ascent rate, prevention strategy). These data were analyzed to better understand the factors affecting the incidence of AMS in this population at this location. This chapter contains the questionnaire data and physiological data ($F_{E_{NO}}$, $S_{P_{O_2}}$, HR) collected from Nepalese pilgrims attending the 2012 Janai Purnima festival at Gosainkunda.

5.3 Introduction

Failure to acclimatise upon ascent to altitudes above 2500 m manifests as acute mountain sickness (AMS), an illness characterized by headache, nausea, dizziness, fatigue, and poor sleep quality (Roach et al. 1993). The symptoms of AMS are usually mild and self-limiting, but symptoms can become incapacitating (Imray et al. 2011). In some cases, AMS may even progress to high-altitude cerebral edema, a rare but life-threatening condition (Hackett and Roach 2001). Annually, AMS affects millions of high-altitude sojourners (Wilson et al. 2009), impacting their health, travel, and economic productivity.

Ascent profile and individual characteristics determine one's likelihood of developing AMS. Strong positive correlations exist between the incidence of AMS and both the altitude attained and the rate of ascent (Hackett and Roach 2001). Yet, for a given ascent regimen, individuals differ greatly in terms of their susceptibilities to AMS, with some developing AMS and others acclimatising well to hypoxia. The basis of these individual differences in susceptibility to AMS is not well understood (reviewed in MacInnis et al. 2011), and little progress has been made in establishing predictive tools for AMS.

The incidence of AMS might be reduced if the most susceptible individuals were identified pre-ascent and provided with precautionary advice (Luks et al. 2010). Several physiological variables have been tested for the potential to predict AMS susceptibility (*e.g.*, chemosensitivity, heart rate variability), but most variables (excluding perhaps those variables measured as part of Richalet's hypoxic exercise test; Richalet et al. 2012) are not sufficiently reliable and many procedures are not feasible in all settings and populations, especially regions with limited medical resources. Demographic factors and family history of AMS may provide a simpler assessment of an individual's susceptibility; however, these factors must be strongly

associated with the incidence of AMS if they are to be useful predictors of AMS. Furthermore, risk factors previously identified in studies of tourists (Hackett et al. 1976; Kayser 1991; S.-H. Wang et al. 2010), mountaineers (Maggiorini et al. 1990; Mairer et al. 2010), and high-altitude laborers and soldiers (Li et al. 2011) must be re-assessed in unspecialized populations to ensure that these results can be generalized.

Janai Purnima is an annual religious festival occurring in the Nepalese Himalaya on the full moon of Shrawan (the forth month in the Nepali calendar). During the festival, thousands of pilgrims ascend rapidly (*i.e.*, in 1-2 days) from Dhunche (1950 m) to Gosainkunda (4380 m). The goals of this study were (i) to ascertain the incidence of AMS in a large population of Nepalese pilgrims at high altitude and (ii) to investigate factors related to AMS susceptibility in this general population.

5.4 Methods

5.4.1 Overview

This study used a prospective, longitudinal design: subjects were recruited in Dhunche (1950 m) over a 5-day period preceding the 2012 Janai Purnima festival and assessed in Gosainkunda (4380 m) over a similar period of time. Interested subjects were able to provide verbal or written informed consent. Based on previous experiences at this location, verbal consent was the preference of most pilgrims. While verbal consent was not documented, data were not collected until informed consent was obtained. The University of British Columbia Clinical Research Ethics Board and the Nepal Health Research Council granted ethical approval for this study (including verbal consent), and the district health, public health, and chief district officers of Rasuwa provided regional approval.

5.4.2 Recruitment

Most pilgrims traveled to Dhunche from the Kathmandu Valley (~1400 m) by motor vehicle (*e.g.*, bus or motorcycle) and by foot. In Dhunche, the main street in the village was canvassed to recruit subjects. Potential subjects were excluded if they had spent any time above 2500 m in the two months prior to the study. A Nepalese medical student or intern conducted an interview with each subject to collect demographic data (*i.e.*, age, sex), family information (*i.e.*,

name, relatives participating in study), and a baseline LLS. Subjects wore numbered paper bracelets on their wrists for identification at the high-altitude site.

5.4.3 Assessment

All subjects traveled on the same trail from Dhunche to Gosainkunda (Figure 5.1), but ascent rates were self-selected. Subjects were assessed upon arrival to Gosainkunda, and those who stayed over night were asked to report the next morning for a follow-up assessment. At both time points, a Nepalese medical student or intern administered the LLS questionnaire (Roach et al. 1993) in Nepali under the supervision of an experienced physician. Subjects with a LLS ≥ 3 (including a headache score ≥ 1) were considered to be positive for AMS (AMS+), while subjects without a headache or with a LLS < 3 were considered to be negative for AMS (AMS-). Subjects also provided information about their ascent profiles (number of days and sleeping locations), strategies for prophylaxis, general health, and current medications.

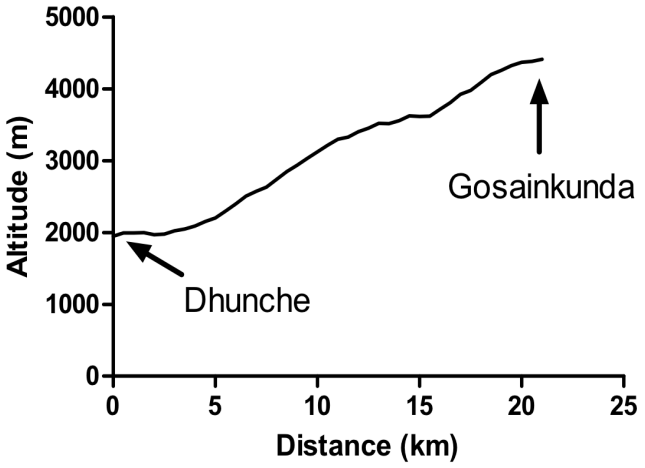

Figure 5.1 The ascent profile from Dhunche (1950 m) to Gosainkunda (4380 m). All subjects ascended to Gosainkunda via this route in 1, 2, or 3 days. The average grade of the ascent was approximately 12%.

5.4.4 Fraction of exhaled nitric oxide and AMS

The F_{ENO} was measured using a NIOX MINO handheld electrochemical analyzer (Aerocrine AB; Solna, Sweden) according to the manufacturer's recommendations and the established guidelines (American Thoracic Society and European Respiratory Society 2005). Briefly, subjects inhaled NO-free air through a mouthpiece connected to the analyzer. Upon

reaching total lung capacity, subjects exhaled at ~50 mL/s for 10s against a pressure of 10 cm H_20. A single F_{ENO} value was measured for each subject.

5.4.5 Heart rate and oxygen saturation

The $S_{P_{O_2}}$ and HR were measured while subjects were seated using a portable pulse oximeter (Go2 Finger Oximeter; Nonin Medical Inc.; Plymouth, MN). A single reading from the index finger was recorded for each subject.

5.4.6 Statistical analysis

Continuous data are presented as means and (standard deviations) and categorical data are presented as counts and/or percentages. A p-value < 0.05 was considered statistically significant for all statistical tests. Independent t-tests were used for continuous variables, and Chi-squared (χ^2) tests were used to assess categorical variables. Risk ratios (RR) were calculated to interpret the strength of associations between categorical variables and the incidence of AMS. Multiple logistic regression was performed to interpret the combined effect of individually significant variables. Mean F_{ENO}, $S_{P_{O_2}}$, and HR values were compared with independent t-tests.

For siblings and parent-offspring pairs, the first subject to be enrolled in the study was regarded as the proband. The relative risk ratio (the risk in siblings of affected probands relative to the risk in siblings of unaffected probands) and the recurrence risk ratio (the risk in siblings of affected probands relative to the population risk) were calculated for siblings. The agreement among the LLS of siblings and parent-offspring pairs was determined using one-way random effects intraclass correlations (ICC).

A post-hoc classification of biogeographical group was performed using the subjects' names (Thornton et al. 2011). Four Nepalese researchers familiar with Nepali names and society independently scored the names as being Indo-Caucasian, Tibeto-Mongolian, or unknown. Those subjects whom at least three of four scorers placed in the same category were used in the analysis. The frequency of AMS in the two main groups (*i.e.,* Indo-Caucasian and Tibeto-Mongolian) was compared with a χ^2 test.

5.5 Results

5.5.1 Subject characteristics and AMS incidence

A total of 538 subjects recruited in Dhunche were included in the study. Baseline LLS were low in all subjects (97% of subjects were below a LLS of 2). Of those initially recruited, 501 (93%) presented for follow-up at Gosainkunda (37 subjects (7%) were lost to follow-up; Table 1). Ten of these subjects were not assessed upon arrival to Gosainkunda (*i.e,* they were assessed the morning after arrival), and they were excluded from further analyses. Dropouts and retained subjects had similar ages (35.6 (12.9) and 36.8 (13.2) years, respectively; t = -0.521; p = 0.60), but dropouts were more likely to be male (92% vs. 69%; χ^2 = 8.07; p = 0.005) and more likely to have traveled to Gosainkunda previously (32% vs. 17%; χ^2 = 5.21; p = 0.022).

Ninety-one percent (n = 491) of subjects were assessed upon arrival to Gosainkunda, and a group of these subjects (n = 125; 25.5%) was assessed a second time the morning after arrival. Subjects assessed at both time points were similar to subjects only assessed upon arrival based on age, sex, and LLS (on arrival; data not shown). Analyses of risk factors, family data, and prophylaxis are based only on data collected upon arrival.

Subject characteristics are presented in Table 5.1. The majority of subjects (n = 366) trekked to Gosainkunda in 2 days (others ascended in 1 or 3 days). The incidence of AMS upon arrival to Gosainkunda was 34.0%, and the distribution of LLS is displayed in Figure 5.2. For those subjects assessed at both time points, the incidence of AMS decreased from 40.0% on arrival to 20.0% on the morning after arrival (χ^2 = 11.7; p = 0.001), and the LLS was significantly lower in the morning (1.8 (2.0)) than on arrival (2.5 (2.0); t = 3.42; p < 0.001), largely due to decreases in the severity of headache and dizziness symptoms (Figure 5.3).

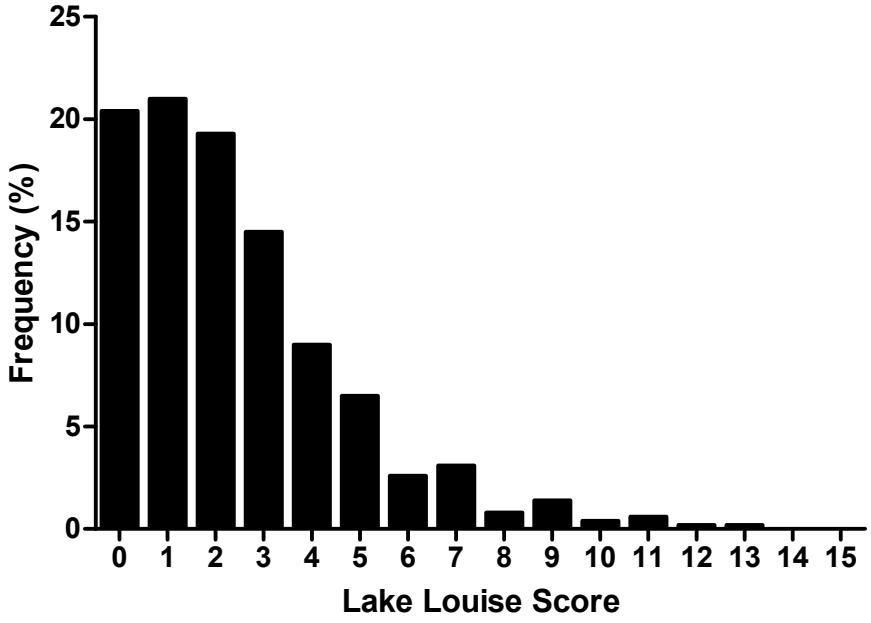

Figure 5.2 The frequency of Lake Louise Scores in Nepalese pilgrims (n = 491) upon arrival to Gosainkunda (4380 m).

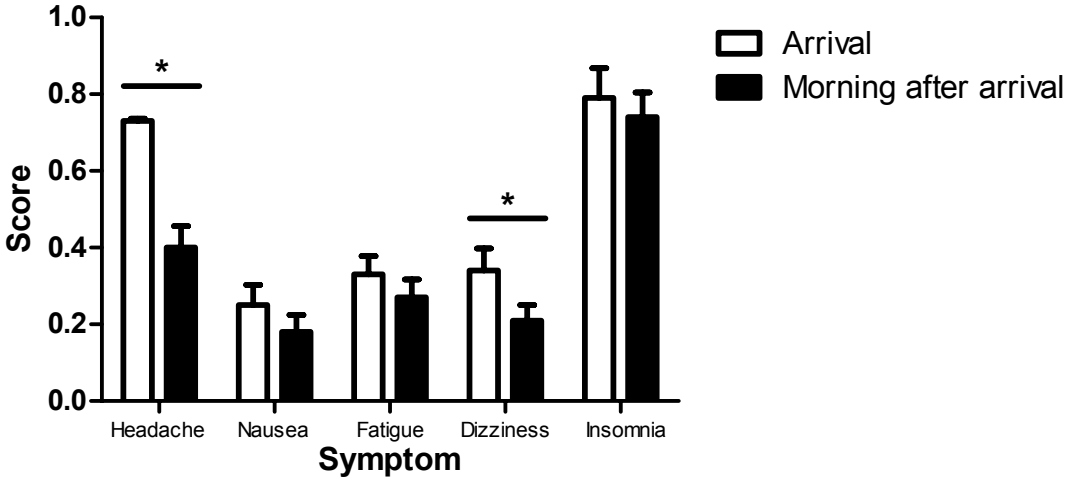

Figure 5.3 A comparison of the AMS symptoms of Nepalese pilgrims who were assessed upon arrival to, and on the morning after arrival to, Gosainkunda (n=125). Headache and dizziness scores were significantly lower the morning after arrival ($t = 4.46$; $p < 0.001$; $t = 2.33$; $p = 0.02$, respectively). Error bars represent one standard error of the mean.

Table 5.1 Characteristics of Nepalese pilgrims assessed upon arrival to Gosainkunda (4380 m).

Variable	Arrival dataset
Sample size	491
Age (years)	36.7 (13.2)
Sex (% male)	70.1
Smoking history (% yes)	29.3
First trek to Gosainkunda? (% yes)	82.7
Ascent rate (days above 3000 m)[#]	1.9 (0.48)
Sleeping altitude of previous night (m)	3566 (652)
Lake Louise Score	2.5 (2.0)

Data are presented as mean (standard deviation) or as a percent.

5.5.2 Individual risk factors

Age was divided at the median (≤ 35 years and > 35 years) to create a dichotomous variable. The 3-day ascent group was removed from the ascent-rate analysis because this group was much smaller (n = 27) and significantly older than the two groups that ascended more rapidly (data not shown).

Age positively correlated with LLS (rho = 0.251; $p < 0.001$), and age was the single strongest predictor of AMS: subjects > 35 years were 63% more likely to develop AMS than subjects ≤ 35 years of age (Table 5.2). Females (57%) and subjects ascending in 1 day (37%) were more likely to develop AMS than males and subjects ascending in 2 days, respectively (Table 5.2). Smoking status and previous travel to Gosainkunda were not significantly associated with the incidence of AMS (Table 5.2); however, the incidence of AMS was ~11% lower in smokers than in non-smokers and ~8% lower in subjects who previously visited Gosainkunda compared to first-time travelers.

Table 5.2 Statistical relationships between dichotomous variables and the incidence of acute mountain sickness (AMS) in Nepalese pilgrims upon arrival to Gosainkunda (4380 m).

Category	Sub-category	Sample size (n (%))		χ^2		Relative Risk	
		Total	AMS+	Stat.	p-value	Stat.	95% CI
Sex	Male	344 (70.1)	100 (29.1)				
	Female	147 (29.9)	67 (45.5)	12.5*	<0.001	1.57*	1.23, 2.00
Age †	≤ 35 years	261 (53.5)	63 (24.1)				
	> 35 years	227 (46.5)	104 (45.8)	25.3*	<0.001	1.63*	1.36, 1.95
Smoking	Yes	147 (29.9)	42 (25.1)				
	No	344 (70.1)	125 (36.3)	2.77	0.096	1.27	0.95, 1.70
Ascent ‡	2 days	366 (80.3)	113 (30.9)				
	1 day	90 (19.7)	38 (42.2)	4.20*	0.040	1.37*	1.03, 1.82
First ascent §	No	85 (17.3)	23 (27.1)				
	Yes	406 (82.7)	144 (35.5)	2.21	0.137	1.31	0.90, 1.90

† The age indicator was missing 3 values due to incomplete data forms.
‡ The 3-day ascent group was removed from the analysis of ascent rate data (see text).
§ First ascent to Gosainkunda (whether subjects had ascended to high altitude elsewhere was not recorded).
*This result is statistically significant (*i.e.*, $p < 0.05$).

Despite the lack of independence among the significant predictor variables (sex, age, ascent rate; data not shown), each variable was a significant predictor of AMS in a multiple logistic regression equation (Table 5.3), suggesting that the effects of each variable persisted when the other variables were controlled.

Table 5.3 The results of multiple binary logistic regression for the individual predictors of AMS in Nepalese pilgrims upon arrival to Gosainkunda (4380 m).

Variable †	B (SE)	Odds ratio		p-value
		Statistic	95% CI	
Constant	-1.57 (0.19)	0.60		0.003
Sex	0.57 (0.23)	1.77*	1.14, 2.75	0.011
Age	1.06 (0.22)	2.89*	1.89, 4.39	<0.001
Ascent rate	0.81 (0.17)	2.26*	1.35, 3.76	0.002

† Males, subjects ≤ 35 years, and the 2-day ascent group were used as the reference categories for the calculation of odds ratios.
* This result is significant (*i.e.*, $p < 0.05$).

5.5.3 Prophylaxis

Pharmaceutical prophylaxis was uncommon (acetazolamide: 7%; paracetamol: 7%; non-steroidal anti-inflammatory drugs (NSAID): 3%) and not associated with a decreased occurrence of AMS (Table 5.4); however, many pilgrims (70%) ingested one or more of the following foods to prevent AMS: garlic, ginger, lemon, or mountain pepper (*Zanthoxylum* sp.). Of these foods, garlic and mountain pepper were associated with a greater risk of AMS (Table 5.4). Only the deliberate ingestion of these foods to prevent AMS was recorded (*i.e.,* subjects were not asked about their diets), and details of dosage and timing were not obtained for pharmaceutical or dietary prophylaxis.

Table 5.4 Statistical relationships between the use of pharmaceutical and dietary prophylaxis and the incidence of acute mountain sickness (AMS) in Nepalese pilgrims upon arrival to Gosainkunda (4380 m).

Prophylactic agent	Use	Sample size (n (%))		χ^2		Relative Risk	
		Total	AMS+	Stat.	p-value	Stat.	95% CI
Acetazolamide	No	457	155 (33.9)				
	Yes	34	12 (35.3)	0.027	0.87	1.04	0.65, 1.67
Paracetamol	No	459	154 (33.6)				
	Yes	32	13 (40.6)	0.67	0.41	1.21	0.78, 1.88
NSAID	No	478	162 (33.9)				
	Yes	13	5 (38.5)	0.12	0.73	1.14	0.56, 2.28
Garlic	No	178	42 (23.6)				
	Yes	313	125 (39.9)	13.50*	< 0.001	1.69*	1.26, 2.28
Ginger	No	371	120 (32.3)				
	Yes	120	47 (39.2)	1.88	0.17	1.21	0.93, 1.58
Lemon	No	366	117 (31.5)				
	Yes	125	50 (40.0)	2.68	0.10	1.25	0.96, 1.63
Mountain pepper	No	448	147 (32.8)				
	Yes	43	20 (47.6)	3.28	0.07	1.42*	1.00, 2.1

* This result is statistically significant (*i.e.*, $p < 0.05$).

5.5.4 Family data

Forty-eight pairs of siblings were identified in the dataset. The relative risk ratio was 1.38 (95% CI = 0.68 – 2.83), and the sibling recurrence risk ratio was 1.47 (95% CI = 0.82 – 2.62), but neither ratio was significantly different from 1.0 ($p > 0.05$). The ICC was significant for the LLS of brothers (0.40; $p = 0.023$) but not for all siblings (0.16; $p = 0.13$). Probands (36.8 (12.9) years) and their siblings (38.0 (13.1) years) were of similar ages ($t = -0.421$; $p = 0.67$).

Twenty-nine parent-offspring pairs were identified in the dataset, but there was no relationship between the LLS of parents and their offspring (ICC = 0.12; $p = 0.26$). Ten parents

developed AMS compared to only five offspring who developed AMS, but parents (54.4 (10.7) years) were significantly older than their offspring (31.7 (12.0) years; $t = 7.48$; $p < 0.001$).

5.5.5 Biogeographical group

Subjects taking Diamox (n = 36) were excluded from the biogeographical analysis. The distribution of subjects (n = 455) by ethnicity was 60.8% Indo-Caucasian, 7.7% Tibeto-Mongolian, and 31.4% undetermined. Indo-Caucasian subjects (36.1%) were more likely to develop AMS than Tibeto-Mongolian subjects (17.1%; $\chi^2 = 4.98$; $p = 0.026$). Similarly, those of Indo-Caucasian ancestry had a significantly greater mean LLS (2.5 (2.2)) relative to those of Tibeto-Mongolian ancestry (1.8 (1.6); $t = -2.1$; $p = 0.042$; see Figure 5.4).

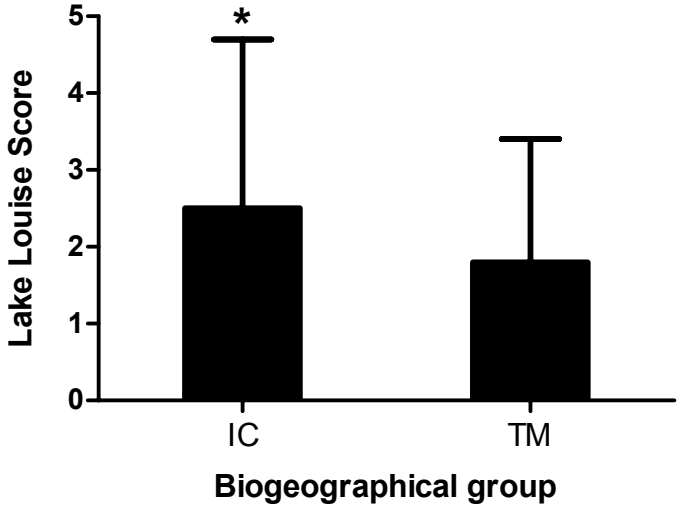

Figure 5.4 The severity of acute mountain sickness in individuals with Indo-Caucasian (IC) and Tibeto-Mongolian (TM) ancestry. The asterisk (*) denotes a statistically significant difference between the two groups. Error bars represent 1 standard deviation.

5.5.6 Fraction of exhaled nitric oxide, oxygen saturation, heart rate and AMS

Subject characteristices for those who provided F_{ENO} samples are shown in Table 5.5. AMS+ and AMS- subjects had similar mean F_{ENO} values (Figure 5.4), and F_{ENO} did not correlate with LLS ($r = 0.03$; $p = 0.86$). AMS+ and AMS- subjects had similar $S_{P_{O_2}}$ and HR values (Table 5.6).

Table 5.5 Characteristics of the subset of subjects who were in the F_{ENO} component of this study.

Variable	Total	AMS+	AMS-	Statistic; p-value
n	46	12	34	NA
Age (years)	33.8 (11.7)	39.5 (12.3)	31.8 (11.0)	-2.03; 0.049*
Sex (M:F)	36:10	3:9	7:27	0.101; 0.75
Ascent rate (1:2:3 days)	3:38:5	1:8:3	2:30:2	3.56; 0.17
Lake Louise Score	2.07 (1.62)	4.08 (1.24)	1.35 (1.04)	-7.43; < 0.001*
F_{ENO} (ppb)	14.5 (9.4)	13.8 (8.9)	14.8 (9.6)	0.30; 0.76

n, sample size; F_{ENO}, fraction of exhaled nitric oxide.

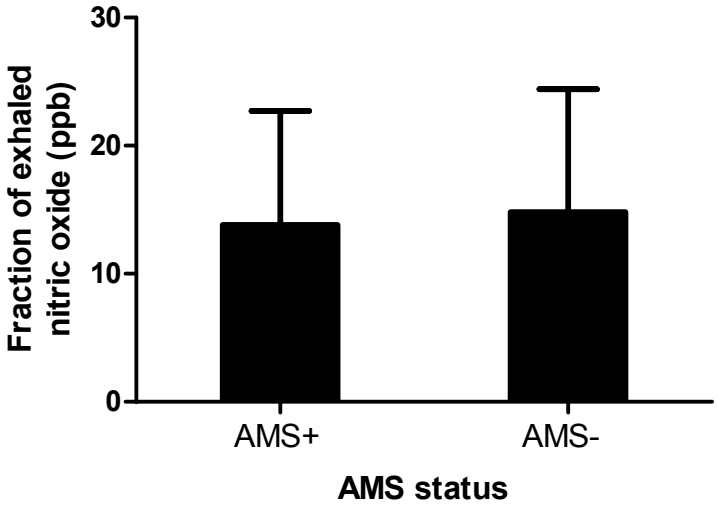

Figure 5.5 The fraction of exhaled nitric oxide (F_{ENO}) in individuals with (AMS+) and without (AMS-) acute mountain sickness. Summary data for these groups is provided in Table 5.5. Error bars represent 1 standard deviation.

Table 5.6 The mean (standard deviation) oxygen saturation (S_PO_2) and heart rate (HR) of subjects with (AMS+) and without (AMS-) acute mountain sickness (AMS).

Variable	Sample size (AMS-: AMS+)	AMS+	AMS-	Statistic; p-value
$S_{P_{O_2}}$ (%)	322:167	81.1 (5.5)	82.1 (5.0)	0.07
HR (bpm)	322:167	106 (15)	105 (15)	0.33

$S_{P_{O_2}}$, oxygen saturation; HR, heart rate; bpm, beats per minute.

Mean (SD)

5.6 Discussion

To our knowledge, this is the largest study of AMS in pilgrims traveling to high-altitude. Our main findings were (1) sex, age, and ascent rate were significant risk factors for the development of AMS upon arrival to 4380 m; (2) the use of traditional preventatives (*i.e.,* garlic

and mountain pepper) was unexpectedly associated with an increased risk of AMS; and (3) sibling AMS status was a weak predictor of AMS, despite a moderate correlation in the LLS of brothers.

Ninety-one percent of subjects who were recruited in Dhunche completed the study. This completion rate likely reflects the religious devotion of the pilgrims, who are known to carry out their religious duties despite significant hardship and AMS symptoms. The few subjects who dropped out of the study may have descended for health reasons (*e.g.,* altitude illness), but even if all of those subjects lost to follow-up were attributed to the AMS+ group, that hypothetical AMS incidence (38%) would not be much greater than our reported incidence.

Previous studies at the Janai Purnima festival in Gosainkunda reported AMS incidences of 5% (Basnyat 1993) and 68% (Basnyat et al. 2000) in Nepalese pilgrims. The former study may have underestimated the true incidence of AMS at the festival because only visibly ill pilgrims were counted as having AMS, while all other passersby were assumed to be free of AMS. The latter study used a random sampling protocol at the high-altitude site, and the incidence might have been greater as a consequence of the cross-sectional design. The AMS incidence reported in this study is similar to incidences measured at different locations with comparable altitudes (*e.g.,* Basnyat et al. 1999; S.-H. Wang et al. 2010).

The severity of AMS is reportedly greater after sleeping at a new altitude than upon arrival (Eichenberger et al. 1996); however, the subjects in this study who were assessed upon arrival and the morning after arrival were symptomatically better the morning after arriving at Gosainkunda. While those subjects who presented for the morning-after-arrival assessment were similar to those who did not present (based on demographic and ascent data collected upon arrival), we cannot rule out the possibility that pilgrims who awoke feeling unwell chose to forego the morning assessment. If this scenario were true, the morning-after-arrival dataset could be biased with respect to LLS (*i.e.,* a lower incidence and severity of AMS would be expected), and this aspect of our study should be interpreted cautiously. Alternatively, these subjects may have had sufficient time to acclimatise, given that the night spent at Gosainkunda was the second night at an altitude above 3000 m for 57% of subjects. Improvements in LLS were largely the result of decreased headache and dizziness scores, but the sleep quality score did not improve. In studies at 4559 m, poor subjective ratings of sleep quality persisted for days, despite decreases in the LLS (Nussbaumer-Ochsner et al. 2011; Nussbaumer-Ochsner et al. 2012).

Similar to earlier studies at the Janai Purnima festival (Basnyat et al. 2000 and M. Freeman, Unpublished data), the incidence of AMS was significantly greater in females than males. This finding is supported by a large prospective study (Richalet et al. 2012) and several smaller studies (Kayser 1991; Honigman et al. 1993), but not all studies reported sex as a risk factor for AMS (*e.g.*, Hackett et al. 1976; Maggiorini et al. 1990; Schneider et al. 2002; Gaillard et al. 2004). Given the agreement among studies of Nepalese women, sex seems to be a strong risk factor for AMS in this population. One reason for the sex difference could be cultural, with females possibly more likely to admit AMS than males (Basnyat et al. 2000). Alternatively, females may have been more likely to avoid food and drink (for religious reasons) than men. Hypoglycemia and dehydration can be confused with AMS, as the symptoms of both conditions overlap considerably (Litch 1996; Hackett and Roach 2001).

The severity and incidence of AMS increased with age. This finding is contrary to many previous studies, including a large prospective study (Richalet et al. 2012) and several smaller studies (Honigman et al. 1993; Gaillard et al. 2004). The relationship between age and AMS reported in our study might be partly due to the religious nature of the Janai Purnima festival: the older pilgrims may have become more dehydrated than the younger pilgrims by avoiding food and drink on the ascent (Basnyat et al. 2000). Alternatively, there may be differences (*e.g.*, health and socioeconomic status) in individuals who travel to altitude for recreation and pilgrims who feel compelled to travel to altitude for religious purposes. Specifically, older pilgrims likely had significant co-morbidities (*e.g.* neurological disorders and pulmonary diseases) that could increase their risk of AMS (Baumgartner et al. 2007; West 2009), and many pilgrims believe that blessings from Lord Shiva can cure these ailments. In studies of mountaineering populations, individuals who are susceptible to AMS may be less likely to continue mountaineering, possibly making older mountaineers a self-selected group that is relatively resistant to AMS. This selection process may partially account for the previously reported lower incidence of AMS in older individuals.

As expected, pilgrims who ascended to Gosainkunda from below 3000 m in one day were more likely to develop AMS than those who ascended in two days, which is likely a result of insufficient time to acclimatise (Hackett et al. 1976). Because subjects chose their own ascent rates, we cannot rule out factors that may have been related to this choice; however, the

relationship between ascent rate and AMS incidence is supported by the literature (Hackett et al. 1976; Schneider et al. 2002).

Two recent studies reported that smokers were less likely to develop AMS than non-smokers (Wu et al. 2012b; You et al. 2012), and, although not statistically significant, our results support these findings. Smoking had a relatively small effect on the incidence and severity of AMS, and it is not recommended for the prevention of AMS for many reasons, including its deleterious effects on overall health, exercise capacity, and frostbite susceptibility (Wu et al. 2012b).

The rate of pharmaceutical prophylaxis amongst Nepalese pilgrims is typically low, although it may be increasing, as the proportion of pilgrims taking acetazolamide in this study was 2.5 times higher than in a previous report (Basnyat et al. 2000). Those who chose to take pharmaceuticals had a similar AMS incidence compared to those who did not, which strongly disagrees with multiple studies (Gertsch et al. 2010; Gertsch et al. 2012) and a recent meta-analysis (Kayser et al. 2012); however, our study was not designed to test the effectiveness of pharmaceutical prophylaxis, and the lack of effect may be due to inappropriate dosages and strategies or confounding factors associated with a non-random design.

Traditional AMS preventatives (*i.e.*, garlic and mountain pepper) were associated with a higher incidence of AMS relative to those who did not consume these foods with the purpose of preventing AMS. It is not likely that these preventatives caused AMS through a physiological pathway, as all subjects likely ingested some amount of each food through their regular diets. While studies have not tested the effects of garlic on AMS, the ingestion of garlic prior to hypoxic exercise did not affect oxygen consumption, oxygen saturation, heart rate, blood pressure, or exercise performance relative to placebo (Morris et al. 2013). Pilgrims who consumed foods that they expected to prevent AMS might have ignored other means of AMS prophylaxis (*e.g.*, slow ascent rate), increasing the probability that they would experience AMS. Until a randomized control trial can demonstrate that these foods prevent AMS, Nepalese pilgrims (and others) should be discouraged from relying solely on traditional Nepalese methods as a means to prevent AMS. Abstention from these foods is not likely necessary while ascending to high altitude.

Family history was not a significant risk factor for AMS in this population, but signs of familial aggregation were still evident. Sibling, parent and offspring AMS status were weak

predictors of AMS. The LLS of brothers had a moderate correlation, but correlations for the LLS of other siblings and parent-offspring pairs were weak. Differences in age and sex may have contributed to the weak correlations. Establishing whether or not AMS aggregates in families is essential for genetic studies of AMS susceptibility, as familial aggregation is a criterion for the genetic basis of AMS (MacInnis et al. 2011). Until larger family studies are conducted, family history of AMS will not be useful for counseling travelers to high altitude.

Subjects identified as Tibeto-Mongolian were approximately half as likely to develop AMS compared to subjects identified as Indo-Caucasian. This reduced susceptibility is consistent with previous studies that showed individuals of Tibetan ancestry to be at a lower risk of AMS than individuals of Japanese and Han Chinese (Wu et al. 2005) and individuals of Han Chinese and Hui Muslim (Li et al. 2011). It is well known that Tibetans living at high altitude are genetically adapted to life in hypoxia (reviewed in MacInnis and Rupert 2011), but individuals of Tibetan ancestry who have resided at low altitude also seem to acclimatise better to high altitude than other groups residing at similar low altitudes. It is possible that these low-altitude populations have the same genetic advantage as the high-altitude Tibetans, although this hypothesis has not been tested.

Biogeographical ancestry was determined using surnames. This approach was previously used by Thornton et al. (2011) to accurately categorize individuals as Indo-Caucasian or Tibeto-Mongolian: all subjects of Tibeto-Mongolian ancestry and 93% of subjects of Indo-Caucasian ancestry were correctly identified. To ensure the reliability of our categorization, we had four researchers independently determine the ancestry of each subject, and we only included subjects whom had agreement from at least three of four researchers. This approach was likely conservative, but it also strengthens our result.

The F_{ENO} was not associated with AMS in the subgroup that provided a sample in Dhunche. This result is in contrast with those of MacInnis et al. and You et al., but they support the results of Brown et al. The sample size was larger than that in two previous studies by our research group; however, this study had a lot more 'noise.' Subjects chose their own ascent rates, represented a much larger range of ages, and potentially had more undiagnosed respiratory conditions, all factors that could affect the F_{ENO} or AMS susceptibility

Our data collection was limited by the austere environment of Gosainkunda (*e.g.,* no electricity) and the short amount of time we had with each pilgrim. We were unable to control

for several variables that could be related to AMS susceptibility, such as underlying health conditions. There has been significant interest in the utility of pulse oximetry in the diagnosis of AMS (Windsor 2012), and we previously demonstrated that pulse oximetry was associated with AMS status at this particular field site (Koehle et al. 2010); however, because of the inclement weather, limited indoor facilities, and large number of subjects who visited our site in a short period of time, we were not able to accurately record heart rate and oxygen saturation data from all subjects. These data are included, but the data may not be true resting values because of the testing environment.

5.7 Conclusions

In a large prospective study of Nepalese pilgrims, we observed an AMS incidence of 34.0% after a rapid ascent from 1950 m to 4380 m. Females and older pilgrims were more likely to develop AMS than male and younger pilgrims, and ascent rate was a risk factor for AMS. The ineffectiveness of dietary supplements suggests that traditional preventative strategies (*e.g.*, garlic) should not be relied on as the sole means of AMS prevention. Weak to moderate relationships were identified between the LLS and AMS status of siblings and parent-offspring pairs, consistent with a modest role for genetics in this cohort.

Chapter 6 A genome-wide association study of AMS susceptibility in a group of Nepalese pilgrims ascending to 4380 m

6.1 Summary

While there is anecdotal and scientific evidence that some people are more tolerant of changes in altitude than others, the basis for individual differences in acute mountain sickness (AMS) susceptibility is unknown. Candidate gene association studies have attempted to identify genetic variants that contribute to AMS susceptibility with limited success (reviewed in MacInnis et al 2010); however, only a few genes have been investigated. We used a genome-wide association study to interrogate the entire genome, thereby probing all known genes rather than choosing genes based on *a priori* hypotheses. Saliva samples were collected from subjects who ascended rapidly to Gosainkunda, Nepal (4380 m) as part of the 2010 and 2012 Janai Purnima festivals (see Appendix B and Chapter 5) and in whom AMS status and AMS severity had been determined using the Lake Louise Score (LLS). Analysis was based on 99 male subjects, representing the 'tails' of the LLS severity distribution (*i.e.*, LLS ≤1 (none/very mild) and LLS ≥4 (severe) and genotyping was performed using Infinium Human Core Exome Bead Chips, which assay 542,556 single nucleotide polymorphisms (SNPs). Data analysis was performed using the R package "GenABEL." In total, 270,389 SNPs passed quality control. Four linked SNPs in the *FAM149A* gene were associated with AMS severity after correcting for multiple-hypothesis testing (p = 2e-7). One SNP was in an intron and the other three were nonsynonymous SNPs. Individuals with the 'susceptible' genotype had a significantly greater mean LLS relative to those with the 'resistant' genotype (Mean (SD); 5.2 (2.8) *vs.* 1.7 (2.2), respectively). Although the function of the *FAM149A* gene is not well characterized, its highest expression is in the trigeminal ganglion, nervous tissue that is hypothesized to be involved in AMS pathophysiology, and the superior cervical ganglion, part of the sympathetic nervous system. While encouraging, attempts should be made for this finding to be replicated in other Nepalese samples, women, and other biogeographical populations before it can be integrated into our understanding of AMS.

6.2 Rationale for this experiment

One of the central hypotheses for my dissertation is that there is a genetic basis to the variation in hypoxia tolerance in humans. Familial and biogeographical data presented in

Chapter 5 supported the postulate that AMS susceptibility is genetic to some extent. Evidence for familial aggregation in AMS severity (Kriemler et al. 2014) and biogeographical differences in AMS susceptibility from other studies support a genetic basis to AMS susceptibility as well (Wu et al. 2005; Li et al. 2011). These data did not implicate any specific genes, and identifying those genes that influence the acute hypoxia tolerance phenotype would improve our understanding of the molecular and physiological mechanisms associated with hypoxia acclimatisation. This chapter reports the results of the first genome-wide association study (GWAS) undertaken to identify genetic variants that may contribute to AMS susceptibility. The study was performed on subsets of samples from Chapter 5 (the 2012 Gosainkunda) and the 2010 Gosainkunda Expedition. As this is the first GWAS for AMS, we opted to maximize the number of genes screened and therefore genotyped >500,000 single nucleotide polymorphisms (SNPs). The large number of tested hypotheses drastically reduces statistical power. In other words, statistical power to detect minor associations was somewhat compromised to increase the coverage of the genome; therefore, this study was largely intended to identify genes for future hypothesis-based experiments.

6.3 Introduction

The physiological basis of acute mountain sickness (AMS) is not well understood (Imray et al. 2010), and whether variation in AMS susceptibility is due to genetic variation, environmental variation, or genetic x environment variation is unclear (MacInnis et al. 2011). The cause of AMS is failure to acclimatise sufficiently to hypoxia, but it is not clear why some individuals develop AMS and others do not, even when similarly exposed. As the hypoxia response likely involves most systems in the body to some extent, identifying the specific physiological pathways that could explain the inter-individual variation in hypoxia tolerance is challenging.

Comparing physiological measurements between individuals who are susceptible to AMS and resistant to AMS is one method to identify the mechanisms underlying AMS; however, this approach is difficult to apply to complex traits like hypoxia tolerance, as numerous systems – and a plethora of tissues, processes, and molecules within these systems – could explain the variation in the phenotype. Therefore, choosing the correct physiological variable to investigate is challenging. Many studies of AMS susceptibility choose easily measured traits (e.g. oxygen

saturation (Chen et al. 2012; MacInnis et al. 2012a), heart rate (MacInnis et al. 2012a; Wagner et al. 2012a), ventilatory parameters (Richard et al. 2014), but the identification of the physiological differences explaining variation in hypoxia tolerance may require more invasive and challenging procedures. AMS symptoms likely result from some degree of cerebral dysfunction (Imray et al. 2010). Studying cerebral structures and physiology is challenging in a well-equipped laboratory, and many of these procedures may be nearly impossible to implement in the mountainous and remote regions where AMS often occurs. While such studies are possible (although maybe in animal models only or using simulated altitude), more *a priori* data might be needed to justify their implementation. An alternative, and conceptually distinct, approach to identify the mechanisms underlying AMS is the genetic association study. This approach identifies genetic differences between individuals who differ in their susceptibilities to AMS, and aims to identify those variables that cause physiological differences that influence hypoxia tolerance. DNA can be easily collected at any point in time, transported back to the lab, and studied under ideal conditions. This approach is predicated on the variation in AMS susceptibility being influenced by genetic variation.

A number of genes have been investigated for a role in human acute hypoxia tolerance (MacInnis et al. 2010; MacInnis et al. 2011). These studies have primarily used a candidate gene approach: genes were investigated because of *a priori* hypotheses related to their known or supposed physiological functions. Uncertainties about the function of most genes in conjunction with limited knowledge of the mechanisms explaining AMS susceptibility hinder the candidate gene approach: potentially associated genes are likely to go untested. Genome-wide association studies avoid the problem of choosing the causal gene(s)/variant(s), as they do not require *a priori* hypotheses of the biological mechanisms (Hirschhorn and Daly 2005). Instead, polymorphisms distributed throughout the genome are simultaneously interrogated to identify genes and regions that are associated with the phenotype (Hakonarson and Grant 2011). Putative associations can then be followed with genetic studies in other populations to confirm the association (Hirschhorn and Daly 2005) and physiological studies to try to determine the physiological causality underlying the association.

The study described in this chapter is the first GWAS to investigate AMS susceptibility. The subjects were Nepalese who had ascended rapidly to Gosainkunda (4380 m) to participate in a sacred pilgrimage. AMS symptoms were assessed and DNA was collected at Gosainkunda.

Our hypothesis was that alleles at one or more genes would be over-represented in afflicted individuals.

6.4 Methods

6.4.1 Subjects

We partitioned our samples into two groups: one with AMS (AMS+; a LLS ≥4 and a headache score ≥1) and one without (AMS-; LLS ≤1 and a headache score of 0). Subjects with LLS of 2 or 3 were not included in the analysis. Subjects were initially chosen from the 2012 expedition dataset; however, after all suitable subjects with a DNA sample were selected, additional subjects were chosen from the 2010 Gosainkunda expedition to fill our quota. A total of 144 subjects were genotyped (39 from 2010 and 105 from 2012). All subjects ascended to Gosainkunda from Dhunche in 2 days. AMS status (binary trait) and LLS (qualitative trait) were investigated separately using the same genotype data. While the binary approach is more common in GWAS, treating AMS as a qualitative trait is more appropriate, as AMS occurs on a continuum, and cut-off scores for differentiating between sick and well are somewhat arbitrary.

6.4.2 DNA sample collection and isolation

6.4.2.1 2010 Gosainkunda Expedition

During the 2010 Janai Purnima festival, buccal cells were collected from Nepalese pilgrims at Gosainkunda (4380 m). Samples were obtained by firmly swabbing the inner cheek of each subject with a cytobrush (Fisher Scientific, Ottawa, ON, Canada). After swabbing, cytobrushes were placed in paper envelopes to dry. Samples were transported to UBC for analysis.

DNA was isolated from buccal cells according to a standard lab procedure (Saftlas et al. 2004). Briefly, brush heads were incubated at 55ºC overnight in lysis buffer containing proteinase K. Following incubation, RNAse A was added to the solution, which was incubated for an additional hour at 55ºC. After the addition of potassium acetate, the solution was cooled on ice and centrifuged to precipitate cellular debris. DNA from the supernatant was precipitated by centrifugation after the addition of isopropnol (and glycogen). Ethanol-rinsed DNA pellets were eluted in TE buffer and then stored at -20ºC until further use.

6.4.2.2 2012 Gosainkunda Expedtion

During the 2012 Janai Purnima festival, saliva samples were collected from Nepalese pilgrims in Gosainkunda (4380 m). Subjects were given a tube attached to a mouthpiece and asked to spit ~2 mL of saliva into the tubes (Saliva DNA Isolation Kit; Norgen Biotek Corp., Thorold, ON, Canada). A preserving reagent was added to the tube by the researcher, and the solution was mixed by inversion. Samples were stored at room temperature and transported to UBC for analysis.

The manufacturer's protocol was followed to isolate DNA from the saliva samples (Saliva DNA Isolation Kit; Norgen Biotek Corp). Briefly, preserved saliva samples were mixed with lysis buffer and incubated at 55ºC for 30 min. Proteinase K was added to an aliquot of the sample, and the solution was vortexed before being incubated at 55ºC for an additional hour. DNA was precipitated in isopropanol, pelleted by centrifugation, and rinsed in 70% ethanol. The resultant DNA pellets were eluted in TE buffer before being stored at -20ºC until further use.

6.4.3 Genotyping

6.4.3.1 DNA quality

The quality and quantity of DNA samples were determined using a spectrophotometer (Nanodrop 2000c, Thermo Fisher Scientific Inc.). The QIAamp DNA micro kit (Qiagen, Venlo, The Netherlands) was used to improve the purity of DNA isolated from saliva samples, as these DNA samples were of lower quality (but much higher quantity) than DNA isolated from buccal samples. The quality and quantity of saliva DNA samples were rechecked after purification using the same spectrophotometer. Samples were diluted according to the specifications of the Centre for Molecular Medicine and Therapeutics at UBC, where a trained technician subsequently performed the genotyping.

6.4.3.2 Genotyping

DNA samples were genotyped using Infinium Human Core Exome Bead Chips (Illumina, Inc.; San Diego, CA) run on the Illumina 500GX Bead Station. This chip allows for the genotyping of 542,556 polymorphisms distributed throughout the genome including in mtDNA.

6.4.4 Data quality control and statistical analysis

Genotype data were checked for quality and analysed for associations using the statistical framework R (r-package.org) and the package "GenABEL" (Aulchenko et al. 2007). The Illumina output file was loaded into the GenABEL package. A marker was excluded from analysis if (i) its call rate was <98 %; (ii) its minor allele frequency (MAF) was < 1%; or (iii) it was not in Hardy-Weinberg Equilibrium (HWE). Applying these criteria limits the impact that poor genotyping could have on the results (Teo 2009). A subject was excluded from analysis if his/her (i) call rate was <98%; (ii) autosomal heterozygosity was too high (False Discovery Rate (FDR) = 0.01); or (iii) proportion of alleles that were identical-by-state compared to another sample was ≥ 0.95. Applying these criteria controls for poor quality samples, contamination, and the possibility of duplicated or related samples, respectively. Only those markers and individuals who passed quality control were analysed.

Polymorphisms were tested for associations with AMS using χ^2 tests and for associations with LLS using linear regression. Lambda was determined by measuring the slope of the line in a q-q plot (observed vs. expected χ^2 values). The possibility of genetic substructure being present in the dataset was explored using classical multi-dimensional scaling (CMDS) to plot the genetic relatedness of subjects.

Because sex and age were both associated with AMS in Chapter 5, these relationships were tested with a Fisher's exact test and a t-test, respectively. As both variables were again associated with AMS in the GWAS dataset, two rounds of analyses were performed. First, tests of association for AMS status and LLS were assessed in the full dataset controlling for age and sex. Due to the very strong relationship between sex and AMS and to the smaller number of female subjects, females were removed from the dataset for the second round of analyses (but age was still used as a covariate in the analyses). Only data from the male dataset are discussed in this chapter.

6.5 Results

6.5.1 Genotype data quality control

Characteristics for the 144 subjects that were chosen for analysis are reported in Appendix C. The distributions of SNPs with respect to HWE, MAF, and genotyping call rate and

the distribution of subjects for genotyping call rate prior to quality control are also reported in Appendix C.

Of the >500,000 SNPs, 265,063 SNPs were removed because of violations of the HWE, MAF, and/or genotyping call rate requirements. Of the 144 subjects, three subjects were removed from the analysis because of unsatisfactory genotyping call rates. A *post-hoc* decision was made to remove 42 female subjects from the analysis because of the strong influence of sex on AMS susceptibility (Chapter 5). Subject characteristics for the 99 subjects in the male dataset are presented in Table 6.1. Post-quality control distributions of SNPs and individuals for the male dataset are reported in Tables 6.2-6.5.

Table 6.1 Characteristics of subjects with and without acute mountain sickness (AMS+ and AMS-) in the male dataset following quality control.

Variable	AMS-	AMS+	Stat. (p-value)
n	56	43	NA
Age (years)	32.1 (10.7)	38.8 (12.5)	0.006
LLS	0.3 (0.4)	5.2 (1.6)	<0.001

Table 6.2 The number and proportion of SNPs below different p-value thresholds for tests of Hardy-Weinberg Equilibrium in the male dataset following quality control.

	Hardy-Weinberg p-value threshold					
Measure	**X ≤ 0.001**	**X ≤ 0.01**	**X ≤ 0.05**	**X ≤ 0.1**	**X ≤ 0.5**	**X ≤ 1.0**
Number	353	2758	11966	23162	109352	270389
Proportion	0.001	0.01	0.044	0.086	0.404	1

Table 6.3 The number and proportion of SNPs at different call rate thresholds in the male dataset following quality control.

	Single nucleotide polymorphism call rate threshold				
Measure	**X ≤ 0.9**	**0.9 < X ≤ 0.95**	**0.95 < X ≤ 0.98**	**0.98 < X ≤ 0.99**	**X > 0.99**
Number	0	0	171	3672	266546
Proportion	0	0	0.001	0.014	0.986

Table 6.4 The number and proportion of SNPs at different minor allele frequency thresholds in the male dataset following quality control.

	Minor allele frequency threshold				
Measure	**X ≤ 0.01**	**0.01 < X ≤ 0.05**	**0.05 < X ≤ 0.1**	**0.1< X ≤ 0.2**	**X >0.2**
Number	64	12732	20218	53368	184007
Proportion	0	0.047	0.075	0.197	0.681

Table 6.5 The number and proportion of individuals at different call rate thresholds in the male dataset following quality control.

	Individual call rate threshold				
Measure	**X ≤ 0.9**	**0.9 < X ≤ 0.95**	**0.95 < X ≤ 0.98**	**0.98 < X ≤ 0.99**	**X > 0.99**
Number	0	0	0	0	99
Proportion	0.000	0.000	0.000	0.000	1.000

6.5.2 Genome-wide association study (AMS status; male dataset)

As Lambda was <1 (Figure 6.1), genomic control was not used to correct the resulting p-values. CMDS was used to plot subjects according to genetic relatedness (Figure 6.2); however, when subjects were grouped into two, three, or four clusters, the incidence of AMS did not differ between clusters (Table 6.6), although one of four clusters had a notably (but not significantly) higher incidence of AMS. Thus there did not appear to be any genetic stratification in the sample. Results were similar with and without statistically controlling for genetic stratification.

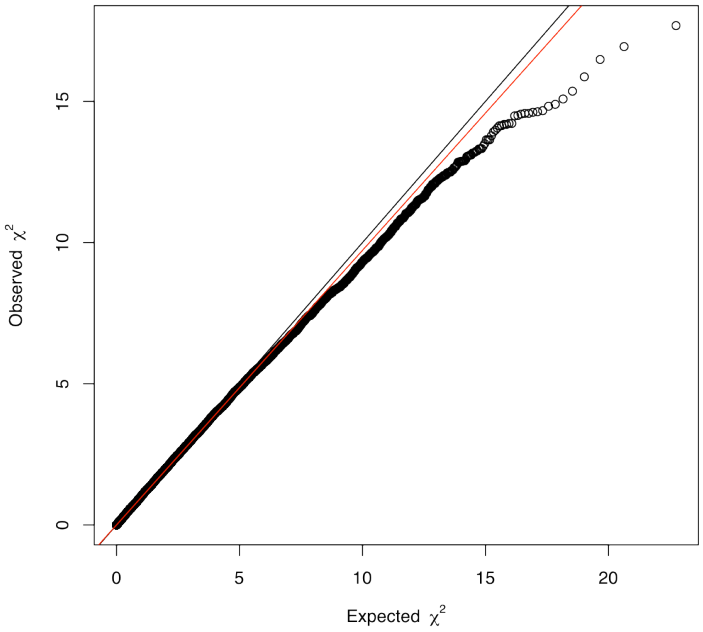

Figure 6.1 A q-q plot displaying the relationship between expected and observed χ^2 values for the AMS status phenotype in the male dataset. The black line is the line of identity, and the red line represents the actual slope of the relationship (lambda = 0.97, standard error = 5.20 e-05).

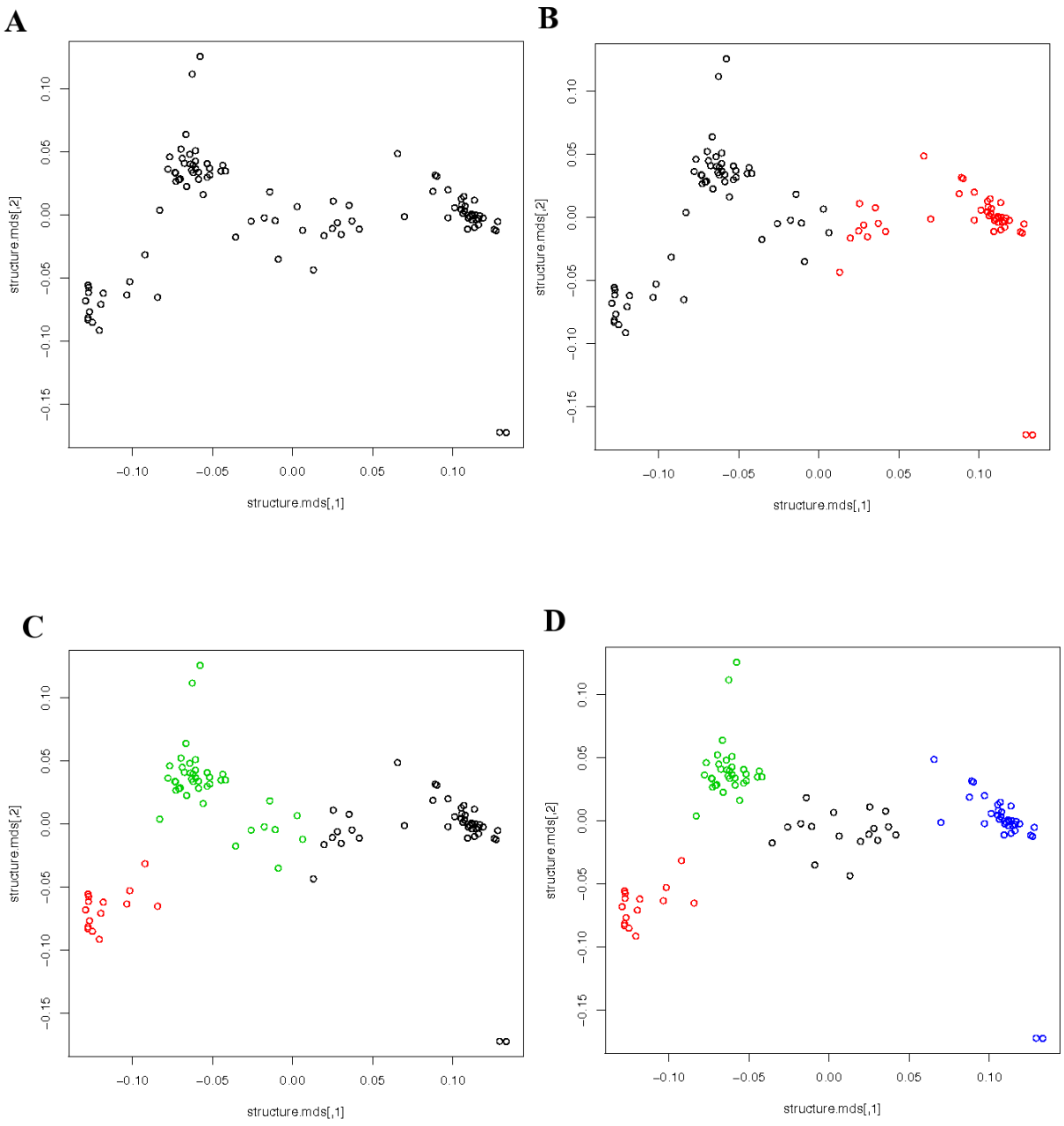

Figure 6.2 The individual sample values for the first two principal components resulting from analysis of genomic kinship. The panels depict the samples as (a) unclustered, (b) clustered in two groups, (c) clustered in three groups, and (d) clustered in four groups.

Table 6.6 The proportion of individuals with AMS in each cluster when individuals in the male dataset were grouped into two, three, and four clusters.

Clusters	Cluster ID	n	AMS+ (%)	χ^2 (p-value)	LLS	F (p-value)
2	Black	56	23 (41%)	0.29 (0.59)	2.3 (2.6)	0.23 (0.64)
	Red	43	20 (47%)		2.6 (2.8)	
3	Black	43	20 (47%)	0.30 (0.86)	2.6 (2.8)	0.14 (0.87)
	Red	15	6 (40%)		2.5 (2.9)	
	Green	41	17 (41%)		2.2 (2.6)	
4	Black	17	12 (71%)	6.62 (0.09)	3.6 (2.8)	1.66 (0.18)
	Red	15	6 (40%)		2.5 (2.9)	
	Green	33	11 (33%)		1.9 (0.3)	
	Blue	34	14 (41%)		2.3 (0.3)	

The Manhattan plot showing the results of the χ^2 tests is presented in Figure 6.3. None of the polymorphisms reached genome-wide statistical significance. The 10 SNPs with the smallest p-values are reported in Table 6.7. Summary information for the 100 SNPs with the lowest p-values are reported in Appendix C.

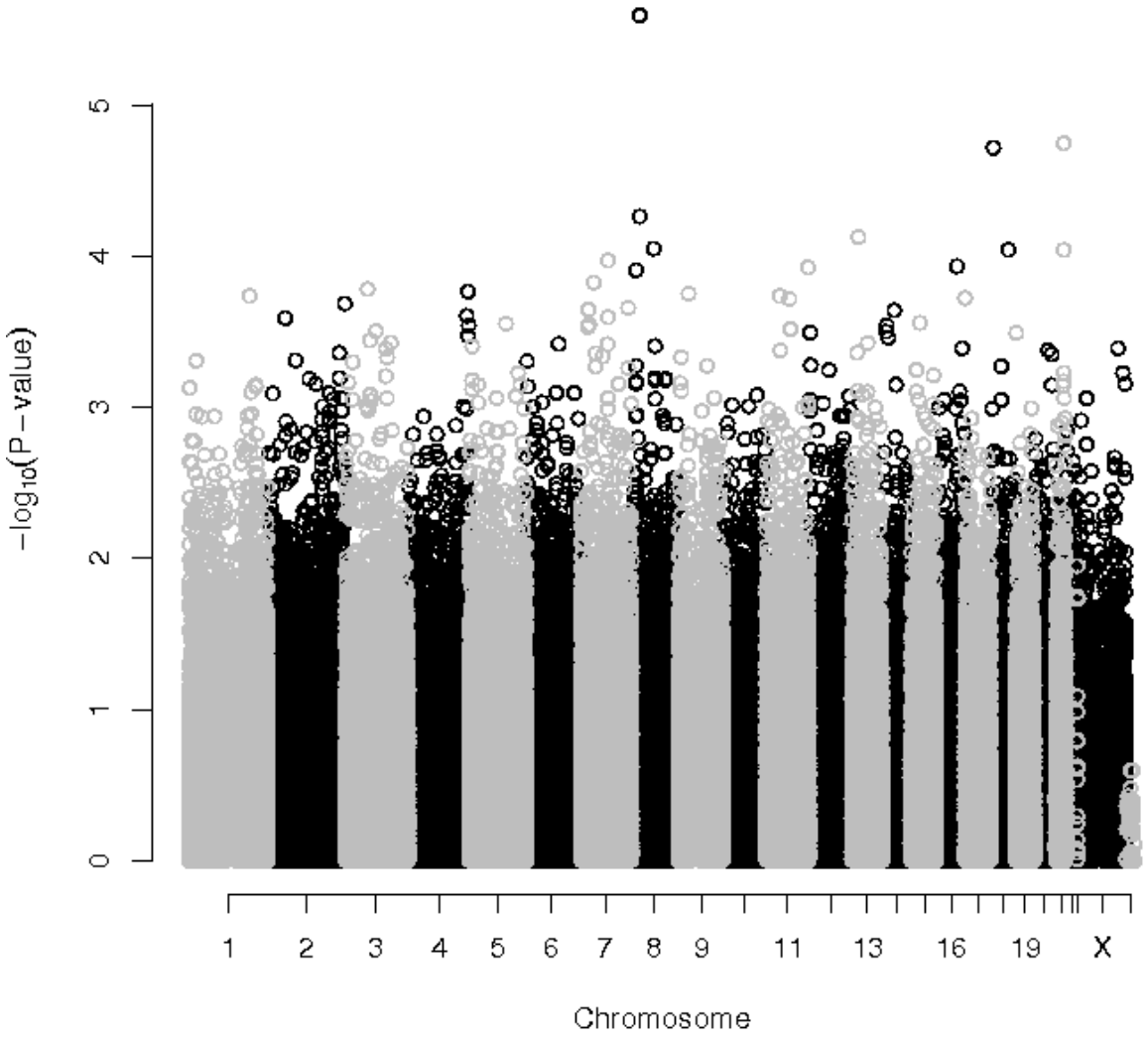

Figure 6.3 A Manhattan plot of the p-values for statistical tests of association between each SNP in the male dataset and the AMS status. Genomic location (organized by chromosome and then by position on the chromosome) is on the x-axis; the p-value is on the y-axis.

Table 6.7 Summary of SNPs with the 10 lowest p-values for the AMS status phenotype and the male dataset.

Polymorphism	Chromosome	Position*	Gene	χ^2	p-value	Corrected p-value
rs7010000	8	24663706	Intergenic	22.12	0.0000026	0.20
rs1209952	21	40175827	*ETS2*	18.39	0.000018	0.81
rs6506564	18	8237063	*PTPRM*	18.27	0.000019	0.82
rs2607605	8	24644749	Intergenic	16.28	0.000055	1.00
rs9548985	13	40502985	Intergenic	15.68	0.000075	1.00
rs13279743	8	68751611	Intergenic	15.35	0.000089	1.00
rs12545592	8	68762313	Intergenic	15.35	0.000089	1.00
rs12961130	18	55260070	Intergenic	15.31	0.000091	1.00
rs457705	21	40191431	*ETS2*	15.31	0.000091	1.00

*Based on NCBI

6.5.3 Genome-wide association study (LLS; male dataset)

Similar to section 6.5.2, Lambda was <1 when the LLS was used for analysis (Figure 6.4); therefore, genomic control was not used to correct the resulting p-values. CMDS was used to group subjects by genetic relatedness, and again subjects were grouped into two, three, and four clusters. The severity of AMS did not differ between clusters (Table 6.6). Thus there did not appear to be any genetic stratification in the sample. Controlling for genetic relatedness did not affect the results.

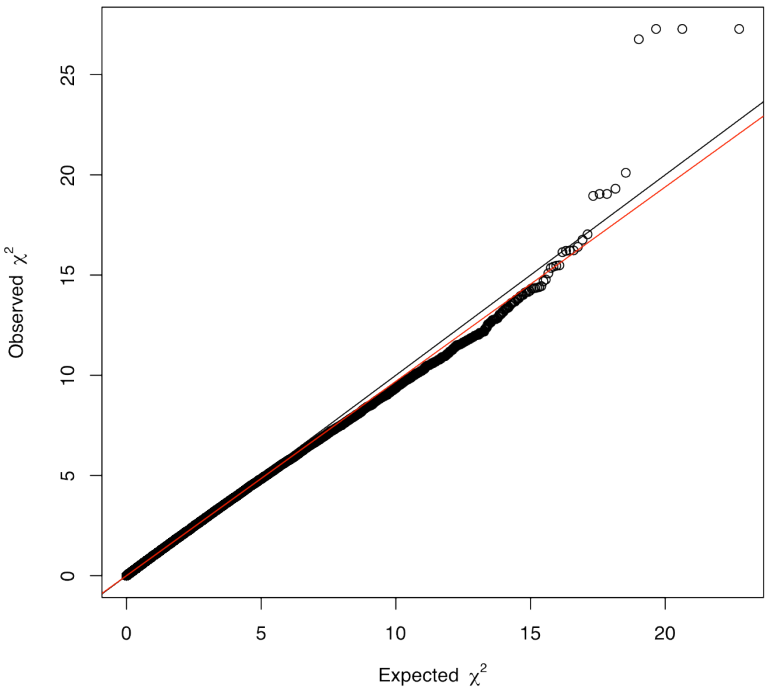

Figure 6.4 A q-q plot displaying the relationship between expected and observed χ^2 values for the Lake Louise Score phenotype in the male dataset. he black line is the line of identity, and the red line represents the actual slope of the relationship (lambda = 0.97, standard error = 4.92 e-05).

The Manhattan plot showing the results of the linear regression tests is presented in Figure 6.5. Four of the polymorphisms reached genome-wide statistical significance. Summary information for the 10 SNPs with the smallest p-values is reported in Table 6.8. Summary information for the 100 SNPs with the smallest p-values is reported in Appendix C. A closer examination of the SNPs located in the 1-Mbp region containing the statistically significant SNPs is presented in Figure 6.6. Using the bioinformatics program GLIDERS (Genome-wide linkage disequilibrium repository and search engine), additional SNPs in linkage disequilibrium with rs7684126 were identified in Table 6.9, including the other three SNPs associated with the LLS in this study.

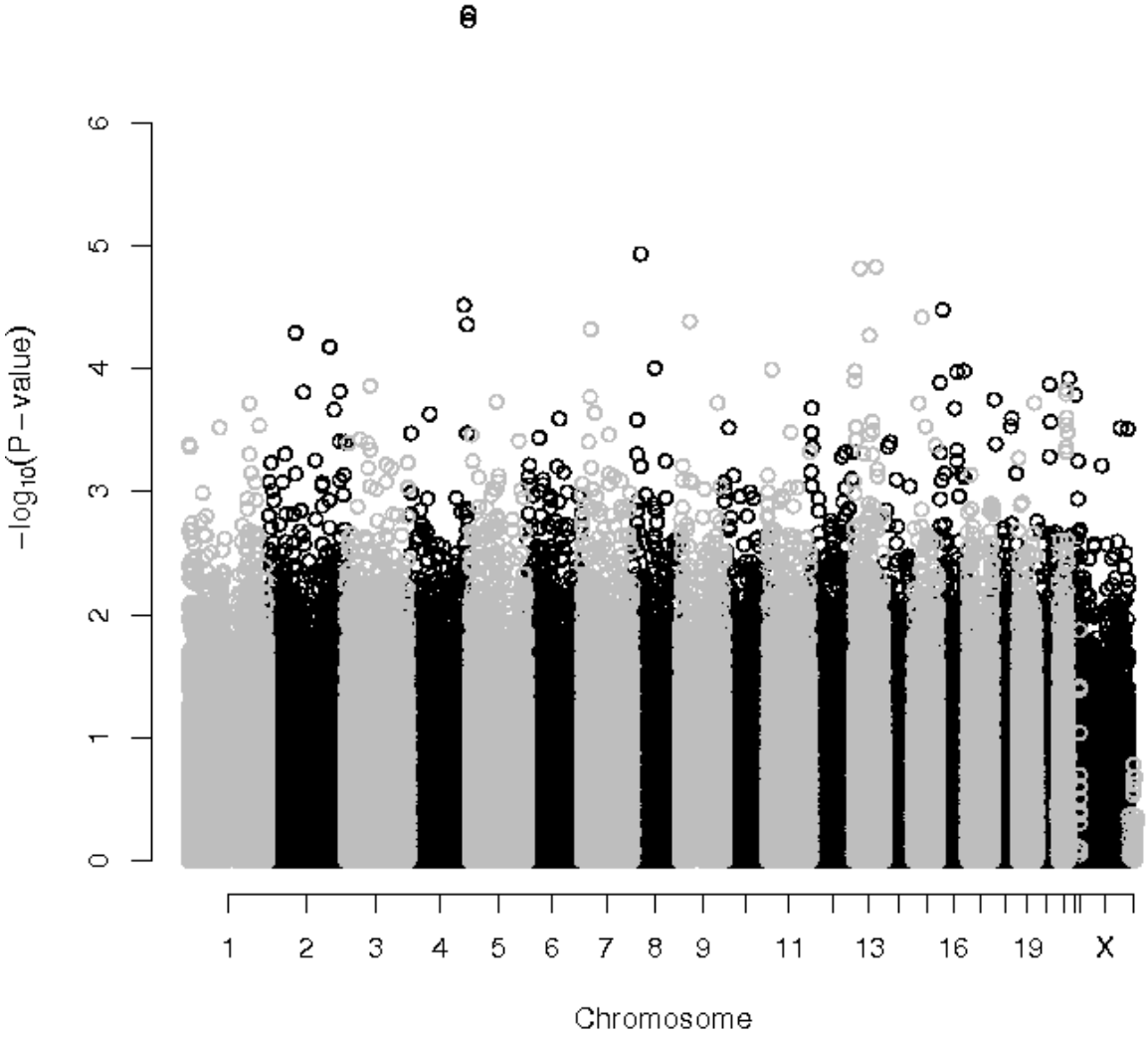

Figure 6.5 A Manhattan plot of the p-values for statistical tests of association between each SNP in the male dataset and the Lake Louise Score. Genomic location (organized by chromosome and then by position on the chromosome) is on the x-axis; the p-value is on the y-axis.

Table 6.8 Summary of SNPs with the 10 lowest p-values for the Lake Louise Score phenotype and the male dataset.

Polymorphism	Chromosome	Position*	Gene	χ^2	p-value	Corrected p-value
rs7684126	4	187072383	*FAM149A*	27.89	0.00000013	0.01
rs4862653	4	187077206	*FAM149A*	27.89	0.00000013	0.01
rs2276924	4	187078785	*FAM149A*	27.89	0.00000013	0.01
rs4862650	4	187074833	*FAM149A*	27.62	0.00000015	0.01
rs7010000	8	24663706	Intergenic	19.22	0.000011	0.83
rs2166202	13	89855595	Intergenic	18.77	0.000015	0.91
rs9548985	13	40502985	Intergenic	18.70	0.000015	0.91
rs7664076	4	172388728	Intergenic	17.39	0.000030	0.99
rs12444395	16	13297348	*SHISA9*	17.22	0.000033	1.00
rs12595633	15	50770917	*USP8*	16.95	0.000038	1.00

*Based on NCBI

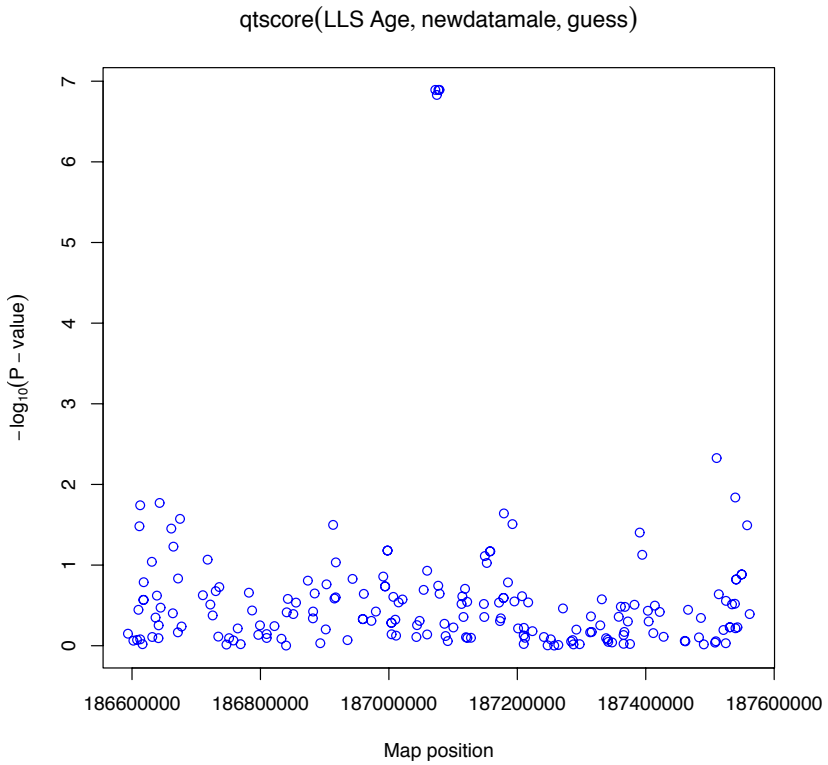

Figure 6.6 The p-values of SNPs in the 1-Mbp region surrounding the single nucleotide polymorphism (SNP) that reached genome-wide statistical significance. The x-axis represents a 1 Mbp region of chromosome, centered on the SNP of interest, rs7684126.

Table 6.9 Summary of the SNPs that were in linkage disequilibrium with rs7684126 according to the program GLIDERS.

SNP (rs)	Position [a]	MAF [b]	Distance (bp) [c]	r^{2} [b]	Gene	Region [a]
rs7668227	187061084	0.156	-11299	0.95	*FAM149A*	Not specified
rs6858112	187062426	0.153	-9957	0.95	*FAM149A*	Not specified
rs13141920	187064553	0.159	-7830	0.95	*FAM149A*	Upstream variant
rs2276914	187072932	0.171	549	1.00	*FAM149A*	Intron variant
rs2276912	187073030	0.171	647	1.00	*FAM149A*	Intron variant
rs4862650	187074833	0.171	2450	1.00	*FAM149A*	Missense (Lys41Glu)
rs4862653	187077206	0.173	4823	0.98	*FAM149A*	Missense (Lys146Glu)
rs2276924	187078785	0.174	6402	0.96	*FAM149A*	Missense (His214Arg)
rs1398008	187110096	0.238	37713	0.92	*FLJ38576*	Downstream variant

[a] According to the dbSNP database.
[b] For a CHB+JPT sample in HapMap phase 3, build 36.
[c] Relative to rs7684126.

The genotype associated with more severe AMS was G/A, Glu/Lys, Glu/Lys, Arg/His and the genotype associated with less severe AMS was G/G, Glu/Glu, Glu/Glu, and Arg/Arg (at the rs7684126, rs4862650, rs4862653, rs2276924 loci, respectively). The mean LLS for male subjects with these genotypes are shown in Figure 6.7. Individuals with the 'susceptible' genotype (5.2 [2.8]) had a significantly greater LLS relative to the 'resistant' genotype (1.7 [2.2]; $t = 6.1$, $p = 0.00000002$), but age was similar between the two genotypes (36.1 [11.0] vs. 35.2 [11.6] years, respectively; $t = 0.28$, $p = 0.78$). Those with the 'susceptible' genotype reported significantly greater headache, gastrointestinal, fatigue, and dizziness symptoms relative to the 'resistant' genotype; however, sleep difficulty was similar between the two groups (Figure 6.8). When female subjects were divided into two groups, the genotypes of interest were not associated with AMS severity (Appendix C).

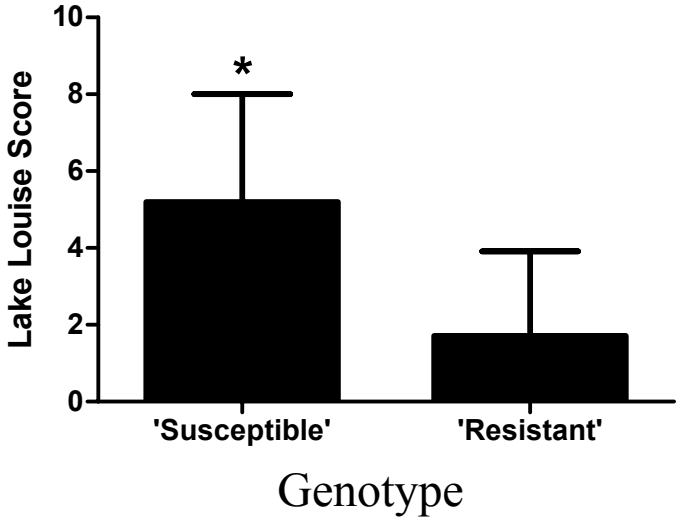

Figure 6.7 The mean Lake Louise Score for male individuals with the 'susceptible' and 'resistant' genotypes. The susceptible genotype was G/A, Glu/Lys, Glu/Lys, Arg/His and the resistant genotype was G/G, Glu/Glu, Glu/Glu, Arg/Arg for the rs7684126, rs4862650, rs4862653, and rs2276924 SNPs, respectively. The asterisk (*) denotes that the mean LLS was statistically greater for the 'susceptible' genotype relative to the 'resistant' genotype. Error bars represent 1 standard deviation.

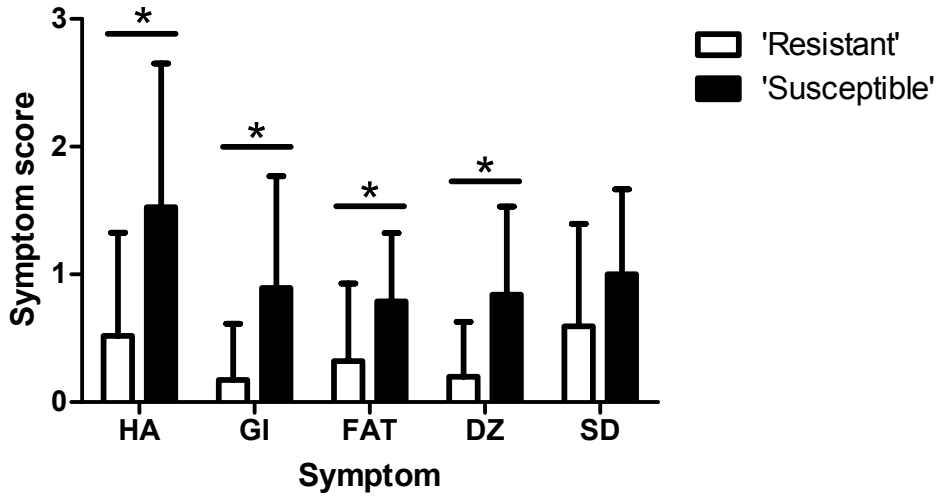

Figure 6.8 The individual acute mountain sickness symptom scores for male individuals with the 'susceptible' and 'resistant' genotypes. The susceptible genotype was G/A, Glu/Lys, Glu/Lys, Arg/His and the resistant genotype was G/G, Glu/Glu, Glu/Glu, Arg/Arg for the rs7684126, rs4862650, rs4862653, and rs2276924 SNPs, respectively. The asterisk (*) denotes that the mean symptom score was statistically greater for the 'susceptible' genotype relative to the 'resistant' genotype. HA, headache; GI, gastrointestinal; FAT, fatigue; DZ, dizziness; SD, sleep difficulty. Error bars represent 1 standard deviation.

6.6 Discussion

This is the first GWAS of AMS susceptibility. Four linked polymorphisms (one intronic SNP and three nonsynonymous SNPs) located in the *FAM149A* gene were associated with AMS severity. The *FAM149A* gene has not been investigated for a role in hypoxia tolerance previously, likely owing to its uncharacterized function. Although follow-up studies in independent samples and different populations are needed to confirm this finding, this result supports a genetic basis for AMS susceptibility, with variants in *FAM149A* gene playing a role. No other gene, including those previously associated with acute hypoxia tolerance (see Chapter 1), were associated with AMS in this study (at genome-wide statistical significance).

The *FAM149A* gene is conserved in amniotes. While little is known about its function, the *FAM149A* gene has markedly greater expression in the trigeminal ganglion relative to most other tissues (Su et al. 2004), and the trigeminal ganglion is implicated in the headache symptoms of AMS (Hackett and Roach 2001; Bartsch and Bailey 2013). According to the "traditional model" of AMS (Bailey et al. 2009a), hypoxia causes vasogenic edema that increases intracranial volume, leading to brain swelling. For individuals with relatively less cranial reserve volume (*i.e.,* a 'tight-fitting brain'), the swelling leads to stretching of pain-sensitive fibers in the trigeminovascular system (Sanchez del Rio and Moskowitz 1999). As there is limited evidence to support vasogenic edema being the cause of AMS (Kallenberg et al. 2007; Kallenberg et al. 2008; Lawley et al. 2012; Cushing et al. 2013; Keyes et al. 2013; Lawley et al. 2013), Bailey and colleagues (Bailey et al. 2009a) have proposed a revised model in which free radicals and not vasogenic edema are thought to cause the condition. While the two models of AMS differ considerably, both involve the trigeminal ganglion, as the revised model postulates that redox activation of the trigeminovascular vascular system in response to hypoxia leads to the symptoms of AMS.

Direct evidence to implicate the trigeminovascular system in AMS is lacking. Metabolism of the calcitonin gene-related peptide (CGRP; a biomarker for trigeminal vascular system activation) was not altered (relative to baseline) after 9 hours of normobaric hypoxia (Bailey et al. 2009b), suggesting that sustained release of the CGRP molecule (and therefore activation of the trigeminal vascular system) is not responsible for AMS. While this study seems to contradict the involvement of the trigeminovascular system, the authors acknowledge that their study could not rule out the possibility of acute CGRP release (*i.e.*, prior to sampling at

hour 9) or the possibility that CGRP might not enter the extracerebral circulation from the intrathecal spaces (Bailey et al. 2009a). Furthermore, the sample size was very small (n=10), not all subjects developed AMS, and AMS symptoms were mild, making it somewhat difficult to draw any firm conclusions regarding the involvement of CGRP in the development of AMS.

Headache is the cardinal symptom of AMS (Imray et al. 2010), and the trigeminal ganglion has been linked to the pathophysiology of migraine, cluster headache, and paroxysmal hemicrania (Goadsby et al. 2002). Given that our grouping of subjects into AMS+ and AMS- simultaneously grouped subjects according to the presence/absence of high-altitude headache, the association between AMS and the *FAM149A* gene is particularly interesting. Although the pathophysiology of migraine is not fully understood, it is hypothesized that pain might originate from the large cranial vessels, proximal intracranial nerves, or dura mater – structures that are innervated by the trigeminal ganglion (reviewed by Goadsby et al. 2002). Thus, it is possible that there could be some overlap between AMS and these other cerebral conditions. In a large GWAS meta-analysis of migraine, 12 loci were associated with susceptibility (Anttila et al. 2013); however, *FAM149A* was not. Whether the variants identified as being associated with AMS severity play any role in sea-level headache susceptibility would be an interesting investigation.

The second tissue in which *FAM149A* gene expression was markedly higher relative to most other tissues was the superior cervical ganglion (Su et al. 2004). The superior cervical ganglion is part of the sympathetic nervous system, and it innervates numerous organs and tissues in the head and trunk, including cerebral blood vessels (Mitchell et al. 2009). Sympathetic activity is increased at altitude (Mazzeo and Reeves 2003), and altered sympathetic activity has been implicated as a risk factor and/or cause of AMS in a couple of studies (Bartsch and Bailey 2013). Subjects with AMS had greater plasma epinephrine concentrations, and higher blood pressure and heart rate relative to subjects without AMS (Loeppky et al. 2003), and the arterial epinephrine concentration correlated with LLS (Kamimori et al. 2009). Although AMS+ subjects consistently had a heart rate that was greater than AMS- subjects, in Chapters 3, 4, and 5 of this dissertation, the differences were not statistically significant. Muscle sympathetic nerve activity was positively correlated with pulmonary artery pressure in a group of HAPE-prone and HAPE-susceptible subjects, and the authors of this study suggested that overactivation of the sympathetic nervous system may contribute to the development of HAPE (Duplain et al. 1999). Performing a superior cervical ganglion block improved cerebral perfusion in patients with

cerebral vasospasm after aneurismal subarachnoid hemorrhage (Treggiari et al. 2003). While it is difficult to speculate how changes in the FAM149A protein could affect the superior cervical ganglia, given the role of this tissue in regulating cerebral circulation, there is a possible association between *FAM149A* and AMS.

The four polymorphisms that were associated with AMS severity were all within ~6500 bp and were tightly linked in this sample. Three of the polymorphisms were in the coding region of the *FAM149A* gene and led to amino acid substitutions, and the other polymorphism was an intronic variant. As the polymorphisms were tightly linked, it was apparent that a genotype was associated with AMS severity. Three simultaneous amino acid substitutions may have an effect on the FAM149A protein, but it is difficult to speculate what the effect might be given that the function of the protein is unknown. The *FAM149A* gene is located on chromosome 4 near several other genes: *TLR3*, *CYP4V2*, *KLKB1*, and *F11*. It is possible that the polymorphisms associated with AMS severity in this study are in linkage disequilibrium with polymorphisms in these genes; however, as the polymorphisms identified in this study are mostly nonsynonymous polymorphisms, it would be more likely that this gene – rather than a neighbouring gene – plays a causal role in the hypoxia tolerance phenotype.

As already mentioned, this is the first GWAS of AMS susceptibility. While weak associations between AMS and variants at a number of genes have been reported in the past (MacInnis et al. 2010), this is the first study to report a strong association in a gene that could play a role in the actual pathology of the condition. This result is very encouraging despite FAM149A having an unknown role; however, before this result influences our understanding of AMS, it must be replicated in other samples and other populations. Despite the very stringent requirements for statistical significance, the *FAM149A* gene may be a false positive. Alternatively, the association may be real, but only in this population (i.e., Nepalese individuals may have different linkage disequilibrium patterns than other populations, and the causal variant(s) may not be linked to those polymorphisms identified in this study). Future studies should focus on confirming this genetic association and determining the function of *FAM149A*.

There are several limitations to our analysis. Firstly, our sample size was limited by the cost of the genotyping. GWAS normally require sample sizes in the range of 1,000-100,000 subjects to overcome the necessary corrections for multiple-hypothesis testing; therefore, small sample sizes normally prevent any SNPs from reaching genome-wide statistical significance.

Knowing this before the analysis began, we planned to use our data as an exploratory study from which interesting genes could be chosen for future experiments. While polymorphisms of one gene did reach genome-wide statistical significance, our study may have been underpowered to detect other genes involved in AMS susceptibility. Thus, our results should not be over-interpreted to rule out the possibility that other genes contribute to the variation in AMS susceptibility. The results of this GWAS must be validated in additional samples from the same population and in other populations before this result can be accepted or applied. If the hits in the *FAM149A* gene are real, future work should focus on identifying its physiological function and the role of the trigeminal ganglion in the symptomology of AMS. Secondly, this study used the LLS to quantify AMS severity and diagnose AMS, but this questionnaire is not an objective measure of hypoxia tolerance. While we are confident in our categorization of cases and controls in terms of AMS status (as defined by the LLS), our results may not generalize to other definitions of acute hypoxia tolerance. Lastly, subjects in this study were assessed upon reaching 4380 m, and their symptoms may have changed with more time spent at altitude. Thus, whether or not this study can be generalized to other ascent profiles and hypoxic exposure durations is unknown.

6.7 Conclusion

This is the first GWAS of AMS. Four SNPs in the *FAM149A* gene reached genome-wide statistical significance for an association with AMS severity. The function of this gene is unknown; however, its highest expression was in the trigeminal and superior cervical ganglia, which are sites plausibly involved in acute hypoxia tolerance or in AMS pathology (e.g. headaches). While our findings provide some support for the involvement of the trigeminal and/or superior cervical ganglia in the pathophysiology of AMS, additional studies are needed to test these hypotheses. The results of our GWAS must be validated to decrease the risk of a false positive association. Additionally, further studies are needed to determine if any of the associated variants are in fact causal. If any variant in this genotype has a causal relationship with AMS susceptibility, the results of this study will significantly aid our understanding of acute hypoxia tolerance in humans.

Chapter 7 Is previous history a reliable predictor for acute mountain sickness susceptibility? A meta-analysis of diagnostic accuracy

7.1 Summary

The goal of this meta-analysis was to determine the clinical utility of acute mountain sickness (AMS) history to predict future incidents of AMS. Seventeen studies (n = 7921 subjects) were included after a systematic review of the literature. A bivariate random-effect model was used to calculate the summary sensitivity and specificity of the diagnostic test, and moderator variables were tested to explain the heterogeneity across studies. The QUADAS-2 method was used to assess the quality of the included studies. History of AMS had a low diagnostic accuracy for the prediction of future AMS incidents: the summary sensitivity was 0.50 (95% CI [0.40, 0.59]) and the summary specificity was 0.72 (95% CI [0.66, 0.78]). There was significant heterogeneity in the sensitivity and specificity across studies, which we modeled using moderator analysis. Studies that restricted the use of acetazolamide and dexamethasone had a higher sensitivity (0.66) relative to those that did not (0.44; p = .03), but also an increased false positive rate (0.39 versus 0.23, p = .03). Analysis of included studies showed AMS histories were insufficiently detailed, and few studies controlled for the prophylactic medication use or recent altitude exposure, leading to high risks of bias and concerns for applicability. The use of AMS history to guide prophylactic strategies for high-altitude ascent is not supported by the literature; however, the low sensitivity and specificity of this diagnostic test could reflect the quality of the available studies.

7.2 Rationale for this experiment

At first, the results of Chapter 4 were concerning: of all the potential risk factors for acute mountain sickness (AMS), a previous history is often reported to be among the best indicators of susceptibility to AMS. That is, individuals who get AMS are expected to get AMS on subsequent exposures to hypoxia, and individuals who do not get AMS are expected to remain AMS free on subsequent exposures to hypoxia. Our results were suggesting that a previous history of AMS was not very useful in predicting future AMS outcomes. Initially, I thought our study might be an outlier, possibly due to the mode of hypoxia (*i.e.,* most previous studies were performed at altitude, and our study was performed in a normobaric hypoxia chamber). The method I chose to determine how our study fit with the literature was a meta-analysis; however, in this case, a

meta-analysis of diagnostic accuracy was used to determine the sensitivity and specificity of a previous AMS diagnosis for future AMS outcomes.

7.3 Introduction

Acute mountain sickness (AMS) affects many of the millions of people who ascend above 2500 m each year (Wu et al. 2012a). The severity of AMS symptoms is often mild-moderate, but the environment in which AMS usually occurs (*i.e.,* austere mountainous regions where medical services are limited) augments its impact on the health, productivity, and travel of high-altitude sojourners. Furthermore, AMS often precedes high-altitude cerebral edema (HACE), a rare but potentially lethal form of altitude illness (Hackett and Roach 2001). While established evidence-based guidelines to treat AMS are available (Luks et al. 2010), preventing AMS is preferable. The often-cited risk factors for AMS are the ascent rate, the altitude attained (or the sleeping altitude), and the previous AMS history of the individual (Hackett and Roach 2001; Basnyat and Murdoch 2003).

There is strong evidence demonstrating that the incidence of AMS is positively correlated with the ascent rate and the altitude attained. With respect to the ascent rate, quicker ascents were associated with significantly greater AMS incidences in large groups of subjects en route to 4559 m (Schneider et al. 2002) and 6962 m (Pesce et al. 2005). With respect to the altitude attained, the incidences of AMS in trekkers were 9%, 13%, 34%, and 53% at 2850 m, 3050 m, 3650 m, and 4559 m, respectively (Maggiorini et al. 1990). That the ascent rate and the altitude attained strongly influence the incidence of AMS is congruent with the primary cause of AMS: insufficient acclimatisation to hypoxia.

Many reviews suggest that a previous history of AMS is a strong predictor of AMS on a future ascent (Hackett and Roach 2001; Schoene 2007; Imray et al. 2011); however, the utility of previous AMS history in predicting future AMS incidence is not clear. Although multiple studies have demonstrated that a previous AMS history is statistically associated with an increased likelihood of developing AMS (Honigman et al. 1993; Wagner et al. 2008), many of the odds ratios that were statistically significant were also relatively small – possibly too small to be clinically useful (Pepe et al. 2004). To determine the utility of AMS history in predicting future AMS incidence, AMS history can be treated as a diagnostic test for future AMS outcomes. In

this context, the diagnostic accuracy of AMS history can be described by its sensitivity and specificity for predicting future AMS outcomes.

Meta-analysis of diagnostic accuracy (MADA) is a statistical technique to combine data from multiple studies of diagnostic accuracy (Jones and Athanasiou 2009). Using MADA, a consensus estimate of the sensitivity and specificity of a diagnostic test can be obtained, and possible sources of heterogeneity across studies can be examined. QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) is a qualitative tool used in conjunction with MADA to determine the quality of included studies and to identify the strengths and weaknesses of included studies (Whiting et al. 2011). QUADAS-2 guides the researcher to create study-specific signaling questions, which are used to determine the risks of bias and the concerns for applicability (*i.e.,* relevance to the specific review question) of four domains: subject selection, index test, reference standard, and timing of assessments. Using these two methods, researchers can synthesize comprehensive results for diagnostic tests and suggest modifications to improve the accuracies of diagnostic tests. The purpose of this examination was to use MADA and QUADAS-2 to systematically determine the utility of a previous history of AMS for the prediction of a future AMS outcome. We hypothesized that AMS history would be a reliable tool for predicting future AMS outcomes.

7.4 Methods

7.4.1 Inclusion criteria

Potential studies were identified by searching PubMed and Google Scholar with combinations of the following keywords as queries: “acute mountain sickness,” “previous history,” “repeatability,” and “reproducibility.” All identified studies published in English before May 2013 were reviewed for inclusion. One French study (Richalet et al. 1988) referred to by another publication was included after being translated into English. To be included in the analysis, studies had to report (a) previous AMS histories for subjects; (b) the AMS outcomes for subjects on the investigated ascent(s); and (c) the number of true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN; Table 7.1). Data were independently extracted from the included studies by two researchers.

7.4.2 Data quality

Additional information was extracted from each study to perform a quality assessment using the QUADAS-2 protocol (Whiting et al. 2011); however, reporting this information was not a requirement for inclusion. Each study's risk of bias and concern for applicability were rated as low, high, or unclear for each domain (except for the timing domain, for which concern for applicability does not apply). Subject selection quality was judged from the method of recruitment, whether any subjects took medications to prevent AMS, and whether any subjects spent >1 day above 3000 m in the preceding 2 months. Under the QUADAS-2 protocol, the AMS history assessment was termed the "index test" and the AMS outcome assessment was termed the "reference standard." The quality of each assessment was judged from the following information: timing of assessment in relationship to the occurrence of symptoms (retrospective/prospective), method of diagnosis (and threshold), altitude of assessment, and whether the assessments were performed independently. Finally, the risk of bias in the timing of the index test and the reference standard was based on the time elapsed between the AMS history and AMS outcome.

7.4.3 Data aggregation

For the purpose of this paper, the subjects' AMS history was treated as a binary index test (*i.e.*, positive/negative). The diagnosis of AMS reported in each study was also treated as a binary reference standard (*i.e.*, positive/negative) and is referred to as the "AMS outcome."

For each study, the TP, FP, FN, and TN data were used to calculate the sensitivity, specificity, and the false positive rate (FPR = 1 - specificity). For each proportion, 95% confidence intervals (95% CI) were calculated based on Deeks (2001).

Table 7.1 A 2 x 2 diagnostic table with AMS outcome as a function of AMS history.

	AMS Outcome	
	Positive AMS diagnosis	Negative AMS diagnosis
Positive AMS History	TP	FP
Negative AMS History	FN	TN
Total	Total AMS-positive outcomes	Total AMS-negative outcomes

Note: TP = true positives; FP = false positives; FN = false negatives; TN = true negatives.

7.5 Analysis

Studies of diagnostic accuracy generate pairs of sensitivity and specificity scores as outcomes. Sensitivity (P) is it the ratio of TP to the total number of people with an AMS-positive outcome (TP+FN). Conceptually, sensitivity represents how well AMS history predicts who will get AMS on a future exposure among people who subsequently developed AMS (a score of 1 would be perfect prediction). Specificity (N) is the ratio of TN to the total number of people with an AMS-negative outcome (TN+FP). Conceptually, specificity represents how well AMS history predicts who will not get AMS among people who did not subsequently develop AMS (again, a score of 1 would be perfect prediction). Related to specificity is the false positive rate (FPR = 1 – specificity), for which a score of 0 would indicate perfect prediction (*i.e.*, no false positive cases). All calculations in the meta-analysis are based on the sensitivity and specificity of individual studies (P_i and N_i) and the variability of these parameters between studies.

The main analysis tested a bivariate random-effects model for sensitivity and specificity of using AMS history to predict AMS outcome. We have used a bivariate meta-analysis approach (Reitsma et al. 2005) instead of a univariate analysis (Glas et al. 2003) because bivariate results allow for the estimation of sensitivity and FPR and the correlation between the two parameters. Simultaneous estimation of these parameters is more clinically relevant than the single diagnostic odds ratio provided by univariate analysis. In order to be analyzed statistically, sensitivity and specificity must be transformed into logits (the logit is the natural log of the odds ratio, which allows for statistical analysis through a general linear model). Following analysis, an inverse logit can be used to return point estimates and confidence intervals to their original dimensions. We used R (r-project.org) to test a bivariate random-effects model of diagnostic accuracy using the ‘mada’ package (Doebler and Holling 2013).

Building on this main analysis, we tested a number of moderator variables to explain the heterogeneity of sensitivity and FPR found between studies. First, heterogeneity tests were performed to determine whether sufficient variation was present to warrant analyzing moderator variables. Limited data for moderator variables were available in the original studies and thus only three moderator variables were tested: (a) altitude of the AMS diagnosis (in km), (b) whether all subjects had previous altitude exposure prior to the AMS outcome ascent (coded as 0 = no, 1 = yes), and (c) whether any subject used prophylactic medications (coded as 0 = no, 1 = yes). Details of the models are explained in Appendix G.

7.6 Results

7.6.1 Study quality

The 17 studies included in this meta-analysis are described briefly in Table 7.2. Results from the QUADAS-2 analysis are displayed in Figure 7.1. There was a low risk of bias in subject selection, but a high concern for the applicability of subjects due to prophylactic medication use and recent altitude exposure. Because most studies determined AMS history retrospectively, did not describe the characteristics of previous ascents, and did not describe the method of determining AMS history, the index test had a high risk of bias and an unclear concern for applicability. In contrast, most studies determined AMS outcome prospectively with an acceptable method, leading to a low risk of bias and low concern for applicability with the reference standard. Finally, the time between the index test and reference standard was not stated in 15 studies (and many studies included subjects with recent altitude exposure), indicating a high risk of bias for the timing of assessments.

Table 7.2 A description of the subjects, index test, reference standard, and flow of included studies.

Reference	Subjects			History (Index test)				Diagnosis (Reference standard)			Flow
	Case-control design?	Any subjects used medications? [d]	Any subjects recently exposed to altitude? [e]	Timing	Method	Altitude (m)	All subjects experienced altitude/ hypoxia? [f]	Timing	Method	Altitude (m)	Time between exposures
Alizadeh et al. 2012	No	No	Yes	R	SSQ	NS	NS	P	LLS > 5	4200	NS
Honigman et al. 1993	No	NS	NS	R	NS	NS	No	P	HS	1920-2957	NS
Lanfranchi et al. 2005	No	NS	NS	R	NS	NS	NS	P	LLS ≥ 3	4559	NS
MacInnis et al. 2014 (Chap. 4)	No	No	No	P	LLS ≥ 3	4000	Yes	P	LLS ≥ 3	4000	14-138 days
Mairer et al. 2009	No	NS	Yes	R	SSQ	NS	NS	P	LLS ≥ 4	2200-3500	NS
Mairer et al. 2010 [a]	No	Yes	Yes	R	SSQ	NS	NS	P	LLS ≥ 4	3454	NS
Mairer et al. 2010 [b]	No	Yes	Yes	R	SSQ	NS	NS	P	LLS ≥ 4	3817	NS
Moore et al. 1986	Yes	NS	NS	R	SSQ	3000+	Yes	P	SSQ	4800	NS
Nilles et al. 2009	No	Yes	NS	R	NS	NS	NS	P	LLS ≥ 3	4328	NS
Rexhaj et al. 2011	No	No	No	P	LLS ≥ 3	3450	Yes	P	LLS ≥ 3	3450	9-12 months
Richalet et al. 1988	No	NS	Yes	R	NS	3500+	Yes	R	HS	6119-8848	NS
Richalet et al. 2012 [c]	No	Yes	NS	R	SSQ	4000+	Yes	R	HS	5079*	NS

Reference	Subjects			History (Index test)				Diagnosis (Reference standard)			Flow
	Case-control design?	Any subjects used medications? [d]	Any subjects recently exposed to altitude? [e]	Timing	Method	Altitude (m)	All subjects experienced altitude/ hypoxia? [f]	Timing	Method	Altitude (m)	Time between exposures
Roach et al. 1995	No	NS	NS	R	NS	NS	Yes	P	LLS ≥ 3	2500	NS
Schneider et al. 2002	No	No	Yes	R	SSQ	3000+	Yes	P	ESQ>0.7	4559	NS
Wagner et al. 2008	No	Yes	Yes	R	SSQ	NS	No	P	LLS ≥ 3	2500-4419	NS
Wagner et al. 2008	No	Yes	Yes	R	SSQ	3779+	Yes	R	LLS ≥ 3	4260-5640	NS
Wang et al. 2010	No	Yes	Yes	R	SSQ	NS	No	R	LLS ≥ 3	3952	NS
Ziaee et al. 2003	No	Yes	NS	R	SSQ	NS	NS	P	LLS ≥ 3	4200	NS

[a] Subjects from the Western Alps.
[b] Subjects from the Eastern Alps.
[c] Subjects with HAPE and HACE were included.
[d] Medications intended to prevent AMS or reduce AMS symptom severity
[e] > 1 day above 3000 in previous two months before AMS outcome
[f] All negative AMS histories indicate that subjects have been to altitude without developing AMS (i.e. negative histories do not include subjects without any altitude/hypoxia experience).
[g] The mean altitude of study participants.
NS, not stated; R, retrospective; P, prospective; SSQ, study-specific questionnaire; LLS, Lake Louise Score; HS, Hackett Score; ESQ, Environmental Symptom Questionnaire.

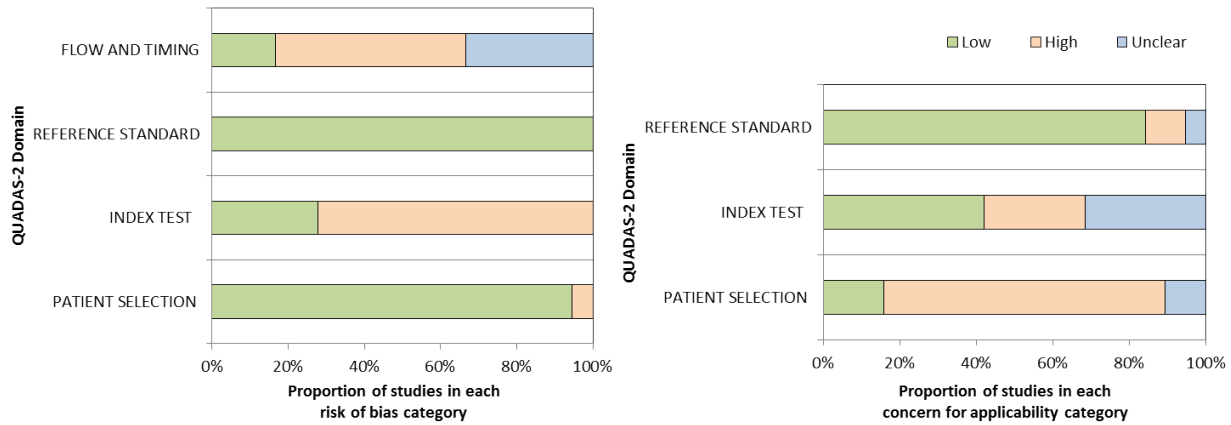

Figure 7.1 The proportion of studies with low, high, and unclear risks of bias (left) and concerns for applicability (right). Studies were categorized following questions tailored to this analysis using the QUADAS-2 protocol.

7.6.2 Descriptive statistics

The raw data from each study are provided in Table 7.3, and the descriptive statistics for each study are shown in Figure 7.2, which plots the sensitivity and specificity (with 95% CI) separately for each study. A total of 7921 subjects were included, and the mean and median sample sizes were 466 and 138, respectively. The test for heterogeneity of sensitivity was significant, $\chi^2(17) = 486.20$, $p < .0001$, as was the test for heterogeneity of specificity, $\chi^2(17) = 436.38$, $p < .0001$. Significant heterogeneity in both factors validates the use of a random-effects modeling procedure and suggests sufficient variability between studies to model this variability using meta-regression.

Table 7.3 The number of true positives (TP), false positives (FP), false negatives (FN), true negatives (TN), and total subjects in each included study.

Reference	TP	FP	FN	TN	Total
Alizadeh et al. 2012	79	64	88	118	349
Honigman et al. 1993	514	978	149	1070	2711
Lanfranchi et al. 2005	7	1	10	23	41
MacInnis et al. 2014 (Chapter 4)	12	9	2	2	25
Mairer et al. 2009	24	91	46	270	431
Mairer et al. 2010 [a]	9	16	21	33	79
Mairer et al. 2010 [b]	13	16	16	38	83
Moore et al. 1986	8	0	0	4	12
Nilles et al. 2009	8	11	6	26	51
Rexhaj et al. 2011	14	10	4	28	56
Richalet et al. 1988	32	23	33	50	138
Richalet et al. 2012 [c]	132	103	74	420	729
Roach et al. 1995	7	10	9	70	96
Schneider et al. 2002	125	175	78	328	706
Wagner et al. 2008	145	163	231	345	884
Wagner et al. 2008	17	6	16	17	56
Wang et al. 2010	82	92	302	590	1066
Ziaee et al. 2003	61	24	209	155	449

[a] Subjects from the Western Alps.
[b] Subjects from the Eastern Alps.
[c] Subjects with HAPE or HACE were included.

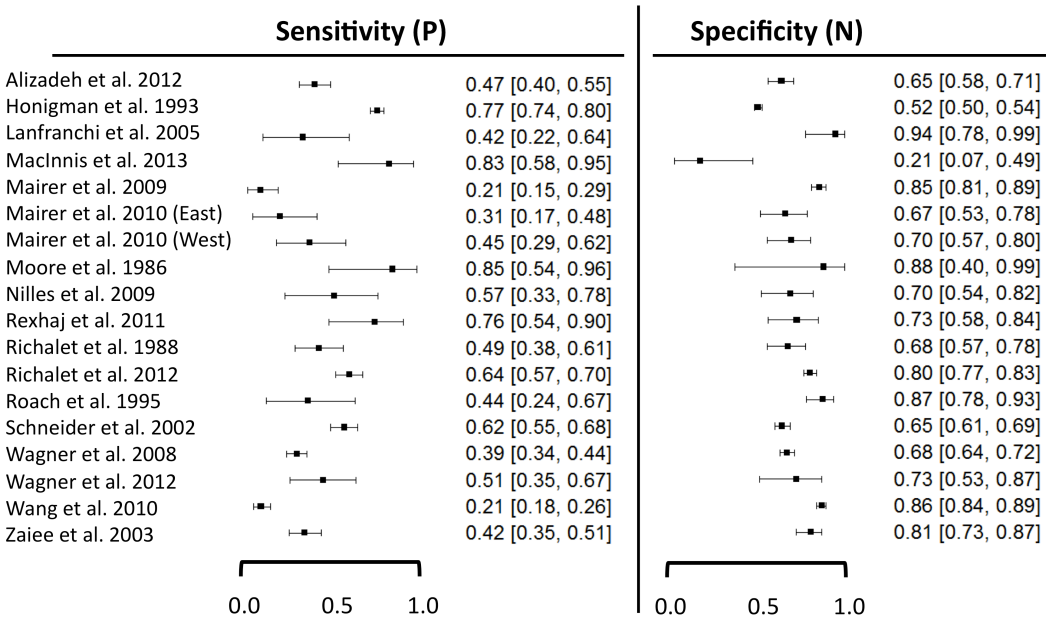

Figure 7.2 Sensitivity and specificity values for each study, shown as a point estimate and 95% confidence interval.

Table 7.4 Bivariate diagnostic random effects-model for Acute Mountain Sickness (estimation method = REML).

Fixed-effect coefficients			
	Estimate	Std. Error	95% CI
Logit(SENS)	-0.019	0.20	[-0.41, 0.37]
Logit(FPR)	-0.955	0.15	[-1.25, -0.66]***
SENS	0.495	--	[0.40, 0.59]
FPR	0.278	--	[0.22, 0.34]
Random-effects coefficients			
	Std. Dev	Corr(logit(FPR))	
Logit(SENS)	0.77	0.76	
Logit(FPR)	0.57	1.00	
Log(Likelihood)	***AIC***	***BIC***	
20.95	-32.07	-24.15	

Note. Analyses are conducted on the logit transform of sensitivity (SENS) and the false positive rate (FPR). Following analysis, point estimates are returned to their original units using an inverse logit. REML = restricted maximum likelihood; Corr(logit(FPR) = correlation of the logit(FPR) to other random-effects coefficients; AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion. ***denotes $p < .001$.

7.6.3 Bivariate random-effects model

Combining data from 7921 subjects in the bivariate meta-analysis led to a summary sensitivity of 0.50 (95% CI [0.40, 0.59]) and summary specificity of 0.72 (95% CI [0.66, 0.78]). The details of the model are presented in Table 7.4, and a summary receiver operator characteristic (ROC) curve is shown in Figure 7.3.

The random-effect model calculates a mean sensitivity and FPR, the amount of between-study variation, and the strength/direction of the correlation between sensitivity and FPR. From these statistics, we calculated a 95% confidence ellipse around a summary estimate of sensitivity and specificity, shown in Figure 7.3. As mentioned above, the model analyzes the logit of sensitivity and the false positive rate, in order to display these estimates in their original units, the results were reverse-transformed using an inverse logit.

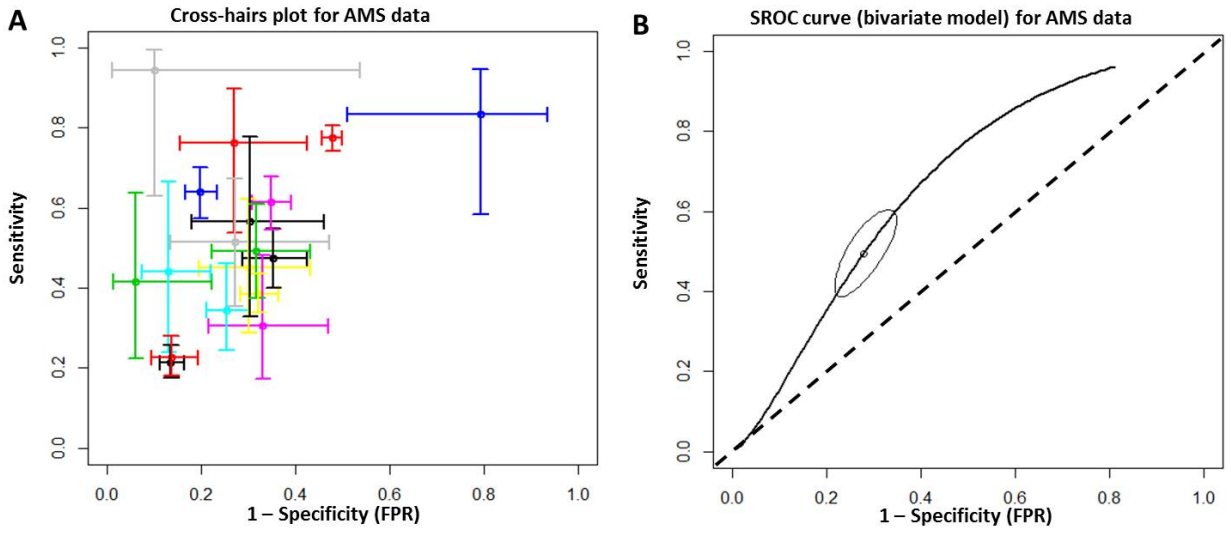

Figure 7.3 Cross-hairs plot for the AMS data showing sensitivity as a function of the false positive rate (FPR) for each study. Error bars show unique estimates of the 95% confidence interval for sensitivity and FPR. *B*: The summary ROC curve based on the bivariate model. The black line is the summary ROC curve; the dashed line is the positive diagonal (shown for reference). The open circle represents the point estimate for the summary sensitivity and FPR. The ellipse represents the 95% confidence region based on the variability and correlation between sensitivity and FPR.

Table 7.5 The results of moderator analysis for peak altitude at diagnosis, previous altitude exposure, and the use of prophylactic medications.

	Sensitivity intercept		Sensitivity slope		FPR intercept		FPR slope	
Moderator	***β***	**P**	***β***	**p**	***β***	**P**	***β***	**p**
Peak altitude diagnosis	-0.07	.92	0.012	.94	-1.01	.06	0.01	.91
Altitude exposure	-0.17	.73	0.72	.24	-0.90	.03	0.01	.98
Medication use	0.66	.10	-1.07	.03	-0.46	.10	-0.75	.03

Note. Peak altitude of the AMS diagnosis was in km. Previous altitude exposure prior to AMS outcome was coded as "0" = exposure not controlled, 1 = exposure controlled. Whether or not any subject used prophylactic medications was coded as 0 = no medications allowed, 1 = medication use allowed. FPR, false positive rate.

7.6.3.1 Tests of moderator variables

For reasons of statistical power, each moderator was tested separately (see Appendix G). The results of the moderator variable analysis are shown in Figure 7.3. Slopes and intercepts in Table 7.5 are reported in logits; point estimates in the text have been transformed back to the original units using inverse logits.

7.6.3.2 Altitude

There was no evidence that the altitude at diagnosis had any effect on test sensitivity or FPR (Appendix G). The estimated sensitivity and FPR at sea level (0.48 and 0.27, respectively) were very similar to values estimated for 8 km above sea level (0.46 and 0.29, respectively).

7.6.3.3 Exposure

Controlling for altitude exposure in the AMS history (*i.e.*, ensuring all subjects had previously been exposed to altitude) did not significantly affect the sensitivity of AMS history as an index test (Appendix G); studies that did not control for previous exposure thus has similar sensitivities (0.46) to studies that did (0.64). Controlling for altitude exposure had a significant effect on the FPR intercept but no effect on the slope, suggesting that FPR was significantly non-zero, but that FPR was comparable between studies that did control (0.29) and did not control (0.29) for altitude exposure.

7.6.3.4 Medication

Finally, controlling for prophylactic medications had a significant effect on sensitivity and FPR (Appendix G). Those studies that did not allow prophylactic medications had a better sensitivity (0.66) relative to those studies that did not control for prophylactic medications use (0.40; p = .03). The FPR was significantly higher in studies that did (0.39) control for medications relative to studies that did not (0.23; p = .03).

7.7 Discussion

Our meta-analysis indicates that a positive AMS history is not very useful in predicting a positive AMS outcome (*i.e.*, low sensitivity), but a negative AMS history is moderately useful in predicting a negative AMS outcome (*i.e.*, moderate specificity/FPR); however, neither the sensitivity nor the specificity was sufficient to rely on AMS history to plan ascents to high altitudes (*e.g.*, recommending medications, ascent rates, etc.). The low utility for AMS history as a predictor of AMS outcome may reflect the quality of the included studies (many studies had high risks of bias, especially for the index test, and high concerns for applicability for all QUADAS-2 domains) or it may suggest that AMS history is not a useful predictor of AMS outcomes.

We used QUADAS-2 to qualitatively describe the quality of the included studies and identify their weaknesses and strengths. Our analysis demonstrated that subject recruitment was not likely biased, but many subjects may not have been applicable to the research question given that some were using one or more prophylactic medications or had spent recent time at altitude to prevent AMS on their AMS outcome ascents. These substantial differences between samples in each study contribute to high diagnostic variability (*i.e.*, significant effects of heterogeneity for sensitivity and specificity).

Our analysis also demonstrated that many studies had poor quality index tests. The majority of studies reported little or no information related to subjects' previous ascents or how they determined AMS history, providing little confidence in the quality of AMS history assessments in most studies. In contrast, most studies used an acceptable method and threshold to diagnose AMS (*i.e.*, Lake Louise Score Questionnaire with a LLS > 3 or > 4 (Roach et al. 1993); Environmental Symptom Questionnaire with an AMS cerebral score > 0.70 (Sampson et al.

1983). Also, many studies diagnosed AMS prospectively, eliminating problems related to remembering past symptoms. Finally, the timing between the index test and reference standard was not reported in most studies, making it unclear whether or not the gap between ascents was appropriate. Given that recent exposure to altitude is associated with a decrease in AMS symptoms on subsequent exposures (Schneider et al. 2002; Pesce et al. 2005; Muza et al. 2010), the timing between ascents is critical.

Our QUADAS-2 analysis examined the included studies in the context of our specific research question, and it is necessary to stress that our results are not demonstrating that the included studies are of overall poor quality. Rather, most studies tested the association between AMS and multiple variables, and most were not designed specifically to test the association between AMS history and AMS outcome. These studies are still relevant to the meta-analysis, as they are frequently cited as evidence for the association between AMS history and AMS outcome.

Given the issues with the quality of the studies included in the meta-analysis, it is not clear if poor sensitivity and specificity reflect problems with predicting AMS outcomes from AMS histories *per se* or a problem with the quality of the available data. Sensitivity and specificity will be high when false negatives and false positives are reduced. A higher rate of FN and FP could be expected if the altitude, ascent rate, acclimatisation status, and drug use were not consistent between the history ascent(s) and outcome ascent. For example, FN may be high (creating low sensitivity) when the altitude and rate of ascent are lower on the AMS history ascent(s) than the AMS outcome ascents or when there was no history ascent and subjects' histories were recorded as negative. This idea is supported by the trend for higher sensitivities in studies that only included subjects with altitude experience (relative to studies that included subjects without any previous altitude experience).

Similarly, the ratio of TP to FP may be affected when subjects develop AMS on their history ascents and then take a prophylactic medication (*e.g.*, acetazolamide) or pre-acclimatise on their outcome ascents. This idea is supported by the significant effect showing that studies excluding the use of medications had higher sensitivities than studies that did not control this variable. Conversely, FN may be high if subjects took acetazolamide or pre-acclimatised for the AMS history ascent(s) but not on the AMS outcome ascent. This information was not provided in most studies, but it is very likely that the majority of studies did not control for these potential

confounding factors given their opportunistic recruitment strategies and observational designs. We attempted to control for acetazolamide use in our analysis because of its capacity to prevent AMS (Luks et al. 2010); however, potential side effects of acetazolamide overlap considerably with the symptoms of AMS (e.g., nausea and lethargy Ellsworth et al. 1987) further complicating the analysis of studies that permitted acetazolamide use.

Only two studies attempted to match the ascent rate, altitude attained, use of medications, and acclimatisation across the AMS history ascent and AMS outcome ascent. Rexhaj et al.(2011) reported a much higher sensitivity and a similar specificity to the estimates provided in our analysis; MacInnis et al. (2014; Chapter 4) reported a much higher sensitivity but also a much lower specificity compared to the estimates provided by our analysis. Increased familiarity with the environment was suggested to explain the low specificity in the latter study. Based on these studies, it is possible that AMS history could be a useful predictor of a positive AMS outcome if the ascents were better matched.

While FP may have occurred because of a lack of methodological controls, an alternative explanation is possible for the moderate FPR: individuals may be less susceptible to AMS on subsequent ascents to altitude simply because they are familiarized with the high altitude environment and the physiological responses to hypoxia and report less severe symptoms. MacInnis et al. (2014) reported a significant decrease in AMS severity across two 12-hour exposures to normobaric hypoxia. The likelihood of acclimation was low in this study, as subjects only experienced 12 hours of hypoxia (>2500 m) in the 10 weeks preceding the second hypoxic exposure. In a study of acclimatisation and re-acclimatisation at altitude, MacNutt et al. (2012) suggested that previous exposures to high altitude may increase one's psychological tolerance of altitude, which could lower self-reported AMS symptom severity at altitude on subsequent exposures.

The Wilderness Medical Society consensus guidelines (Luks et al. 2010) for preventing AMS are based partly on the AMS history of individuals. Our meta-analytic results (based on a large and systematically collected dataset) do not suggest that there is utility in this strategy. It is important to note that our analysis does not demonstrate that AMS history *cannot* predict AMS outcome, rather it demonstrates that the predictive utility of AMS history is insufficient for AMS history to be a diagnostic tool. As discussed above, a possible reason for the lack of evidence is that most studies classified the AMS history of subjects without any appreciation for the

conditions of previous ascents (*e.g.* ascent characteristics, use of medications, and pre-acclimatisation strategies): A subject developing AMS only above 8000 m and a subject developing AMS at 2000 m are both considered to have a positive AMS history. In this case, the positive AMS history at 2000 m may indicate that the subject is at high risk for developing AMS at 3000 m, but the latter subject's response to 8000 m will not likely be a good predictor of that subject's response to 3000 m. Similarly, an individual who has not developed AMS at 3000 m should not be considered to have a negative AMS history when planning an ascent to 5000 m (an 'uninformative history' would probably be a better classification). Thus, AMS history should be evaluated with an appreciation of the previous ascent(s) and the future ascent(s); there is no reason to necessarily expect AMS history to accurately predict an AMS outcome when the conditions are considerably different.

7.8 Conclusion

Currently, AMS history is not clinically useful in predicting future AMS outcomes based on the available published data. The low sensitivity and specificity may reflect the quality of the included studies, and large well-designed studies are needed to clarify the potential utility of previous AMS history in predicting AMS outcomes. Most importantly, AMS history should be considered in the context of the previous and future ascents, and extrapolations to novel altitudes and conditions may not be possible. More consideration of rate of ascent (at outcome and history), altitude of assessment (at outcome and history), medication use, and detailed reporting of demographic data are important for future research. Given the low cost and speed of using previous AMS history as a diagnostic tool, it is tremendously important to extricate these variables and determine if the sensitivity and specificity of AMS history improve as a result. Based on available data, there is significant variability in the predictive utility of different studies, but controlling for existing moderators did little to improve the sensitivity or specificity of AMS history as a diagnostic test. Further research is needed to see if this variability can be modeled systematically, creating a more nuanced, but more accurate, relationship between AMS history and the likelihood of developing AMS in the future.

Chapter 8 Is poor sleep quality at high altitude separate from acute mountain sickness? Factor structure and internal consistency of the Lake Louise Score Questionnaire

8.1 Summary

The factor structure and internal consistency of the Lake Louise Score Questionnaire (LLSQ) have not been determined in a large population at high-altitude; however, a single-factor structure and a high internal consistency are preferable for accurate clinical and research applications of the LLSQ. A large group of Nepalese pilgrims (n = 491) were assessed for acute mountain sickness with a verbal Nepali translation of the LLSQ after rapidly ascending from 1950 m to 4380 m. The factor structure and internal consistency of the LLSQ were determined with a confirmatory factor analysis (CFA) and the ordinal alpha coefficient, respectively. A one-factor structure with all five items of the LLSQ was accepted. Four items (headache, gastrointestinal upset, fatigue/weakness, and dizziness/lightheadedness) loaded strongly on this factor (> 0.70), but sleep quality had a low factor loading (0.33). The internal consistency (ordinal alpha coefficient) was 0.79, but removing the sleep quality item improved this value to 0.84. The sleep quality item of the LLSQ was weakly related to the other items of the LLSQ. Future research should further investigate whether impaired sleep at altitude should be considered separately from other symptoms of AMS.

8.2 Rationale for this experiment

The final data chapter of my dissertation is an analysis of the principle questionnaire used for assessing AMS, the Lake Louise Score Questionnaire (LLSQ). Although the LLSQ has been used for the past 2 decades, many of its key parameters have not been analysed thoroughly. In this chapter, I present an analysis of the factor structure and internal consistency of the LLSQ, two key parameters of any psychometric questionnaire. Prior to this investigation, it was assumed that the LLSQ was measuring one condition and that the symptoms were related to each other. To understand the pathology and etiology of AMS, it is necessary to understand the manifestation of symptoms. The physiological response to hypoxia is very complex, and multiple pathophysiological processes could be occurring simultaneously.

8.3 Introduction

Acute mountain sickness (AMS) is a syndrome that can occur in individuals ascending to altitudes greater than 2500 m. The presence of multiple non-specific symptoms defines AMS, necessitating self-report questionnaires for the diagnosis of AMS. Two questionnaires are commonly used for this purpose: the Environmental Symptom Questionnaire III (ESQ III; Sampson et al. 1983) and the Lake Louise Score Questionnaire (LLSQ; Roach et al. 1993).

The LLSQ is used more frequently than the ESQ III because the LLSQ is easier to administer and evaluate in field settings. The LLSQ is a relatively simple questionnaire, requiring individuals to rate five symptoms from 0 (not present) to 3 (severe; Roach et al. 1993). The five symptom scores are summed to give the 'Lake Louise Score' (LLS), and the diagnosis of AMS requires a recent gain in altitude, the presence of a headache (*i.e.*, a score $\geq$1 for the headache item), and a LLS $\geq$ 3. In comparison, the ESQ III requires individuals to rate 68 symptoms from 0 (not at all) to 5 (extreme; Sampson et al. 1983). To determine an individual's 'cerebral AMS score' (AMS-C), 11 of the 68 individual symptom scores are multiplied by unique constants, the products are summed, and the sum is divided by a constant. An AMS-C $\geq$ 0.70 is diagnostic of AMS. A shortened 11-question version of the ESQ III specific to AMS has been validated, but for quick and accurate use in the field a computer is required (Beidleman et al. 2007).

Despite the widespread use of the LLSQ, its factor structure and internal consistency have not been determined. Factor structure refers to the number of latent factors (*i.e.,* the unobservable variables) that the items of the questionnaire (*i.e.,* the observable variables) measure and the interactions between these latent factors (Schreiber et al. 2006). For example, the ESQ III contains 68 items that measure nine latent factors. Only 11 of these items relate to the cerebral AMS latent factor, while the other 54 items relate to additional latent factors such as 'respiratory AMS' and 'fatigue' (Sampson et al. 1983). The LLSQ is assumed to have a single latent factor (*i.e.,* the five symptoms are believed to contribute to one syndrome), but this assumption has not been verified statistically. Similar to factor analysis, internal consistency is a measure of the relationships among questionnaire items, and it can demonstrate the degree to which items are measuring the same condition (Henson 2001). Cronbach's alpha can be used to measure internal consistency of a questionnaire, but the ordinal alpha is more appropriate for the LLSQ (*i.e.,* a Likert-type scale with fewer than 5 data points per item; Zumbo et al. 2007). Each

statistic is a 0-1 measure of internal consistency, with values near 1 indicating excellent internal consistency.

A single-factor structure and a high internal consistency are preferable for accurate clinical and research applications of the LLSQ. Using a questionnaire without establishing these psychometric properties could cause errors in interpretation, leading to incorrect medical advice and treatment for individuals at high altitude or the inappropriate grouping of subjects in research studies. Both scenarios have serious implications for those practicing and researching high-altitude medicine and physiology. The objectives of this analysis were to determine the factor structure and internal consistency of the LLSQ (verbally administered in Nepali) in a large group of Nepalese pilgrims who ascended rapidly to Gosainkunda, Nepal (4380 m).

8.4 Methods

A total of 538 Nepalese pilgrims were recruited in Dhunche (1950 m) over a 5-day period preceding the 2012 Janai Purnima festival. The study was explained to pilgrims traveling through Dhunche, and those who provided informed consent were enrolled in the study if they resided below 2500 m and had not traveled above 2500 m in the preceding 2 months. Subjects ascended to Gosainkunda (4380 m) in 1-3 days on foot by following the same route. Enrolled subjects were assessed in Gosainkunda over a 6-day period. A Nepalese medical student or intern (under the supervision of a trained physician) verbally administered the self-report LLSQ (Roach et al. 1993) to subjects in the Nepali language immediately upon arrival to Gosainkunda. A LLS ≥ 3 (with a headache score ≥ 1) was considered a positive diagnosis for AMS. All questionnaires were complete (*i.e.*, there were no missing data).

An exploratory factor analysis (EFA) and a confirmatory factor analysis (CFA) were conducted with MPlus software (Version 6.1). Because the five items of the LLSQ were categorical, a polychoric correlation matrix and a weighted least squares mean and variances adjusted (WLSMV) estimator were used for the analyses. Polychoric correlations are calculated for pairs of ordinal variables. All factors from the EFA with an eigenvalue greater than 1.0 were retained (Guttman 1954; Kaiser 1960), and all items with loading scores > 0.4 were retained for the CFA. The model fit of the CFA was assessed with a chi-squared test of model fit, the Comparative Fit Index (CFI), the Tucker-Lewis Index (TLI), and the Root Mean Square Error of Approximation (RMSEA). A good model is indicated by a non-significant chi-squared value (*i.e.*

$p \geq 0.05$), a CFI > 0.95, a TLI > 0.95, and a RMSEA near 0.06 (Hu and Bentler 1999). For each item, a factor loading, which indicates the strength of the correlation between the item (the observable variable) and the factor (the latent variable measured by the questionnaire), was calculated. To determine the internal consistency of the LLSQ, the ordinal alpha coefficient, which is based on a polychoric correlation matrix, was calculated following syntax provided by Gadermann et al. (2012)

8.5 Results

Of the 538 recruited subjects, 491 were assessed immediately upon arrival to Gosainkunda. The mean age of subjects was 36.7 (standard deviation: 13.2) years and 70.1% were male. Seventy-five percent of subjects ascended to Gosainkunda in 2 days, and the mean sleeping altitude the night prior to assessment was 3600 m (standard deviation: 650 m). None of the 491 subjects met the criteria for AMS at 1950 m. Upon arrival to Gosainkunda, the mean LLS was 2.5 (standard deviation: 2.0), 34% of subjects were diagnosed with AMS, and individual LLSQ item scores were mostly right-skewed and kurtotic (Table 8.1) with small-medium strength polychoric correlations between each item (Table 8.2). Figure 8.1 is a graphical representation of the relationships between AMS symptoms.

Table 8.1 Descriptive statistics for the items of the Lake Louise Score Questionnaire (LLSQ) in a sample of Nepalese pilgrims upon arrival to 4380 m.

Item	n	M	SD	Range	Skewness	Kurtosis
1 (HA)	491	0.69	0.78	0-3	0.98	0.47
2 (GI)	491	0.27	0.63	0-3	2.38	5.12
3 (FW)	491	0.32	0.58	0-3	1.79	2.72
4 (DL)	491	0.33	0.62	0-3	1.74	1.95
5 (SQ)	491	0.83	0.84	0-3	0.52	-0.90

n, sample size; M, mean; SD, standard deviation.
HA, headache; GI, gastrointestinal symptoms; FW, fatigue/weakness; DL, dizziness/lightheadedness; SQ, sleep quality

Table 8.2 The polychoric correlation matrix for the items of the Lake Louise Score Questionnaire (LLSQ) in a sample of Nepalese pilgrims upon arrival to 4380 m.

Item	Item				
	1 (HA)	2 (GI)	3 (FW)	4 (DL)	5 (SQ)
1 (HA)	**1.00**	-	-	-	-
2 (GI)	0.52 (0.05)	**1.00**	-	-	-
3 (FW)	0.55 (0.05)	0.65 (0.05)	**1.00**	-	-
4 (DL)	0.55 (0.05)	0.52 (0.06)	0.60 (0.07)	**1.00**	-
5 (SQ)	0.24 (0.05)	0.20 (0.07)	0.26 (0.06)	0.28 (0.06)	**1.00**

Polychoric correlation (standard error)
HA, headache; GI, gastrointestinal symptoms; FW, fatigue/weakness; DL, dizziness/lightheadedness; SQ, sleep quality

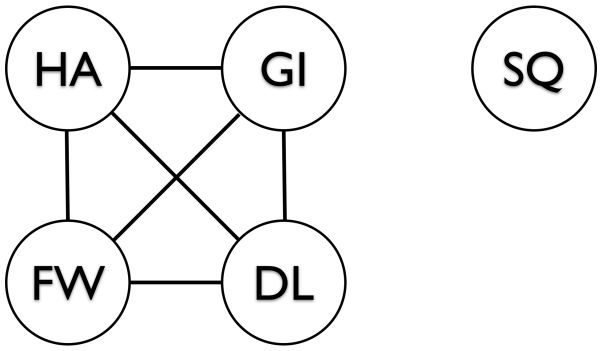

Figure 8.1 A graphical representation of the relationships amongst the items of the Lake Louise Score. Lines are drawn between items with a correlation coefficient ≥ 0.50 (see Table 8.2).

Only one factor had an eigenvalue greater than 1.0 in the EFA, and this factor explained 56.5% of the variance in the dataset. All items of the LLSQ loaded significantly on this factor; therefore, a one-factor structure with all five items of the LLSQ was retained for the CFA. Modifications of the one-factor model with all five items were not performed because it had a good fit: χ^2 (5) = 4.74, p = 0.45, CFI = 1.00, TLI = 1.00, RMSEA = 0.000 (90% confidence interval = 0.000, 0.061). The unstandardized and standardized factor loadings are presented in Table 8.3. Items 1-4 had relatively high factor loadings (> 0.70), but item 5 had a low factor loading (0.33).

The ordinal alpha coefficient for the LLSQ in the arrival dataset was 0.79. Individually deleting any of items 1-4 decreased the ordinal alpha coefficient (range: 0.72-0.74), whereas deleting item 5 increased the ordinal alpha coefficient to 0.84. For comparison, Cronbach's alpha was 0.68 in this dataset.

Table 8.3 The unstandardized and standardized factor loadings of the confirmatory factor analysis (CFA) of the Lake Louise Score Questionnaire (LLSQ).

Item	Unstandardized (SE)	Standardized	R^2
1 (HA)	1.000 (0.00)	0.703*	0.494
2 (GI)	1.066 (0.08)	0.749*	0.561
3 (FW)	1.157 (0.08)	0.813*	0.661
4 (DL)	1.063 (0.08)	0.747*	0.557
5 (SQ)	0.466 (0.08)	0.327*	0.107

*$p < 0.001$
HA, headache; GI, gastrointestinal symptoms; FW, fatigue/weakness; DL, dizziness/lightheadedness; SQ, sleep quality

8.6 Discussion

This is the first study to assess the factor structure and the ordinal alpha coefficient of the LLSQ. Our main finding is that sleep quality was not strongly related to other symptoms of AMS. This result is evident from the weak correlations between sleep quality and other AMS symptoms, the small factor loading for sleep quality, and the improvement in the internal consistency of the LLSQ with the removal of the sleep quality item. Based on our results, it may be useful to consider sleep impairment at altitude separately from other AMS symptoms.

While the one-factor model had a good fit, overall our data suggest that the LLSQ measured two factors: how well subjects felt (*i.e.,* headache, nausea, fatigue/weakness, and dizziness/lightheadedness) and how well subjects slept (*i.e.,* sleep difficulty). In support of our results, the ESQ III does not include sleep difficulty in the AMS-C component: items related to sleep quality in the full ESQ III loaded on a 'fatigue' factor, not the 'cerebral AMS' factor (Sampson et al. 1983). The inclusion of sleep quality in the LLS but not in the AMS-C may be partly responsible for different results obtained from the LLSQ and the ESQ III (Dellasanta et al. 2007; Wagner et al. 2012b): subjects who are not very sick but have trouble sleeping may be identified as having AMS with the LLSQ but not with the ESQ III.

In this study, subjects slept at altitudes (mean: 3600 m) below the altitude at which they were assessed for AMS (4380 m). The difference in altitude may have partially explained the division of symptoms: our results may have been due to the sleeping altitude being below the altitude at which AMS was diagnosed. As expected, sleep quality was poorly correlated to other AMS symptoms in those subjects who slept below 3900 m (n = 215; polychoric correlations < 0.26); however, our findings also held true in the subset of subjects who slept ≥ 3900 m (n = 276; polychoric correlations < 0.32). In both datasets, removing the sleep quality item improved the internal consistency of the LLSQ. Therefore, the weak relationship between sleep quality and other AMS symptoms is not likely a result of subjects sleeping far below the assessment altitude.

Although high-altitude sleep impairment is a frequent symptom of high-altitude exposure (e.g. Nussbaumer-Ochsner et al. 2011; Nussbaumer-Ochsner et al. 2012), sleep quality may not be a good indicator of AMS. There are several reasons to explain why sleep quality may be a poor indicator of AMS. Firstly, unless the LLSQ is administered upon waking, the items may pertain to different altitudes and time periods. This potential separation in the timing of symptoms makes the sleep quality item less relevant (or possibly irrelevant) while individuals are ascending or descending. In addition, many chamber studies that occur during the day (e.g., Roach et al. 2000; MacInnis et al. 2012) must exclude the sleep quality item from the LLSQ. Secondly, the resolution of sleep quality symptoms seems to differ temporally from the resolution of other AMS symptoms, as subjective ratings of poor sleep quality based on the LLSQ persisted for days at 4559 m despite decreases in the overall LLS (Nussbaumer-Ochsner et al. 2011; Nussbaumer-Ochsner et al. 2012). In support of this point, several indicators of sleep quality at altitude, including periodic breathing (Bloch et al. 2009; Insalaco et al. 2012), an elevated apnea/hypopnea index (Nussbaumer-Ochsner et al. 2012), nocturnal desaturation events (Nussbaumer-Ochsner et al. 2012), and low sleep efficiency (Nussbaumer-Ochsner et al. 2012) remained disturbed or worsened over days at altitude despite signs of acclimatisation. Thirdly, events contributing to poor sleep quality (*e.g.* ambient noise, bed comfort, nocturia) may only affect sleep quality, but contributors to headache, weakness, dizziness, or nausea may be more likely to affect all items. Finally, rating sleep quality may be more difficult than rating other AMS symptoms: self-assessed sleep quality (*e.g.,* total sleep time and sleep latency) was unrelated to objective measurements made with polysomnography (Nussbaumer-Ochsner et al. 2011).

Zumbo et al. (2007) have demonstrated that Cronbach's alpha (the measure used in previous studies to determine the internal consistency of the LLSQ) underestimated the true reliability (i.e. internal consistency) of Likert-type scales with fewer than five points while the ordinal alpha coefficient accurately estimated reliability regardless of the number of scale points. Likewise in our analysis, the ordinal alpha coefficient provided a greater estimate of internal consistency than did Cronbach's alpha. The ordinal alpha reported here indicated moderate internal consistency, and similar to the study by Carod-Artal et al. (2011), removing the sleep quality item improved the internal consistency of the LLSQ.

It is important to acknowledge that our study was performed using a Nepali translation of the LLSQ in a population of Nepalese pilgrims; therefore, our results cannot necessarily be generalized to the English LLSQ or to other populations. Two previous studies (Vera Calzaretta et al. 2006; Carod-Artal et al. 2011) have examined the internal consistency of a Spanish version of the LLSQ, and although they only reported Cronbach's alpha, their results are very similar to ours. These similar findings in different language translations of the LLSQ may be an indication that the low internal consistency of the LLSQ is independent of language; however, similar analyses should be performed using the English (or other languages) version of the LLSQ before our findings can be generalized.

To increase the likelihood of retaining subjects, AMS assessments were performed immediately upon arrival to Gosainkunda. It is possible that the physical exertion from completing the trek influenced the subjects' LLS; however, most of the subjects spent the previous night near the assessment site (*i.e.,* 1-4 hour walk; < 500 vertical meters below), the last segment of the trek was gradual, and the subjects generally walked slowly, which should have limited the influence of physical exertion on the subjects' LLS. To support this reasoning, self-reported fatigue scores were relatively low. Assessing subjects immediately upon arrival may have limited the influence of alcohol use on the LLS. Although we cannot completely rule out any alcohol use, based on our experiences at this site, alcohol use is uncommon during the ascent and was unlikely to have had a substantial effect on our results.

In conclusion, our analysis of the LLSQ administered in a large Nepalese population that rapidly ascended to altitude revealed that sleep quality was weakly related to other symptoms of AMS. Furthermore, the internal consistency of the LLSQ was only moderate. Future research

should investigate whether impaired sleep at altitude should be considered separately from other symptoms of AMS.

Chapter 9 General discussion and conclusions

9.1 Overview of discussion

By addressing a number of fundamental questions related to high-altitude acclimatisation, the research projects comprising my dissertation make significant contributions to the field of high-altitude biology. It was my intention to elucidate the basis of AMS susceptibility, and I implemented a variety of approaches in pursuit of this goal. I posed several questions related to genetic susceptibility and physiological markers of susceptibility (as well as demographic and dietary variables), and I also investigated the utility of the Lake Louise Score, the most frequently used questionnaire for measuring hypoxia tolerance in humans. In this chapter of my dissertation, I will briefly summarize the findings of each project, integrate my results to address the hypotheses of my dissertation, discuss the general limitations of my research, and provide some recommendations and conclusions.

9.2 Main findings

9.2.1 Genetic susceptibility to AMS

My dissertation was primarily based on the hypothesis that at least some of the variation in susceptibility to AMS was due to genetic variation. Consequently, I hypothesized that AMS would (i) be repeatable across two identical exposures; (ii) aggregate in families; (iii) be related to biogeographical origins; and (iv) be associated with genetic polymorphisms. These hypotheses were addressed in Chapters 4, 5, 6, and 7.

9.2.1.1 AMS repeatability

The study described in Chapter 4 was the first blinded and sham-controlled investigation of AMS repeatability. In this experiment, susceptibility to AMS was not repeatable in a group of subjects who were twice exposed to identical hypoxia conditions in a NH chamber: the severity of AMS was significantly lower on the second hypoxic exposure relative to the first, but physiological signs of acclimation to hypoxia were not evident. Thus, I concluded that familiarity with the experiment (or with the exposure to hypoxia) decreased the severity of AMS symptoms. Furthermore, several symptoms of AMS (according to the LLS) were infrequently reported in NH, the chamber reduced sleep quality (independent of hypoxia), and only headache was elevated above baseline in both hypoxic exposures. As this study was conducted in a

laboratory setting, it was unclear whether these findings could be extrapolated to high-altitude settings.

As previous history of AMS is commonly regarded as the strongest individual risk factor for AMS susceptibility (Hackett and Roach 2001; Imray et al. 2011), the results of Chapter 4 were unexpected; however, after performing an intensive review of the literature, I realized that only three studies had actually measured AMS repeatability with a prospective design (Robinson et al. 1971; Forster 1984b; Rexhaj et al. 2011), and that these studies were unconvincing. I performed a meta-analysis of studies that measured AMS susceptibility and previous history of AMS, looking to determine whether there was evidence to suggest AMS was repeatable. In line with the results of Chapter 4, the analysis described in Chapter 7 showed that AMS history was not a strong predictor of future AMS outcomes. In other words, the literature did not strongly support the notion that AMS was repeatable despite review articles frequently reporting that AMS history was a strong risk factor for AMS. The meta-analysis had some limitations, as the number of high-quality studies available was low. Accordingly, more research is needed to determine test the repeatability of AMS in high-altitude settings. The findings of Chapter 4 were also supported to some degree by the results of Chapter 5: first-timers were 31% ($p = 0.07$) more likely to develop AMS than those who had previously been to the festival. (*N.b.,* It cannot be ruled out that those who develop AMS choose not to return to the festival, causing selection for AMS resistance in those who visit routinely). This effect might be experiental and not psychological; those who experienced symptoms of acute altitude exposure previously might alter their plans to lessen these symptoms. Although this finding was not statistically significant, it supports my observation that AMS severity is lessened by previous exposure to hypoxia in the absence of physiological acclimatisation, which is in agreement with a hypothesis put forward by MacNutt et al. (2012).

That AMS was not strictly repeatable in Chapter 4 and Chapter 7 is suggestive that variation in susceptibility to AMS is not primarily due to genetic variation. As one's genetic information is stable over time, their susceptibilities to AMS should also be stable over time if genetic differences are the primary cause of variation in AMS susceptibility. It should be noted that Chapter 4 reported decreased AMS severity on the second exposure relative to the first, which is indicative of an influence of familiarity; however, it could be that the influence of familiarity was greater than the influence of genetics. It is possible that the severity of AMS

across multiple exposures would eventually plateau, after which an individual could respond similarly to hypoxia, making AMS susceptibility repeatable. As I only studied AMS repeatability across two hypoxic exposures, I cannot rule out this possibility. Alternatively, it may be that some individuals respond similarly to each exposure but that other individuals respond differently (potentially due to issues with familiarization, anxiety, or some other factor). In this scenario, the individuals who respond differently would introduce 'noise' into a study.

Overall, these findings do not necessarily exclude the possibility that genetic variation contributes to variation in AMS susceptibility, but they are not supportive of a genetic contribution. Using just one hypoxic exposure to determine susceptibility may lead to more 'noise' in the dataset; however, if genetic variation strongly influences the phenotype, it may still be detectable even when one exposure to hypoxia is used to determine AMS susceptibility.

9.2.1.2 Familial aggregation

While the primary research goal of the 2012 Gosainkunda Expedition was to collect DNA for genetic association studies, I took the opportunity to measure the intraclass correlations for LLS between sibling pairs and parent-offspring pairs. There was a moderate-strength relationship between pairs of brothers, but the relationship was smaller in sister-sister, brother-sister, and parent-offspring pairs. Brother-brother pairs were the best-matched group with respect to age and sex; these variables may have confounded the AMS severity intraclass correlations among parent-offspring and brother-sister pairs (Chapter 5). Note that few sister-sister pairs were obtained.

While I was able to demonstrate an association between the severities of AMS in pairs of brothers, the importance of a family history (whether measured on the same ascent or a previous ascent) in AMS susceptibility remains mostly unknown. Only one published study had investigated family history prior to our study (Ziaee et al. 2003), and their familial data are not well explained (although they report no association between family history and AMS). Recently, a prospective study has demonstrated that the children of parents who developed AMS were significantly more likely to develop AMS than the children of parents who did not develop AMS (Kriemler et al. 2014). These data provide strong evidence for the familial aggregation of AMS, a finding that my dissertation also supports, albeit to a smaller degree.

While not proof *per se*, familial aggregation of a trait is consistent with there being a genetic basis to that trait. My data provide some evidence for similarities between relatives with respect to AMS severity, complementing the more compelling findings of Kriemler et al. (2014) and supporting at least some contribution of genetics to the variation in AMS susceptibility.

9.2.1.3 Biogeographical differences

This was the first study to compare the incidence of AMS in individuals of Tibeto-Mongolian and Indo-Caucasian ancestries, but Tibetans have been compared to other biogeographical groups previously (Wu et al. 2005; Li et al. 2011). This analysis was initially conducted using surnames as markers of ancestry. As presented in Chapter 5, those with presumed Tibeto-Mongolian ancestry were significantly less likely to develop AMS relative to those of presumed Indo-Caucasian ancestry. This was the hypothesized outcome, as those of Tibetan ancestry are reportedly genetically adapted to altitude (Beall et al. 2010; Simonson et al. 2010; Yi et al. 2010). While altitude adaptation and acclimatisation are not equivalent, there could be some physiological overlap: those who are adapted to high-altitude may acclimatise better to hypoxia than those who are not adapted to high altitude, even without recent exposure to hypoxia (Wang et al. 2010a). There is already some epidemiological evidence to support this hypothesis (Wu et al. 2005; Li et al. 2011). A second, arguably more precise, analysis of ancestry was performed using genome-wide polymorphisms and a subgroup of the sample. Individuals studied in Chapter 6 were clustered according to genetic kinship, but the incidence of AMS was not significantly different across clusters. It should be noted that the genetic analysis had a sample size of < 1/3 of the surname analysis and may have been underpowered to detect an association of the same extent as that detected by surnames. Thus, it seems likely that there was a differential susceptibility across the two groups; however, this result should be confirmed with a follow-up study in the same population.

Similar to family history, a difference in AMS susceptibility across biogeographical groups is insufficient on its own to demonstrate that variation in a phenotype is a result of genetic variation. The ancestry results of Chapter 5 support a genetic basis of AMS. If the ancestry results of Chapter 6 are considered separately from Chapter 5, the genetic analysis of ancestry does not support a genetic basis of AMS. As the datasets overlapped, it is likely that the difference between groups was not statistically detectable in Chapter 6 because of the much

smaller sample size. My data, considered with the limited number of studies available, suggests that Tibetans are less susceptible to AMS, consistent with there being a genetic basis to the variation in AMS susceptibility; however, environmental and cultural differences could also explain differential AMS susceptibility across biogeographical groups.

9.2.1.4 Genetic variants

The GWAS presented in Chapter 6 demonstrated a strong association between variants of the *FAM149A* gene and the severity of AMS. While this gene has not been functionally characterized, when compared to other tissues in humans, it has markedly higher expression in the trigeminal ganglion, which is the location from which symptoms of AMS are thought to originate (Bailey et al. 2009a), and the superior cervical ganglion, which is a component of the sympathetic nervous system (Mitchell et al. 2009).

Candidate gene association studies have attempted to identify genes contributing to AMS susceptibility with limited success (MacInnis et al. 2010). These studies require the selection of genes based on knowledge of their functions. Given that the pathophysiology of AMS is far from understood and there are >20,000 genes (many of which are also not well understood), this is a challenging task (MacInnis et al. 2010). That *FAM149A* has not been investigated for an association with AMS susceptibility demonstrates one of the strengths of GWAS: *a priori* hypotheses are not needed to choose the polymorphisms that are analyzed. While we had a relatively small sample size for a GWAS (Pearson and Manolio 2008), four variants reached genome-wide statistical significance. These are the first variants to be strongly associated with AMS susceptibility, and this study provides evidence that genetic variation contributes to variation in AMS susceptibility.

While very encouraging, this finding must be replicated in Nepalese and other populations before it can be integrated into our understanding of AMS. Re-testing this association in a similar sample (*e.g.,* Nepalese) would help determine whether or not it was a false positive. While we controlled for multiple hypotheses, there is always a chance of statistically significant results being false positives. If the result can be replicated, then the association should be tested in different populations to determine whether or not it can be generalized. If *FAM149A* is associated with AMS severity in multiple populations, the next step would be to investigate additional SNPs in the gene to determine which are causal with respect to

the AMS phenotype. If *FAM149A* is not associated with AMS susceptibility in other populations, before the result is discounted as a false positive, the possibility of linkage disequilibrium specific to Nepalese populations should be investigated. If *FAM149A* is truly associated with AMS severity, the function of the *FAM149A* gene must be characterized so that its influence on AMS susceptibility can be understood. Because *FAM149A* is known to be highly expressed in the trigeminal ganglion and the superior cervical ganglion (Su et al. 2004), physiological studies of these regions would be a logical follow-up to this study.

Despite four variants reaching statistical significance, the small sample size of this study may have limited our ability to identify other polymorphisms that are associated with AMS. Specifically, those polymorphisms that weakly contribute to the variation in AMS would not be detectable in our study. Thus, we cannot rule out that other polymorphisms contribute to the variation in AMS susceptibility.

9.2.2 Physiological markers of AMS

In addition to the genetics hypotheses, I also hypothesized that individuals susceptible to AMS would be identifiable prior to and/or during hypoxic exposures. Specifically, I hypothesized that F_{ENO}, HR, and S_{PO_2} would be associated with AMS susceptibility. To test these hypotheses, I measured F_{ENO}, HR, and S_{PO_2} in three separate experiments (Chapters 3, 4, and 5).

9.2.2.1 The fraction of exhaled nitric oxide and AMS

The first experiment I conducted as part of my dissertation measured the F_{ENO} before and during a 6-hour exposure to normobaric hypoxia. Those who developed AMS had significantly lower F_{ENO} than those who remained well during the exposure. This study was a follow-up study to a previous candidate gene association study from our lab group (Wang et al. 2009) that suggested variants in one of the NOS genes were a genetic risk factor for AMS (and potentially a genetic marker for AMS susceptibility). Two experiments were planned to validate this finding, one in the UBC NH chamber and one in Nepal.

In the second laboratory-based study of my dissertation, F_{ENO} was measured prior to and at the end of a 12-hour exposure. Again, those who had AMS had a lower mean F_{ENO} relative to those who did not develop AMS. While not statistically significant ($p = 0.07$), this result still supported the hypothesis that F_{ENO} was a marker of AMS susceptibility. This study had a longer

exposure to hypoxia and was conducted overnight, making it more similar to a high-altitude exposure relative to the study described in Chapter 3. Additionally, as this study was sham-controlled, the design allowed me to demonstrate that NH did not affect the F_{ENO}. This finding was contrary to some reports in the literature (Schmetterer et al. 1997; Brown et al. 2006), but a meta-analysis of available studies demonstrated that NH did not affect the F_{ENO} (Appendix E). The disagreement was likely over the mode of hypoxia (Hemmingsson et al. 2009): it seems that hypobaric hypoxia does affect the F_{ENO}, meaning that altitude must be considered when measuring F_{ENO}.

I collected F_{ENO} data in Dhunche (~2000 m) during the 2012 Gosainkunda Expedition. In this study, those who subsequently developed AMS had slightly lower F_{ENO} than those who did not, but the difference was very small and not statistically significant. While the field study was larger than the two laboratory experiments, it had more 'noise,' as I could not control the rate of ascent to altitude (which would affect AMS; Chapter 5) or the diet of the subjects (which would affect F_{ENO}; Olin et al. 2001). The population was also more heterogeneous, as subjects in the laboratory studies were primarily healthy students and those in the field study were older and potentially had more undiagnosed medical conditions.

Examining these results collectively, I cannot recommend using F_{ENO} as a measure of an individual's likelihood of developing AMS upon exposure to high altitude. In my studies, the difference was statistically significant or nearly so in two laboratory studies, but not in a field study. A much larger field study has since been completed by another group (You et al. 2012), and the authors reported that F_{ENO} was statistically associated with AMS susceptibility; however, they also suggested that F_{ENO} had relatively low predictive value. Thus, overall it seems very possible that F_{ENO} explains some of the variation in AMS susceptibility, but that it will not be reliable in identifying those who are susceptible to AMS. The weak association may still help elucidate the physiological basis of hypoxia acclimatisation, so follow-up studies are warranted.

9.2.2.2 Oxygen saturation and AMS

Oxygen saturation (via pulse oximetry) is easy to measure and often assessed in individuals at high altitude. Hypoxia lowers S_{PO_2}, which was the case in all studies in which I measured S_{PO_2}. Intuitively, variation in S_{PO_2} may explain the variation in AMS susceptibility:

hypoxia lowers the S_{PO_2}, which reduces the availability of oxygen to tissues; there is variation in S_{PO_2} at altitude; those who have a lower S_{PO_2} should be the ones who develop AMS. Yet, this intuitive explanation is not borne out in the results of many studies (Chen et al. 2012; Wagner et al. 2012a), potentially because of limitations associated with the measure of oxygen saturation via pulse oximetry (Windsor 2012).

As described in Chapter 3, those who developed AMS had a lower mean S_{PO_2} at each time point of the 6-hour exposure, but the difference was not statistically significant. In that study, S_{PO_2} was measured with an instantaneous reading (*i.e.,* a stabilized value from a ~10-s period), not an average of a longer duration. This is not an ideal method, as S_{PO_2} can vary considerably over short periods of time (Windsor 2012). Also, the exposure to hypoxia was relatively short, limiting the generalization of this finding to high-altitude settings, where exposures are often longer.

The second laboratory study (Chapter 4) corrected many of the potential limitations of the first study. Oxygen saturation was measured during 5-minute periods prior to entering and exiting the UBC NH chamber. The exposure was also extended to 12 hours and was overnight instead of during the day, providing a more accurate simulation of an ascent to high altitude. In addition, S_{PO_2} was measured while subjects slept (as a safety precaution, but also for scientific interest). Despite the better design, an association between AMS and S_{PO_2} was not detected at any time point. These data were convincing evidence that S_{PO_2} was not a strong marker of AMS status.

In contrast to the two laboratory studies described in Chapters 3 and 4, S_{PO_2} was significantly lower in those who had AMS compared to those who did not during the 2010 Gosainkunda Expedition (Appendix B); however, we were unable to replicate this finding in our 2012 expedition (Chapter 5 and Appendix B). For both expeditions, we were only able to make instantaneous measurements of S_{PO_2} (similar to that for the Chapter 3), as there was no power to supply a larger pulse oximeter, power lab data acquisition system, and computer. In this study, those with AMS had a lower S_{PO_2} than those without AMS, but the difference was less than 1%; therefore, the association between AMS and S_{PO_2} in the 2010 Gosainkunda Expedition may have been a false positive.

Collectively, the S_{PO_2} data collected for my dissertation suggests that differences in S_{PO_2} between those with and without AMS are likely to be very small if they exist at all. It is unlikely that the difference in S_{PO_2} would be meaningful for diagnosing AMS, and it is unlikely that the difference in S_{PO_2} would be sufficient to explain the variation in AMS susceptibility. As breathing patterns and cold temperature influence measurements of S_{PO_2} (Windsor 2012), it would not be an ideal marker of AMS susceptibility in high-altitude regions.

9.2.2.3 Heart rate and AMS

The assessment of heart rate in high-altitude settings is as common as that of S_{PO_2}, probably because the two variables are often measured using the same device. Again, the literature is ambiguous as to whether HR can distinguish those who do and do not have AMS. Given that hypoxia is stressful, heart rate could increase due to the release of catecholamines (Mazzeo and Reeves 2003). I measured heart rate in all studies where I measured S_{PO_2}, using the same pulse oximeter in each study. As noted already, methodological limitations may have affected several of the studies (*i.e.,* HR was often measured over a very short time period).

Those who developed AMS during 6-hour of hypoxia had higher HR (~5 bpm) values than those who did not develop AMS, but the difference was not significant at any time point. A power calculation showed that 200 subjects would be needed to find a significant difference in heart rate between those with and without AMS (data not shown). Prior to entering and exiting the UBC NH chamber and during a 6-hour period of sleep (on their second exposure to NH), subjects who developed AMS had a HR ~6 bpm above those who did not, but, again, the differences were not statistically significant. In field settings, those who had AMS in the 2010 Gosainkunda sample had a significantly higher mean HR than those who did not develop AMS; however, the larger 2012 expedition did not replicate these findings.

Overall, it seems likely that those who develop AMS might have a greater HR than those who do not develop AMS; however, HR may not be very useful in diagnosing AMS because of the relatively small difference between individuals with and without AMS and the high variability in heart rate.

9.2.3 Epidemiological risk factors of AMS

To understand the factors contributing to AMS in the field sample, it was necessary to investigate other variables that could influence AMS susceptibility. As the literature is ambiguous with respect to the relationship between age, sex, and AMS, I hypothesized that demographic variables would not be associated with AMS susceptibility. Similarly, based on a lack of evidence in the literature, I also hypothesized that traditional Nepalese strategies to prevent AMS would not be effective. These hypotheses were addressed in Chapter 5.

9.2.3.1 Sex, age, ascent rate, and garlic

Sex, age, and ascent rate were associated with the incidence of AMS in the 2012 Nepalese dataset. Those who were female, older, and ascended more quickly were more likely to develop AMS. As discussed in Chapter 5, the relationship between AMS and ascent rate is well known, and the relationships between AMS and sex and age are ambiguous in the literature. While these results are interesting on their own, they were most useful for attempting to control variables that might influence the GWAS in Chapter 6. Specifically, females were dropped from the analysis, subjects who ascended in 2 days were the preferred samples, and age was used as a covariate. That being said, these results have some bearing on the other hypotheses of my dissertation. If AMS is genetic, it seems that it is modified by sex and age. Controlling for these variables to reduce 'noise' in future genetic studies will be important. These variables could also impact on the physiological variables measured as part of this dissertation and should be controlled in these studies as well.

Given the Nepali belief that consumption of garlic and mountain pepper prevents AMS, I was surprised to show the opposite: both foods were associated with a greater incidence of AMS. It is unclear whether garlic and mountain pepper are actual physiological risk factors or whether the increased incidence of AMS was a result of behavioral changes (*e.g.,* subjects thought they were protected from AMS and took less caution while ascending, or subjects were more worried about becoming ill and therefore more likely to report more severe symptoms). As these foods represent a common AMS preventative method in the Himalayas, this information could have important public health implications.

9.2.4 The Lake Louise Score

Separate from my two main hypotheses, I investigated the factor structure and internal consistency of the primary questionnaire for assessing hypoxia tolerance, the Lake Louise Score (LLS). This analysis is the concluding data chapter of my dissertation (Chapter 8). I used this questionnaire to measure acute hypoxia tolerance in humans; however, my results suggested that the LLS needs to be modified to improve the ability of scientists to assess this phenotype (and for physicians to diagnose and treat AMS).

Evidence suggesting that the current definition of AMS encompasses multiple syndromes is beginning to mount. I demonstrated that the LLS had a single factor structure, with sleep quality weakly related to this factor and poorly correlated with the other four symptoms of the LLS (Chapter 8). Independently, Hall et al. (2014), using visual analog scales to record the severity of AMS symptoms, reported that three distinct clinical syndromes were apparent in 1100 altitude-exposure days (n = 269 independent subjects). The major clusters were: (1) sleep difficulty, headache, and fatigue symptoms; (2) sleep difficulty and fatigue symptoms; and (3) headache and fatigue symptoms. These studies indicate that high-altitude sleep impairment could represent a separate condition from AMS, which is supported by the Environmental Symptom Questionnaire, as it does not include sleep quality as a symptom of AMS (Sampson et al. 1983).

In addition to the statistical evidence above, the inclusion of sleep quality in the LLS is unwarranted for multiple reasons. Firstly, sleep quality from the previous night is unchanged after waking, whereas other symptoms can intensify or resolve during the day. Continuing to use one's sleep quality score from the previous night, regardless of changes in the altitude at which they are residing, is unreasonable. Secondly, sleep quality can only be assessed if individuals spend the night at altitude. Upon arrival to altitude and in many chamber studies (*e.g.,* Chapter 3), sleep quality is not an applicable symptom. Finally, as demonstrated in Chapter 4, a novel environment, independent of hypoxia, can influence sleep quality. Separating sleep problems at altitude from other symptoms of altitude exposure would help improve our understanding of the effects of altitude on humans.

Although the remaining symptoms of the LLS were reasonably well correlated in my study and the study from Hall and colleagues, these symptoms do not necessarily represent one syndrome. Until these symptoms can be shown to manifest from a common mechanism, it would be prudent to assess each symptom separately. The grouping of potentially unrelated symptoms

could increase the noise in research studies, reducing statistical power. The inability of researchers to elucidate the pathophysiology of AMS may be partly attributable to difficulties in diagnosing and quantifying AMS. The definition of AMS must change for our understanding of acute hypoxia tolerance to progress.

9.3 Limitations

Like all science, this thesis is not without limitations, which the reader should appreciate when interpreting the main findings and their significance to the field. The specific limitations of each project are discussed in the respective chapters, and these will not be reiterated here. The general limitations to my research projects were the relatively small sample sizes, which may have allowed for type I and type II errors. A greater limitation to my research, although it is not specific to my research, is the lack of an objective method to measure hypoxia tolerance. The general limitations of each hypothesis are outlined in Table 9.1.

Table 9.1 The general limitations of specific hypotheses of this dissertation.

Hypothesis	Chapter(s)	Limitations
AMS susceptibility will be repeatable on multiple hypoxic exposures	4, 7	Hypoxia tolerance measure
AMS susceptibility will aggregate in families	5	Hypoxia tolerance measure; small sample size
AMS susceptibility will differ across biogeographical groups	5, 6	Hypoxia tolerance measure; small sample size
Genetic variants will be associated with AMS susceptibility.	6	Hypoxia tolerance measure; small sample size
AMS susceptible individuals will have a lower F_{ENO} than resistant subjects prior to hypoxia exposure	3, 4, 5	Hypoxia tolerance measure
AMS susceptible individuals will have a lower S_{PO_2} than resistant subjects during hypoxia exposure	3, 4, 5	Hypoxia tolerance measure
AMS susceptible individuals will have a higher HR than resistant subjects during hypoxia exposure	3, 4, 5	Hypoxia tolerance measure
AMS susceptible individuals will not be identifiable based on demographic variables	5	Hypoxia tolerance measure
AMS susceptible individuals not be protected from AMS by traditional Nepalese preventative foods	5	Hypoxia tolerance measure
The Lake Louise Score Questionnaire will have a single-factor structure and high internal consistency	8	Hypoxia tolerance measure

AMS, acute mountain sickness; F_{ENO}, fraction of exhaled nitric oxide; S_{PO_2}, oxygen saturation; HR, heart rate.

Several of the studies in this thesis had relatively small sample sizes, even if they were normal or large for this area of research. To overcome these issues, I attempted to follow-up as many studies as possible with independent studies. For example, in Chapter 3, I reported that F_{ENO} was associated with AMS susceptibility, but the sample size was 18 subjects. This result was followed up in two studies, one in the UBC NH chamber (n = 24) and one in the Himalaya (n = 46). Ultimately, what appeared to be a strong relationship in the results of Chapter 3 was not quite significant in the results of Chapter 4 and was not associated with AMS in the field (Chapter 5). The sample size for the epidemiology study presented in Chapter 5 was rather large (n = 491), providing sufficient statistical power to test associations between AMS susceptibility and a number of demographic variables; however, in the GWAS presented in Chapter 6, the high

cost of the analysis restricted the sample size. The sample sizes needed to adequately power GWAS are very large in comparison to the numbers available for my analysis (Pearson and Manolio 2008). As a result, the GWAS results, despite the statistical significance, are still somewhat exploratory. The association between AMS severity and the *FAM149A* gene must be tested in additional samples.

The lack of an objective method for assessing hypoxia tolerance is a limitation in the field of high-altitude biology. I accepted this limitation when I designed my research projects; however, I may not have been critical enough of the LLS at the outset of my dissertation. The LLS was used to assess hypoxia tolerance in all chapters of this dissertation; therefore, the results of my dissertation are specific to the current LLS definition of AMS and specific to AMS as measured using self-reported LLS. It is not necessarily correct to extrapolate the results of my dissertation to alternative definitions of acute hypoxia tolerance: if hypoxia tolerance were assessed another way, different results than those reported in my dissertation might have been obtained.

9.4 Future directions

After spending nearly 5 years studying AMS, I have a number of suggestions for future directions. While additional physiological and genetic studies are warranted, much of this research will continue to be hindered by the current methods for assessing acute hypoxia tolerance in humans. Thus, my suggestions are, in order of importance, (i) redefine AMS; (ii) change the scale on which AMS symptoms are measured; and (iii) include sham exposures in research designs.

While the LLS is widely accepted and commonly used by researchers and physicians worldwide, it has several flaws, and the LLS definition of AMS should be reconsidered. The most pressing issue with respect to understanding the basis of variation in acute hypoxia tolerance is the validity of the tools used to measure AMS severity. While numerous subjective symptoms occur in response to hypoxia, it is not clear that these symptoms share a common pathology. I demonstrated that sleep quality is not strongly related to other AMS symptoms (Chapter 8) and that being in a hypoxic chamber negatively affects sleep quality independent of hypoxia (Chapter 4). Investigating symptoms of acute hypoxia exposure separately might reduce the noise and potentially allow greater insights into their pathophysiology. Based on my

dissertation, I recommend that the most attention be given to high-altitude headache. This was the only symptom in Chapter 4 that was significantly greater on the second hypoxic exposure relative to the sham exposure. It is also the most commonly reported symptom at high altitude (Sampson et al. 1983), and it is recognized as a requirement for an AMS diagnosis based on the LLS (Roach et al. 1993; Roach et al. 2011). Separating AMS into separate symptoms of high-altitude exposure and adding more questions about each symptom (*i.e.,* several questions about headache as opposed to a single headache symptom severity question) may be helpful. As sleep difficulty is a frequent complaint during hypoxia exposure (Sampson et al. 1983) and a potentially separate condition from AMS (Chapter 8), it is also deserving of more direct attention. As an example, in a randomized control trial, Temazepam, a drug prescribed to treat insomnia, improved sleep quality relative to acetazolamide in a group at 3450 m (Tanner et al. 2013). Treating sleep quality symptoms separate from other AMS symptoms may be practical and beneficial.

My second suggestion is related to the scale by which symptom severity is measured. The current LLS is not ideal for analysis or interpretation: (i) the items are measured on different scales; and (ii) points on the scales are not evenly spaced. For example, headache is currently measured on a scale of none, mild, moderate, severe (incapacitating), whereas gastrointestinal symptoms are measured on a scale of good appetite, poor appetite (or nausea), moderate nausea or vomiting, and severe, incapacitating nausea and vomiting. These scales are not equivalent, and the points are not necessarily evenly spaced (it is difficult to put the possible responses on a linear scale). To further demonstrate this point, the scale by which sleep is measured is different as well: slept as well as usual, did not sleep as well as usual, woke many times (poor night's sleep), and could not sleep at all. When the symptoms are added into the overall LLS, the difference between 'none' and 'mild' on the headache scale is the same as the difference between 'moderate' and 'severe' on the nausea scale or the difference between 'woke many times' and 'could not sleep at all' on the sleep scale. It seems inappropriate to attribute each change to 1 point in the overall LLS. Visual analog scales or discrete scales with more items than the LLS (and the same scale for each item [*i.e.,* 0-10 from 'no symptom' to 'extreme']) could prove more useful for measuring symptom severity. (Note that when AMS was assessed with the visual analog scale, multiple clinical syndromes were still apparent; Hall et al. 2014).

The results in Chapter 4 exposed two potential flaws with some previous research: individuals do not report consistent AMS symptoms across two identical hypoxia exposures, and sham conditions can induce symptoms of AMS, namely difficulty sleeping. As discussed above, it seems likely that a previous exposure to hypoxia reduces the reported symptom severity on a subsequent exposure without inducing acclimatisation; therefore, all studies using within-subject designs should randomize treatment orders. Including sham conditions in all studies should be the standard, as symptoms can be reported for reasons independent of hypoxia. Given that the symptoms of AMS are entirely subjective and not specific to AMS, researchers must ensure that the reported AMS symptoms are due to hypoxia, not to an unfamiliar environment, observation effects, or some other factor. It may also be inappropriate to classify individuals as 'susceptible' or 'resistant' based on a single exposure to hypoxia. The number of exposures needed to classify an individual as AMS susceptible or resistant is currently unknown, but this should be a focus of future work.

9.5 Conclusions

Humans can tolerate extremely high altitudes if they are given sufficient time to acclimatise; however, failure to acclimatise manifests as AMS (and possibly HAPE, HACE, or death). Acclimatisation to hypoxia is a complex process that evokes molecular, cellular and physiological responses throughout the body. The primary hypothesis of this dissertation was that variation in AMS susceptibility was due, at least in part, to genetic variation, and this dissertation provides evidence to support this hypothesis: brothers had similar AMS severities on ascent to 4380 m, those of Tibeto-Mongolian ancestry were less susceptible to AMS than those of Indo-Caucasian ancestry, and variants of the *FAM149A* gene were associated with AMS susceptibility. Most notably, the *FAM149A* gene is expressed in regions (trigeminal ganglion and superior cervical ganglion) plausibly associated with the pathophysiology of AMS. That AMS was not strictly repeatable (i) in identical exposures to normobaric hypoxia and (ii) in a meta-analysis of (mostly) high-altitude studies does not support a genetic basis for the variation in AMS susceptibility; however, these results could indicate that previous experiences (with an experiment, hypoxia, or high altitude) modify AMS susceptibility. Aside from the influence of genetic variation on hypoxia tolerance, my dissertation investigated several other variables hypothesized to influence AMS susceptibility. Several physiological variables were investigated

as being either predictive (F_{ENO}) or characteristic (HR, and S_{PO_2}) of AMS, but they had little utility in those roles. In a large prospective study, the age and sex of subjects and the rate of exposure influenced susceptibility to AMS, and these factors should be considered in studies of AMS. In the same study, traditional Nepalese strategies to prevent AMS were associated with a higher incidence of AMS, suggesting that these foods do not prevent AMS. Lastly, my dissertation demonstrated that the Lake Louise Score was not an ideal questionnaire for assessing hypoxia tolerance, as high-altitude sleep impairment appears to be a separate condition from AMS. Therefore, the current grouping of AMS symptoms into a syndrome may not be warranted. Modifying this questionnaire and/or developing new questionnaires to assess hypoxia tolerance would contribute greatly to high-altitude biology research.

References

Ahsan A, Charu R, Pasha MAQ, Norboo T, Charu R, Afrin F, Ahsan A, Baig MA. 2004. eNOS allelic variants at the same locus associate with HAPE and adaptation. Thorax 59:1000–1002.

Ainslie PN, Smith KJ. 2011. Integrated human physiology: breathing, blood pressure and blood flow to the brain. J Physiol 589:2917–2917.

Akiyama Y, Nishimura M, Suzuki A, Yamamoto M, Kawakami Y. 1991. Peripheral chemosensitivity assessed by the modified transient O2 test in female twins. Chest 100:102–105.

Aldenderfer M. 2011. Peopling the Tibetan Plateau: insights from archaeology. High Alt Med Biol 12:141–147.

Alizadeh R, Ziaee V, Frooghifard L-A, Mansournia M-A, Aghsaeifard Z. 2012. The effect of path and beginning time of ascending on incidence of acute mountain sickness around Mount Damavand in Iran (5671 m). Neurology Research International 2012:1–6.

Allemann Y, Rotter M, Hutter D, Lipp E, Sartori C, Scherrer U, Seiler C. 2004. Impact of acute hypoxic pulmonary hypertension on LV diastolic function in healthy mountaineers at high altitude. Am J Physiol Heart Circ Physiol 286:H856–H862.

Allemann Y, Sartori C, Lepori M, Pierre S, Mélot C, Naeije R, Scherrer U, Maggiorini M. 2000. Echocardiographic and invasive measurements of pulmonary artery pressure correlate closely at high altitude. Am J Physiol Heart Circ Physiol 279:H2013–H2016.

Alving K, Weitzberg E, Lundberg JM. 1993. Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 6:1368–1370.

American Thoracic Society, European Respiratory Society. 2005. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care 171:912–930.

Anand IS, Prasad BA, Chugh SS, Rao KR, Cornfield DN, Milla CE, Singh N, Singh S, Selvamurthy W. 1998. Effects of inhaled nitric oxide and oxygen in high-altitude pulmonary edema. Circulation 98:2441–2445.

Anttila V, Winsvold BS, Gormley P, et al. 2013. Genome-wide meta-analysis identifies new susceptibility loci for migraine. Nature 45:912–917.

Aoki VS, Robinson SM. 1971. Body hydration and the incidence and severity of acute mountain sickness. J Appl Physiol 31:363–367.

Aulchenko YS, Ripke S, Isaacs A, van Duijn CM. 2007. GenABEL: an R library for genome-wide association analysis. Bioinformatics 23:1294–1296.

Bailey DM, Roukens R, Knauth M, et al. 2005. Free radical-mediated damage to barrier function is not associated with altered brain morphology in high-altitude headache. J Cereb Blood Flow Metab 26:99–111.

Bailey DM, Bartsch P, Knauth M, Baumgartner RW. 2009a. Emerging concepts in acute mountain sickness and high-altitude cerebral edema: from the molecular to the morphological. Cell Mol Life Sci 66:3583–3594.

Bailey DM, Taudorf S, Berg RMG, Jensen LT, Lundby C, Evans KA, James PE, Pedersen BK, Moller K. 2009b. Transcerebral exchange kinetics of nitrite and calcitonin gene-related peptide in acute mountain sickness: evidence against trigeminovascular activation? Stroke 40:2205–2208.

Balding DJ. 2006. A tutorial on statistical methods for population association studies. Nat Rev Genet 7:781–791.

Banchero N, Sime F, Penaloza D, Cruz JC, Gamboa R, Marticorena EA. 1966. Pulmonary pressure, cardiac output, and arterial oxygen saturation during exercise at high altitude and at sea level. Circulation 33:249–262.

Barcroft J. 1924. Mountain sickness. Nature 114:90–92.

Barry PW, Pollard AJ. 2003. Altitude illness. BMJ 326:915–919.

Bärtsch P, Maggiorini M, Ritter M, Noti C, Vock P, Oelz O. 1991. Prevention of high-altitude pulmonary edema by nifedipine. N Engl J Med 325:1284–1289.

Bärtsch P, Maggiorini M, Mairbäurl H, Vock P, Swenson ER. 2002. Pulmonary extravascular fluid accumulation in climbers. Lancet 360:571–authorreply571–2.

Bärtsch P, Bailey DM, Berger MM, Knauth M, Baumgartner RW. 2004. Acute mountain sickness: controversies and advances. High Alt Med Biol 5:110–124.

Bärtsch P, Gibbs JSR. 2007. Effect of altitude on the heart and the lungs. Circulation 116:2191–2202.

Bärtsch P, Saltin B. 2008. General introduction to altitude adaptation and mountain sickness. Scand J Med Sci Sports 18:1–10.

Bärtsch P, Bailey DM. 2013. Acute Mountain Sickness and High Altitude Cerebral Oedema. In: New York, NY: Springer New York. pp. 379–403.

Basnyat B. 1993. Acute mountain sickness in local pilgrims to a high altitude lake (4154 m) in Nepal. J Wilderness Med 4:286–292.

Basnyat B, Lemaster J, Litch JA. 1999. Everest or bust: a cross sectional, epidemiological study of acute mountain sickness at 4243 meters in the Himalayas. Aviat Space Environ Med 70:867–873.

Basnyat B, Murdoch DR. 2003. High-altitude illness. Lancet 361:1967–1974.

Basnyat B, Subedi D, Sleggs J, Lemaster J, Bhasyal G, Aryal B, Subedi N. 2000. Disoriented and ataxic pilgrims: an epidemiological study of acute mountain sickness and high-altitude cerebral edema at a sacred lake at 4300 m in the Nepal Himalayas. Wilderness Environ Med 11:89–93.

Basnyat B, Gertsch JH, Holck PS, et al. 2006. Acetazolamide 125 mg BD is not significantly different from 375 mg BD in the prevention of acute mountain sickness: the prophylactic acetazolamide dosage comparison for efficacy (PACE) trial. High Alt Med Biol 7:17–27.

Basnyat B. 2010. Gosainkunda's highs and lows. Nepali Times [Internet]. Available from: http://nepalitimes.com/news.php?id=17354#.Ux6gQ17e6zJ

Basnyat B. 2013. Rejuvenation Time. High Alt Med Biol 14:1–2.

Baumgartner RW, Bärtsch P, Maggiorini M, Waber U, Oelz O. 1994. Enhanced cerebral blood flow in acute mountain sickness. Aviat Space Environ Med 65:726–729.

Baumgartner RW, Siegel AM, Hackett PH. 2007. Going high with preexisting neurological conditions. High Alt Med Biol 8:108–116.

Beall CM, Laskowski D, Strohl KP, Soria R, Villena M, Vargas E, Alarcon AM, Gonzales C, Erzurum SC. 2001. Pulmonary nitric oxide in mountain dwellers. Nature 414:411–412.

Beall CM. 2003. High-altitude adaptations. Lancet 362 Suppl:s14–s15.

Beall CM. 2011. Genetic changes in Tibet. High Alt Med Biol 12:101–102.

Beall CM, Cavalleri GL, Deng L, et al. 2010. Natural selection on EPAS1 (HIF2) associated with low hemoglobin concentration in Tibetan highlanders. Proc Natl Acad Sci USA 107:11459–11464.

Beall CM, Laskowski D, Erzurum SC. 2012. Nitric oxide in adaptation to altitude. Free Radic Biol Med52:1123–1134.

Beckman JS, Koppenol WH. 1996. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 271:C1424–C1437.

Beidleman BA, Muza SR, Fulco CS, et al. 2004. Intermittent altitude exposures reduce acute mountain sickness at 4300 m. Clin Sci 106:321–328.

Beidleman BA, Muza SR, Fulco CS, Rock PB, Cymerman A. 2007. Validation of a shortened electronic version of the environmental symptoms questionnaire. High Alt Med Biol 8:192–199.

Bigham AW, Kiyamu M, León-Velarde F, Parra EJ, Rivera-Ch M, Shriver MD, Brutsaert TD. 2008. Angiotensin-converting enzyme genotype and arterial oxygen saturation at high

altitude in Peruvian Quechua. High Alt Med Biol 9:167–178.

Bigham A, Bauchet M, Pinto D, et al. 2010. Identifying signatures of natural selection in Tibetan and Andean populations using dense genome scan data. PLoS Genet 6:e1001116.

Bilo G, Revera M, Bussotti M, et al. 2012. Effects of slow deep breathing at high altitude on oxygen saturation, pulmonary and systemic hemodynamics. PLoS ONE 7:e49074.

Bland JM, Altman DG. 1990. A note on the use of the intraclass correlation coefficient in the evaluation of agreement between two methods of measurement. Comput Biol Med20:337–340.

Blitzer ML, Loh E, Roddy MA, Stamler JS, Creager MA. 1996. Endothelium-derived nitric oxide regulates systemic and pulmonary vascular resistance during acute hypoxia in humans. J Am Coll Cardiol 28:591–596.

Bloch KE, Turk AJ, Maggiorini M, et al. 2009. Effect of ascent protocol on acute mountain sickness and success at Muztagh Ata, 7546 m. High Alt Med Biol 10:25–32.

Bouchard C, Sarzynski MA, Rice TK, Kraus WE, Church TS, Sung YJ, Rao DC, Rankinen T. 2011. Genomic predictors of the maximal O2 uptake response to standardized exercise training programs. J Appl Physiol 110:1160–1170.

Brown DE, Beall CM, Strohl KP, Mills PS. 2006. Exhaled nitric oxide decreases upon acute exposure to high-altitude hypoxia. Am J Hum Biol 18:196–202.

Buroker NE, Ning X-H, Zhou Z-N, et al. 2010. Genetic associations with mountain sickness in Han and Tibetan residents at the Qinghai-Tibetan Plateau. Clin Chim Acta:1–8.

Burtscher M. 2004. Exercise capacity for mountaineering: how much is necessary? Res in Sports Med 12:241–250.

Busch T, Bärtsch P, Pappert D, Grünig E, Hildebrandt W, Elser H, Falke KJ, Swenson ER. 2001. Hypoxia decreases exhaled nitric oxide in mountaineers susceptible to high-altitude pulmonary edema. Am J Respir Crit Care 163:368–373.

Carod-Artal FJ, Echávarri DE, Peral ALG. 2011. Metric properties of the Spanish version of the Lake Louise Acute Mountain Sickness Questionnaire. Neurología (English Edition) 26:337–342.

Caspersen C, Stang J, Thorsen E, Stensrud T. 2012. Exhaled nitric oxide concentration upon acute exposure to moderate altitude. Scand J Med Sci Sports 23:e102-e107.

Cermak NM, Gibala MJ, van Loon LJC. 2012. Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. Int J Sport Nutr Exerc Metab 22:64–71.

Chen H-C, Lin W-L, Wu J-Y, Wang S-H, Chiu T-F, Weng Y-M, Hsu T-Y, Wu M-H. 2012. Change in oxygen saturation does not predict acute mountain sickness on Jade Mountain.

Wilderness Environ Med 23:122–127.

Clarenbach CF, Senn O, Christ AL, Fischler M, Maggiorini M, Bloch KE. 2012. Lung function and breathing pattern in subjects developing high altitude pulmonary edema. PLoS ONE 7:e41188.

Clarke C. 1988. High altitude cerebral oedema. Int J Sports Med 9:170–174.

Collins DD, Scoggin CH, Zwillich CW, Weil JV. 1978. Hereditary aspects of decreased hypoxic response. J Clin Invest 62:105–110.

Conkin J, Wessel JH. 2008. Critique of the equivalent air altitude model. Aviat Space Environ Med 79:975–982.

Conkin J, Gernhardt ML, Abercromby AF, Feiveson AH. 2013. Probability of hypobaric decompression sickness including extreme exposures. Aviat Space Environ Med 84:661–668.

Consolazio CF, Matoush LO, Johnson HL, Krzywicki HJ, Daws TA, Isaac GJ. 1969. Effects of high-carbohydrate diets on performance and clinical symptomatology after rapid ascent to high altitude. Fed Proc 28:937–943.

Cushing T, Paterson R, Haukoos J, Harris NS. 2013. Intraocular pressure is not associated with acute mountain sickness. High Alt Med Biol 14:342–345.

Daly AK, Day CP. 2001. Candidate gene case-control association studies: advantages and potential pitfalls. Br J Clin Pharmacol 52:489–499.

Daniels J. 2005. Daniels' Running Formula. 2nd ed. Windsor, ON: Human Kinetics Publishers.

Dauncey, S. 2012. Can dietary nitrate supplements improve tolerance to hypoxia? Journal of Intensive Care Society 13:198–205.

Deeks JJ. 2001. Systematic reviews in health care: Systematic reviews of evaluations of diagnostic and screening tests. BMJ 323:157–162.

Dehnert C, Weymann J, Montgomery HE, Woods D, Maggiorini M, Scherrer U, Gibbs JSR, Bartsch P. 2002. No association between high-altitude tolerance and the ACE I/D gene polymorphism. Med Sci Sports Exerc 34:1928–1933.

Dehnert C, Luks AM, Schendler G, et al. 2010. No evidence for interstitial lung oedema by extensive pulmonary function testing at 4,559 m. Eur Respir J 35:812–820.

Dellasanta P, Gaillard S, Loutan L, Kayser B. 2007. Comparing questionnaires for the assessment of acute mountain sickness. High Alt Med Biol 8:184–191.

DerSimonian R. 1986. Meta-analysis in clinical trials. Control clin trials 7:177–188.

Deykin A, Massaro AF, Drazen JM, Israel E. 2002. Exhaled nitric oxide as a diagnostic test for

asthma: online versus offline techniques and effect of flow rate. Am J Respir Crit Care 165:1597–1601.

Ding H, Liu Q, Hua M, Ding M, Du H, Zhang W, Li Z, Zhang J. 2011. Polymorphisms of hypoxia-related genes in subjects susceptible to acute mountain sickness. Respir 81:236–241.

Ding H, Liu Q, Hua M, Ding M, Du H, Zhang W, Li Z, Zhang J. 2012. Associations between vascular endothelial growth factor gene polymorphisms and susceptibility to acute mountain sickness. J Int Med Res 40:2135–2144.

Doebler P, Holling H. 2013. Meta-analysis of diagnostic accuracy with mada. [Internet]:1–20. Available from: http://cran.r-project.org/web/packages/mada/index.html.

Droma Y, Hanaoka M, Ota M, Katsuyama Y, Koizumi T, Fujimoto K, Kobayashi T, Kubo K. 2002. Positive association of the endothelial nitric oxide synthase gene polymorphisms with high-altitude pulmonary edema. Circulation 106:826–830.

Droma Y, Hanaoka M, Hotta J, Katsuyama Y, Ota M, Kobayashi T, Kubo K. 2003. The r506 Q mutation of coagulation factor V gene in high altitude pulmonary-edema-susceptible subjects. High Alt Med Biol 4:497–498.

Droma Y, Hanaoka M, Basnyat B, et al. 2008. Adaptation to high altitude in Sherpas: association with the insertion/deletion polymorphism in the Angiotensin-converting enzyme gene. Wilderness Environ Med 19:22–29.

Donnelly J, Cowan DC, Yeoman DJ, Lucas SJE, Herbison GP, Thomas KN, Ainslie PN, Taylor DR. 2011. Exhaled nitric oxide and pulmonary artery pressures during graded ascent to high altitude. Respir Physiol Neurobiol 177:213–217.

Duffin J. 1990. The chemoreflex control of breathing and its measurement. Can J Anaesth 37:933–942.

Duplain H, Vollenweider L, Delabays A, Nicod P, Bärtsch P, Scherrer U. 1999. Augmented sympathetic activation during short-term hypoxia and high-altitude exposure in subjects susceptible to high-altitude pulmonary edema. Circulation 99:1713–1718.

Duplain H, Sartori C, Lepori M, Egli M, Allemann Y, Nicod P, Scherrer U. 2000. Exhaled nitric oxide in high-altitude pulmonary edema: role in the regulation of pulmonary vascular tone and evidence for a role against inflammation. Am J Respir Crit Care 162:221–224.

Dweik RA, Laskowski D, Abu-Soud HM, Kaneko F, Hutte R, Stuehr DJ, Erzurum SC. 1998. Nitric oxide synthesis in the lung. Regulation by oxygen through a kinetic mechanism. J Clin Invest 101:660–666.

Dweik RA. 2006. The lung in the balance: arginine, methylated arginines, and nitric oxide. Am J Physiol Lung Cell Mol Physiol 292:L15–L17.

Eichenberger U, Weiss E, Riemann D, Oelz O, Bärtsch P. 1996. Nocturnal periodic breathing and the development of acute high altitude illness. Am J Respir Crit Care 154:1748–1754.

Ellsworth AJ, Larson EB, Strickland D. 1987. A randomized trial of dexamethasone and acetazolamide for acute mountain sickness prophylaxis. Am J Med 83:1024–1030.

Epstein M, Saruta T. 1972. Effects of simulated high altitude on renin-aldosterone and Na homeostasis in normal man. J Appl Physiol 33:204–210.

Erba P, Anastasi S, Senn O, Maggiorini M, Bloch KE. 2004. Acute mountain sickness is related to nocturnal hypoxemia but not to hypoventilation. Eur Respir J 24:303–308.

Fagenholz PJ, Gutman JA, Murray AF, Noble VE, Camargo CA, Harris NS. 2009. Optic nerve sheath diameter correlates with the presence and severity of acute mountain sickness: evidence for increased intracranial pressure. J Appl Physiol 106:1207–1211.

Faiss R, Pialoux V, Sartori C, Faes C, Dériaz O, Millet GP. 2012. Ventilation, Oxidative Stress and Nitric Oxide in Hypobaric vs. Normobaric Hypoxia. Med Sci Sports Exerc 45: 253-260.

Fischler M, Maggiorini M, Dorschner L, et al. 2009. Dexamethasone but not tadalafil improves exercise capacity in adults prone to high-altitude pulmonary edema. Am J Respir Crit Care 180:346–352.

Forster P. 1984a. Health and work at high altitude: A study at the Mauna Kea observatories. Publications of the Astronomical Society of the Pacific 96:478–487.

Forster P. 1984b. Reproducibility of individual response to exposure to high altitude. Br Med J (Clin Res Ed) 289:1269.

Forster P. 1985. Effect of different ascent profiles on performance at 4,200 m elevation. Aviat Space Environ Med 56:758–764.

Forwand SA, Landowne M, Follansbee JN, Hansen JE. 1968. Effect of acetazolamide on acute mountain sickness. N Engl J Med 279:839–845.

Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WM. 1991. Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. Circulation 83:2038–2047.

Fred HL, Schmidt AM, Bates T, Hecht HH. 1962. Acute pulmonary edema of altitude clinical and physiologic observations. Circulation 25:929–937.

Fulco CS, Rock PB, Cymerman A. 1998. Maximal and submaximal exercise performance at altitude. Aviat Space Envion Med 69:793–801.

Fulco CS, Muza SR, Beidleman BA, Demes R, Staab JE, Jones JE, Cymerman A. 2011. Effect of repeated normobaric hypoxia exposures during sleep on acute mountain sickness, exercise performance, and sleep during exposure to terrestrial altitude. Am J Physiol Regul Integr

Comp Physiol 300:R428–R436.

Gabry AL, Ledoux X, Mozziconacci M, Martin C. 2003. High-altitude pulmonary edema at moderate altitude (<2,400 m; 7,870): a series of 52 patients. Chest 123:49–53.

Gadermann, M A, Guhn, Zumbo MBD. 2012. Estimating ordinal reliability for Likert-type and ordinal item response data: A conceptual, empirical, and practical guide. Practical Assessment, Research & Evaluation 17:1–13.

Gaillard S, Dellasanta P, Loutan L, Kayser B. 2004. Awareness, prevalence, medication use, and risk factors of acute mountain sickness in tourists trekking around the Annapurnas in Nepal: A 12-year follow-up. High Alt Med Biol 5:410–419.

Gallagher SA, Hackett PH. 2004. High-altitude illness. Emerg Med Clin North Am 22:329–355, viii.

Garske LA, Brown MG, Morrison SC. 2003. Acetazolamide reduces exercise capacity and increases leg fatigue under hypoxic conditions. J Appl Physiol 94:991–996.

George SC. 2008. How accurately should we estimate the anatomical source of exhaled nitric oxide? J Appl Physiol 104:909–911.

Gerlach H, Rossaint R, Pappert D, Falke KJ. 1993. Time-course and dose-response of nitric oxide inhalation for systemic oxygenation and pulmonary hypertension in patients with adult respiratory distress syndrome. Eur J Clin Invest 23:499–502.

Gertsch JH, Lipman GS, Holck PS, et al. 2010. Prospective, double-blind, randomized, placebo-controlled comparison of acetazolamide versus ibuprofen for prophylaxis against high altitude headache: the Headache Evaluation at Altitude Trial (HEAT). Wilderness Environ Med 21:236–243.

Gertsch JH, Corbett B, Holck PS, et al. 2012. Altitude Sickness in Climbers and Efficacy of NSAIDs Trial (ASCENT): randomized, controlled trial of ibuprofen versus placebo for prevention of altitude illness. Wilderness Environ Med 23:307–315.

Girard O, Koehle MS, MacInnis MJ, et al. 2012. Comments on Point:Counterpoint: Hypobaric hypoxia induces/does not induce different responses from normobaric hypoxia. J Appl Physiol 112:1788–1794.

Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PMM. 2003. The diagnostic odds ratio: a single indicator of test performance. J Clin Epidemiol 56:1129–1135.

Goadsby PJ, Lipton RB, Ferrari MD. 2002. Migraine--current understanding and treatment. N Engl J Med 346:257–270.

Goldfarb-Rumyantzev AS, Alper SL. 2014. Short-term responses of the kidney to high altitude in mountain climbers. Nephrol Dial Transplant 29:497–506.

Golja P, Flander P, Klemenc M, Maver J, Princi T. 2008. Carbohydrate ingestion improves oxygen delivery in acute hypoxia. High Alt Med Biol 9:53–62.

Gore CJ, Little SC, Hahn AG, et al. 1997. Reduced performance of male and female athletes at 580 m altitude. Eur J Appl Physiol Occup Physiol 75:136–143.

Govoni M, Jansson EA, Weitzberg E, Lundberg JO. 2008. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide 19:333–337.

Grover RF, Weil JV, Reeves JT. 1986. Cardiovascular adaptation to exercise at high altitude. Exerc Sport Sci Rev 14:269–302.

Groves BM, Reeves JT, Sutton JR, Wagner PD, Cymerman A, Malconian MK, Rock PB, Young PM, Houston CS. 1987. Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen. J Appl Physiol 63:521–530.

Guo FH, de Raeve HR, Rice TW, Stuehr DJ, Thunnissen FB, Erzurum SC. 1995. Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo. Proc Natl Acad Sci USA 92:7809–7813.

Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. 1991. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. Biochem Biophys Res Commun 181:852–857.

Guttman L. 1954. Some necessary conditions for common-factor analysis. Psychometrika 19:149–161.

Hackett PH, Rennie D, Levine HD. 1976. The incidence, importance, and prophylaxis of acute mountain sickness. Lancet 2:1149–1155.

Hackett PH, Roach RC. 2001. High-altitude illness. N Engl J Med 345:107–114.

Hackett PH, Roach RC. 2004. High altitude cerebral edema. High Alt Med Biol 5:136–146.

Hakonarson H, Grant SFA. 2011. Planning a genome-wide association study: Points to consider. Ann Med 43:451–460.

Hall DP, MacCormick IJC, Phythian-Adams AT, et al. 2014. Network Analysis Reveals Distinct Clinical Syndromes Underlying Acute Mountain Sickness.Schulz C, editor. PLoS ONE 9:e81229.

Hambraeus-Jonzon K, Bindslev L, Mellgård AJ, Hedenstierna G. 1997. Hypoxic pulmonary vasoconstriction in human lungs. A stimulus-response study. Anesthesiology 86:308–315.

Hanaoka M, Kubo K, Yamazaki Y, Miyahara T, Matsuzawa Y, Kobayashi T, Sekiguchi M, Ota M, Watanabe H. 1998. Association of high-altitude pulmonary edema with the major histocompatibility complex. Circulation 97:1124–1128.

Hanaoka M, Droma Y, Hotta J, Matsuzawa Y, Kobayashi T, Kubo K, Ota M. 2003a. Polymorphisms of the tyrosine hydroxylase gene in subjects susceptible to high-altitude pulmonary edema. Chest 123:54–58.

Hanaoka M, Droma Y, Naramoto A, Honda T, Kobayashi T, Kubo K. 2003b. Vascular endothelial growth factor in patients with high-altitude pulmonary edema. J Appl Physiol 94:1836–1840.

Hatzenbuehler J, Glazer J, Kuhn C. 2009. Awareness of altitude sickness among visitors to a North American ski resort. Wilderness Environ Med 20:257–260.

Hemmingsson T, Horn A, Linnarsson D. 2009. Measuring exhaled nitric oxide at high altitude. Respir Physiol Neurobiol 167:292–298.

Hemmingsson T, Linnarsson D. 2009. Lower exhaled nitric oxide in hypobaric than in normobaric acute hypoxia. Respir Physiol Neurobiol 169:74–77.

Hennis PJ, Bussell C, Darlison MG. 2010. The lack of associations between alleles at the hypoxia-inducible factor 1A C1772T loci and responses to acute hypoxia. Wilderness Environ Med 21:219–228.

Henson RK. 2001. Understanding internal consistency reliability estimates: A conceptual primer on coefficient alpha. Measurement and Evaluation in Counseling and Development 34:177–189.

Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. Higgins JPT, Green S, editors.

Hildebrandt W, Alexander S, Bartsch P, Dröge W. 2002. Effect of N-acetyl-cysteine on the hypoxic ventilatory response and erythropoietin production: linkage between plasma thiol redox state and O(2) chemosensitivity. Blood 99:1552–1555.

Hirschhorn JN, Daly MJ. 2005. Genome-wide association studies for common diseases and complex traits. Nat Rev Genet 6:95–108.

Högman M, Strömberg S, Schedin U, Frostell C, Hedenstierna G, Gustafsson LE. 1997. Nitric oxide from the human respiratory tract efficiently quantified by standardized single breath measurements. Acta Physiol Scand 159:345–346.

Hoit BD, Dalton ND, Erzurum SC, Laskowski D, Strohl KP, Beall CM. 2005. Nitric oxide and cardiopulmonary hemodynamics in Tibetan highlanders. J Appl Physiol 99:1796–1801.

Honigman B, Theis MK, Koziol-McLain J, Roach R, Yip R, Houston C, Moore LG, Pearce P. 1993. Acute mountain sickness in a general tourist population at moderate altitudes. Ann Intern Med 118:587–592.

Hotta J. 2004. Polymorphisms of renin-angiotensin system genes with high-altitude pulmonary edema in Japanese subjects. Chest 126:825–830.

Hu LT, Bentler PM. 1999. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. Structural Equation Modeling: A Multidisciplinary Journal 6:1–55.

Hultgren HN, Spickard WB, Hellriegel K, Houston CS. 1961. High altitude pulmonary edema. Medicine (Baltimore) 40:289–313.

Hultgren HN, Grover RF, Hartley LH. 1971. Abnormal circulatory responses to high altitude in subjects with a previous history of high-altitude pulmonary edema. Circulation 44:759–770.

Hultgren HN, Honigman B, Theis K, Nicholas D. 1996. High-altitude pulmonary edema at a ski resort. West. J. Med. 164:222–227.

Imray C, Wright A, Subudhi A, Roach R. 2010. Acute mountain sickness: pathophysiology, prevention, and treatment. Prog Cardiovasc Dis 52:467–484.

Imray C, Booth A, Wright A, Bradwell A. 2011. Acute altitude illnesses. BMJ 343:d4943–d4943.

Insalaco G, Romano S, Salvaggio A, Pomidori L, Mandolesi G, Cogo A. 2012. Periodic breathing, arterial oxyhemoglobin saturation, and heart rate during sleep at high altitude. High Alt Med Biol 13:258–262.

Iwamoto J, Pendergast DR, Suzuki H, Krasney JA. 1994. Effect of graded exercise on nitric oxide in expired air in humans. Respir Physiol 97:333–345.

Jacobs KA, Kressler J, Stoutenberg M, Roos BA, Friedlander AL. 2011. Sildenafil has little influence on cardiovascular hemodynamics or 6-km time trial performance in trained men and women at simulated high altitude. High Alt Med Biol 12:215–222.

Jacobs RA, Lundby C, Robach P, Gassmann M. 2012. Red blood cell volume and the capacity for exercise at moderate to high altitude. Sports Med 42:643–663.

Jain SC, Bardhan J, Swamy YV, Krishna B. 1980. Body fluid compartments in humans during acute high-altitude exposure. Aviat Space Environ Med 51:234–236.

Janocha AJ, Koch CD, Tiso M, Ponchia A, Doctor A, Gibbons L, Gaston B, Beall CM, Erzurum SC. 2011. Nitric oxide during altitude acclimatization. N Engl J Med 365:1942–1944.

Jiang C, Li F, He M, Sun S. 2005. Glutathione S-transferase M1, T1 genotypes and the risk of mountain sickness. Chin J Ind Hyg Occup Dis 23: 188-190.

Jones CM, Athanasiou T. 2009. Diagnostic accuracy meta-analysis: review of an important tool in radiological research and decision making. Br J Radiol 82:441–446.

Kaiser HF. 1960. The application of electronic computers to factor analysis. Educational and Psychological Measurement 20:141–151.

Kallenberg K, Bailey DM, Christ S, Mohr A, Roukens R, Menold E, Steiner T, Bartsch P, Knauth M. 2007. Magnetic resonance imaging evidence of cytotoxic cerebral edema in acute mountain sickness. J Cereb Blood Flow Metab 27:1064–1071.

Kallenberg K, Dehnert C, Dörfler A, Schellinger PD, Bailey DM, Knauth M, Bärtsch PD. 2008. Microhemorrhages in nonfatal high-altitude cerebral edema. J Cereb Blood Flow Metab 28:1635–1642.

Kalson NS, Thompson J, Davies AJ, Stokes S, Earl MD, Whitehead A, Tyrrell-Marsh I, Frost H, Montgomery H. 2008. The effect of angiotensin-converting enzyme genotype on acute mountain sickness and summit success in trekkers attempting the summit of Mt. Kilimanjaro (5,895 m). Eur J Appl Physiol 105:373–379.

Kamimori GH, Ryan EJ, Otterstetter R, Barkley JE, Glickman EL, Davis HQ. 2009. Catecholamine levels in hypoxia-induced acute mountain sickness. Aviat Space Environ Med 80:376–380.

Karinen HM, Peltonen JE, Kähönen M, Tikkanen HO. 2010. Prediction of acute mountain sickness by monitoring arterial oxygen saturation during ascent. High Alt Med Biol 11:325–332.

Kaufmann BA, Bernheim AM, Kiencke S, Fischler M, Sklenar J, Mairbaurl H, Maggiorini M, Brunner-La Rocca HP. 2008. Evidence supportive of impaired myocardial blood flow reserve at high altitude in subjects developing high-altitude pulmonary edema. Am J Physiol Heart Circ Physiol 294:H1651–H1657.

Kawakami Y, Yoshikawa T, Shida A, Asanuma Y, Murao M. 1982. Control of breathing in young twins. J Appl Physiol 52:537–542.

Kawakami Y, Yamamoto H, Yoshikawa T, Shida A. 1984. Chemical and behavioral control of breathing in adult twins. Am Rev Respir Dis 129:703–707.

Kayser B. 1991. Acute mountain sickness in western tourists around the Thorong pass (5400 m) in Nepal. J Wilderness Med 2:110–117.

Kayser B, Dumont L, Lysakowski C, Combescure C, Haller G, Tramèr MR. 2012. Reappraisal of acetazolamide for the prevention of acute mountain sickness: a systematic review and meta-analysis. High Alt Med Biol 13:82–92.

Keyes LE, Paterson R, Boatright D, Browne V, Leadbetter G, Hackett P. 2013. Optic nerve sheath diameter and acute mountain sickness. Wilderness Environ Med 24:105–111.

Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. 2003. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. Eur Respir J 21:433–438.

King SJ, Greenlee RR. 1990. Successful use of the Gamow hyperbaric bag in the treatment of altitude illness at Mount Everest. J Wilderness Med 1:193–202.

Klausen K. 1966. Cardiac output in man in rest and work during and after acclimatization to 3,800 m. J Appl Physiol 21:609–616.

Kleger GR, Bärtsch P, Vock P, Heilig B, Roberts LJ, Ballmer PE. 1996. Evidence against an increase in capillary permeability in subjects exposed to high altitude. J Appl Physiol 81:1917–1923.

Kobayashi S, Nishimura M, Yamamoto M, Akiyama Y, Kishi F, Kawakami Y. 1993. Dyspnea sensation and chemical control of breathing in adult twins. Am Rev Respir Dis 147:1192–1198.

Kobayashi N, Hanaoka M, Droma Y, Ito M, Katsuyama Y, Kubo K, Ota M. 2013. Polymorphisms of the Tissue Inhibitor of Metalloproteinase 3 gene are associated with resistance to high-altitude pulmonary edema (HAPE) in a japanese population: a case control study using polymorphic microsatellite markers. PLoS ONE 8:e71993.

Koehle MS, Wang P, Guenette JA, Rupert JL. 2006. No association between variants in the ACE and angiotensin II receptor 1 genes and acute mountain sickness in Nepalese pilgrims to the Janai Purnima Festival at 4380 m. High Alt Med Biol 7:281–289.

Koehle MS, Guenette JA, Warburton DER. 2010. Oximetry, heart rate variability, and the diagnosis of mild-to-moderate acute mountain sickness. Eur J Emerg Med 17:119–122.

Koller EA, Drechsel S, Hess T, Macherel P, Boutellier U. 1988. Effects of atropine and propranolol on the respiratory, circulatory, and ECG responses to high altitude in man. Eur J Appl Physiol Occup Physiol 57:163–172.

Koshland DE. 1992. The molecule of the year. Science 258:1861.

Kriemler S, Jansen C, Linka A, Kessel-Schaefer A, Zehnder M, Schurmann T, Kohler M, Bloch K, Brunner-La Rocca HP. 2008. Higher pulmonary artery pressure in children than in adults upon fast ascent to high altitude. Eur Respir J 32:664–669.

Kriemler S, Bürgi F, Wick C, et al. 2014. Prevalence of acute mountain sickness at 3500 m within and between families: a prospective cohort study. High Alt Med Biol 15:28–38.

Kumar R, Pasha Q, Khan AP, Gupta V. 2004. Renin angiotensin aldosterone system and ACE I/D gene polymorphism in high-altitude pulmonary edema. Aviat Space Environ Med 75:981–983.

Kundel HL, Polansky M. 2003. Measurement of Observer Agreement. Radiology 228:303–308.

Küpper T, Milledge JS, Hillebrandt D, Kubalová J, Hefti U, Basnyat B, Gieseler U, Pullan R, Schöffl V. 2011. Work in hypoxic conditions--consensus statement of the Medical Commission of the Union Internationale des Associations d'Alpinisme (UIAA MedCom). Ann Occup Hyg 55:369–386.

Kwon NS, Nathan CF, Gilker C, Griffith OW. 1990. L-citrulline production from L-arginine by

macrophage nitric oxide synthase. The ureido oxygen derives from dioxygen. J Biol Chem 265:13442–13445.

Lanfranchi PA. 2005. Autonomic cardiovascular regulation in subjects with acute mountain sickness. Am J Physiol Heart Circ Physiol 289:H2364–H2372.

Lawley JS, Oliver SJ, Mullins P, Morris D, Junglee NA, Jelleyman C, Macdonald JH. 2012. Optic nerve sheath diameter is not related to high altitude headache: a randomized controlled trial. High Alt Med Biol 13:193–199.

Lawley JS, Oliver SJ, Mullins PG, Macdonald JH. 2013. Investigation of whole-brain white matter identifies altered water mobility in the pathogenesis of high-altitude headache. J Cereb Blood Flow Metab 33:1286–1294.

Le Cras TD, McMurtry IF. 2001. Nitric oxide production in the hypoxic lung. Am J Physiol Lung Cell Mol Physiol 280:L575–L582.

Leach RM, Treacher DF. 1998. Oxygen transport-2. Tissue hypoxia. BMJ 317:1370–1373.

Leaf DE, Goldfarb DS. 2007. Mechanisms of action of acetazolamide in the prophylaxis and treatment of acute mountain sickness. J Appl Physiol 102:1313–1322.

Li R, Lyn D, Lapu-Bula R, et al. 2004. Relation of endothelial nitric oxide synthase gene to plasma nitric oxide level, endothelial function, and blood pressure in African Americans. Am J Hypertens 17:560–567.

Li X, Tao F, Pei T, You H, Liu Y, Gao Y. 2011. Population level determinants of acute mountain sickness among young men: a retrospective study. BMC Public Health 11:740.

Lim KG, Mottram C. 2008. The use of fraction of exhaled nitric oxide in pulmonary practice. Chest 133:1232–1242.

Litch JA. 1996. Drug-induced hypoglycemia presenting as acute mountain sickness, after mistaking acetohexamide for acetazolamide. Wilderness Environ Med 7:232–235.

Loeppky JA, Icenogle MV, Maes D, Riboni K, Scotto P, Roach RC. 2003. Body temperature, autonomic responses, and acute mountain sickness. High Alt Med Biol 4:367–373.

Loeppky JA, Roach RC, Maes D, Hinghofer-Szalkay H, Roessler A, Gates L, Fletcher ER, Icenogle MV. 2005. Role of hypobaria in fluid balance response to hypoxia. High Alt Med Biol 6:60–71.

Lorenzo F V, Yang Y, Simonson TS, Nussenzveig R, Jorde LB, Prchal JT, Ge R-L. 2009. Genetic adaptation to extreme hypoxia. Blood Cells Mol Dis 43:221–225.

Lorenzo VF, Yang Y, Simonson TS, Nussenzveig R, Jorde LB, Prchal JT, Ge RL. 2010. Erratum to: Genetic adaptation to extreme hypoxia: Study of high-altitude pulmonary edema in a three-generation Han Chinese family [Blood Cells Mol. Dis. 43:3 (2009) 221--225]. Blood

Cells Mol Dis 44:198.

Lovering AT, Romer LM, Haverkamp HC, Pegelow DF, Hokanson JS, Eldridge MW. 2008. Intrapulmonary shunting and pulmonary gas exchange during normoxic and hypoxic exercise in healthy humans. J Appl Physiol 104:1418–1425.

Luks AM, Swenson ER. 2007. Travel to high altitude with pre-existing lung disease. Eur Respir J 29:770–792.

Luks AM. 2009. Should travelers with hypertension adjust their medications when traveling to high altitude? High Alt Med Biol 10:11–15.

Luks AM, McIntosh SE, Grissom CK, Auerbach PS, Rodway GW, Schoene RB, Zafren K, Hackett PH, Wilderness Medical Society. 2010. Wilderness Medical Society consensus guidelines for the prevention and treatment of acute altitude illness. Wilderness Environ Med 21:146–155.

Lundberg JON, Weitzberg E, Nordvall SL, Kuylenstierna R, Lundberg JM, Alving K. 1994. Primarily nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome. Eur Respir J 7:1501–1504.

Lundberg JO, Farkas-Szallasi T, Weitzberg E, Rinder J, Lidholm J, Anggård A, Hökfelt T, Lundberg JM, Alving K. 1995. High nitric oxide production in human paranasal sinuses. Nat Med 1:370–373.

Lundberg JON, Weitzberg E, Lundberg JM, Alving K. 1996. Nitric oxide in exhaled air. Eur Respir J 9:2671–2680.

Lundberg JO, Weitzberg E, Gladwin MT. 2008. The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov 7:156–167.

MacInnis MJ, Koehle MS, Rupert JL. 2010. Evidence for a Genetic Basis for Altitude Illness: 2010 Update. High Alt Med Biol 11:349–368.

MacInnis MJ, Rupert JL. 2011. 'ome on the range: altitude adaptation, positive selection, and Himalayan genomics. High Alt Med Biol 12:133–139.

MacInnis MJ, Wang P, Koehle MS, Rupert JL. 2011. The genetics of altitude tolerance: the evidence for inherited susceptibility to acute mountain sickness. J Occup Environ Med 53:159–168.

MacInnis MJ, Carter EA, Koehle MS, Rupert JL. 2012a. Exhaled nitric oxide is associated with acute mountain sickness susceptibility during exposure to normobaric hypoxia. Respir Physiol Neurobiol 180:40–44.

MacInnis MJ, Rupert JL, Koehle MS. 2012b. Evaluation of the Balance Error Scoring System (BESS) in the diagnosis of acute mountain sickness at 4380 m. High Alt Med Biol 13:93–97.

MacInnis MJ, Carter EA, Freeman MG, et al. 2013 a. A prospective epidemiological study of acute mountain sickness in Nepalese pilgrims ascending to high altitude (4380 m). PLoS ONE 8:e75644.

MacInnis MJ, Lanting SC, Rupert JL, Koehle MS. 2013 b. Is poor sleep quality at high altitude separate from acute mountain sickness? Factor structure and internal consistency of the Lake Louise Score Questionnaire. High Alt Med Biol 14:334–337.

MacInnis MJ, Lohse KR, Strong JK, Koehle MS. 2013 c. Is previous history a reliable predictor for acute mountain sickness susceptibility? A meta-analysis of diagnostic accuracy. Br J Sports Med. Epub ahead of print: doi:10.1136/bjsports-2013-092921.

MacInnis MJ, Koch S, MacLeod KE, Carter EA, Jain R, Koehle MS, Rupert JL. 2014. Acute mountain sickness is not repeatable across two 12-hour normobaric hypoxia exposures. Wilderness Envion Med. 25:143–151.

MacLeod KE, MacInnis MJ, Manokhina I, Rupert JL. 2013. Twin studies in altitude and hypoxia research. Aviat Space Environ Med 84:613–619.

MacNutt MJ, Laursen PB, Kedia S, Neupane M, Parajuli P, Pokharel J, Sheel AW. 2012. Acclimatisation in trekkers with and without recent exposure to high altitude. Eur J Appl Physiol 112:3287–3294.

Madjdpour C, Jewell UR, Kneller S, et al. 2003. Decreased alveolar oxygen induces lung inflammation. Am J Physiol Lung Cell Mol Physiol 284:L360–L367.

Maggiorini M, Bühler B, Walter M, Oelz O. 1990. Prevalence of acute mountain sickness in the Swiss Alps. BMJ 301:853–855.

Maggiorini M, Mélot C, Pierre S, et al. 2001. High-altitude pulmonary edema is initially caused by an increase in capillary pressure. Circulation 103:2078–2083.

Maggiorini M, Bruner-La Rocca HP, Peth S, et al. 2006. Both tadalafil and dexamethasone may reduce the incidence of high-altitude pulmonary edema. Ann Intern Med 145:497-506.

Mairbäurl H, Weymann J, Möhrlein A, Swenson ER, Maggiorini M, Gibbs JSR, Bartsch P. 2003. Nasal epithelium potential difference at high altitude (4,559 m): evidence for secretion. Am J Respir Crit Care 167:862–867.

Mairer K, Wille M, Bucher T, Burtscher M. 2009. Prevalence of acute mountain sickness in the Eastern Alps. High Alt Med Biol 10:239–245.

Mairer K, Wille M, Burtscher M. 2010. The prevalence of and risk factors for acute mountain sickness in the Eastern and Western Alps. High Alt Med Biol 11:343–348.

Manolio TA, Collins FS. 2009. The HapMap and genome-wide association studies in diagnosis and therapy. Annu Rev Med 60:443–456.

Manolio TA. 2010. Genomewide association studies and assessment of the risk of disease. N Engl J Med 363:166–176.

Mansoor JK, Morrissey BM, Walby WF, Yoneda KY, Juarez M, Kajekar R, Severinghaus JW, Eldridge MW, Schelegle ES. 2005. L-arginine supplementation enhances exhaled NO, breath condensate VEGF, and headache at 4342 m. High Alt Med Biol 6:289–300.

Martin D, Windsor J. 2008. From mountain to bedside: understanding the clinical relevance of human acclimatisation to high-altitude hypoxia. Postgrad Med J 84:622–627; quiz626.

Martin DS, Ince C, Goedhart P, Levett DZH, Grocott MPW, Caudwell Xtreme Everest Research Group. 2009. Abnormal blood flow in the sublingual microcirculation at high altitude. Eur J Appl Physiol 106:473–478.

Martin DS, Levett DZH, Grocott MPW, Montgomery HE. 2010. Variation in human performance in the hypoxic mountain environment. Exp Physiol 95:463–470.

Mazzeo RS, Reeves JT. 2003. Adrenergic contribution during acclimatization to high altitude: perspectives from Pikes Peak. Exerc Sport Sci Rev 31:13–18.

McIntosh SE, Campbell AD, Dow J, Grissom CK. 2008. Mountaineering Fatalities on Denali. High Alt Med Biol 9:89–95.

Millet GP, Faiss R, Pialoux V. 2012a. Point: Hypobaric hypoxia induces different physiological responses from normobaric hypoxia. J Appl Physiol 112:1783–1784.

Millet GP, Faiss R, Pialoux V. 2012b. Last Word on Point: Counterpoint: Hypobaric hypoxia induces different responses from normobaric hypoxia. J Appl Physiol 112:1795–1795.

Mishra A, Ali Z, Vibhuti A, Kumar R, Alam P, Ram R, Thinlas T, Mohammad G, Pasha MAQ. 2011. CYBA and GSTP1 variants associate with oxidative stress under hypobaric hypoxia as observed in high-altitude pulmonary oedema. Clin Sci 122:299–309.

Mishra A, Mohammad G, Thinlas T, Pasha MQ. 2013. EGLN1 variants influence its expression and SaO2 levels to associate with high-altitude pulmonary edema and adaptation. Clin Sci 124: 479-489.

Mitchell DA, Lambert G, Secher NH, Raven PB, van Lieshout J, Esler MD. 2009. Jugular venous overflow of noradrenaline from the brain: a neurochemical indicator of cerebrovascular sympathetic nerve activity in humans. J Physiol 587:2589–2597.

Moore GWK, Semple JL. 2006. Weather and death on Mount Everest: an analysis of The Into Thin Air storm. Bull Amer Meteor Soc 87:465–480.

Moore LG, Harrison GL, McCullough RE, McCullough RG, Micco AJ, Tucker A, Weil JV, Reeves JT. 1986. Low acute hypoxic ventilatory response and hypoxic depression in acute altitude sickness. J Appl Physiol 60:1407–1412.

Moore LG. 2001. Human Genetic Adaptation to High Altitude. High Alt Med Biol 2:257–279.

Moraga FA, Pedreros CP, Rodríguez CE. 2008. Acute Mountain Sickness in Children and Their Parents After Rapid Ascent to 3500 m (Putre, Chile). Wilderness Environ Med 19:287.

Morris DM, Beloni RK, Wheeler HE. 2013. Effects of garlic consumption on physiological variables and performance during exercise in hypoxia. Appl Physiol Nutr Metab 38:363–367.

Muggeridge DJ, Howe CCF, Spendiff O, Pedlar C, James PE, Easton C. 2014. A single dose of beetroot juice enhances cycling performance in simulated altitude. Med Sci Sports Exerc 46:143–150.

Murdoch D. 1995. Altitude illness among tourists flying to 3740 meters elevation in the Nepal Himalayas. J Travel Med 2:255–256.

Muza SR, Kaminsky D, Fulco CS, Banderet LE, Cymerman A. 2004. Cysteinyl leukotriene blockade does not prevent acute mountain sickness. Aviat Space Environ Med 75:413–419.

Muza SR, Beidleman BA, Fulco CS. 2010. Altitude preexposure recommendations for inducing acclimatization. High Alt Med Biol 11:87–92.

Naeije R, Mélot C, Mols P, Hallemans R. 1982. Effects of vasodilators on hypoxic pulmonary vasoconstriction in normal man. Chest 82:404–410.

Naeije R. 2010. Physiological adaptation of the cardiovascular system to high altitude. Progress in Cardiovascular Diseases 52:456–466.

Nakagawa S, Schielzeth H. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev Camb Philos Soc 85:935–956.

Nespoulet H, Wuyam B, Tamisier R, Saunier C, Monneret D, Remy J, Chabre O, Pépin J-L, Levy P. 2012. Altitude illness is related to low hypoxic chemoresponse and low oxygenation during sleep. Eur Respir J 40:673–680.

Nilles E, Sayward H, D'Onofrio G. 2009. Vascular endothelial growth factor and acute mountain sickness. J Emerg Trauma Shock 2:6–9.

Norboo T, Saiyed HN, Angchuk PT, et al. 2004. Mini review of high altitude health problems in Ladakh. Biomed. Pharmacother 58:220–225.

Nussbaumer-Ochsner Y, Schuepfer N, Siebenmann C, Maggiorini M, Bloch KE. 2011. High altitude sleep disturbances monitored by actigraphy and polysomnography. High Alt Med Biol 12:229–236.

Nussbaumer-Ochsner Y, Ursprung J, Siebenmann C, Maggiorini M, Bloch KE. 2012. Effect of short-term acclimatization to high altitude on sleep and nocturnal breathing. Sleep 35:419–423.

Oelz O, Maggiorini M, Ritter M, Waber U, Jenni R, Vock P, Bärtsch P. 1989. Nifedipine for high altitude pulmonary oedema. Lancet 2:1241–1244.

Olin AC, Aldenbratt A, Ekman A, Ljungkvist G, Jungersten L, Alving K, Torén K. 2001. Increased nitric oxide in exhaled air after intake of a nitrate-rich meal. Respi Med 95:153–158.

Ozkan M, Dweik RA. 2001. Nitric oxide and airway reactivity. Clin Pulm Med 8:199–206.

Pagani L, Ayub Q, MacArthur DG, et al. 2012. High altitude adaptation in Daghestani populations from the Caucasus. Hum Genet 131:423–433.

Partie Lange D. 2001. Climbing high, but feeling low. The New York Times [Internet]. Available from: http://www.nytimes.com/2001/02/06/health/06ALTI.html?pagewanted=all

Patel S, Woods DR, Macleod NJ, Brown A, Patel KR, Montgomery HE, Peacock AJ. 2003. Angiotensin-converting enzyme genotype and the ventilatory responseto exertional hypoxia. Eur Respir J 22:755–760.

Pearson TA, Manolio TA. 2008. How to interpret a genome-wide association study. JAMA 299:1335–1344.

Peng Y, Yang Z, Zhang H, et al. 2011. Genetic variations in Tibetan populations and high-altitude adaptation at the Himalayas. Mol Biol Evol 28:1075–1081.

Peoples GE, Gerlinger T, Craig R, Burlingame B. 2005. The 274th Forward Surgical Team experience during Operation Enduring Freedom. Mil Med 170:451–459.

Pepe MS, Janes H, Longton G, Leisenring W, Newcomb P. 2004. Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. Am J Epidemiol 159:882–890.

Pesce C, Leal C, Pinto H, González G, Maggiorini M, Schneider M, Bartsch P. 2005. Determinants of acute mountain sickness and success on Mount Aconcagua (6962 m). High Alt Med Biol 6:158–166.

Pison U, López FA, Heidelmeyer CF, Rossaint R, Falke KJ. 1993. Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction without impairing gas exchange. J Appl Physiol 74:1287–1292.

Powell F. 1998. Time domains of the hypoxic ventilatory response. Respir Physiol 112:123–134.

Puybasset L, Rouby JJ, Mourgeon E, et al. 1994. Inhaled nitric oxide in acute respiratory failure: dose-response curves. Intensive Care Med 20:319–327.

Qi Y, Niu W, Zhu T, Zhou W, Qiu C. 2007. Synergistic effect of the genetic polymorphisms of the renin–angiotensin–aldosterone system on high-altitude pulmonary edema: a study from Qinghai-Tibet altitude. Eur J Epidemiol 23:143–152.

Qi Y, Niu W-Q, Zhu T-C, et al. 2009. Genetic interaction of Hsp70 family genes polymorphisms with high-altitude pulmonary edema among Chinese railway constructors at altitudes exceeding 4000 meters. Clin Chim Acta 405:17–22.

Rajput C, Najib S, Norboo T, Afrin F, Qadar Pasha MA. 2006. Endothelin-1 gene variants and levels associate with adaptation to hypobaric hypoxia in high-altitude natives. Biochem Biophys Res Commun 341:1218–1224.

Reinhart WH, Kayser B, Singh A, Waber U, Oelz O, Bärtsch P. 1991. Blood rheology in acute mountain sickness and high-altitude pulmonary edema. J Appl Physiol 71:934–938.

Rees DD, Palmer RM, Moncada S. 1989. Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc Natl Acad Sci USA 86:3375–3378.

Reeves J, Wagner J, Zafren K, Honigman B. 1994. Seasonal variation in barometric pressure and temperature: effect on altitude illness. In: Sutton J, Houston CS, editors. Hypoxia and Molecular Medicine. Burlington, VT: Queen City Printers. pp. 275–281.

Reitsma JB, Glas AS, Rutjes AWS, Scholten RJPM, Bossuyt PM, Zwinderman AH. 2005. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 58:982–990.

Rexhaj E, Garcin S, Rimoldi SF, Duplain H, Stuber T, Allemann Y, Sartori C, Scherrer U. 2011. Reproducibility of acute mountain sickness in children and adults: a prospective study. Pediatrics 127:e1445–e1448.

Reynafarje C, Lozano R, Valdivieso J. 1959. The polycythemia of high altitudes: iron metabolism and related aspects. Blood 14:433–455.

Richalet JP, Keromes A, Dersch B, et al. 1988. Caractéristiques physiologiques des alpinistes de haute altitude. Science & Sports 3:89–108.

Richalet J-P, Donoso MV, Jiménez D, Antezana A-M, Hudson C, Cortès G, Osorio J, Leòn A. 2002. Chilean miners commuting from sea level to 4500 m: a prospective study. High Alt Med Biol 3:159–166.

Richalet J-P, Gratadour P, Robach P, Pham I, Déchaux M, Joncquiert-Latarjet A, Mollard P, Brugniaux J, Cornolo J. 2005. Sildenafil inhibits altitude-induced hypoxemia and pulmonary hypertension. Am J Respir Crit Care 171:275–281.

Richalet J-P, Gimenez-Roqueplo A-P, Peyrard S, et al. 2009. A role for succinate dehydrogenase genes in low chemoresponsiveness to hypoxia? Clin Auton Res 19:335–342.

Richalet J-P, Larmignat P, Poitrine E, Letournel M, Canouï-Poitrine F. 2012. Physiological risk factors for severe high-altitude illness: a prospective cohort study. Am J Respir Crit Care 185:192–198.

Richard NA, Koehle MS. 2012. Differences in cardio-ventilatory responses to hypobaric and

normobaric hypoxia: a review. Aviat Space Environ Med 83:677–684.

Richard NA, Sahota IS, Widmer N, Ferguson S, Sheel AW, Koehle MS. 2014. Acute mountain sickness, chemosensitivity, and cardiorespiratory responses in humans exposed to hypobaric and normobaric hypoxia. J Appl Physiol 116:945–952.

Roach RC, Bärtsch P, Oelz O, Hackett P. 1993. Lake Louise AMS Scoring Consensus Committee: The Lake Louise acute mountain sickness scoring system. In: Sutton JR, Houston C, Coates G, editors. Hypoxia and molecular medicine. Burlington, VT: Queen City Printers. pp. 272–274.

Roach RC, Houston CS, Honigman B, Nicholas RA, Yaron M, Grissom CK, Alexander JK, Hultgren HN. 1995. How well do older persons tolerate moderate altitude? West. J. Med. 162:32–36.

Roach RC, Loeppky JA, Icenogle MV. 1996. Acute mountain sickness: increased severity during simulated altitude compared with normobaric hypoxia. J Appl Physiol 81:1908–1910.

Roach RC, Maes D, Sandoval D, Robergs RA, Icenogle M, Hinghofer-Szalkay H, Lium D, Loeppky JA. 2000. Exercise exacerbates acute mountain sickness at simulated high altitude. J Appl Physiol 88:581–585.

Roach R, Kayser B, Hackett P. 2011. Pro: Headache should be a required symptom for the diagnosis of acute mountain sickness. High Alt Med Biol 12:21–22.

Robinson SM, King AB, Aoki V. 1971. Acute mountain sickness: reproducibility of its severity and duration in an individual. Aerosp Med 42:706–708.

Rodway GW, Muza SR. 2011. Fighting in thin air: operational wilderness medicine in high Asia. Wilderness Environ Med 22:297–303.

Roscoe C, Baker E, Johnston E, Sandven T, Gustafson C, Arndt T, Dow J. 2008. Carbon Monoxide Exposure on Denali: Comparing the 2004 and 2005 Climbing Seasons. Wilderness Environ Med 19:15–21.

Ross RT. 1985. The random nature of cerebral mountain sickness. Lancet 1:990–991.

Rupert JL, Hochachka PW. 2001. The evidence for hereditary factors contributing to high altitude adaptation in Andean natives: a review. High Alt Med Biol 2:235–256.

Rupert JL, Koehle MS. 2006. Evidence for a genetic basis for altitude-related illness. High Alt Med Biol 7:150–167.

Saftlas AF, Waldschmidt M, Logsden-Sackett N, Triche E, Field E. 2004. Optimizing buccal cell DNA yields in mothers and infants for human leukocyte antigen genotyping. American J Epidemiol 160:77–84.

Salam MT, Bastain TM, Rappaport EB, Islam T, Berhane K, Gauderman WJ, Gilliland FD.

2010. Genetic variations in nitric oxide synthase and arginase influence exhaled nitric oxide levels in children. Allergy 66:412–419.

Sampson JB, Cymerman A, Burse RL, Maher JT, Rock PB. 1983. Procedures for the measurement of acute mountain sickness. Aviat Space Environ Med 54:1063–1073.

Sánchez Crespo A, Hallberg J, Lundberg JO, Lindahl SGE, Jacobsson H, Weitzberg E, Nyrén S. 2010. Nasal nitric oxide and regulation of human pulmonary blood flow in the upright position. J Appl Physiol 108:181–188.

Sanchez del Rio M, Moskowitz MA. 1999. High altitude headache. Lessons from headaches at sea level. Adv Exp Med Biol 474:145–153.

Sarkar S, Banerjee PK, Selvamurthy W. 2003. High altitude hypoxia: an intricate interplay of oxygen responsive macroevents and micromolecules. Mol Cell Biochem 253:287–305.

Sartori C, Vollenweider L, Loffler BM, Delabays A, Nicod P, Bärtsch P, Scherrer U. 1999. Exaggerated endothelin release in high-altitude pulmonary edema. Circulation 99:2665–2668.

Sartori C, Allemann Y, Trueb L, Lepori M, Maggiorini M, Nicod P, Scherrer U. 2000. Exaggerated pulmonary hypertension is not sufficient to trigger high-altitude pulmonary oedema in humans. Schweiz Med Wochenschr 130:385–389.

Sartori C, Allemann Y, Duplain H, et al. 2002. Salmeterol for the prevention of high-altitude pulmonary edema. N Engl J Med 346:1631–1636.

Sartori C, Duplain H, Lepori M, Egli M, Maggiorini M, Nicod P, Scherrer U. 2004. High altitude impairs nasal transepithelial sodium transport in HAPE-prone subjects. Eur Respir J 23:916–920.

Sawka MN, Young AJ, Rock PB, et al. 1996. Altitude acclimatization and blood volume: effects of exogenous erythrocyte volume expansion. J Appl Physiol 81:636–642.

Sawka MN, Convertino VA, Eichner ER, Schnieder SM, Young AJ. 2000. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. Med Sci Sports Exerc 32:332–348.

Saxena S, Kumar R, Madan T, Gupta V, Muralidhar K, Sarma PU. 2005. Association of polymorphisms in pulmonary surfactant protein A1 and A2 genes with high-altitude pulmonary edema. Chest 128:1611–1619.

Scherrer U, Vollenweider L, Delabays A, et al. 1996. Inhaled nitric oxide for high-altitude pulmonary edema. N Engl J Med 334:624–629.

Schmetterer L, Strenn K, Kastner J, Eichler HG, Wolzt M. 1997. Exhaled NO during graded changes in inhaled oxygen in man. Thorax 52:736–738.

Schneider JC, Blazy I, Déchaux M, Rabier D, Mason NP, Richalet JP. 2001. Response of nitric oxide pathway to L-arginine infusion at the altitude of 4,350 m. Eur Respir J 18:286–292.

Schneider M, Bernasch D, Weymann J, Holle R, Bartsch P. 2002. Acute mountain sickness: influence of susceptibility, preexposure, and ascent rate. Med Sci Sports Exerc 34:1886–1891.

Schoene RB. 2007. Illnesses at High Altitude. Chest 134:402–416.

Schoonman GG, Sándor PS, Nirkko AC, et al. 2008. Hypoxia-induced acute mountain sickness is associated with intracellular cerebral edema: a 3 T magnetic resonance imaging study. J Cereb Blood Flow Metab 28:198–206.

Schreiber JB, Nora A, Stage FK, Barlow EA, King J. 2006. Reporting structural equation modeling and confirmatory factor analysis results: A review. J Educ Res 99:323–338.

Scoggin CH, Hyers TM, Reeves JT, Grover RF. 1977. High-altitude pulmonary edema in the children and young adults of Leadville, Colorado. N Engl J Med 297:1269–1272.

Self DA, Mandella JG, Prinzo OV, Forster EM, Shaffstall RM. 2011. Physiological equivalence of normobaric and hypobaric exposures of humans to 25,000 feet (7620 m). Aviat Space Environ Med 82:97–103.

Severinghaus JW, Chiodi H, Eger EI, Brandstater B, Hornbein TF. 1966. Cerebral blood flow in man at high altitude. Role of cerebrospinal fluid pH in normalization of flow in chronic hypocapnia. Circ Res 19:274–282.

Severinghaus JW. 1979. Simple, accurate equations for human blood O2 dissociation computations. J Appl Physiol Respir Environ Exerc Physiol 46:599–602.

Sheel AW, MacNutt MJ, Querido JS. 2010. The pulmonary system during exercise in hypoxia and the cold. Exp Physiol 95:422–430.

Shi G, Boerwinkle E, Morrison AC, Gu CC, Chakravarti A, Rao DC. 2010. Mining gold dust under the genome wide significance level: a two-stage approach to analysis of GWAS. Genet. Epidemiol. 35:111–118.

Simonson TS, Yang Y, Huff CD, et al. 2010. Genetic evidence for high-altitude adaptation in Tibet. Science 329:72–75.

Singh B, Cable GG, Hampson GV, Pascoe GD, Corbett M, Smith A. 2010. Hypoxia awareness training for aircrew: a comparison of two techniques. Aviat Space Environ Med 81:857–863.

Skol AD, Scott LJ, Abecasis GR, Boehnke M. 2006. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 38:209–213.

Srivastava S, Bhagi S, Kumari B, Chandra K, Sarkar S, Ashraf MZ. 2012. Association of

polymorphisms in angiotensin and aldosterone synthase genes of the renin-angiotensin-aldosterone system with high-altitude pulmonary edema. J Renin-Angio-Aldo S 13:155–160.

Staab JE, Beidleman BA, Muza SR, Fulco CS, Rock PB, Cymerman A. 2013. Efficacy of residence at moderate versus low altitude on reducing acute mountain sickness in men following rapid ascent to 4300 m. High Alt Med Biol 14:13–18.

Stamler JS, Loh E, Roddy MA, Currie KE, Creager MA. 1994. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. Circulation 89:2035–2040.

Stanforth PR, Gagnon J, Rice T, Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH. 2000. Reproducibility of resting blood pressure and heart rate measurements. The HERITAGE Family Study. Ann Epidemiol 10:271–277.

Stäubli M, Rohner F, Kammer P, Ziegler W, Straub PW. 1986. Plasma volume and proteins in voluntary hyperventilation. J Appl Physiol 60:1549–1553.

St Croix CM, Wetter TJ, Pegelow DF, Meyer KC, Dempsey JA. 1999. Assessment of nitric oxide formation during exercise. Am J Respir Crit Care 159:1125–1133.

Stobdan T, Ali Z, Khan AP, et al. 2011. Polymorphisms of renin-angiotensin system genes as a risk factor for high-altitude pulmonary oedema. . J Renin-Angio-Aldo S 12:93–101.

Stobdan T, Kumar R, Mohammad G, Thinlas T, Norboo T, Iqbal M, Pasha MAQ. 2010. Probable role of beta2-adrenergic receptor gene haplotype in high-altitude pulmonary oedema. Respirology 15:651–658.

Storm van's Gravesande K, Wechsler ME, Grasemann H, Silverman ES, Le L, Palmer LJ, Drazen JM. 2003. Association of a missense mutation in the NOS3 gene with exhaled nitric oxide levels. Am J Respir Crit Care 168:228–231.

Su AI, Wiltshire T, Batalov S, et al. 2004. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci USA 101:6062–6067.

Sutherland AI, Morris DS, Owen CG, Bron AJ, Roach RC. 2008. Optic nerve sheath diameter, intracranial pressure and acute mountain sickness on Mount Everest: a longitudinal cohort study. Br J Sports Med 42:183–188.

Swenson ER, Duncan TB, Goldberg SV, Ramirez G, Ahmad S, Schoene RB. 1995. Diuretic effect of acute hypoxia in humans: relationship to hypoxic ventilatory responsiveness and renal hormones. J Appl Physiol 78:377–383.

Swenson ER, MacDonald A, Vatheuer M, Maks C, Treadwell A, Allen R, Schoene RB. 1997. Acute mountain sickness is not altered by a high carbohydrate diet nor associated with elevated circulating cytokines. Aviat Space Environ Med 68:499–503.

Swenson ER, Maggiorini M, Mongovin S, Gibbs JSR, Greve I, Mairbäurl H, Bartsch P. 2002. Pathogenesis of high-altitude pulmonary edema: inflammation is not an etiologic factor.

JAMA 287:2228–2235.

Tanner JB, Tanner SME, Thapa GB, Chang Y, Watson KLM, Staunton E, Howarth C, Basnyat B, Harris NS. 2013. A randomized trial of temazepam versus acetazolamide in high altitude sleep disturbance. High Alt Med Biol 14:234–239.

Taylor DR, Pijnenburg MW, Smith AD, Jongste JCD. 2006. Exhaled nitric oxide measurements: clinical application and interpretation. Thorax 61:817–827.

Teo YY. 2009. Exploratory data analysis in large-scale genetic studies. Biostatistics 11:70–81.

Thomas DA, Swaminathan S, Beardsmore CS, McArdle EK, MacFadyen UM, Goodenough PC, Carpenter R, Simpson H. 1993. Comparison of peripheral chemoreceptor responses in monozygotic and dizygotic twin infants. Am Rev Respir Dis 148:1605–1609.

Thornton D, Martin TPC, Amin P, Haque S, Wilson S, Smith MCF. 2011. Chronic suppurative otitis media in Nepal: ethnicity does not determine whether disease is associated with cholesteatoma or not. J Laryngol Otol 125:22–26.

Treacher DF, Leach RM. 1998. Oxygen transport-1. Basic principles. BMJ 317:1302–1306.

Treggiari MM, Romand J-A, Martin J-B, Reverdin A, Rüfenacht DA, de Tribolet N. 2003. Cervical sympathetic block to reverse delayed ischemic neurological deficits after aneurysmal subarachnoid hemorrhage. Stroke 34:961–967.

Tsianos G, Eleftheriou KI, Hawe E, Woolrich L, Watt M, Watt I, Peacock A, Montgomery H, Grant S. 2005. Performance at altitude and angiotensin I-converting enzyme genotype. Eur J Appl Physiol 93:630–633.

Tsuchiya M, Tokai H, Takehara Y, Haraguchi Y, Asada A, Utsumi K, Inoue M. 2000. Interrelation between oxygen tension and nitric oxide in the respiratory system. Am J Respir Crit Care 162:1257–1261.

Tsujino I, Miyamoto K, Nishimura M, Shinano H, Makita H, Saito S, Nakano T, Kawakami Y. 1996. Production of nitric oxide (NO) in intrathoracic airways of normal humans. Am J Respir Crit Care 154:1370–1374.

van Patot MCT, Leadbetter G, Keyes LE, Maakestad KM, Olson S, Hackett PH. 2008. Prophylactic low-dose acetazolamide reduces the incidence and severity of acute mountain sickness. High Alt Med Biol 9:289–293.

Vera Calzaretta A, Sepúlveda Jara R, Contreras Tudela G. 2006. Auto-reporte de síntomas físicos y correlatos psicosocialesen trabajadores de la minería. Cienc Trab 8:74–78.

Verges S, Flore P, Favre-Juvin A, Lévy P, Wuyam B. 2005. Exhaled nitric oxide during normoxic and hypoxic exercise in endurance athletes. Acta Physiol Scand 185:123–131.

Vinnikov D, Brimkulov N, Redding-Jones R, Jumabaeva K. 2011. Exhaled nitric oxide is

reduced upon chronic intermittent hypoxia exposure in well-acclimatized mine workers. Respir Physiol Neurobiol 175:261–264.

Vogel JA, Hartley LH, Cruz JC. 1974. Cardiac output during exercise in altitude natives at sea level and high altitude. J Appl Physiol 36:173–176.

Wagner DR, D'zatko K, Tatsugawa K, Murray K, Parker D, Streeper T, Willard K. 2008. Mt. Whitney: determinants of summit success and acute mountain sickness. Med Sci Sports Exerc 40:1820–1827.

Wagner PD. 2010. The physiological basis of reduced VO2max in Operation Everest II. High Alt Med Biol 11:209–215.

Wagner DR, Knott JR, Fry JP. 2012a. Oximetry fails to predict acute mountain sickness or summit success during a rapid ascent to 5640 meters. Wilderness Environ Med 23:114–121.

Wagner DR, Teramoto M, Knott JR, Fry JP. 2012b. Comparison of scoring systems for assessment of acute mountain sickness. High Alt Med Biol 13:245–251.

Walter R, Maggiorini M, Scherrer U, Contesse J, Reinhart WH. 2001. Effects of high-altitude exposure on vascular endothelial growth factor levels in man. Eur J Appl Physiol 85:113–117.

Wang P, Koehle MS, Rupert JL. 2007. Common Haplotypes in the β-2 Adrenergic Receptor Gene Are Not Associated with Acute Mountain Sickness Susceptibility in Nepalese. High Alt Med Biol 8:206–212.

Wang P, Koehle MS, Rupert JL. 2009. Genotype at the missense G894T polymorphism (Glu298Asp) in the NOS3 gene is associated with susceptibility to acute mountain sickness. High Alt Med Biol 10:261–267.

Wang P, Ha Ayn, Kidd KK, Koehle MS, Rupert JL. 2010a. A variant of the endothelial nitric oxide synthase gene (NOS3) associated with AMS susceptibility is less common in the Quechua, a high altitude Native population. High Alt Med Biol 11:27–30.

Wang P, Koehle MS, Rupert JL. 2010b. No association between alleles of the bradykinin receptor-B2 gene and acute mountain sickness. Exp Biol Med 235:737–740.

Wang Q-Q, Yu L, Huang G-R, et al. 2013. Polymorphisms of angiotensin converting enzyme and nitric oxide synthase 3 genes as risk factors of high-altitude pulmonary edema: a case-control study and meta-analysis. Tohoku J Exp Med 229:255–266.

Wang S-H, Chen Y-C, Kao W-F, Lin Y-J, Chen J-C, Chiu T-F, Hsu T-Y, Chen H-C, Liu S-W. 2010. Epidemiology of acute mountain sickness on Jade Mountain, Taiwan: an annual prospective observational study. High Alt Med Biol 11:43–49.

Weir EK, López-Barneo J, Buckler KJ, Archer SL. 2005. Acute oxygen-sensing mechanisms. N Engl J Med 353:2042–2055.

Weiss J, Haefeli WE, Gasse C, Hoffmann MM, Weyman J, Gibbs S, Mansmann U, Bartsch P. 2003. Lack of evidence for association of high altitude pulmonary edema and polymorphisms of the NO pathway. High Alt Med Biol 4:355–366.

West JB, Lahiri S, Maret KH, Peters RM, Pizzo CJ. 1983. Barometric pressures at extreme altitudes on Mt. Everest: physiological significance. J Appl Physiol 54:1188–1194.

West JB. 1996. Prediction of barometric pressures at high altitude with the use of model atmospheres. J Appl Physiol 81:1850–1854.

West JB. 1999. Barometric pressures on Mt. Everest: new data and physiological significance. J Appl Physiol 86:1062–1066.

West JB. 2002. Highest permanent human habitation. High Alt Med Biol 3:401–407.

West JB. 2009. Who should not go high? High Alt Med Biol 10:1–2.

West JB. 2012. High-Altitude Medicine. Am J Respir Crit Care 186:1229–1237.

Westerterp KR. 2001. Energy and water balance at high altitude. News Physiol Sci 16:134–137.

Whiting PF, Rutjes AWS, Westwood ME, et al. 2011. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 155:529–536.

Wilson MH, Newman S, Imray CH. 2009. The cerebral effects of ascent to high altitudes. The Lancet Neurol 8:175–191.

Wilson MH, Davagnanam I, Holland G, et al. 2013. Cerebral venous system and anatomical predisposition to high-altitude headache. Ann Neurol 73:381–389.

Windsor JS. 2012. Pulse oximetry and predicting acute mountain sickness: are we asking the right questions? Wilderness Environ Med 23:112–113.

Wolfel EE, Selland MA, Mazzeo RS, Reeves JT. 1994. Systemic hypertension at 4,300 m is related to sympathoadrenal activity. J Appl Physiol 76:1643–1650.

Woods DR, Pollard AJ, Collier DJ, Jamshidi Y, Vassiliou V, Hawe E, Humphries SE, Montgomery HE. 2002. Insertion/deletion polymorphism of the angiotensin I-converting enzyme gene and arterial oxygen saturation at high altitude. Am J Respir Crit Care 166:362–366.

Wu T, Li S, Ward MP. 2005. Tibetans at extreme altitude. Wilderness Environ Med 16:47–54.

Wu TY, Ding SQ, Liu JL, et al. 2009. Reduced incidence and severity of acute mountain sickness in Qinghai-Tibet railroad construction workers after repeated 7-month exposures despite 5-month low altitude periods. High Alt Med Biol 10:221–232.

Wu TY, Ding SQ, Zhang SL, et al. 2010. Altitude illness in Qinghai–Tibet railroad passengers. High Alt Med Biol 11:189–198.

Wu TY, Ding SQ, Liu JL, Jia JH, Chai Z-C, Dai RC. 2012a. Who are more at risk for acute mountain sickness: a prospective study in Qinghai-Tibet railroad construction workers on Mt. Tanggula. Chin Med J 125:1393–1400.

Wu TY, Ding SQ, Liu JL, Jia JH, Chai ZC, Dai RC, Zhao JZ, Tang QD, Kayser B. 2012b. Smoking, acute mountain sickness and altitude acclimatisation: a cohort study. Thorax 67:914–919.

Xu S, Li S, Yang Y, et al. 2011. A genome-wide search for signals of high-altitude adaptation in Tibetans. Mol Biol Evol 28:1003–1011.

Yaron M, Niermeyer S, Lindgren KN, Honigman B. 2002. Evaluation of diagnostic criteria and incidence of acute mountain sickness in preverbal children. Wilderness Environ Med 13:21–26.

Yaron M, Waldman N, Niermeyer S, Nicholas R, Honigman B. 1998. The diagnosis of acute mountain sickness in preverbal children. Arch Pediatr Adolesc Med 152:683–687.

Yi X, Liang Y, Huerta-Sanchez E, et al. 2010. Sequencing of 50 human exomes reveals adaptation to high altitude. Science 329:75–78.

You H, Li X, Pei T, Huang Q, Liu F, Gao Y. 2012. Predictive value of basal exhaled nitric oxide and carbon monoxide for acute mountain sickness. Wilderness Environ Med 23:316–324.

Young AJ, Sawka MN, Muza SR, et al. 1996. Effects of erythrocyte infusion on VO2max at high altitude. J Appl Physiol 81:252–259.

Zheng B-H, Li S-Z, He Y, Wang H-B, Li S-S. 2007. [The effect of inhaled nitric oxide on endothelium-derived angiokinetic factors in patients with acute high altitude disease]. Zhonghua Jie He He Hu Xi Za Zhi 30:127–129.

Zhou F, Wang F, Li F, Yuan J, Zeng H, Wei Q, Tanguay RM, Wu T. 2005. Association of hsp70-2 and hsp-hom gene polymorphisms with risk of acute high-altitude illness in a Chinese population. Cell Stress Chaperones 10:349–356.

Ziaee V, Yunesian M, Ahmadinejad Z, Halabchi F, Kordi R, Alizadeh R, Afsharjoo HR. 2003. Acute mountain sickness in Iranian trekkers around Mount Damavand (5671 m) in Iran. Wilderness Environ Med 14:214–219.

Ziegler A, König I, Pahlke F. 2010. A Statistical Approach to Genetic Epidemiology: Concepts and Applications. 2nd ed. Hoboken, NJ: Wiley-Blackwell

Zumbo, D B, Gadermann, M A, Zeisser, C. 2007. Ordinal versions of coefficients alpha and theta for likert rating scales. Journal of Modern Applied Statistical Methods 6:21–29.

Appendices

Appendix A Brief introduction to high-altitude pulmonary edema and high-altitude cerebral edema

A.1 High-altitude pulmonary edema

High-altitude pulmonary edema (HAPE) is an acute altitude illness that primarily affects the lungs (at least initially) after ascent to an altitude above 3000 m (Schoene 2007). Developing after 1-2 days, often (but not necessarily) following the development of AMS, HAPE is characterized by breathlessness, coughing (dry cough progressing to productive cough), and in severe cases, the production of pink frothy sputum (West 2012). Additional signs of HAPE include pulmonary rales, cyanosis, tachycardia, pyrexia, and radiographic evidence of pulmonary edema (West 2012).

Similar to AMS, the incidence of HAPE is dependent on the rate of ascent, altitude attained, individual susceptibility, and exertion (Hackett and Roach 2001); however, cold exposure also increases the likelihood of HAPE (Reeves et al. 1994). Aside from a previous history, individual risk factors include pulmonary hypertension, pulmonary circulation abnormalities, and an exaggerated rise in PAP in response to hypoxia (Imray et al. 2011). Estimates for the incidence of HAPE range between 0.2% (4559 m) to 15% (3500 m; Schoene 2007). For those with a history of HAPE, the recurrence risk has been estimated as ~60% following a rapid ascent to an altitude of 4559 m (Bartsch et al. 2002). See Table A.1 for a list of recurrence rates across multiple studies.

The mechanism of HAPE is better understood than that of AMS. West (2012) has provided a simple diagram of the hypothesized pathophysiological process in a recent review. In this model, the hypoxia of high altitude causes vasoconstriction in the lung that seems to be uneven, causing overperfusion and increased pressure in the capillaries that are unprotected (*i.e.,* not constricted). The resulting stress failure alters the blood-gas barrier, causing blood to leak into the alveolar spaces. That the edema of HAPE is due to increased permeability of the alveoli is consistent with the accumulation of high-molecular-weight proteins in alveolar lavage samples (Swenson et al. 2002). The accumulation of fluid in the lungs further potentiates arterial hypoxemia (Scherrer et al. 1996).

The Wilderness Medical Society guidelines (Luks et al. 2010) suggest that the first step to preventing HAPE is a moderate ascent rate (the same as described for AMS). Pharmaceutical

prophylaxis is only recommended for those with a history of HAPE, and nifedipine is the preferred prophylactic medication (Bartsch et al. 1991). Direct evidence to support a preventative role for acetazolamide is not available, but because acetazolamide expedites acclimatisation, it theoretically would reduce the risk of HAPE (Luks et al. 2010).

If HAPE goes untreated, it is likely to be lethal (Hackett and Roach 2001). A rapid descent is the preferred treatment, but if descent is not possible, supplemental oxygen or artificially increased pressure (*e.g.,* a Gamow bag) can be effective (Luks et al. 2010). Nifedipine can be used if the above strategies are not possible (Oelz et al. 1989).

Many studies have taken HAPE-susceptible subjects to high altitude to investigate factors related to the etiology of the condition (Table A.1), and many of these studies have taken place at the Capanna Margherita (4559 m). The susceptibility of subjects was typically based on the presence of one or more radiographically confirmed HAPE episodes (often limited to the previous 4-5 years), although the recollection of the clinical signs of HAPE was considered sufficient for a positive history of HAPE in a few studies. The recurrence of HAPE was generally confirmed with a chest radiograph, but again, the physical signs of HAPE were considered sufficient evidence for a positive HAPE diagnosis in some studies. There is no exhaustive summary of these studies available in the literature, although in a brief letter to the editor of the Lancet, Bartsch et al. (2002) summarized the data available from studies occurring before 2000: across multiple trips to the Capanna Margherita, 42 of 66 HAPE-susceptible individuals (64%) developed HAPE, but only seven of 122 control subjects (6%) developed HAPE. From these data, the odds ratio for the development of HAPE in those individuals with a previous history of HAPE was 28.75 (CI = 11.54 – 71.64), and the risk ratio for a HAPE episode with a previous history of HAPE was 11.1 (CI = 5.3 – 23.3). The sensitivity and specificity of a previous HAPE episode were 86% and 83%, respectively; the positive and negative predictive values of a previous HAPE episode were 64% and 94%, respectively. In other words, individuals with a previous HAPE episode are much more likely to develop HAPE than individuals without a previous history of HAPE, and having a negative history for HAPE is a good indication of resistance to HAPE. In contrast, the rate of HAPE recurrence was moderate (64%).

The many studies exposing HAPE-susceptible individuals to high-altitude, including those summarized by Bartsch et al. (2002), are described in Table A.1. A total of 303 HAPE-susceptible individuals were exposed to high-altitude (23 of 24 studies were conducted at 4559

m), and HAPE recurred in 173 of these individuals. It is difficult to determine if the same subjects repeatedly volunteered for studies at the Capanna Margherita, or if some studies overlapped (i.e., reported different data from the same subjects on the same ascents). These limitations in analyzing published data prevent an extremely accurate estimate of the recurrence risk of HAPE.

It is important to note that HAPE may take several days to present, and studies are limited by their durations. The incidence of HAPE in the HAPE-susceptible group may have been even higher if subjects stayed at altitude for longer durations (the duration of time spent at 4559 m varied between 18 hours and 3 days), but the same is possibly true for the HAPE-resistant group.

A number of studies have investigated whether or not susceptibility to HAPE has a genetic basis. Unlike AMS (Chapters 4 and 7), there is strong evidence that HAPE is repeatable (Bartsch et al. 2002; Dehnert et al. 2002; Table A.1), which supports the possibility that variation in susceptibility could be a result of variation in genetics. Several small studies have suggested that HAPE aggregates in families; however, sample sizes are small (Table 1.3). Those studies that have investigated individual genes for association with HAPE are described in Table A.2. While not included in this table, one large association study of 400 microsatellites distributed throughout the genome has been performed with HAPE-resistant and HAPE-susceptible individuals. This study reported that several regions of the genome were associated with the variation in HAPE susceptibility. The study had relatively low resolution (~1 marker per 10 million base pairs of the genome), which meant that 100 kb regions surrounding the putatively associated markers had to be scanned for genes that had a plausible association. Thus, this technique did not completely eliminate the need for *a priori* hypotheses. Upon inspection of these regions, the authors noted that *TIMP3* was the most likely identified gene to be associated with HAPE susceptibility. To substantiate this finding, six polymorphisms in the *TIMP3* gene were genotyped. The rs130293 SNP was associated with variation in the *TIMP3* gene; however, this association has not been confirmed in an independent sample.

Table A.1 Methodological characteristics for studies measuring the recurrence rate of high-altitude pulmonary edema (HAPE).

Authors	HAPE-s (n)	Recurrence (n (%))	Altitude (m)	Ascent rate	Time at altitude before assessment	Previous Dx	Current Dx
Maggiorini et al. 2001	16	9 (56%)	4559	<24 h 1715 m/d	12-36 h	≥ 1 case of HAPE*	Chest radiograph; clinical symptoms
Scherrer et al. 1996	18	10 (56%)	4559	2 d 1715 m/d	18-36 h	≥ 1 radiographically confirmed case in the previous 4 years	Chest radiograph; clinical symptoms
Sartori et al. 2000	14	8 (57%)	4559	2 d 1715 m/d	18-24 h	≥ 1 radiographically confirmed case in the previous 4 years	Chest radiograph; clinical symptoms
Sartori et al. 2002	19	14 (74%)	4559	2 d (<22 h) 1715 m/d	≤ 2 d	≥ 1 radiographically confirmed case in the previous 4 years	Chest radiograph; clinical symptoms
Bartsch et al. 1991	11	7 (64%)	4559	2 d (<22 h) 1715 m/d	≤ 4 d	≥ 1 radiographically confirmed case	Chest radiograph; clinical symptoms
Hultgren et al. 1971	5	0 (0%)	3100 (3900)#	8 h (3100m/d)	24 h	History and physical signs observed by physician	Chest radiograph; clinical symptoms
Duplain et al. 2000	28	13 (46%)	4559	2 d (<22 h) 1715 m/d	≤ 2 d	≥ 1 radiographically confirmed case in the previous 4 years	Chest radiograph; clinical symptoms
Mairbaurl et al. 2003	10	10 (100%)	4559	2 d 1730 m/d	18 h	≥ 1 case of HAPE*	Chest radiograph; clinical symptoms
Sartori et al. 2004	21	13 (62%)	4559	2 d (<24 h) 1715 m/d	≤ 2 d	≥ 1 radiographically confirmed case in the previous 5 years	Chest radiograph; clinical symptoms
Maggiorini et al. 2006	9	7 (78%)	4559	2 d (<24 h) 1715 m/d	≤ 2 d	≥ 1 case of HAPE*	Chest radiograph; clinical symptoms
Duplain et al. 1999	6	4 (67%)	4559	2 d (<24 h) 1715 m/d	≤ 2 d	≥ 1 radiographically confirmed case in the previous 4 years	Chest radiograph; clinical symptoms

Authors	HAPE-s (n)	Recurrence (n (%))	Altitude (m)	Ascent rate	Time at altitude before assessment	Previous Dx	Current Dx
Allemann et al. 2000	16	8 (50%)	4559	2 d 1715 m/d	≤ 2 d	≥ 1 radiographically confirmed case	Chest radiograph; clinical symptoms
Siebenmann et al 2011	14	0 (0%)	4559	2 d 1677 m/d	≤ 3 d	≥ 1 radiographically or clinically confirmed case	Chest radiograph; clinical symptoms
Fischler et al. 2009	8	7 (87.5)	4559	2 d 1730 m/d	≤ 2 d	≥ 1 radiographically or clinically confirmed case	Chest radiograph; clinical symptoms
Kleger et al. 1996	8	4 (50%)	4559	2 d	~40 h	≥ 1 documented case	Chest radiograph; clinical symptoms
Clarenbach et al. 2012	8	8 (100%)	4559	2 d 1715 m/d	≤ 3d	≥ 1 radiographical case	Chest radiograph; clinical symptoms
Dehnert et al. 2010	6	4 (67%)	4559	2 d 1730 m/d	~44 h	≥ 1 HAPE case	Chest radiograph; clinical symptoms
Kaufmann et al. 2008	6	5 (83%)	4559	2 d 1715 m/d	?	≥ 1 HAPE case	Chest radiograph; clinical symptoms
Oelz et al. 1989; Reinhart et al. 1991; Eichenberger et al. 1996	12	8 (67%)	4559	2 d 1694 m/d	3 d	≥ 1 radiographically or clinically confirmed case	Chest radiograph; clinical symptoms
Allemann et al. 2004	18	9 (50%)	4559	2 d 1715 m/d	2 d	≥ 1 radiographically or clinically confirmed case	Chest radiograph; clinical symptoms
Swenson et al. 2002	10	9 (90%)	4559	2 d	2 d	≥ 1 HAPE case	Chest radiograph; clinical symptoms
Sartori et al. 1999	16	8 (50%)	4559	2 d 1715 m/d	18–36 h	≥ 1 radiographically confirmed case in the previous 4 years	Chest radiograph; clinical symptoms
Allemann et al. 2006	16	8 (50%)	4559	2 d (<24 h) 1715 m/d	2 d	≥ 1 radiographically confirmed case in the previous 4 years	Chest radiograph; clinical symptoms

Authors	HAPE-s (n)	Recurrence (n (%))	Altitude (m)	Ascent rate	Time at altitude before assessment	Previous Dx	Current Dx
Walter et al. 2001	10	6 (60%)	4559	18-24 h	2 d	Radiographic evidence (n=7)	Chest radiograph

*Method of assessment not described
#Climbed to 3900 m and descended to 3100 m for evaluation.

Table A.2 Summary of candidate gene association studies in high altitude pulmonary edema (HAPE).

Gene	Polymorphism[a]	Population (C:P)[b]	Results	Reference
ACE	Alu insertion/deletion; intron 16 (rs4340)	European (102:50)	no association	Dehnert et al. 2002
ACE	Alu insertion/deletion; intron 16 (rs4340)	Indian (20:19)	no association	Kumar et al. 2004
ACE	Alu insertion/deletion; intron 16 (rs4340)	Japanese (55:49)	no association	Hotta et al. 2004
ACE	Alu insertion/deletion; intron 16 (rs4340)	"Same ethnicity" (53:64)	I/D+D/D genotypes	Rajput et al. 2006
ACE	A(-240)T; promoter (rs4291)	Han Chinese (144:140)	A allele; A/A genotype	Qi et al. 2007
	A(2350)G; silent; exon 17 (rs4343)		no association	
ACE	Alu insertion/deletion; intron 16 (rs4340)	Indian (160:163)	D allele; I/D+D/D genotypes	Stobdan et al. 2011
ACE	Alu insertion/deletion; intron 16 (rs4340)	27:108	no association	Wang et al. 2013
	A(-240)T; promoter (rs4291)		no association	
	A(2350)G; silent; exon 17 (rs4343)		no association	
	CG; intron (rs8066114)		G allele; G/G and C/G genotypes	
	GA; (rs4363)		no association	
	(rs4461142) [c]		no association	
ACE2	A(1075)G, intron 1	Han Chinese (144:140)	no associations	Qi et al. 2007
	G(8790)A, intron 3			
ADRB2	A(-1023)G; promoter (rs2053044)	Indian (143:110)	Dominant model associated	Stobdan et al. 2010
	A(-654)G; promoter (rs12654778)		H10 haplotype (0.002; 0 (na))	
	C(-367)T; promoter (rs11959427)		S6 haplotype (0.006; 0.5 (0.3-0.8))	
	A(46)G; arg16gly; exonic (rs1042713)			
	C(79)G; gln27glu; exonic (rs1042714)			
	C(491)T; thr164ile; exonic (rs1800888)			
	C(523)A; silent; exonic (rs1042718)			
	A(1239)G; silent; exonic (rs1042720)			
AGT	T(704)C; met235thr, exon 2 (rs699)	Han Chinese (144:140)	no associations	Qi et al. 2007
	C(521)T; thr174met, exon 2 (rs4762)			
AGT	A(-6)G; 5'UTR(rs5051)	Indian (160:163)	G/A genotype	Stobdan et al. 2011
	C(521)T; thr174met, exon 2 (rs4762)		no association	
	T(704)C; met235thr, exon 2 (rs699)		M allele; M/T and M/M genotypes	
AGT	C(521)T; thr174met, exon 2 (rs4762)	Indian (80:48)	no associations	Srivastava et al. 2012
	glu53stop			
AGTR1	A(1166)C; 3' UTR (rs5186)	Japanese (55:49)	no association	Hotta et al. 2004
	G(1517)T; 3' UTR		G allele	
AGTR1	A(1166)C; 3' UTR (rs5186)	Indian (160:163)	no association	Stobdan et al. 2011
AGTR1	A(1166)C; 3' UTR (rs5186)	Indian (80:48)	no associations	Srivastava et al. 2012
	A(-777)T; promoter (rs275651)			
AGTR2	G(1498)T; 3'UTR (rs5193)	Indian (160:163)	no association	Stobdan et al. 2011
	G(1504)A; 3' UTR (rs5194)		no association	
	G(2402)C; 3' UTR (rs12845035)		no association	
BDKRB2	C(-58)T; promoter (rs1799722)	Han Chinese (144:140)	no associations	Qi et al. 2007
	±9 bp; exon 1 (rs72348790)			
CYBA	C(242)T; his72tyr; exon 4 (rs4673)	Caucasian (52:51)	no association	Weiss et al. 2003

Gene	Polymorphism[a]	Population (C:P) [b]	Results	Reference
CYBA	A(-930)G; near gene (rs9932581)	Unclear (150:180)	G allele; G/G genotype	Mishra et al. 2011
	C/T; his72tyr; exon (rs4673)		C allele; C/C genotype	
CYP11B2	T(-344)C; promoter (rs1799998)	Indian (64:59)	no association	Ahsan et al. 2004
	conversion; intron 2		con/con genotype	
	A(2713)G; lys173arg, exon 3 (rs4539)		no association	
CYP11B2	C(-344)T; promoter (rs1799998)	Han Chinese (144:140)	T allele; T/T genotype	Qi et al. 2007
	A(2713)G; lys173arg; exon 3 (rs4539)		A (lys) allele; A/A genotype	
CYP11B2	C(-344)T; promoter (rs1799998)	Indian (80:48)	no association	Srivastava et al. 2012
EDN1	(CT)n-(CA)n 27 repeats; 5'UTR (J05008)	"Same ethnicity" (53:64)	no association	Rajput et al. 2006
	T(2288)G; intron 2 (rs2070699)		T/T genotype; G/T+T/T genotypes	
	-/A; 5' UTR (rs10478694)		no association	
	G(594)T; lys198asn; exon 5 (rs5370)		no association	
EGLN1	A(571)G; 5' UTR (rs2153364)	250:210	no association	Mishra et al. 2013
	(rs1538664)		A/A genotype	
	TG; (rs1416911)		no association	
	CT; (rs2491419)		no association	
	AC; (rs7542797)		no association	
	AC; (rs2095935)		no association	
	AG; (rs1572794)		no association	
	(rs2808614)		no association	
	(rs2808611)		no association	
	(rs479200)		T/T genotype	
	(rs479311)		no association	
	(rs546774)		no association	
	(rs545937)		no association	
	(rs519504)		no association	
	(rs516651)		no association	
	(rs537135)		no association	
	(rs2486727)		no association	
	(rs2244986)		no association	
	(rs2244994)		no association	
	(rs2486729)		A/A genotype	
	(rs2790879)		G/G genotype	
	(rs2790882)		no association	
	(rs2486731)		no association	
	(rs480902)		C/C genotype	
	(rs2486732)		no association	
	(rs2024878)		no association	
	(rs2739511)		no association	
	(rs2486736)		A/A genotype	
	(rs973253)		no association	
	(rs973252)		G/G genotype	
EPAS1	chr2:46441523(hg18)	Han Chinese (153: 298)	C allele	Yang et al. 2013

Gene	Polymorphism[a]	Population (C:P) [b]	Results	Reference
F5	G(1691)A; arg506gln (rs6025)	Japanese (51:44)	no association	Droma et al. 2003
GSTP1	A(562)G; ile105val (rs1695)	Unclear (150:180)	G allele; G/G	Mishra et al. 2011
	C(590)T; ala114val (rs1138272)		T/T genotype	
HLA	HLA-DR6	Japanese (30:100)	DR6 allele	Hanaoka et al. 1998
	HLA- DQ4		DQ4 allele	
	HLA-A,B,C, DR, DQ alleles		no associations	
HSPA1A	G(+190)C; 5' UTR (rs1043618)	Han Chinese (483:148)	no association	Qi et al. 2009
	A(-110)C; 5' UTR (rs1008438)		C allele; C/C genotype	
HSPA1B	A(1267)G; silent; exon 1 (rs1061581)	Han Chinese (483:148)	A allele	Qi et al. 2009
	G(2074)C; silent; exon 1 (rs539689)		no association	
HSPA1L	T(2437)C; met493thr; exon 2 (rs2227956)	Han Chinese (483:148)	no association	Qi et al. 2009
NOS3	G(894)T; glu298asp; exon 7 (rs1799983)	Japanese (51: 41)	T (asp) allele	Droma et al. 2002
	27 bp VNTR 4a/4b/4c; intron 4		4a allele	
NOS3	G(894)T; glu298asp; exon 7 (rs1799983)	Caucasian (52:51)	no association	Weiss et al. 2003
	T(-786)C; promoter (rs2070744)			
	CA repeats, intron 13			
NOS3	G(894)T; glu298asp; exon 7 (rs1799983)	Indian (64:59)	T (asp) allele	Ahsan et al. 2004
	27 bp VNTR 4a/4b/4c; intron 4		4a/4b genotype	
NOS3	G(894)T; glu298asp; exon 7 (rs1799983)	27:108	T allele; G/T genotype	Wang et al. 2013
SFTPA1	C(1101)T; va19ala; signal peptide (rs1059047)	Indian (10:9)	C (val) allele	Saxena et al. 2005
	C(1162)T; silent; exon 2		no association	
	C(1193)G; leu50val; exon 2		no association	
	C(1416)T; intron		no association	
	G(1544)A; exon 3		no association	
	T(3138)C; silent; exon 5		T allele	
	T(3192)C; silent; exon 5 (rs1059058)		T allele	
	T(3234)C; silent; exon 5 (rs10351)		T allele	
SFTPA2	C(1382)G; intron 3	Indian (12:7)	no association	Saxena et al. 2005
	T1492)C; intron 3		no association	
	G(1649)C; ala91pro; exon 4		no association	
	A(1660)G; silent; exon 4		no association	
	C(2474)T; silent; exon 5		no association	
	C(2491)A; gln120pro; exon 5		no association	
	T(3018)C; silent; exon 6		no association	
	A(3265)C; gln223lys; exon 6 (rs1965708)		C (lys) allele	
TH	(TCAT)n tetranucleotide repeat; intron 1	Japanese (51:43)	no association	Hanaoka et al. 2003a
	A(2606)G; met81val; exon 2 (rs6356)		no association	
TIMP3	C()T; (rs738992)	Japanese (53:67)	no association	Kobayashi et al. 2013
	A()G; (rs130287)		no association	
	T()G; (rs130293)		C/T genotype	
	A()G; (rs715572)		no association	
	C()T; (rs2071947)		no association	
	C()T; (rs9862)		no association	

Gene	Polymorphism[a]	Population (C:P)[b]	Results	Reference
VEGFA	C(-2578)A; promoter (rs699947) G(-1154)A; promoter (rs1570360) T(-460)C; promoter (rs833061) G(+405)C; 5′-UTR (rs2010963) C(936)T; 3′-UTR (rs3025039)	Japanese (69:53)	no associations	Hanaoka et al. 2003b

[a] SNPs are shown as base (position) base; protein changes are shown as amino acid, position, amino acid; UTR, untranslated region; dbSNP (rs).

[b] Sample size: Control (resistant); Patients (susceptible); if n varied between polymorphisms tested in a study, the smallest sample size for which an association was reported is given.

[c] This SNP does not appear to be in the ACE gene.

A.2 High-altitude cerebral edema

High-altitude cerebral edema (HACE) can occur following ascent to altitudes above 2000 m, and most typically above 3000 m (Hackett and Roach 2004). The incidence has been reported to be very low (*e.g.,* 1.0% in trekkers between 4243 m and 5500 m (Hackett et al. 1976), although one study reported an unusually high incidence of HACE (31%) after rapid ascent to 4380 m in the Himalaya. Clinically, the onset of HACE is characterized by changes in consciousness and ataxia of gait. Individuals with HACE may also be withdrawn, apathetic, confused (maybe extremely so), fatigued, anorexic, and they may develop headache and nausea (Hackett and Roach 2004). HACE may progress to deep coma, after which the mortality rate is greater than 60% (Clarke 1988).

Many researchers consider HACE to be a severe form of AMS, both clinically and pathophysiologically (Hackett and Roach 2004). Normally, AMS precedes HACE, but whether AMS *always* precedes HACE is unknown, and it is not a requirement for a diagnosis of HACE (Hackett and Roach 2004). If it occurs, the progression of AMS to HACE occurs most often over 24-36 hours, but it can occur more rapidly (Hackett and Roach 2004). The development of HAPE may potentiate the progression of AMS to HACE, as it causes severe hypoxemia (Hackett and Roach 2001) that would mimic a rapid ascent to an altitude above that to which the individual is currently exposed. For example, 13% (Gabry et al. 2003) and 14% (Hultgren et al. 1996) of patients with HAPE also had signs of HACE or were diagnosed with HACE, and in a group of individuals who died from HAPE, 50% also had HACE (Hackett and Roach 2004).

Because HACE is considered a severe form of AMS, the pathophysiology and risk factors of HACE are similar to those described above for AMS. Also, because the occurrence of HACE is triggered by the occurrence of HAPE, the risk factors of HAPE also apply to HACE. For these reasons, the pathophysiology and risk factors of HACE will not be described. Similarly, the Wilderness Medical Society guidelines to prevent HACE are the same as those for AMS and HAPE. Dexamethasone (possibly in combination with acetazolamide), supplemental oxygen, and descent are the recommended treatments for HACE.

Appendix B Non-invasive physiological field tests for acute mountain sickness: the utility of oxygen saturation and heart rate in diagnosing and predicting AMS

B.1 Introduction

The purpose of this study was to determine if (a) oxygen saturation (S_{PO_2}) measured at 4380 m was associated with AMS status and LLS at 4380 m and (b) heart rate (HR) measured at 4380 m was associated with AMS status and LLS at 4380 m. These variables were measured at the 2010 and 2012 Janai Purnima festivals. The hypotheses were that each variable would be associated with AMS status and LLS at 4380 m. The University of British Columbia Clinical Research Ethics Board and the Nepal Health Research Council approved both studies.

B.2 Methods

Subjects

At the 2010 Janai Purnima festival, subjects were recruited at the Himalayan Rescue Association temporary medical clinic. This study had a cross-sectional, case-control design, with only pilgrims who presented at the clinic being recruited. (*N.b.,* some subjects visited the clinic for AMS treatment, and other subjects visited the clinic with family or friends, providing both cases and controls for this study). The subjects' ages, sexes, ascent rates, and LLS were collected through interviews conducted in Nepalese. Subjects' HR and S_{PO_2} were measured after the interview (described below). This dataset will be referred to as the "2010-CS" dataset throughout the chapter.

In addition to those subjects recruited for the 2010 study, a cross-sectional study was conducted at Gosainkunda to recruit additional subjects. The method of recruitment was similar to that of the 2010 Nepal study: subjects were recruited from the Himalayan Rescue Association temporary medical camp and from passersby our research site. Interviews were conducted in Nepalese to collect age, sex, ascent rate, and LLS data. Subjects' HR and S_{PO_2} were measured after the interview (described below). This dataset will be referred to as the "2012-CS" dataset throughout this chapter.

Ascent

Most pilgrims attending the Janai Purnima festival traveled to Dhunche from the Kathmandu Valley (~1400 m) by motor vehicle (*e.g.*, bus or motorcycle) and by foot. Others traveled from surrounding communities. From Dhunche to Gosainkunda, all subjects followed the same trail; however, ascent rates were self selected by individuals.

Acute mountain sickness

A Nepalese medical student or intern administered the LLS questionnaire (Roach et al. 1993) in Nepali under the supervision of an experienced researcher or physician. Subjects with a LLS $\geq$ 3 (including a headache score $\geq$ 1) were considered to be positive for AMS (AMS+), while subjects without a headache or with a LLS < 3 were considered to be negative for AMS (AMS-).

Heart rate and oxygen saturation

The SpO_2 and HR were measured while subjects were seated using portable pulse oximeters. In the 2010 Nepal study, the Oximeter Plus Oxi-Go (Oximeter Plus, Inc.; Roslyn, NY) was used to obtain the data, and in the 2012 Nepal studies a Go2 Finger Oximeter (Nonin Medical Inc.; Plymouth, MN) was used to obtain these data. For both studies, a single reading from the index finger was recorded for each subject.

B.3 Results

Subject characteristics

A total of 92 subjects were recruited at Gosainkunda in 2010. The HR and S_{PO_2} were assessed in all of the subjects, but complete data were only available from 90 subjects (Table B.1). The AMS+ group was significantly older and had a greater proportion of females relative to the AMS- group, but ascent rates were similar between groups (Table B.1).

A total of 86 subjects that were recruited at Gosainkunda were eligible for the analyses. Of these subjects, HR and S_{PO_2} were assessed in 82 subjects, and complete information was available for 67 of these subjects (Table B.1). The AMS+ and AMS- groups did not differ significantly in terms of age, sex, or ascent rate (Table B.1).

Table B.1 The characteristics of the two datasets for which heart rate and oxygen saturation were available.

Dataset	Variable	Group			Stat; p-value
		Total	AMS+	AMS-	
2010-CS	n	87	55	32	NA
	Age (years)	36.4 (13.9)	38.9 (15.2)	32.0 (10.3)	-2.54; 0.01*
	Sex (M:F)	62:25	34:21	28:4	6.52; 0.01*
	Ascent rate (1:2:3 days)	11:61:15	4:43:8	7:18:7	5.43; 0.07
	Lake Louise Score	2.90 (2.51)	4.53 (1.61)	0.09 (0.30)	-15.4; < 0.001*
2012-CS	n	66	47	19	NA
	Age (years)	40.6 (13.7)	38.9 (13.1)	44.8 (14.8)	1.61; 0.11
	Sex (M:F)	32:34	20:27	12:7	2.30; 0.13
	Ascent rate (1:2:3 days)	20:45:1	12:34:1	8:11:0	2.05; 0.36
	Lake Louise Score	5.68 (3.21)	7.06 (2.53)	2.26 (1.88)	-7.46; < 0.001*

* $p < 0.05$
NA, not applicable

Table B.2 The heart rate and oxygen saturation for subjects with (AMS+) and without (AMS-) acute mountain sickness subdivided by dataset.

Dataset	Variable	Group			Stat; p-value
		Total	AMS+	AMS-	
2010-CS	HR	93.8 (16.6)	97.1 (16.8)	88.2 (14.8)	-2.51; 0.01*
	S_{PO_2}	83.7 (5.08)	82.2 (5.2)	86.3 (3.8)	3.83; < 0.001*
2012-CS	HR	101.0 (17.6)	99.0 (17.5)	106.0 (17.5)	1.47; 0.15
	S_{PO_2}	81.0 (8.2)	81.2 (8.6)	80.4 (7.4)	-0.35; -0.73

HR, heart rate; S_{PO_2}, oxygen saturation.
* $p < 0.05$

Incidences of acute mountain sickness

The two cross-sectional studies had greater incidences (63.2% in the 2010-CS dataset and 71.2% in the 2012-CS dataset) than that observed in the longitudinal dataset presented in Chapter 5 ($p < 0.05$ for both comparisons).

Heart rate and oxygen saturation for diagnosing AMS

The mean HR of AMS+ subjects was only significantly greater from the mean HR of AMS- subjects in the 2010-CS dataset (Table B.2). In this dataset, HR was positively correlated with LLS ($r = 0.22$, $p = 0.04$) and negatively correlated with S_{PO_2} ($r = -0.24$; $p = 0.03$). Neither HR nor S_{PO_2} were correlated with LLS in the 2012 dataset, but a similar strength (although non-significant correlation) was observed between HR and S_{PO_2} ($r = -0.22$, $p = 0.07$) in this dataset.

The mean S_{PO_2} of AMS+ subjects was only significantly different than the mean S_{PO_2} of AMS- subjects in the 2010-CS dataset (Table B.2). In this dataset, the AMS- subjects had a mean

heart rate that was 4.1% greater than the AMS+ subjects. Also in this dataset, S_{PO_2} was negatively correlated with age ($r = -0.29$, $p = 0.01$) and negatively correlated with LLS ($r = -0.44$, $p < 0.001$). The only other significant correlation was observed in the 2012-CS dataset: S_{PO_2} was negatively correlated with age ($r=-0.29$, $p = 0.02$).

Appendix C GWAS data supplement

C.1 Genotype data prior to quality control

Characteristics of subjects prior to quality control are described in Table C.1. Pre-quality control distributions for SNPs and individuals are reported in Table C.2–Table C.5.

Table C.1 Characteristics of subjects with and without acute mountain sickness (AMS+ and AMS-) prior to quality control.

Variable	AMS-	AMS+	Statistic (p-value)
n	71	73	NA
Male:Female	58:13	43:30	$\chi^2 = 8.923$ (0.003)
Age (years)	32.2 (11.2)	39.9 (12.1)	t = 3.960 (<0.001)
LLS	0.3 (0.5)	5.6 (2.2)	t = 19.806 (<0.001)

AMS-, without AMS; AMS+, with AMS; n, sample size; LLS, Lake Louise Score; NA, not applicable.

Table C.2 The number and proportion of SNPs below different p-value thresholds for tests of Hardy-Weinberg Equilibrium in the full dataset prior to quality control.

	Hardy-Weinberg p-value threshold					
Measure	**X ≤ 0.001**	**X ≤ 0.01**	**X ≤ 0.05**	**X ≤ 0.1**	**X ≤ 0.5**	**X ≤ 1.0**
Number	975	60936	77638	92292	190415	535452
Proportion	0.002	0.114	0.145	0.172	0.356	1.000

Table C.3 The number and proportion of SNPs at different call rate thresholds in the full dataset prior to quality control.

	Single nucleotide polymorphism call rate threshold				
Measure	**X ≤ 0.9**	**0.9 < X ≤ 0.95**	**0.95 < X ≤ 0.98**	**0.98 < X ≤ 0.99**	**X > 0.99**
Number	3577	1877	17393	44511	468094
Proportion	0.000	0.000	0.000	0.000	0.001

Table C.4 The number and proportion of SNPs at different minor allele frequency thresholds in the full dataset prior to quality control.

	Minor allele frequency threshold				
Measure	**X ≤ 0.01**	**0.01 < X ≤ 0.05**	**0.05 < X ≤ 0.1**	**0.1 < X ≤ 0.2**	**X > 0.2**
Number	244489	24077	20479	55481	190926
Proportion	0.457	0.045	0.038	0.104	0.357

Table C.5 The number and proportion of individuals at different call rate thresholds in the full dataset prior to quality control.

	Individual call rate threshold				
Measure	**X ≤ 0.9**	**0.9 < X ≤ 0.95**	**0.95 < X ≤ 0.98**	**0.98 < X ≤ 0.99**	**X > 0.99**
Number	2	1	0	3	138
Proportion	0.014	0.007	0.000	0.021	0.958

C.2 Supporting data for the male dataset presented in Chapter 6

In Chapter 6, the 10 SNPs with the smallest p-values were reported for the male dataset with AMS status as the phenotype and AMS severity as the phenotype. The 100 SNPs with the lowest p-values for these phenotypes are reported in Table C.6 and Table C.7.

Table C.6 The 100 SNPs with the smallest p-values from the male dataset, using AMS status as the phenotype.

Polymorphism	Chromosome	Position*	Gene	χ^2	p-value	Corrected p-value
rs7010000	8	24663706	Intergenic	22.12	0.0000026	0.20
rs1209952	21	40175827	*ETS2*	18.39	0.000018	0.81
rs6506564	18	8237063	*PTPRM*	18.27	0.000019	0.82
rs2607605	8	24644749	Intergenic	16.28	0.000055	1.00
rs9548985	13	40502985	Intergenic	15.68	0.000075	1.00
rs13279743	8	68751611	Intergenic	15.35	0.000089	1.00
rs12545592	8	68762313	Intergenic	15.35	0.000089	1.00
rs12961130	18	55260070	Intergenic	15.31	0.000091	1.00
rs457705	21	40191431	*ETS2*	15.31	0.000091	1.00
rs1228925	7	84136556	Intergenic	15.00	0.00011	1.00
rs9928490	16	64594588	Intergenic	14.84	0.00012	1.00
rs2658828	11	131547887	*NTM*	14.81	0.00012	1.00
rs7817551	8	13438708	Intergenic	14.73	0.00012	1.00
rs17171612	7	39724464	*RALA*	14.38	0.00015	1.00
rs13090606	3	69113153	*UBA3*	14.19	0.00017	1.00
rs7684126	4	187072383	*FAM149A*	14.12	0.00017	1.00
exm437766	4	187077206	*FAM149A*	14.12	0.00017	1.00
exm437786	4	187078785	*FAM149A*	14.12	0.00017	1.00
rs12353109	9	32282392	Intergenic	14.05	0.00018	1.00
rs10800956	1	190632794	*LOC440704*	13.99	0.00018	1.00
rs1157545	11	42424649	Intergenic	13.99	0.00018	1.00
rs9908467	17	90429	*RPH3AL*	13.93	0.00019	1.00
rs7111814	11	72935825	Intergenic	13.91	0.00019	1.00
rs10196581	2	240755338	Intergenic	13.77	0.00021	1.00
rs2527068	7	147731239	*CNTNAP2*	13.65	0.00022	1.00
rs7779573	7	22831386	Intergenic	13.60	0.00023	1.00
rs11849276	14	57734577	*AP5M1/EXOC5*	13.58	0.00023	1.00
rs17072678	4	182964412	Intergenic	13.42	0.00025	1.00
rs4732558	7	84168615	Intergenic	13.38	0.00025	1.00
rs9642186	7	84243643	Intergenic	13.38	0.00025	1.00
rs10164993	2	53530783	Intergenic	13.36	0.00026	1.00
rs12595633	15	50770917	*USP8*	13.22	0.00028	1.00
rs365818	5	115017389	Intergenic	13.20	0.00028	1.00
rs9655257	7	25377346	Intergenic	13.16	0.00029	1.00

Polymorphism	Chromosome	Position*	Gene	χ^2	p-value	Corrected p-value
rs4377642	4	188792218	Intergenic	13.15	0.00029	1.00
rs7141612	14	34140885	*NPAS3*	13.15	0.00029	1.00
rs10486391	7	20376018	*ITGB8*	13.10	0.00030	1.00
rs7927894	11	76301316	Intergenic	13.04	0.00030	1.00
rs7927997	11	76301375	Intergenic	13.04	0.00030	1.00
rs4857185	3	95912089	Intergenic	12.98	0.00031	1.00
rs4981820	14	31531855	*AP4S1*	12.97	0.00032	1.00
rs10744613	12	3554821	*PRMT8*	12.94	0.00032	1.00
rs887030	19	2528705	*GNG7*	12.94	0.00032	1.00
rs4862650	4	187074833	*FAM149A*	12.85	0.00034	1.00
rs8021894	14	38706711	Intergenic	12.80	0.00035	1.00
rs3913578	3	76515427	*ROBO2*	12.72	0.00036	1.00
exm2261397	3	142653680	LOC100507389	12.66	0.00037	1.00
rs6781362	3	142653680	LOC100507389	12.66	0.00037	1.00
rs661827	13	69424751	Intergenic	12.65	0.00038	1.00
rs4495279	6	100257668	Intergenic	12.62	0.00038	1.00
rs17819684	7	82732583	*PCLO*	12.61	0.00038	1.00
rs2116078	8	73363989	Intergenic	12.56	0.00039	1.00
rs2256569	5	9160380	*SEMA5A*	12.55	0.00040	1.00
rs1572973	X	128173087	Intergenic	12.50	0.00041	1.00
rs4468638	16	80531515	Intergenic	12.50	0.00041	1.00
rs11914882	3	126807142	Intergenic	12.48	0.00041	1.00
rs6130667	20	43310251	Intergenic	12.44	0.00042	1.00
rs934178	11	44585933	*CD82*	12.43	0.00042	1.00
rs9545925	13	36535192	*DCLK1*	12.37	0.00044	1.00
rs1899039	2	223910943	Intergenic	12.37	0.00044	1.00
rs3735669	7	45754582	*ADCY1*	12.36	0.00044	1.00
rs1337241	20	54228308	Intergenic	12.32	0.00045	1.00
rs7794902	7	68092473	Intergenic	12.27	0.00046	1.00
rs4568657	9	9246647	*PTPRD*	12.24	0.00047	1.00
rs16831670	3	130499302	Intergenic	12.24	0.00047	1.00
rs11674612	2	85658232	Intergenic	12.15	0.00049	1.00
exm29993	1	22915858	*EPHA8*	12.14	0.00049	1.00
rs1764109	6	938900	Intergenic	12.13	0.00050	1.00
rs698224	3	22946172	Intergenic	12.11	0.00050	1.00
rs11063622	12	5452453	Intergenic	12.02	0.00053	1.00
rs7037401	9	90387296	*CTSL3P*	12.00	0.00053	1.00
rs10091421	8	13468201	Intergenic	12.00	0.00053	1.00
rs8093346	18	32805796	Intergenic	11.99	0.00054	1.00
rs2461108	7	45723834	*ADCY1*	11.97	0.00054	1.00
rs1997530	7	147650411	*CNTNAP2*	11.93	0.00055	1.00
rs2196470	15	40237322	*EIF2AK4*	11.88	0.00057	1.00
rs12823007	12	62367084	*FAM19A2*	11.88	0.00057	1.00
rs469180	21	40217738	Intergenic	11.81	0.00059	1.00

Polymorphism	Chromosome	Position*	Gene	χ^2	p-value	Corrected p-value
rs5945544	X	143716729	Intergenic	11.80	0.00059	1.00
rs462515	5	153744999	*GALNT10*	11.79	0.00059	1.00
rs2168351	15	92983722	*ST8SIA2*	11.73	0.00062	1.00
rs7646841	3	126695927	Intergenic	11.71	0.00062	1.00
rs1430838	8	73360653	Intergenic	11.65	0.00064	1.00
rs9283536	2	223930506	Intergenic	11.65	0.00064	1.00
rs12156003	8	106233193	Intergenic	11.63	0.00065	1.00
rs10166905	2	128780173	*SAP130*	11.63	0.00065	1.00
rs886511	5	9155728	*SEMA5A*	11.59	0.00066	1.00
rs10505035	8	103775175	Intergenic	11.59	0.00066	1.00
exm2266641	8	73379450		11.59	0.00066	1.00
rs1534825	21	40210516	Intergenic	11.56	0.00067	1.00
exm683769	8	13424583	Intergenic	11.54	0.00068	1.00
rs1979582	3	8310990	*LMCD1-AS1*	11.53	0.00069	1.00
rs16923640	9	5898829	*MLANA*	11.51	0.00069	1.00
rs10090349	8	13426508	Intergenic	11.50	0.00070	1.00
rs6430345	2	149584946	Intergenic	11.49	0.00070	1.00
rs1859032	7	117631978	Intergenic	11.48	0.00070	1.00
rs4828625	X	150486962	Intergenic	11.48	0.00070	1.00
rs869834	14	63177144	Intergenic	11.47	0.00071	1.00
rs4844477	1	209685058	Intergenic	11.46	0.00071	1.00

[a] According to the dbSNP database.

Table C.7 The 100 SNPs with the smallest p-values from the male dataset, using AMS severity as the phenotype.

Polymorphism	Chromosome	Position*	Gene	χ^2	p-value	Corrected p-value
rs7684126	4	187072383	*FAM149A*	27.89	0.00000013	0.01
exm437766	4	187077206	*FAM149A*	27.89	0.00000013	0.01
exm437786	4	187078785	*FAM149A*	27.89	0.00000013	0.01
exm437744	4	187074833	*FAM149A*	27.62	0.00000015	0.01
rs7010000	8	24663706	Intergenic	19.22	0.000012	0.83
rs2166202	13	89855595	Intergenic	18.77	0.000015	0.91
rs9548985	13	40502985	Intergenic	18.70	0.000015	0.91
exm2265893	4	172388728	Intergenic	17.39	0.000030	0.99
rs7664076	4	172388728	Intergenic	17.39	0.000030	0.99
newrs12444395	16	13297348	*SHISA9*	17.22	0.000033	1.00
rs12595633	15	50770917	*USP8*	16.95	0.000038	1.00
rs12353109	9	32282392	Intergenic	16.81	0.000041	1.00
rs1987162	4	182278636	Intergenic	16.70	0.000044	1.00
rs11564037	7	27326739	Intergenic	16.53	0.000048	1.00
rs11674612	2	85658232	Intergenic	16.41	0.000051	1.00
rs661827	13	69424751	Intergenic	16.32	0.000054	1.00
rs4267464	2	193050042	*TMEFF2*	15.91	0.000066	1.00
rs13279743	8	68751611	Intergenic	15.16	0.000099	1.00
rs12545592	8	68762313	Intergenic	15.16	0.000099	1.00
rs4436527	11	12987342	Intergenic	15.10	0.00010	1.00
rs4468638	16	80531515	Intergenic	15.06	0.00010	1.00
rs4770402	13	23754744	*SGCG*	15.05	0.00010	1.00
rs2967162	16	57810090	*KIFC3*	15.02	0.00011	1.00
rs4819558	22	17616217	Intergenic	14.80	0.00012	1.00
rs9317535	13	23755892	*SGCG*	14.71	0.00013	1.00
rs13330014	16	5561166	*LOC100287538*	14.64	0.00013	1.00
rs2235590	20	40042321	Intergenic	14.58	0.00013	1.00
rs3913578	3	76515427	*ROBO2*	14.53	0.00014	1.00
rs424372	21	41601422	*DSCAM*	14.40	0.00015	1.00
rs1899039	2	223910943	Intergenic	14.34	0.00015	1.00
rs919943	2	110420681	Intergenic	14.31	0.00016	1.00
rs1209952	21	40175827	*ETS2*	14.24	0.00016	1.00
rs11912647	22	38035076	*SH3BP1*	14.21	0.00016	1.00
rs9655257	7	25377346	Intergenic	14.14	0.00017	1.00
rs7407488	18	4129162	*DLGAP1*	14.04	0.00018	1.00
rs1420655	5	82977843	*HAPLN1*	13.97	0.00019	1.00
rs1326807	9	119585852	*ASTN2*	13.93	0.00019	1.00
rs7254514	19	51678482	Intergenic	13.93	0.00019	1.00
exm1828917	15	40916462		13.92	0.00019	1.00
rs10800956	1	190632794	*LOC440704*	13.91	0.00019	1.00
rs10744613	12	3554821	*PRMT8*	13.74	0.00021	1.00
rs2216054	16	50600877	*NKD1*	13.74	0.00021	1.00
rs6435304	2	206701468	Intergenic	13.69	0.00022	1.00

Polymorphism	Chromosome	Position*	Gene	χ^2	p-value	Corrected p-value
rs13423607	2	206713452	Intergenic	13.69	0.00022	1.00
rs17171612	7	39724464	*RALA*	13.57	0.00023	1.00
rs12639640	4	65592228	Intergenic	13.54	0.00023	1.00
rs444584	21	41595726	*DSCAM*	13.41	0.00025	1.00
rs12327434	18	58679941	Intergenic	13.39	0.00025	1.00
rs4495279	6	100257668	Intergenic	13.37	0.00026	1.00
rs7817551	8	13438708	Intergenic	13.33	0.00026	1.00
rs4885528	13	78886839	*RNF219-AS1*	13.28	0.00026	1.00
rs6130667	20	43310251	Intergenic	13.26	0.00027	1.00
rs469180	21	40217738	Intergenic	13.17	0.00028	1.00
rs17521587	1	221495334	Intergenic	13.14	0.00029	1.00
rs12961130	18	55260070	Intergenic	13.11	0.00029	1.00
rs1406226	15	62459031	*C2CD4B*	13.10	0.00030	1.00
rs4771030	13	27381936	Intergenic	13.08	0.00030	1.00
rs6593639	1	97670325	*DPYD-AS1*	13.06	0.00030	1.00
rs2077150	10	13734850	*FRMD4A*	13.06	0.00030	1.00
rs1572973	X	128173087	Intergenic	13.05	0.00030	1.00
rs4828625	X	150486962	Intergenic	13.01	0.00031	1.00
rs7987265	13	79799834	Intergenic	12.94	0.00032	1.00
rs7111814	11	72935825	*P2RY2*	12.90	0.00033	1.00
rs10849573	12	982321	*WNK1*	12.89	0.00033	1.00
rs956868	12	990912	*WNK1*	12.89	0.00033	1.00
rs9283037	21	44722695	Intergenic	12.89	0.00033	1.00
rs4766119	12	3548756	*PRMT8*	12.87	0.00033	1.00
rs4861947	4	182284224	Intergenic	12.87	0.00033	1.00
rs4302466	4	6755324	Intergenic	12.86	0.00034	1.00
rs637826	13	69070763	Intergenic	12.82	0.00034	1.00
rs4732558	7	84168615	Intergenic	12.81	0.00034	1.00
rs9642186	7	84243643	Intergenic	12.81	0.00034	1.00
rs2964135	5	5398279	Intergenic	12.80	0.00035	1.00
rs7762245	6	36716229	*CPNE5*	12.71	0.00036	1.00
rs2578693	3	45649417	*LIMD1*	12.66	0.00037	1.00
rs9553816	13	27004133	Intergenic	12.63	0.00038	1.00
rs462515	5	153744999	*GALNT10*	12.59	0.00039	1.00
rs9283536	2	223930506	Intergenic	12.57	0.00039	1.00
rs10486391	7	20376018	*ITGB8*	12.56	0.00039	1.00
rs8021894	14	38706711	Intergenic	12.55	0.00040	1.00
rs10196581	2	240755338	Intergenic	12.55	0.00040	1.00
rs13090606	3	69113153	*UBA3*	12.52	0.00040	1.00
rs1979582	3	8310990	*LMCD1-AS1*	12.51	0.00040	1.00
rs6506564	18	8237063	*PTPRM*	12.49	0.00041	1.00
exm269	1	881918		12.46	0.00041	1.00
rs2168351	15	92983722	*ST8SIA2*	12.44	0.00042	1.00
rs6666453	1	4987424	Intergenic	12.38	0.00043	1.00

Polymorphism	Chromosome	Position*	Gene	χ^2	p-value	Corrected p-value
rs4981820	14	31531855	*AP4S1*	12.38	0.00043	1.00
rs457705	21	40191431	*ETS2*	12.38	0.00043	1.00
rs11063622	12	5452453	Intergenic	12.37	0.00044	1.00
rs774610	3	76780594	*ROBO2*	12.28	0.00046	1.00
rs4255775	16	57846851	*LOC388282*	12.27	0.00046	1.00
rs7980145	12	132041924	Intergenic	12.23	0.00047	1.00
rs1534825	21	40210516	Intergenic	12.21	0.00048	1.00
rs10778637	12	108851822	Intergenic	12.20	0.00048	1.00
rs2658828	11	131547887	*NTM*	12.19	0.00048	1.00
rs8058856	16	5563370	*LOC100287538*	12.19	0.00048	1.00
rs7331957	13	51338548	*DLEU7*	12.15	0.00049	1.00
rs10164993	2	53530783	Intergenic	12.14	0.00049	1.00
rs10091421	8	13468201	Intergenic	12.13	0.00050	1.00

[a] According to the dbSNP database.

For the primary analysis in Chapter 6, female subjects were removed from the dataset. The rationale was that females were much more likely to develop AMS than males (Chapter 5), and this association would affect the results. As shown in Table C.8, the association between Lake Louise Score and haplotype presented in Chapter 6 did not hold for female subjects.

Table C.8. The age, acute mountain sickness (AMS) severity, and proportion of individuals with AMS for female subjects with the 'susceptible' and 'resistant' genotypes.

Variable	Total	Genotype		p-value
		Susceptible	Resistant	
n	42	11	31	NA
Age	38.7 (12.5)	38.6 (9.7)	38.7 (13.5)	0.98
Lake Louise Score	4.5 (3.6)	3.9 (3.4)	4.8 (3.6)	0.47
AMS+:AMS-	29:13	6:5	23:8	0.40

NA, not applicable.

Appendix D Acute mountain sickness diagnosis/severity questionnaires

D.1 Lake Louise Score Questionnaire (English)

1.Headache:

No headache 0	____
Mild headache 1	____
Moderate headache 2	____
Severe, incapacitating 3	____

2.Gastrointestinal (GI):

No GI symptoms 0	____
Poor appetite or nausea 1	____
Moderate nausea or vomiting 2	____
Severe N&V, incapacitating 3	____

3.Fatigue/weak:

Not tired or weak 0	____
Mild fatigue/weakness 1	____
Moderate fatigue/weakness 2	____
Severe F/W, incapacitating 3	____

4.Dizzy/lightheaded:

Not dizzy 0	____
Mild dizziness 1	____
Moderate dizziness 2	____
Severe, incapacitating 3	____

5.Difficulty sleeping:

Slept well as usual 0	____
Did not sleep as well as usual 1	____
Woke many times, poor night's sleep 2	____
Could not sleep at all 3	____

Calculation of Lake Louise Score (LLS)

LLS = Headache + GI + Fatigue/Weakness + Dizzy/lightheadedness + Difficulty sleeping

D.2 Lake Louise Score Questionnaire (Phonetic Nepali translation)

1.Headache: (Kapal Dukcha)
No headache (Dukdaina) 0 _____
Mild headache (Ali Ali Dukcha) 1 _____
Moderate headache (Thikai Dukcha) 2 _____
Severe, incapacitating (Ekdam Dukcha) 3 _____

2.GI: (Pet Ko Bare Lachyanharu)
No GI symptoms (Wak-Wak Chhaina, Bhok Lagcha) 0 _____
Poor appetite or nausea (Wak-Wak Lagcha, Bok Chhaina) 1 _____
Moderate nausea or vomiting (Wak-Wak Ra Ali-Ali Banta) 2 _____
Severe N&V, incapacitating (Dherai Banta) 3 _____

3.Fatigue/weakness: (Thakai Lagne)
Not tired or weak (Thakai Chaina) 0 _____
Mild fatigue/weakness (Alikati Thakai) 1 _____
Moderate fatigue/weakness (Thikai-Thikai Thakai) 2 _____
Severe F/W, incapacitating (Dherai Thakai) 3 _____

4.Dizzy/lightheaded: (Ringata Lagne)
Not dizzy (Ringata Chaina) 0 _____
Mild dizziness (Alikati Ringata) 1 _____
Moderate dizziness (Thikai-Thikai Ringata) 2 _____
Severe, incapacitating (Dherai Ringata) 3 _____

5.Difficulty sleeping: (Sutna Garo)
Slept well as usual (Ramrai Steko) 0 _____
Did not sleep as well as usual (Ramrai Nasuteko) 1 _____
Woke many times (Dherai Choti Utheko) 2 _____
Could not sleep at all (Sutdai Nasuteko) 3 _____

Calculation of Lake Louise Score (LLS)

LLS = Headache + GI + Fatigue/Weakness + Dizzy/lightheadedness + Difficulty sleeping

D.3 Environmental Symptom Questionnaire III

Symptoms

		0	1	2	3	4	5
1.	I feel lightheaded	____	____	____	____	____	____
2.	I had a headache	____	____	____	____	____	____
4.	I felt dizzy	____	____	____	____	____	____
5.	I felt faint	____	____	____	____	____	____
6.	My vision was dim	____	____	____	____	____	____
7.	My coordination was off	____	____	____	____	____	____
19.	I felt weak	____	____	____	____	____	____
24.	I felt sick to my stomach (nauseous)	____	____	____	____	____	____
52.	I lost my appetite	____	____	____	____	____	____
53.	I felt sick	____	____	____	____	____	____
54.	I felt hungover	____	____	____	____	____	____

Response Key

0 = Not at all
1 = Slight
2 = Somewhat
3 = Moderate
4 = Quite a bit
5 = Extreme

Calculation of cerebral AMS score

AMS-C = F1 / 5.189, where F1 = (Q1 * 0.489) + (Q2 * 0.465) + (Q4 * 0.446) + (Q5 * 0.346) + (Q6 * 0.501) + (Q7 * 0.519) + (Q19 * 0.387) + (Q24 * 0.347) + (Q52 * 0.413) + (Q53 * 0.692) + (Q54 * 0.584)

Appendix E Field study questionnaires

E.1 Acute mountain sickness (AMS) questionnaire: Gosainkunda Cross-section 2010

Date:_____ Time of day: _____ Sample collected: **YES** **NO**
Subject name: _____ Sample number:_ _____
Sex:……**Male** **Female** Age: _____

1) When did you arrive at Gosainkunda (time of day)? _____

2) Did you come here from a low-altitude area? …….. **YES** **NO**
If yes: How long did it take you to come to the festival from the low-altitude area?
less than 1 day **1 day** **2 days** **3 days** **4 days** **more than 4 days**

3) Where did you stay last night? _____
Where did you stay the night before ?_____

4) Have you been to high-altitude (above 2500) meters in the last 2 months…..**YES** **NO**
(note: this refers to before coming to the festival)?

5) Have you heard of acute mountain sickness (or have you heard that people can get sick when they travel into the mountains because of the altitude/less oxygen/"thin" air)?..... **YES** **NO**
If yes: did you do anything to try to avoid AMS (drugs, diet, travel route/timing, etc)……**YES** **NO**
If yes, please describe _____(or use back of page)

6) Have you been to this festival before?.....**YES** **NO**
If yes: How many times? _____
If yes: Did you get AMS before? **NO** **SOMETIMES (how many times?_____)** **EVERYTIME**

7) Are your parents Nepalese?: **no** **mother only** **father only** **both**

8) How many of your grandparents were Nepalese? **0** **1** **2** **3** **4** **not sure**

9) Are you related to anyone else participating in this study:…….. **YES** **NO**

If yes: Who? _____ How related?_____

Do you or did you previously smoke tobacco?…….. **YES** **NO** How often? _____

Are you pregnant?…….. **YES** **NO**

Are you taking any medications?..... **YES** **NO**

Thank you for volunteering to participate in this research.

E.2 Acute mountain sickness (AMS) questionnaire: Low altitude longitudinal 2010

Date:_____ Time: _____ Sample #: _____ Sex: **M / F** Age: _____ NAME
1) Did you come here from a low-altitude area? **YES / NO**

_____
2) Have you been to high-altitude (above 2500) meters in the last 2 months? **YES / NO**
3) Have you heard of acute mountain sickness (or that people can get sick in the mountains due to "thin" air)? **YES / NO**
If yes: did you do anything to try to avoid AMS (drugs, diet, travel route/timing, etc)? **YES / NO**
If yes, please describe _____
4) Have you been to Gosainkunda? **YES / NO** How many times? _____ Did you get AMS before? **YES / NO**
5) Are your parents Nepalese?: **no** **mother only** **father only** **both**
6) How many of your grandparents were Nepalese? **0** **1** **2** **3** **4** **not sure**
7) Are you related to anyone else participating in this study: **YES / NO**
Who and how?
8) Are you taking any other medications?

E.3 Acute mountain sickness (AMS) questionnaire: High altitude longitudinal 2010

Date:_____ Time: _____ Sample #: _____ Name _____

1) When did you arrive at Gosainkunda (time of day)? _____
2) Where did you stay last night? _____
3) Where did you stay the night before?_____
4) Where did you stay two nights before? _____
4) Have you taken any medication to prevent altitude illness or any medication that might affect the perception of altitude illness (e.g. ibuprofen)?
5) Do you have any family members traveling with you?

E.4 Acute mountain sickness (AMS) questionnaire: Gosainkunda cross-sectional 2010

Date: _____ Time: _____ Sample #: _____ Sex: **M / F** Age: _____ NAME
1) Did you come here from a low-altitude area? **YES / NO**

_____
2) Have you been to high-altitude (above 2500) meters in the last 2 months? **YES / NO**
3) Have you heard of acute mountain sickness (or that people can get sick in the mountains due to "thin" air)? **YES / NO**
If yes: did you do anything to try to avoid AMS (drugs, diet, travel route/timing, etc)? **YES / NO**
If yes, please describe _____
4) Have you been to Gosainkunda? **YES / NO** How many times? _____ Did you get AMS before? **YES / NO**
5) Are your parents Nepalese?: **no** **mother only** **father only** **both**
6) How many of your grandparents were Nepalese? **0** **1** **2** **3** **4** **not sure**
7) Are you related to anyone else participating in this study: **YES / NO**
Who and how?

1) When did you arrive at Gosainkunda (time of day)? _____
2) Where did you stay last night? _____
3) Where did you stay the night before?_____
4) Where did you stay two nights before? _____
5) Have you taken any medication to prevent altitude illness or any medication that might affect the perception of altitude illness (e.g. ibuprofen)?

Appendix F Acute normobaric hypoxia exposure does not affect exhaled nitric oxide: A meta-analysis

F.1 Summary

The effect of hypoxia on the exhaled nitric oxide (NO) of humans is unresolved. Many studies have measured the fraction of exhaled NO ($F_{E_{NO}}$) or the partial pressure of exhaled NO ($P_{E_{NO}}$) in normobaric and hypobaric hypoxia, with differing results. To better understand NO physiology and altitude acclimatisation, we employed a random effects meta-analysis to determine the effect of acute normobaric hypoxia on the $P_{E_{NO}}$ of humans. A total of 117 subjects from nine published studies (with 11 groups) were included. The median duration of exposure was 30 minutes, and the mean hypoxic $P_{I_{O_2}}$ was 89 (SD = 13) mmHg. The weighted standardised mean difference (SMD) in $P_{E_{NO}}$ measured at baseline and during an acute exposure to normobaric hypoxia was not significantly different from zero (SMD = -0.05; 95% CI = -0.32, 0.23; z = 0.33; p = 0.74), indicating that acute normobaric hypoxia did not affect the $P_{E_{NO}}$. This result should be considered for interpretations of high-altitude (and hypobaric) measurements of exhaled NO. As the $P_{E_{NO}}$ is a potential biomarker for altitude-illness susceptibility, recognizing that normobaric hypoxia does not affect the $P_{E_{NO}}$ will be important for understanding previous associations between low exhaled NO and poor acclimatisation to hypoxia.

F.2 Rationale for this study

That the fraction of exhaled nitric oxide ($F_{E_{NO}}$) did not decrease in either of my chamber experiments (Chapters 3 and 4) was very surprising. One of the main studies I was using as a guide had demonstrated that the $F_{E_{NO}}$ decreased significantly as the $F_{I_{O_2}}$was decreased from 0.30 to 0.05 (Dweik et al. 1998). After reading this manuscript over and over, I realized that (a) the authors measured $F_{E_{NO}}$ during tidal breathing but did not control for hyperventilation [*n.b.,* the partial pressure of CO_2 decreased as $F_{I_{O_2}}$decreased, indicating that hyperventilation occurred], which lowers $F_{E_{NO}}$ independent of hypoxia; and (b) only a small proportion of subjects could tolerate the 0.05 $F_{I_{O_2}}$due to the strong hyperventilation response, meaning that the dataset was incomplete at the lowest $F_{I_{O_2}}$condition and that fitting a curve through the data was not

appropriate. Rather than repeating this experiment with a single-breath method to measure the $F_{E_{NO}}$, I chose to perform a meta-analysis of previous studies that measured $F_{E_{NO}}$ in normobaric hypoxia. This analysis allowed me to synthesize all of the available data to formally test whether or not normobaric hypoxia decreases the $F_{E_{NO}}$.

F.3 Introduction

Nitric oxide (NO) is a gaseous signaling molecule with a diverse set of functions in the human body, including airway and vascular smooth muscle relaxation, ventilation-perfusion matching, neurotransmission, and host defense (reviewed in; Dweik 2006). The main sites of NO production are the endothelium of the vasculature and the epithelium of the lungs and conducting airways (Le Cras and McMurtry 2001). Some of this endogenous NO is exhaled in the breath of humans, and it can be measured at the mouth or nose (American Thoracic Society and European Respiratory Society 2005). Exhaled NO is typically measured as a fraction of exhaled NO ($F_{E_{NO}}$, often measured in parts per billion, ppb) or as a partial pressure of exhaled NO ($P_{E_{NO}}$, often measured in nmHg). The concentration of NO in exhaled breath summarizes the production, transfer, and consumption of NO in the lungs (Brown et al. 2006).

Exhaled NO is usually measured at or near sea level; however, there is much interest in the role that exhaled NO might have in hypoxia adaptation (Beall et al. 2001) and hypoxia acclimatisation (MacInnis et al. 2012a). The hypoxia of high altitude (hypobaric hypoxia) results from a lower barometric pressure (P_B), which decreases the ambient partial pressure of oxygen (P_{O_2}). In contrast, normobaric hypoxia can be generated in a laboratory by lowering the fraction of inspired oxygen ($F_{I_{O_2}}$) and maintaining the ambient P_B. While not necessarily eliciting equivalent physiological responses (reviewed by Conkin and Wessel 2008 and Richard and Koehle 2012), equivalent inspired partial pressures of oxygen ($P_{I_{O_2}}$) are obtainable from the two modes of hypoxia. Because O_2 is a substrate in the production of NO via the L-arginine pathway, cellular O_2 concentrations are thought to regulate the enzymatic production of NO (Dweik et al. 1998). Consequently, if hypoxia limits the endogenous production of NO, exposure to either mode of hypoxia could be expected to result in lower rates of NO production and lower values of $P_{E_{NO}}$.

That hypobaric hypoxia decreases the $F_{E_{NO}}$ is a common finding among many studies (e.g., Brown et al. 2006; Hemmingsson and Linnarsson 2009); however, studies of $F_{E_{NO}}$ in response to normobaric hypoxia have produced varied results (e.g., Schmetterer et al. 1997; Donnelly et al. 2011). Thus, the factors (e.g., hypoxia, hypobaria, an interaction of the two conditions, or some other factor(s)) responsible for the decreased $P_{E_{NO}}$ observed in hypobaric hypoxia are unclear.

To date, there has been no systematic review of the literature pertaining to the effect of acute normobaric hypoxia on the $F_{E_{NO}}$ measured from humans. To investigate this aspect of NO physiology, we employed a random effects meta-analysis using the summary data from the available published studies.

F.4 Methods

Collection of data

We investigated available published studies examining the effects of acute normobaric hypoxia on oral exhaled NO from healthy conscious humans (Table F.1 and Table F.2). Studies were identified through searches of PubMed and Google Scholar using combinations of the following terms as queries: "normobaric," "hypoxia," and "exhaled nitric oxide." Additional studies were obtained from the references of identified papers. All identified studies published in English before November 2012 were reviewed. To be included in the meta-analysis, each study needed to report the effect sizes (or the means and standard deviations) for comparisons of groups measured at baseline (normoxia) and during normobaric hypoxia. As the techniques for measuring exhaled NO varied between the studies, we limited our analysis to those studies with consistent protocols across conditions: we excluded studies that measured exhaled NO during tidal breathing without controlling for the differences in flow rate that would be expected from exposure to hypoxia, as exhaled NO varies greatly depending on the exhaled flow rate (Iwamoto et al. 1994) and flow rate is greater in hypoxia than normoxia.

Conversion of data to PENO

Exhaled NO is typically measured as a $F_{E_{NO}}$ or a $P_{E_{NO}}$. To allow for comparisons across studies, we converted all $F_{E_{NO}}$ values to $P_{E_{NO}}$ values using the provided P_B and the following equation:

$$P_{E_{NO}} \text{ (nmHg)} = F_{E_{NO}} \text{ (ppb)} * (P_B \text{ (mmHg)} - 47 \text{ (mmHg)}) / 1000$$

If P_B was not stated, and the study took place at or near sea level, a value of 760 mmHg was assumed. As this value was used for the calculation of baseline and hypoxic $P_{E_{NO}}$ measures, it did not affect the relative difference between the two measurements.

Analysis of summary data

A random-effects meta-analysis was used to determine whether acute normobaric hypoxia affected the $P_{E_{NO}}$. The duration of exposure to hypoxia was less than 30 minutes for six of the nine studies. For the studies with a longer duration, the mean $P_{E_{NO}}$ values from the first hours of a 6-hour exposure (MacInnis et al. 2012a; Chapter 3) and a 24-hour exposure (Faiss et al. 2012) were used in the analysis. The mean $P_{E_{NO}}$ from hour 12 was used from the ninth study (MacInnis et al. 2014; Chapter 4), as no intermediate measures of exhaled NO were collected. If exhaled NO was measured at multiple $P_{I_{O_2}}$, the lowest $P_{I_{O_2}}$ was chosen to maximize the potential effect of hypoxia.

Using the random-effects model (DerSimonian 1986) from RevMan 5.0 (Review Manager, Copenhagen), the standardized mean differences (SMD; Hedges' adjusted G), the 95% confidence intervals of the SMD, and the weight of each study were calculated (see Higgins and Green (2011) for formulas). A p-value less than 0.05 was considered statistically significant. The I^2 index was used to quantify heterogeneity. Data are presented as means (standard deviation) unless otherwise stated.

F.5 Results

Included studies

A total of 11 studies reporting the effects of acute normobaric hypoxia on exhaled NO from conscious humans were identified. Nine of these studies were included in the meta-analysis. Two of the included studies had two groups, resulting in the inclusion of 11 groups.

Two studies (Dweik et al. 1998; Busch et al. 2001) were excluded because exhaled NO was measured during tidal breathing without controlling for differences in flow rates across levels of $F_{I_{O_2}}$ (Iwamoto et al. 1994). Details of the protocols of each study are provided in Table F.1.

Table F.1 Summary of the protocols used in the included studies.

Study	Description of technique and analysis					
	Breathing pattern	Flow rate (mL/s)	On-line/ Off-line?	Duration (minutes)	Reported adherence to ATS/ERS guidelines?	Nitric oxide analyzer
Donnelly et al. 2011	SB	50	On	25	Yes[b]	NIOX MINO
Faiss et al. 2012	SB	50	On	60	No	NIOX MINO
Hemmingsson and Linnarsson 2009	SB	50	On	10	Yes[b]	NIOX MINO
MacInnis et al. 2012 (Chapter 3)	SB	50	On	60	Yes[b]	Bedfont NObreath
MacInnis et al. 2014 (Chapter 4)	SB	50	On	720	Yes[b]	NIOX MINO
Schmetterer et al. 1997	SB/BH	NR	On	10	No	Model 8840, Monitor Labs
St. Croix et al. 1999	SB	46	On	5	No	Sievers Model 280 NOA
Tsujino et al. 1996	TB[a]	NR	On	3	No	Model 42S
Verges et al. 2005	SB	170	On	30	Yes[c]	Cosma analyzer

[a] Breathing patterns were controlled across the two conditions.
[b] ATS/ERS 2005
[c] ATS 1999
SB, single breath; BH, breath hold; TB, tidal breathing; NR, not reported.

Analysis of summary data for exposure to hypoxia and PENO

In total, the nine included studies assessed the effect of acute normobaric hypoxia on the $P_{E_{NO}}$ of 117 different subjects (the same 24 subjects were assessed twice by MacInnis et al. unpublished). The average baseline and hypoxic $P_{I_{O_2}}$ values were 149 (3) mmHg and 89 (13) mmHg, respectively (Table F.2). The median duration of exposure was 30 minutes (**Table F.2**). The included studies did not report effect sizes; therefore, all calculations are based on summary data. For 10 of the 11 groups, the SMDs in the $P_{E_{NO}}$ between baseline and normobaric hypoxia were not significantly different from zero (Table F.2; Figure F.1). The overall SMD for the 11 groups was -0.05 (95% CI = -0.32, 0.23; Figure F.1), which was also not significantly different from an SMD of zero ($z = 0.33$; $p = 0.74$). Thus, acute normobaric hypoxia did not affect the $P_{E_{NO}}$ relative to baseline. The I^2 value was 22%, suggesting low variation in the effects of normobaric hypoxia across studies. Removing the one study that reported a significant decrease in the $P_{E_{NO}}$ in response to acute normobaric hypoxia **(Schmetterer et al. 1997)** decreased the I^2 value to 0%, suggesting the results from the remaining eight studies were highly similar.

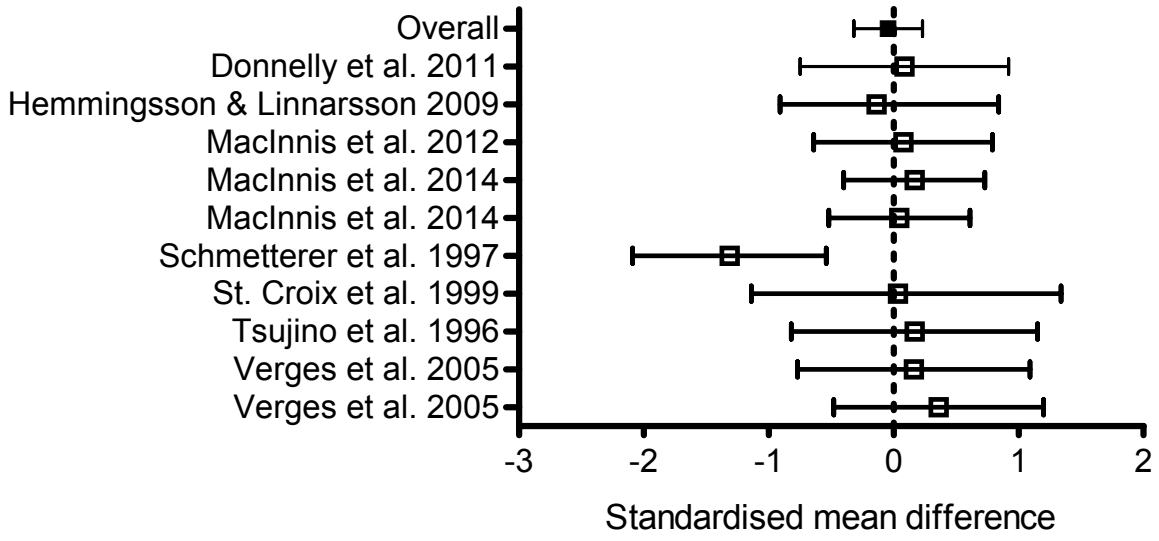

Figure F.1 A forest plot of the standardised mean differences (SMD) of the $P_{E_{NO}}$ between baseline and acute exposure to normobaric hypoxia. Lines represent 95% confidence intervals of the SMD. The black box represents the average SMD for all included studies (see Table F.2 for each study's weight in the calculation of the overall SMD). A positive SMD indicates that mean $P_{E_{NO}}$ increased during exposure to acute normobaric hypoxia and *vice versa*.

Table F.2 The summary data collected from each study and the random effects meta-analysis data that were generated for each study.

Authors	Summary Data					Meta-analysis data		
	n	$F_{I_{O_2}}$	$P_{I_{O_2}}$ (mmHg)[a]	$P_{E_{NO}}$ (nmHg)	$P_{E_{NO}}$ SD (nmHg)	SMD	95% CI of SMD	Weight (%)
Donnelly et al. 2011	11	0.21	150	18.8	11.4	0.09	-0.75, 0.92	8.5
	11	NR[b]	NR[b]	21.0[d]	12.4[d]			
	11	NR[c]	NR[c]	19.9	12.8			
Faiss et al. 2012	10	0.21	140	15.2	8.5	-0.03	-0.91, 0.84	7.9
	10	0.15	99	14.9	9.2			
Hemmingsson and Linnarsson 2009	8	0.21	149	18.2	2.2	-0.34	-1.33, 0.65	6.5
	8	0.15	104	17.6[d]	2.6[d]			
	8	0.11	80	17.4	2.3			
MacInnis et al. 2012	15	0.21	150	8.8	5.6	0.08	-0.64, 0.79	10.8
	15	0.12	86	9.3	6.9			
MacInnis et al. 2014	24	0.21	150	15.4	7.7	0.04	-0.52, 0.61	15.0
	24	0.14	90	15.7	6.2			
	24	0.21	150	15.8	6.4	0.17	-0.40, 0.73	15.0
	24	0.14	90	16.9	6.5			
Schmetterer et al. 1997	16	0.21	150	21.2[e]	1.9[e]	-1.31	-2.09, -0.54	9.6
	16	0.10	71	18.5	2.1			
St. Croix et al. 1999	5	0.21	150	22.4	17.3	0.10	-1.14, 1.34	4.4
	5	0.14	100	24.5	19.7			
Tsujino et al. 1996	8	0.21	150	9.6	8.9	0.17	-0.82, 1.15	6.6
	8	0.10	71	11.3	10.5			
Verges et al. 2005	11	0.21	150	8.9	4.8	0.36	-0.48, 1.20	8.4
	11	0.15	107	11.1	6.8			
	9[f]	0.21	150	14.0	12.4	0.16	-0.77, 1.09	7.2
	9[f]	0.15	107	16.0	11.5			

[a] If P_B was not reported, $P_{I_{O_2}}$ was calculated from an assumed P_B of 760 mmHg;

[b] Subjects' pulse oxygen saturations were maintained at ~90% ($P_{I_{O_2}}$ was not provided);

[c] Subjects' pulse oxygen saturations were maintained at ~80% ($P_{I_{O_2}}$ was not provided);

[d] These data were not used in the meta-analysis;

[e] These values are the mean of three means calculated from repeated baseline measures;

[f] Subjects were diagnosed with exercise induced arterial hypoxemia, but this diagnosis was independent of the concentration of exhaled NO measured under resting conditions.

n, sample size; SD, standard deviation; SMD, standardised mean difference of the $P_{E_{NO}}$ between baseline and hypoxia; CI, confidence interval of the SMD; NR, not reported.

F.6 Discussion

Acute normobaric hypoxia did not affect the $P_{E_{NO}}$ measured at the mouth from humans in this meta-analysis. This finding is supported by the individual results of many of the included studies: acute normobaric hypoxia did not significantly affect the $P_{E_{NO}}$ relative to baseline measures in eight of nine studies (and 10 of 11 groups). The one study that reported a decreased $P_{E_{NO}}$ in normobaric hypoxia (Schmetterer et al. 1997) was difficult to interpret: the $F_{E_{NO}}$ was lower while subjects breathed 10% compared to baseline (21% oxygen), but the $F_{E_{NO}}$ was also lower while subjects breathed 20% oxygen, which is not a strong a hypoxic stimulus.

Intra-study variation in environmental conditions between low- and high-altitude sampling sites, inter-study differences in measurement techniques, and questions regarding the suitability of different NO analyzers for the measurement of the $P_{E_{NO}}$ in hypobaric conditions precluded a meta-analysis of the effects of hypobaric hypoxia on the $P_{E_{NO}}$; however, that the $P_{E_{NO}}$ is reduced in hypobaric hypoxia relative to sea level is a common finding at high (e.g. Brown et al. 2006) and moderate (e.g. Caspersen et al. 2012) altitudes.

The decreased $P_{E_{NO}}$ in hypobaric hypoxia is often attributed to the hypoxia (i.e. the low $P_{I_{O_2}}$); however, the present meta-analysis (and the majority of individual studies) demonstrated that acute normobaric hypoxia does not affect the $P_{E_{NO}}$. Extrapolating this finding to hypobaric hypoxia, a causal relationship between the $P_{I_{O_2}}$ and the $P_{E_{NO}}$, independent of an effect of P_B, would be unexpected. It is more likely that the decreased $P_{E_{NO}}$ observed in hypobaric hypoxia is caused by a relatively low P_B, an interaction between a low P_B and a low $P_{I_{O_2}}$, or some other factor(s). The possibility that P_B and not $P_{I_{O_2}}$ affects the $P_{E_{NO}}$ is further supported by three repeated-measures studies that reported decreased $P_{E_{NO}}$ in hypobaric hypoxia but a similar $P_{E_{NO}}$ in an equivalent normobaric hypoxia (Hemmingsson and Linnarsson 2009; Donnelly *et al*. 2011; Faiss et al. 2012). While studies of hypobaric hypoxia typically use greater durations of hypoxic exposure than studies of normobaric hypoxia (e.g., Donnelly et al. 2011: ~3 weeks of hypobaric hypoxia, and 25 minutes of normobaric hypoxia), Hemmingsson and Linnarsson (2009) and Faiss et al. (2012) exposed subjects to equal durations of normobaric and hypobaric hypoxia, controlling for this potential confounding factor.

It is outside the scope of this study to discuss the possible explanations for a role of P_B on the $P_{E_{NO}}$; however, that the two modes of hypoxia could elicit different physiological responses is not unprecedented (Girard et al. 2012; Millet et al. 2012b).

Exhaled NO has been investigated as a factor in the development of high altitude pulmonary edema (HAPE) and acute mountain sickness (AMS). After a rapid ascent to 4559 m, subjects who developed HAPE exhaled less NO compared to HAPE-resistant subjects at the same altitude (Duplain et al. 2000). Additional subjects with a history of HAPE (but without signs of HAPE on that particular ascent) also had a significantly lower mean exhaled NO compared to HAPE-resistant subjects. Similarly, male subjects who developed AMS during a brief normobaric hypoxia exposure had a lower $F_{E_{NO}}$ than subjects who did not develop AMS (MacInnis et al. 2012a). There was no association between $F_{E_{NO}}$ and AMS upon exposure to hypobaric hypoxia (Brown et al. 2006), but the duration of exposure (3 hours) was relatively short, and the incidence of AMS was relatively low.

The physiological mechanisms linking NO production in the lungs and conducting airways with susceptibility to altitude illness have yet to be fully elucidated. One possibility is that innate variation in exhaled NO production is related to differences in blood oxygenation (Tsuchiya et al. 2000). To support this hypothesis, the inhalation of NO increased the blood oxygen saturation of patients with HAPE and also reduced the severity of HAPE (Scherrer et al. 1996; Anand et al. 1998). Similarly, the inhalation of NO reduced the severity of AMS, although subjects' oxygen saturations were not measured before and after NO inhalation (Zheng et al. 2007). More research is needed to understand the physiological significance of innate differences in exhaled NO.

There are several limitations to our analysis. Firstly, slightly different methods were used to measure exhaled NO across studies, and we could not control for all of this variation; however, excluding those studies that measured exhaled NO during tidal breathing without controlling for differences in expiratory flow rates eliminated the most significant differences in measurement technique. Secondly, the data included in the meta-analysis were from studies of different durations; therefore, we cannot speculate on the effect of prolonged exposure to normobaric hypoxia on the $P_{E_{NO}}$ relative to baseline. Studies of 12 hours (MacInnis et al. 2014) and 24 hours (Faiss et al. 2012) suggest that there is no effect of normobaric hypoxia on the $P_{E_{NO}}$

in at least the first 24 hours of exposure. Thirdly, our results are likely specific to the $F_{I_{O_2}}$ of included studies; therefore, our results cannot be extrapolated to more extreme hypoxic exposures (e.g., an $F_{I_{O_2}}$ of 0.05).

F.7 Conclusions

This meta-analysis indicates that acute exposure to normobaric hypoxia does not affect the $P_{E_{NO}}$ measured orally from humans. As several studies have reported decreased $P_{E_{NO}}$ in hypobaric hypoxia, our analysis suggests that a factor other than hypoxia might mediate the decrease in $P_{E_{NO}}$ at altitude. Further studies of the $P_{E_{NO}}$ in hypobaric hypoxia and hypobaric normoxia are necessary to understand the effects of P_B on the $P_{E_{NO}}$. The results of this study will aid in the interpretation of the association between altitude-illness susceptibility and exhaled NO, but more research is needed to elucidate this potential relationship.

Appendix G Bivariate meta-regression models for Chapter 7

All of the meta-regressions detailed below build on a bivariate random-effects model that estimates the logit transforms of sensitivity and false positive rate (FPR, which is simply 1 - specificity; Reitsma et al. 2005) and were implemented using the MADA package in R (Doebler and Holling 2013). This model assumes that the logit sensitivity ($\theta_{P,i}$) and FPR ($\theta N_{,i}$) are normally distributed around a common mean value:

$$\begin{pmatrix} \hat{\theta}_{P,i} \\ \hat{\theta}_{N,i} \end{pmatrix} \sim N\left(\begin{pmatrix} \theta_{P,i} \\ \theta_{N,i} \end{pmatrix}, \Sigma + C_i \right)$$

where $\Sigma = \begin{pmatrix} \sigma^2_{P,i} & \sigma_{PN,i} \\ \sigma_{PN,i} & \sigma^2_{N,i} \end{pmatrix}$ and $C_i = \begin{pmatrix} s^2_{P,i} & 0 \\ 0 & s^2_{N,i} \end{pmatrix}$

Moderating variables can then be added to the model as in a multivariate regression and these models can be fit using likelihood estimation. Given concerns about statistical power with only 18 studies, the three predictors were tested one at a time. The fit of the models should be compared with caution however, as different numbers of data points contributed to the summary model and the regression models. Regression models included fewer studies due to incomplete reporting of data (*i.e.,* studies with not stated, NS, entries in Table 7.2 were omitted from analysis). Thus, there are three bivariate meta-regressions that we tested: (i) controlling for the altitude at diagnosis (all studies included), (ii), controlling for exposure to altitude in the AMS history (studies that did not report exposure were omitted), and (iii) controlling for medications during the AMS history and outcome assessments (studies that did not report medical prophylaxis use were omitted).

The output of the bivariate model includes the logit sensitivity (θ_P) and the logit FPR (θ_N), estimates of between study variability for sensitivity (σ_P^2) and FPR (σ_N^2) and the correlation between these factors (based on the covariance, σ_{PN}). Logit sensitivity and FPR are reverse transformed to give point estimates for the overall sensitivity and FPR. Similarly, estimates of the variance are used to calculate 95% confidence intervals for sensitivity and FPR. These estimates of variance can be combined with the covariance to estimate the summary receiver

operator characteristic (ROC) curve that accounts for the relationship between sensitivity and FPR.

G.1 Model 1: Controlling for altitude at diagnosis.

In order to control for the effects of altitude at diagnosis in our model, we regressed the maximum reported altitude (in km) from each study onto the sensitivity and FPR. These results are shown in Table G.1. There were no significant effects of diagnosis altitude on either sensitivity or the false positive rate, shown by the relatively flat lines in Figure G.1 The modeled effects of altitude on the logit sensitivity (solid line) and FPR (dashed line).. In the figure, the logit-value is shown on the y-axis and diagnosis altitude is shown on the x-axis. A logit of zero is equivalent to a sensitivity or FPR = 0.5 (that is a logit of zero represents chance for binary outcomes). The solid line shows the sensitivity at each altitude predicted by the model. The dashed line shows the predicted FPR as a function of altitude.

Model 1: (logit(SENS),logit(FPR)) ~ altitude (in kilometres), estimation method = REML.

Table G.1 Bivariate meta-regression of altitude (in km) onto logit sensitivity and FPR.

Fixed-effect coefficients			
	Estimate	Std. Error	95% CI
Intercepts			
$\beta_{sens.int}$	-0.068	0.69	[-1.43, 1.29]
$\beta_{fpr.int}$	-1.014	0.53	[-2.05, 0.03]$^{\#}$
Effects of altitude at diagnosis			
$\beta_{sens.alt}$	0.012	0.15	[-0.29, 0.31]
$\beta_{fpr.alt}$	0.014	0.12	[-0.22, 0.24]
Random-effects coefficients			
	Std. Dev	Corr(logit(FPR))	
Logit(SENS)	0.79	0.76	
Logit(FPR)	0.60	1.00	
Log(Likelihood)	***AIC***	***BIC***	
18.53	-23.06	-11.96	

$^{\#}$denotes $p = .06$.

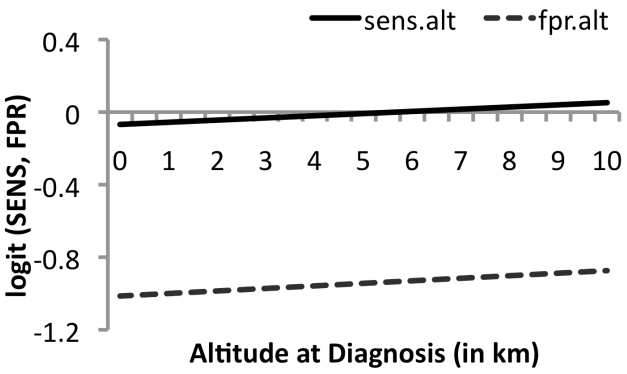

Figure G.1 The modeled effects of altitude on the logit sensitivity (solid line) and FPR (dashed line).

G.2 Model 2: Controlling for exposure to altitude in AMS history

In order to control for the exposure to altitude in our model, we regressed a dummy coded variable of altitude exposure onto the sensitivity and FPR. In this model, we coded the predictor variable such that 0 = no control for previous exposure (i.e., subjects may or may not have been exposed) and 1 = all subjects had previous exposure. Studies that did not explicitly state whether subjects were exposed to altitude were omitted from the analysis. Statistical results are presented in Table G.2. There were no significant effects of altitude exposure on either sensitivity or the FPR. The FPR intercept was significant, but this simply suggests that when altitude exposure was controlled, the logit(FPR) was significantly different from zero which translates into an FPR significantly lower than chance. The significant intercept and a lack of significant slope suggests that FPR was quite low across studies, regardless of controlling for previous exposure to altitude. Figure G.2 shows the predicted logit for studies that did control for previous exposure ("0") and the predicted logit when all subjects had previous exposure to altitude ("1").

Model 2: (logit(SENS),logit(FPR)) ~ altExp {0 = subjects may/may not have experienced previous exposure, 1 = all subjects had previous exposure}, estimation method = REML.

Table G.2 Bivariate meta-regression of controlling for exposure to altitude onto logit sensitivity and FPR.

Fixed-effect coefficients			
	Estimate	Std. Error	95% CI
Intercepts			
$\beta_{sens.int}$	-0.173	0.51	[-1.17, 0.82]
$\beta_{fpr.int}$	-0.898	0.42	[-1.71, -0.08]*
Effects of controlling for exposure			
$\beta_{sens.exp}$	0.723	0.61	[-0.48, 1.93]
$\beta_{fpr.exp}$	0.012	0.51	[-0.98, 1.01]
Random-effects coefficients			
	Std. Dev	Corr(logit(FPR))	
Logit(SENS)	0.87	0.84	
Logit(FPR)	0.72	1.00	
Log(Likelihood)	***AIC***	***BIC***	
13.43	-12.86	-5.22	

*denotes $p < .05$

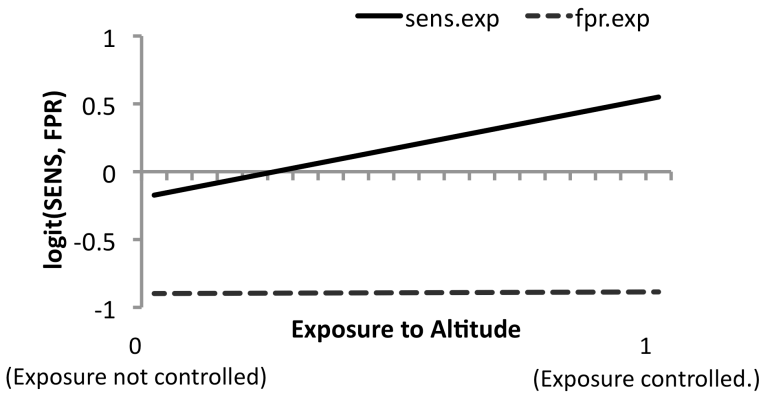

Figure G.2 The modeled effects of controlling for exposure on the logit sensitivity (solid line) and FPR (dashed line). Studies in which exposure was not controlled were coded as "0"; controlled studies were coded as "1" in the model.

G.3 Model 3: Controlling for medications

In order to control for the effects of prophylactic medication use in our analysis, we coded studies based on control of acetazolamide and dexamethasone (0 = did not allow use, 1 = did not control for use), these results are shown in Table G.3 and Figure G.3.

We also performed this analysis controlling for the use of any medications that might affect Lake Louise Scores (i.e., not just acetazolamide and dexamethasone, but also analgesics such as ibuprofen and acetominophen; 0 = did not allow use, 1 = did not control for use), these results

are shown in Table G.4. Studies that did not explicitly state medication use were omitted from both analyses.

The results of these different analyses were congruent in the direction of the effects although effects differed slightly in magnitude. For sensitivity and FPR, the intercepts of neither model were significant, although the sensitivity intercept approached significance. In Model 3A, the slopes for both sensitivity and FPR were significant, suggesting that controlling acetazolamide/dexamethasone use significantly increased the sensitivity of diagnosis, but also increased the FPR as well. Results suggested that controlling for pharmaceutical prophylaxis generally increased sensitivity but also increased the FPR. Details of both models are reported below, but only the results controlling for acetazolamide/dexamethasone are reported in the main paper.

Model 3A: (logit(SENS),logit(FPR)) ~ drugUse {0 = did not allow use of acetazolamide / dexamethasone, 1 = did not control for use of medications}, estimation method = REML.

Table G.3 Model 3A: Bivariate meta-regression of controlling for acetazolamide use onto logit sensitivity and FPR.

Fixed-effect coefficients			
	Estimate	Std. Error	95% CI
Intercepts			
$\beta_{sens.int}$	0.661	0.40	[-0.12, 1.44]
$\beta_{fpr.int}$	-0.458	0.28	[-1.01, 0.09]
Effects of controlling for medication			
$\beta_{sens.drug}$	-1.068	0.48	[-2.00, -0.14]*
$\beta_{fpr.drug}$	-0.748	0.34	[-1.41, -0.09]*
Random-effects coefficients			
	Std. Dev	Corr(logit(FPR))	
Logit(SENS)	0.68	0.52	
Logit(FPR)	0.47	1.00	
Log(Likelihood)	***AIC***	***BIC***	
16.33	-18.66	-10.42	

*denotes $p < .05$.

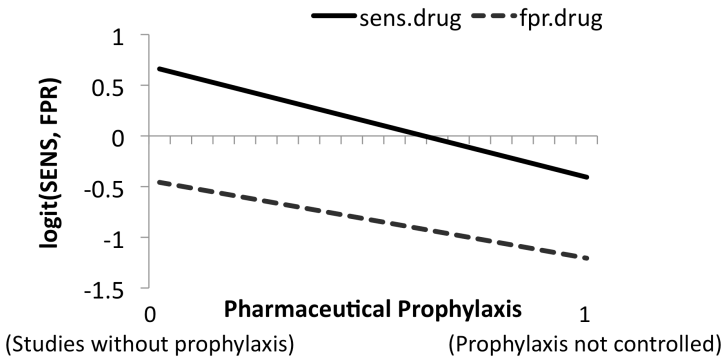

Figure G.3 The modeled effects of controlling for acetazolamide/dexamethasone use on the logit sensitivity (solid line) and FPR (dashed line). Studies in which prophylaxis was not allowed during AMS outcome measures are coded as "0"; studies where prophylaxis was uncontrolled are coded as "1" in the model.

Model 3B: (logit(SENS),logit(FPR)) ~ drugUse {0 = did not allow use of any prophylactic medication, 1 = did not control for use of medications}, estimation method = REML.

Table G.4 Model 3B: Bivariate meta-regression of controlling for all pharmaceutical prophylaxis onto logit sensitivity and FPR.

Fixed-effect coefficients			
	Estimate	Std. Error	95% CI
Intercepts			
$\beta_{sens.int}$	0.787	0.55	[-0.29, 1.86]
$\beta_{fpr.int}$	-0.292	0.42	[-1.11, 0.52]
Effects of controlling for medication			
$\beta_{sens.drug}$	-0.943	0.61	[-2.14, 0.25]
$\beta_{fpr.drug}$	-0.795	0.46	[-1.69, 0.10]$^{\#}$
Random-effects coefficients			
	Std. Dev	Corr(logit(FPR))	
Logit(SENS)	0.82	0.73	
Logit(FPR)	0.61	1.00	
Log(Likelihood)	***AIC***	***BIC***	
17.51	-21.01	-11.68	

$^{\#}$denotes $p = .08$.