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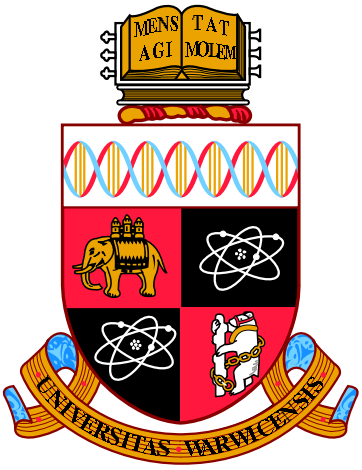

Hypoxia Adaptation and Exercise Performance at Altitude

by

Heather Louise Riley

Thesis

Submitted to the University of Warwick
for the degree of

Doctor of Philosophy
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Declarations

This thesis is presented in accordance with the regulations for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree. The work in this thesis has been undertaken by myself except where otherwise stated. The following paper has been published during the course of this PhD project:

Denny Z Levett, Bernadette O Fernandez, **Heather L Riley**, Daniel S Martin, Kay Mitchell, Carl A Leckstrom, Can Ince, Brian J Whipp, Monty G Mythen, Hugh E Montgomery, Mike P Grocott, and Martin Feelisch. The role of nitrogen oxides in human adaptation to hypoxia. *Sci Rep-Uk*, 1:109, Jan 2011.

Abstract

Hypoxia is defined as a deficiency in the amount of oxygen reaching the tissues, and is a common problem in critically ill patients. It is not currently possible to predict how well an individual will adapt to hypoxic conditions, and patients presenting with hypoxia are often treated with supplemental oxygen. However, this blanket-treatment approach is not suitable in all cases and a more personalised approach is required.

My thesis project builds on information acquired during the Caudwell Xtreme Everest (CXE 2007) expedition, where over 200 volunteers trekked to Everest Base Camp. CXE uses studies on healthy volunteers exposed to extreme environments to aid in the understanding of the complicated issues concerned with critical illness, and aims to use these findings to improve the treatment of critically ill patients, without putting them directly at risk.

My thesis project has combined physiological information acquired during CXE with biochemical information measured in plasma samples taken during CXE. Performance at altitude has been used as a proxy for hypoxia adaptation, with individuals who show a small loss of performance at altitude compared to London assumed to be adapting better compared to individuals who show a larger loss.

Analysis of the physiological and biochemical data for a core group of 24 individuals has culminated in the application of multiple linear regression to produce a number of models capable of predicting the key changes in physiological response as a function of a number of biochemical metabolites. These models have been used to identify a set of biochemical metabolites to measure in a further 190 individuals, to allow validation and training of the models on a larger sample size. These models can then be adapted for use in a critical illness environment, to allow the prediction of how well an individual will adapt to hypoxic conditions.

Abbreviations

8-isoPGF$_{2\alpha}$	8-iso-Prostaglandin F$_{2\alpha}$
AMS	Acute Mountain Sickness
ANOVA	Analysis of Variance
ANP	Atrial Natriuretic Peptide
ATP	Adenosine Triphosphate
BNP	Brain Natriuretic Peptide
C_V	Coefficient of Variation
cGMP	Cyclic Guanine Monophosphate
CI	Critical Illness
CNP	C-type Natriuretic Peptide
CPX	Cardiopulmonary Exercise Testing
CRP	C-Reactive Protein
CXE	Caudwell Xtreme Everest 2007
DBP	Diastolic Blood Pressure
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid), also known as Ellman's reagent
EBC	Everest Base Camp

EDA	Exploratory Data Analysis
EPO	Erythropoietin
ET-1	Endothelin-1
GIP	Gastric Inhibitory Polypeptide
GLP-1	Glucagon-Like Peptide-1
GR	Glutathione Reductase
GSH	Reduced Glutathione
GSSG	Oxidised Glutathione
H_0	Null hypothesis
H_1	Alternate hypothesis
HACE	High Altitude Cerebral Edema
HAPE	High Altitude Pulmonary Edema
HIF	Hypoxia-inducible Factor
HNE	4-hydroxy-2-nonenal
HSP-70	70 kilodalton Heat Shock Protein
ICU	Intensive Care Unit
IFN-γ	Interferon-Gamma
IL	Interleukin
LaT	Lactate Threshold
MIF	Macrophage Migration Inhibitory Factor
MLR	Multiple Linear Regression

MS	Mean square
NO	Nitric Oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NOS	Nitric Oxide Synthase
NOx	Addition of nitrate and nitrite
PAI-1	Plasminogen Activator Inhibitor-1
proCO	Protein Carbonyls
R^2	Coefficient of determination
RNNO	N-nitroso compounds
ROS	Reactive Oxygen Species
RSNO	S-nitrisothyols
RxNO	Addition of RSNO and RNNO
SBP	Systolic Blood Pressure
SLR	Simple Linear Regression
SS	Sum of Squares
TNF-α	Tumor Necrosis Factor Alpha
VEGF	Vascular Endothelial Growth Factor
VO_2max	Maximal physical output during CPX testing
XA	Xtreme Alps
XE2	Xtreme Everest 2

Chapter 1

Introduction

1.1 Project motivation

Intensive care units (ICUs) treat patients with life-threatening conditions. Patients admitted to ICUs require constant monitoring and treatment from specialised equipment to keep the body functioning. Critical care is increasingly becoming a part of people's lives, with one in four people expected to receive treatment in an ICU during their lifetime. Over 120 000 patients were admitted to ICUs in the UK in 2006 alone [CASE, 2012].

Many critically ill patients suffer from oxygen starvation of one kind or another, known as *hypoxia*, which can be caused by a lack of oxygen delivery to the cells, or by an inability to utilise the oxygen delivered to the cells, due to conditions such as mitochondrial dysfunction. It is not currently possible to predict when an individual will have difficulty adapting to these conditions. An inability to adapt to a lack of oxygen can become increasingly serious in a very short period of time, and is even more dangerous when combined with other factors associated with critical illness. Currently, the main treatment for cellular hypoxia is to increase oxygen delivery via an oxygen mask. However, in some cases an overload of oxygen to the system can have no effect, or even cause harm, such as retinopathy of prematurity in newborn babies, which causes severe scarring and blindness due to both oxygen toxicity and hypoxia. It is therefore vital that doctors are able to track how well an individual is coping with hypoxic exposure, and to be able to foresee any difficulties they may have in adapting to these lower oxygen levels.

A more *personalised* approach to treating critically ill patients would be preferable to the current blanket-treatment approach, but is complicated by the lack of understanding of the process of cellular hypoxia adaptation, and by the

lack of data from critically ill patients. Invasive studies on critically ill patients are difficult to perform due to the highly unstable and serious nature of their condition. Any studies carry a high risk of making the patient's condition worse, and may be of no benefit to that individual in the short or longer-term.

Studies using cultured cells are of little use here, as the response varies significantly between cell and tissue types. The exposure to multiple stress pathways means that critically ill patients are not exposed to hypoxia in isolation, and many stress response pathways overlap and interact with one another, to produce a complex systems response, which we may not be able to replicate in an animal model system. Similarly, chamber studies expose individuals to hypoxia in isolation, and miss out on looking at interactive systems responses associated with critical illness.

Caudwell Xtreme Everest (CXE) is a research project coordinated by University College London (UCL) Centre for Altitude, Space and Extreme Environment Medicine (CASE). CXE uses studies on healthy volunteers exposed to extreme environments to aid in the understanding of the complicated issues concerned with critical illness, and aims to use these findings to improve the treatment of critically ill patients, without putting them directly at risk. During CXE 2007, individuals climbed to Everest Base Camp, with exercise performance tests and blood sampling administered during the ascent. Exercise performance (as measured via cardiopulmonary exercise testing) was used as a *proxy* for hypoxia adaptation, with individuals showing similar performance levels to those seen at London assumed to be the better adapters [CASE, 2012].

This thesis project builds on the work carried out during CXE 2007, by combining information from exercise performance tests carried out during the expedition with biochemical data derived from blood samples collected during the ascent. Several stages of statistical analysis have been carried out to increase the understanding of why some individuals adapt to hypoxia better than others. The information has been analysed together, with the aim of producing a model capable of predicting how well an individual will *perform*, and therefore *adapt* to the extreme environment at high altitude. It is hoped that these models can be adapted for use in a critical care environment, helping to track how well an individual is adapting to hypoxia, and being able to take necessary interventional treatment to aid individuals who are having difficulty in adapting to hypoxic exposure.

1.2 Background of the problem

1.2.1 Hypoxia

In humans, oxygen is required for respiration and energy generation within cells, and is vital for the life of most multicellular organisms on Earth. *Hypoxia* generally describes several conditions caused by the starvation of oxygen, and can occur naturally, for example during periods of strenuous exercise or during ascent to high altitude, where oxygen availability is reduced.

Most humans can ascend to around 2500m without experiencing any problems with hypoxic stress [McArdle et al., 2007]. However, when ascending further, individuals may begin to suffer from acute mountain sickness (AMS), and display symptoms similar to those of a mild case of flu or a hangover, which usually abate after a few days of acclimatisation [McArdle et al., 2007]. AMS can progress to more severe conditions such as high altitude cerebral edema (HACE), and high altitude pulmonary edema (HAPE). HACE is usually preceded by progressive AMS symptoms, and is a condition where the brain tissue swells due to leakage of fluid from the capillaries, and can cause death due to brain herniation [Bärtsch and Saltin, 2008]. HAPE is a condition where fluid collects in the lung, impairing gas exchange, and can lead to fatal respiratory failure. Both of these conditions are life-threatening, and individuals suffering from HACE or HAPE would need to be immediately moved to a lower altitude to recover.

Exposure to even a moderate altitude can cause significant problems for some individuals. The problem of adapting to altitude is of great interest due to millions of people travelling to high altitudes each year for business and holidays worldwide. During such travels, individuals rarely take sufficient time to acclimatise to the reduced oxygen availability and other challenges at altitude, resulting in a high susceptibility to illness [Bezruchka, 1992].

High altitude research has been carried out for well over 100 years, and as such, the general response to hypoxic exposure is well documented [Bärtsch and Saltin, 2008]. However, it is still not understood why some individuals seem prone to AMS when ascending to altitude and others are not. The ability to *predict* how well an individual will respond to hypoxic exposure could be invaluable in allowing the better preparation for exposure to higher altitude, and limiting the cases of HACE and HAPE that occur at altitude [Richalet et al., 2012; Maggiorini, 2001].

1.2.2 Hypoxia and critical illness

Hypoxia is a common problem in critically ill patients, and can contribute to, or be a result of the illness itself [Grocott et al., 2007]. Individuals vary in how well they can adapt to hypoxic conditions, and there is currently no way to predict how well an individual will cope when exposed to a hypoxic environment, in the same way that it is not possible to predict when an individual will become ill at altitude. The ability to track how well an individual is coping with increased hypoxic exposure could be very useful in a critical illness environment, where patients' conditions can deteriorate quickly, and swift responses to any lack of adaptation could mean the difference between a patient surviving or not.

1.2.3 General response to hypoxia

Acclimatisation is the process of an organism adapting to a gradual change in its environment, such as temperature, UV or hypoxic exposure, which enables it to continue to function in the altered environment. Full acclimatisation to high altitude usually requires at least a few weeks, and the time needed varies significantly between individuals, with some being unable to adapt to certain extreme altitudes at all.

Acute exposure to high altitude results in an increase in respiration (hyperventilation) and an increase in blood flow, to compensate for the lower oxygen levels available. The increase in blood flow seen at altitude is due to an increase in both blood pressure and heart rate, but not stroke volume (the volume of blood pumped from one ventricle of the heart with each beat) [McArdle et al., 2007]. Any change in ventilation leads to an alteration in the acid-base balance of the blood, and is strictly controlled.

Carbon dioxide is produced as a by-product of respiration within cells and diffuses into the blood, where it is transported to the lung and exhaled. Carbon dioxide enters the red blood cells and reacts with water to form carbonic acid (H_2CO_3). This reaction is catalysed by the enzyme carbonic anhydrase, which is found in virtually every cell, and in particularly high concentrations within red blood cells. Carbonic acid dissociates into bicarbonate (HCO_3^-) and hydrogen ions (H^+), as shown in equation 1.1. This reaction also occurs outside red blood cells, however, at a much slower pace. Any hydrogen ions formed combine with haemoglobin within the red blood cell, limiting the reduction in pH within the red blood cell and acting as a natural buffer. Bicarbonate ions diffuse out of the red blood cells into the plasma, whilst being replaced by chloride (Cl^-) ions, known as the *chloride shift*. At the lung, these reactions reverse; bicarbonate ions move into red blood cells and

combine with hydrogen ions to form carbonic acid. This acid is then broken down into carbon dioxide and water, and the carbon dioxide formed diffuses out of the red blood cells and into the alveoli, where it can be expelled via the lungs.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \tag{1.1}$$

Blood pH is tightly regulated to between 7.35-7.45, and hyperventilation removes carbon dioxide from the blood, shifting the chemical equilibrium shown in equation 1.1 towards the left to replace the CO_2 removed. This in turn raises the pH of the blood, known as *respiratory alkalosis*, which limits the extent to which ventilation can be increased in response to hypoxic exposure.

Continued exposure to altitude results in further acclimatisation processes occurring within the body. These adaptations to prolonged altitude exposure take longer to occur than initial exposure responses, and improve the body's tolerance to long-term altitude exposure. Long-term acclimatisation includes an increased excretion of bicarbonate, which removes excess base from the body and allows ventilation to remain elevated without causing alkalosis. Synthesis of haemoglobin and erythrocytes is increased due to increased production of the erythrocyte production-stimulating hormone erythropoietin (EPO). This increases the blood's oxygen carrying capacity, and is the most important long-term adaptation to altitude exposure [McArdle et al., 2007]. Bicarbonate, haemoglobin and EPO levels have been measured in individuals to track this acclimatisation during CXE 2007. A summary of the adaptive responses to hypoxic exposure is shown in Table 1.1.

Studies on native highlanders living on different continents have given insights into long-term evolutionary adaptation to high altitude. Interestingly, adaptation to hypoxia differs between populations. Andean highlanders from South America are characterised by high numbers of red blood cells and haemoglobin concentrations, whereas Tibetan highlanders show haemoglobin levels that are similar to comparable US sea level individuals [Beall et al., 1998]. Tibetans are therefore thought to have adapted other mechanisms for long-term adaptation to altitude, such as higher forearm blood flow and circulating nitric oxide products [Erzurum et al., 2007]. Further study of generational adaptation such as that seen in Tibetan highlanders may aid in our understanding of acclimatisation to chronic hypoxia.

	Acclimatisation		Adaptation
	Acute Response (minutes / hours)	Chronic Response (days / weeks)	Evolutionary Response (generations)
Cardiovascular	Increased heart rate Increased blood pressure Decreased plasma volume Increased haemoglobin	Stably increased heart rate Increased blood pressure Decreased plasma volume Increased haemoglobin	Normal plasma volume Normal haemoglobin (Tibetans) Increased haemoglobin (Andeans)
Ventilation	Hyperventilation Increased bicarbonate	Stable hyperventilation Decreased bicarbonate	Hypoventilation Normal bicarbonate
Performance	Decreased VO_2max^* Increased anaerobic respiration Increased catecholamines Increased mitochondrial density	Decreased VO_2max^* Increased anaerobic respiration Decreased catecholamines	Stable VO_2max^* Aerobic respiration

Table 1.1: A summary of the human response to exposure to high altitude. $^*VO_2max$ is the maximum capacity of an individual to utilise oxygen during incremental exercise, and is a measure of the fitness of that individual. Adapted from [McArdle et al., 2007]

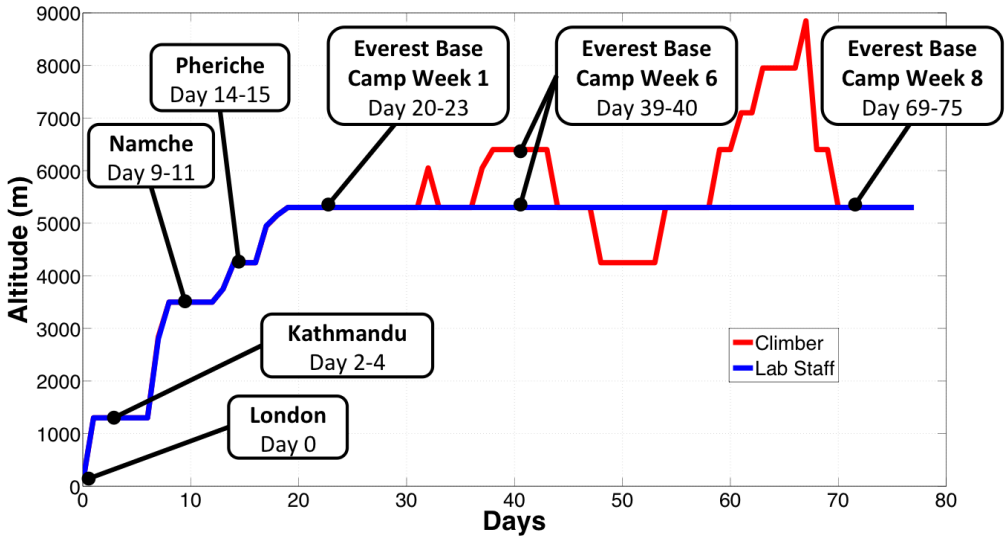

Figure 1.1: The ascent profile for participants of the Caudwell Xtreme Everest Expedition, 2007. The blue line indicates the general ascent profile for all individuals. Individuals denoted *lab staff* remained at Everest Base Camp at 5300m until the end of the expedition, and did not ascend further. The red line indicates where the 14 individuals of the *climber* group descended back down to Pheriche, and also ascended further than Base Camp, with 8 individuals summiting at 8848m. The labelled boxes indicate where and when testing took place during the ascent, and show the days over which sampling took place; London (75m), Kathmandu (1300m), Namche (3500m), Pheriche (4250m) and Everest Base Camp (5300m).

1.3 Caudwell Xtreme Everest 2007

Caudwell Xtreme Everest (CXE) 2007 was the largest ever field-study of human adaptation to hypoxia, and saw over 200 volunteers climb to Everest base camp (5300m). Various measurements such as cardiopulmonary exercise testing, blood sampling and muscle biopsies were taken at five different laboratories on the way up. A second *core* group of 24 doctors and scientists also took part in the ascent, consisting of 10 *lab staff* individuals, who stayed at Base Camp with the volunteer group, and 14 *climber* individuals, who ascended further and attempted to summit, with 8 succeeding [Grocott et al., 2007]. The ascent profile from the expedition is shown in Figure 1.1, which shows the basic ascent profile for all participants in blue, and where the climber group ascended further than base camp, in red. The laboratories where testing took place during the ascent are also marked; London (75m), Kathmandu (1300m), Namche (3500m), Pheriche (4250m) and Everest Base Camp (5300m).

The ascent exposed the team to multiple stresses, including reduced oxygen levels and increased exposure to cold and UV light. Critically ill patients are similarly exposed to multiple stresses, such as trauma, tissue hypoxia and infection [Grocott, 2008; Grocott et al., 2007]. As the responses to these different stresses would occur simultaneously, isolating one single aspect (such as hypoxia adaptation) is very difficult. Adaptive stress-response pathways also overlap and communicate with one another, to produce a complicated systems response. CXE 2007 aimed to increase the understanding of a very complex adaptation process, with the aim of using the results to aid the treatment of critically ill patients.

During the expedition only a small number of individuals suffered with altitude sickness. However, three became seriously ill and had to be taken to a lower altitude to recover. An additional individual also became ill, but later recovered. It was hoped that these four individuals would show some differences to other core team members in their response to altitude in one or more of the measurements. It was hoped that these differences may help identify why some individuals adapt to hypoxia better than others, to help in the understanding of differences in individual's adaptability to low oxygen levels.

The value of the data from CXE 2007 is that it shows how metabolites from many different biochemical pathways change with increasing altitude, including those associated with hypoxia adaptation, inflammation, metabolism and homeostasis. No other study has looked at such a vast array of biochemical metabolites at altitude, or has had the ability to combine this data with so many physiological

measurements. Therefore, this project offers a unique opportunity to gain insight into what is happening in the body as altitude increases, in multiple biochemical and physiological pathways at the same time.

1.3.1 Exercise performance testing

During CXE 2007, Cardiopulmonary Exercise Testing (CPX/CPET) was performed on each individual at different locations during the ascent. CPX testing measures an individual's ability to do exercise or *work* as a measure of their aerobic fitness, by assessing pulmonary and cardiac function as a measure of the whole-body response to performing exercise. CPX testing was used as part of the CXE study to assess each individual's ability to do work compared to sea level, and analyse how this changed as they ascended.

Performance was expected to decrease as the expedition progressed, due to decreasing oxygen availability as the climbers ascended. Oxygen is required by cells for respiration, the process by which complex organic substances such as carbohydrates, lipids and proteins are broken down in the presence of oxygen to produce adenosine triphosphate (ATP) [McArdle et al., 2007]. ATP is the *energy currency* of the cell and used to perform many of the cell's energy-requiring processes (such as *work*), and is therefore *vital* to the survival of the cell [Voet and Voet, 2004].

In the presence of sufficient oxygen, glucose is broken down via aerobic respiration, where 38 ATP molecules are generated from one glucose molecule, as shown in equation 1.2. However, if insufficient oxygen is available then anaerobic respiration is stimulated, which generates only 2 ATP per glucose molecule and 2 lactate molecules, as shown in equation 1.3.

$$C_6H_{12}O_6 + 38ADP + 38P_i + 6O_2 \rightarrow 6CO_2 + 44H_2O + 38ATP \tag{1.2}$$

$$C_6H_{12}O_6 + 2ADP + 2P_i \rightarrow 2C_3H_6O_3 + 2H^+ + 2H_2O + 2ATP \tag{1.3}$$

Aerobic breakdown of glucose is obviously preferred due to the much higher yield of ATP obtained, and supplies the majority of ATP up to the *anaerobic threshold*, where anaerobic breakdown of glucose occurs to supplement the aerobic breakdown of glucose as the work rate increases. As the climbers ascended during CXE 2007, oxygen availability decreased. CPX testing was used to assess how well each individual was adapting to the decreased oxygen levels available, with exercise performance being used as a model for hypoxia adaptation. Individuals who show a

large decrease in the ability to do work at high altitude were considered to be adapting poorly, as they are not able to do as much work compared to sea level with less oxygen available. Individuals who performed in a similar manner or showed a small decrease in their ability to do work compared to sea level were considered to be adapting well, as they were able to do a similar amount of physical work with less oxygen available, and therefore must be adapting to use the lower levels of oxygen available more productively.

1.3.2 Other methods of studying human adaptation to hypoxia

Hypobaric chamber studies have been used by some groups as a highly-controlled simulation of the ascent to Everest base camp and beyond [Wagner, 2010]. A hypobaric chamber experiment would not have been possible on the scale of CXE 2007, as the expedition took place over 78 days and involved a very large group of individuals. Chamber studies are very expensive to run, whereas climbing participants usually contribute financially to the expedition. In addition, hypobaric chambers only expose individuals to hypoxic stress and occasional exercise testing, and do not encompass systemic responses to multiple stresses encountered during an expedition such as CXE 2007. Both types of study are useful, depending on the questions being asked, and in this case an expedition was more suitable due to the large number of participants and the application of the results to critically ill patients.

1.3.3 Biomarkers

Biomarkers are characteristics that can be measured to give information on the *normal* state of an organism, to track the progress of an illness, the body's response to an illness or to monitor the response to a treatment. Plasma biomarkers in particular are of great interest, due to the ease in which blood samples can be obtained in the clinical environment. Biomarkers are already used as markers of ischemia, heart failure and inflammation in the field of cardiac medicine. However, it is only recently that single biomarkers have started to be combined into multimarker strategies [Maisel et al., 2006].

Figure 1.1 shows the ascent profile for CXE 2007, and identifies the five laboratories where exercise performance testing and blood sampling were performed. The blood samples were spun down in each laboratory, and the plasma frozen and stored for later analysis. These plasma samples were used at The University of Warwick to measure multiple biochemical metabolites, including metabolites known to play a role in hypoxia adaptation, markers of inflammation and metabolism-

Dataset	Parameter type	Number of variables	Collected	Analysed	Number of individuals	Number of measurements
Core	Physiological	65	CXE 2007	CXE 2007	24	7
	Biochemical	59	CXE 2007	University of Warwick	24	7
Trekker	Physiological	62	CXE 2007	CXE 2007	190	5
	Biochemical	-	CXE 2007	Yet to be analysed	190	5
Diary	Physiological	16	CXE 2007	CXE 2007	24	78

Table 1.2: A summary of the different datasets collected during CXE 2007. Plasma samples were measured at The University of Warwick.

related metabolites.

Biochemical and physiological information have been analysed together in this thesis, to see if changes in physiological measurements (used as an indicator of hypoxia adaptation) can be predicted by changes in biochemical measurements at lower altitudes. This would therefore enable the *prediction* of how well an individual will adapt to a higher altitude by taking a blood sample at a lower altitude.

1.3.4 Data available

Three different datasets have been used during this thesis project, which contain measurements for the 24 core team members obtained during different stages of the expedition. An overview of the different datasets is shown in Table 1.2.

The *core team* data is the main dataset that has been used during this thesis project, and contains information about the 24 core team members only. The core dataset contains physiological information collected during CXE 2007, as well as biochemical information measured by myself and other members of the Feelisch laboratory at The University of Warwick in plasma samples collected during CXE 2007. This dataset has been used to understand the overall changes occurring in each of the 24 core team individuals during the expedition, and all modelling efforts have been based on this dataset.

The *diary data* is an additional dataset collected for the 24 core team members, consisting of daily measurements taken during the expedition, and shows the day-to-day variation of each measurement. It contains only a small number of physiological measurements such as heart rate and respiratory rate before and after a simple 2-minute step exercise test, designed to look at how the body was adapting to changes in altitude. This dataset has been used to look at the reliability and day-to-day variability of some of the physiological measurements, i.e. if the same

Response pathway	Parameters involved
Oxygen regulation	HIF-1α targets
Redox regulation	Nitric oxide and reactive oxygen species metabolites
Inflammation / stress	Cytokines, acute phase proteins
Metabolism / organ function	Hormones

Table 1.3: A summary of the different biochemical pathways assessed during this thesis project, giving an idea of different response pathways measured, and which biochemical metabolites were used as anologues for the different pathways.

measurement is taken on two subsequent days, how similar are they?

The *trekker data* contains information about the 190 volunteers (individuals from the general population, not core team members) who took part in the expedition, and stayed at Everest Base Camp. This dataset only contains physiological information about the exercise performance tests carried out during the ascent. Plasma samples are available for these individuals, and are being kept in storage at -80^{o}C until use. The trekker plasma samples can be measured for various biochemical metabolites identified during data analysis, to validate the models formed from the core team dataset.

1.4 Biochemical adaptation to hypoxia

During CXE 2007, plasma samples were collected at five different laboratories during the ascent, as detailed in Figure 1.1. These plasma samples were measured by myself and other members of the Feelisch laboratory at The University of Warwick, to investigate how the biochemical makeup of the plasma changed during the ascent. Metabolites measured include readouts of hormonal activity, acid-base balance, inflammation, regulatory pathways and redox regulation. A summary of the biochemical pathways measured is shown in Table 1.3. Many of the metabolites measured are known targets of HIF-1α, the *master gene regulator* for hypoxia adaptation. Other metabolites were measured to gain insight into how related metabolites may be changing. This section gives an overview of which biochemical metabolites were measured and the reasoning behind these selections.

1.4.1 Oxygen regulation

Oxygen levels are constantly monitored by redox-sensitive and oxygen-responsive transcription factors and cytokines within the body [Haddad, 2002]. Any change in the amount of oxygen available leads to a pre-defined response mediated primarily

by hypoxia inducible factor (HIF)-1α, the *master gene regulator* for hypoxia adaptation, which has over 300 confirmed downstream targets [Haddad, 2002; Wenger, 2002]. In the presence of oxygen, the HIF-1α protein undergoes continuous proteasomal degradation. During hypoxia the protein is stabilised and binds to hypoxia-responsive element (HRE) sites on DNA to cause activation of hypoxia-inducible targets. Examples of targets include erythropoietin [Semenza and Wang, 1992], transferrin, energy metabolism genes, cell proliferation genes and vascular development genes, for example vascular endothelial growth factor (VEGF) and inducible nitric oxide synthase (iNOS). This allows the cell to *switch* from using oxygen to produce energy during aerobic respiration, to anaerobic respiration when oxygen is scarce. HIF-1α is also known to be regulated by several other molecules, including nitric oxide (NO) and reactive oxygen species (ROS) [Wenger, 2002]. Several HIF-1α targets have been measured in this study, to monitor HIF-1α activity and track the progress of cellular hypoxia adaptation. These include NO metabolites, VEGF, erythropoetin and other related metabolites.

1.4.1.1 Nitric oxide

Nitric oxide (NO) is produced enzymatically by mammalian cells and has been found to have roles in hypoxia adaptation, as well as in the gastrointestinal tract, immune system, central nervous system, respiratory system, cardiovascular system and cell proliferation [Erzurum et al., 2007; Moncada et al., 1991]. NO is produced from L-arginine via 3 isoforms of Nitric Oxide Synthase (NOS) and via reduction of endogenous inorganic nitrite, as shown in Figure 1.2. Neuronal NOS (nNOS) and endothelial NOS (eNOS) are both constitutively expressed, whereas inducible NOS (iNOS) is only expressed upon exposure to specific stimuli, such as pro-inflammatory cytokines and HIF-1α binding during hypoxia [Melillo et al., 1995]. Both eNOS and nNOS play roles in hypoxia adaptation and the immune response, primarily by increased blood flow [Erzurum et al., 2007; Delannoy et al., 2010]. This is achieved by causing vascular smooth muscle to relax; NO diffuses into smooth muscle cells and activates soluble guanylate cyclase (sGC), which leads to the production of the secondary messenger molecule cyclic guanosine monophosphate (cGMP). cGMP then acts on smooth muscle cells, causing them to relax. eNOS knockout mice show high blood pressure, indicating that NO also plays a role in general blood pressure regulation [Huang, 1999; Stauss et al., 1999].

NO is oxidised in a step-wise manner to nitrite (NO_2^-) and nitrate (NO_3^-), and in blood NO is almost completely converted to nitrite and nitrate [Yoshida et al., 1983]. The main source of nitrate is from the diet, with green leafy vegetables

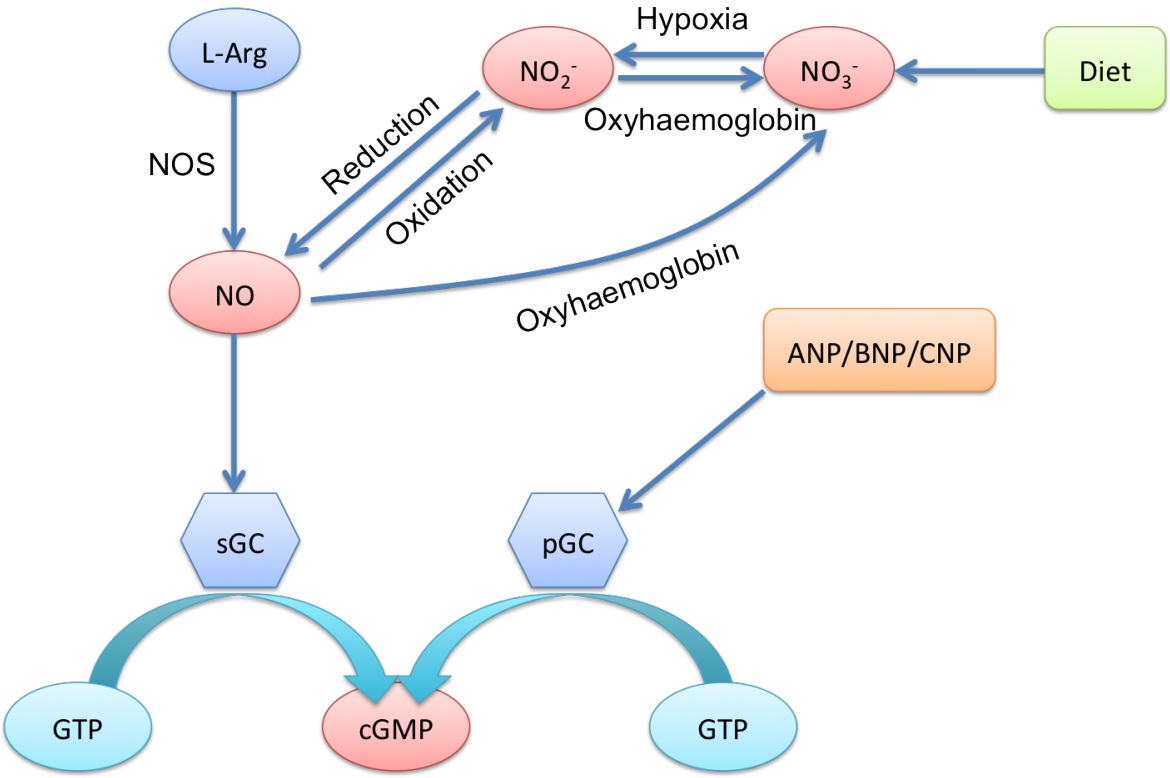

Figure 1.2: A brief summary of part of the NO pathway in plasma. Nitrite and nitrate are oxidation products of NO, but may also act as an alternative NO source. NO binds to soluble guanylate cyclase (sGC), increasing the production of cGMP from GTP. cGMP then acts on vascular tissue to cause smooth muscle relaxation. Membrane-bound particulate GC (pGC) produces cGMP following stimulation by either atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) or C-type natriuretic peptide (CNP), independently of NO. They are measured in this study to determine whether any change seen in cGMP is due to a change in NO, ANP, BNP or CNP.

being a particularly rich source. Nitrite and nitrate have also been shown to be reduced back to NO in vivo, and may also provide an alternative source of NO in the human cell [Lundberg and Weitzberg, 2005; Lundberg et al., 2008], as shown in Figure 1.2.

In plasma, the half-life of NO is in the region of milliseconds [Hakim et al., 1996]. Therefore, the amount of NO in plasma cannot currently be measured directly. Alternatively, the quantification of NO metabolites can give information about NO production, availability and metabolism, such as those shown in Figure 1.2. Therefore, the measurement of metabolites such as nitrite, nitrate and cGMP can give information on pathways downstream from HIF-1α, and therefore give information about how the body is responding to lower oxygen availability.

Several cytokines were also measured as they are known to interact with NO and its products to facilitate inflammation, fight infection and regulate metabolism. For example, NO is known to be generated by phagocytes as part of the human immune response via iNOS, which can be activated by interferon-gamma (IFN-γ) or tumour necrosis factor (TNF). The activity of iNOS is inhibited by other cytokines such as interleukin (IL)-4 and IL-10 [Moncada et al., 1991].

1.4.2 Redox regulation

The so-called *oxygen paradox* is that oxygen is inherently dangerous to cells, however, aerobic organisms require oxygen to generate ATP and cannot survive without it. Each oxygen atom has two unpaired electrons in its outer shell, making it a free radical. The reduction of oxygen to generate ATP by mitochondria is essential to the cell, however, the univalent reduction of oxygen generates highly reactive intermediate species, the formation of which are promoted due to the reductive atmosphere of the cell. Reduction of this kind can generate hydrogen peroxide (H_2O_2) and the hydroxyl radical ($\bullet$OH), which are known as reactive oxygen species (ROS), and can cause large amount of damage to the cell. This is also a major problem in critically ill patients, who have difficulty in responding to increased levels of oxidative stress.

During hypoxia, there is thought to be an accumulation of reducing equivalents in the mitochondrial transport chain, due to a reduction in oxygen availability to act as an *electron acceptor*, which may in turn lead to an increase in the production of ROS [Kehrer and Lund, 1994; Chandel et al., 1998]. If the cell is unable to remove the excess ROS, it is said to be in a state of *oxidative stress*. ROS are known to have an effect on the stability and signalling of HIF-1α [Brüne and Zhou, 2007], and have been shown to interact with NO to form secondary reactive nitric oxide species and reduce the bioavailability of free NO [Brüne and Zhou, 2007; Feel-

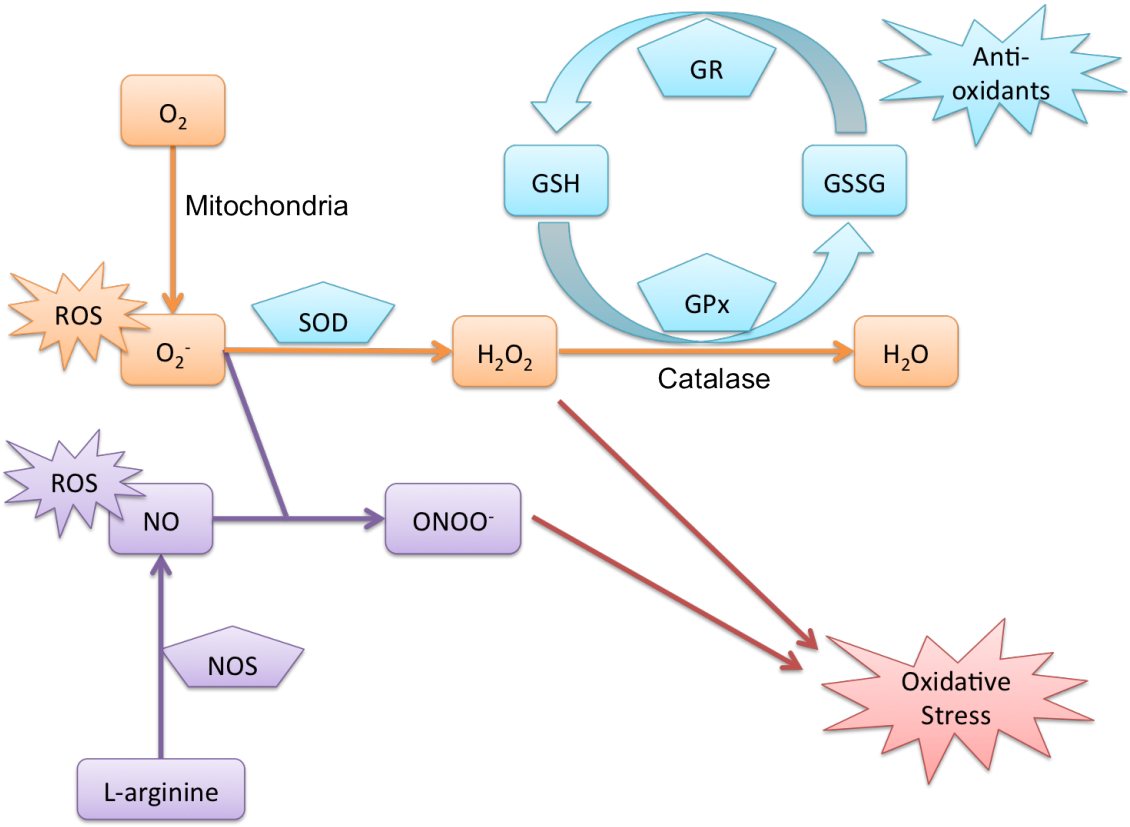

Figure 1.3: Reactive Oxygen Species (ROS) summary, showing oxygen becoming a free radical. This free radical can then react with nitric oxide (NO) to form reactive nitrogen oxygen species (RNOS) such as $ONOO^-$, which can stimulate oxidative stress within the cell. Superoxide dismutase (SOD) catalyses the dismutation/disproportionation of superoxide free radical oxygen (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen, acting as an important antioxidant. H_2O_2 is then converted into water via the action of catalase and glutathione-dependent peroxidase (GPx), which leads to the formation of oxidised glutathione (GSSG). GSSG is then readily converted back to the reduced form (GSH) by the action of glutathione reductase (GR).

isch, 2007], as shown in Figure 1.3. Therefore, measurement of ROS would provide information on how the body is responding to a lower availability of oxygen.

To combat the formation of ROS within the cell, cells produce *antioxidants* such as glutathione, which react with ROS and remove them from the cell safely, as shown in Figure 1.3. It is not currently possible to measure the amount of ROS directly. Therefore, the levels of products formed during the interaction of ROS with biological targets such as hydroxynonenal (HNE) and isoprostanes, as well as antioxidant levels are measured in this study as indicators of oxidative stress.

1.4.3 Inflammation

Acute-phase proteins are a class of proteins that show changes in expression in response to inflammation, known as the *acute-phase response*. Inflammatory responses were expected to occur during CXE 2007 due to the high levels of stress the team were subjected to. During injury, inflammatory cells such as macrophages and neutrophils secrete a number of cytokines into the bloodstream, to mediate the inflammatory response. Inflammation typically accompanies disease, and is present in many cases of critical illness. Cytokines such as IL-1, IL-6, IL-8 and TNF-α were measured during this study to monitor the level of inflammatory response seen during the expedition. Measurement of these proteins was also of interest due to the cross-talk that occurs with NO-related metabolites during inflammatory and immune responses, as previously mentioned in Section 1.4.1.1.

1.4.4 Metabolism and organ function

Cytokines and hormones are small protein molecules that act as signalling molecules between cells. A large number of signalling molecules were measured to monitor any cross-talk between cells occurring during the expedition. The measurement of hormones such as insulin and glucagon provides information on changes in cell metabolism during CXE 2007. Molecules such as glucose and lactate provide information on the energy production and use of cells. Organ function was monitored by the measurement of molecules such as creatinine, which provides information on kidney function.

1.5 Purpose of the study

The main aim of my thesis project was to develop a series of statistical models capable of predicting how an individual will respond to hypoxic stress, from measured

changes in certain biochemical metabolites. The models were constructed using a population response, with the aim of applying the final models to individual cases. There is substantial data available, including 65 physiological measurements and 59 biochemical metabolites, providing a good possibility of finding novel relationships for modelling.

1.6 Assumptions

The general assumptions for this thesis project are that:

1. Data collected from CXE 2007 can be used to design predictive models;
2. The physiological data can be used as a reliable indicator of how well an individual is adapting to hypoxic exposure (i.e. that exercise performance at altitude can be used as a proxy for hypoxia adaptation);
3. The biochemical data can be used to predict a physiological response;
4. The information we extract from this data can be useful for the application to critically ill patients;
5. The information we extract from this data can be used to inform the Xtreme Everest 2 (XE2) follow-up study in 2013.

1.7 Research questions

In order to test the hypotheses for this thesis project, the following questions were posed:

1. Is there a difference between how the lab group and climber groups respond to altitude?
2. Are there differences in the physiological responses seen between individuals?
3. Are there differences in the biochemical responses seen between individuals?
4. Which physiological measurements appear to be the most suitable for measuring how well an individual is adapting to altitude/hypoxia?
5. Is there a *pattern* of biochemical metabolites that can be used to predict how well an individual will adapt to hypoxia?

6. Can this data be used to create a model capable of predicting how well a critically ill patient will respond to hypoxic stress?
7. Can the predictive models formed be used to inform the planned XE2 follow-up study?
8. Can any of these predictions be validated during the planned XE2 follow-up study?

1.8 Scope of the study

Systems Biology is an integrative inter-disciplinary approach to answering biological questions. During this thesis project, biological experimentation has been integrated with statistical analysis to extract as much information from the data as possible. A Systems Biology approach is *cyclic*, as shown in Figure 1.4, where data is used to inform a model, and the resulting model informs further data collection. It was not possible to complete the cycle of data and modelling during this thesis project, however, the results from this project can be used to inform further data collection and analysis by other members of the Feelisch laboratory and the CXE team.

1.9 Outline of thesis

This thesis project will look at:

- The materials and methods used during this thesis project;
- The results for the biochemical measurements carried out by myself;
- The analysis of the 16 physiological measurements collected on each day of the expedition, denoted the *diary data*;
- Exploratory statistical analysis of the physiological and biochemical information available in the main dataset, denoted the *core data*;
- Exploratory modelling analysis, looking at relationships between physiological and biochemical metabolites;
- A targeted modelling analysis, looking at the prediction of a *change* in a small number of biologically relevant physiological measurements with a combination of biochemical metabolites;
- A discussion of the results and applications of the models formed.

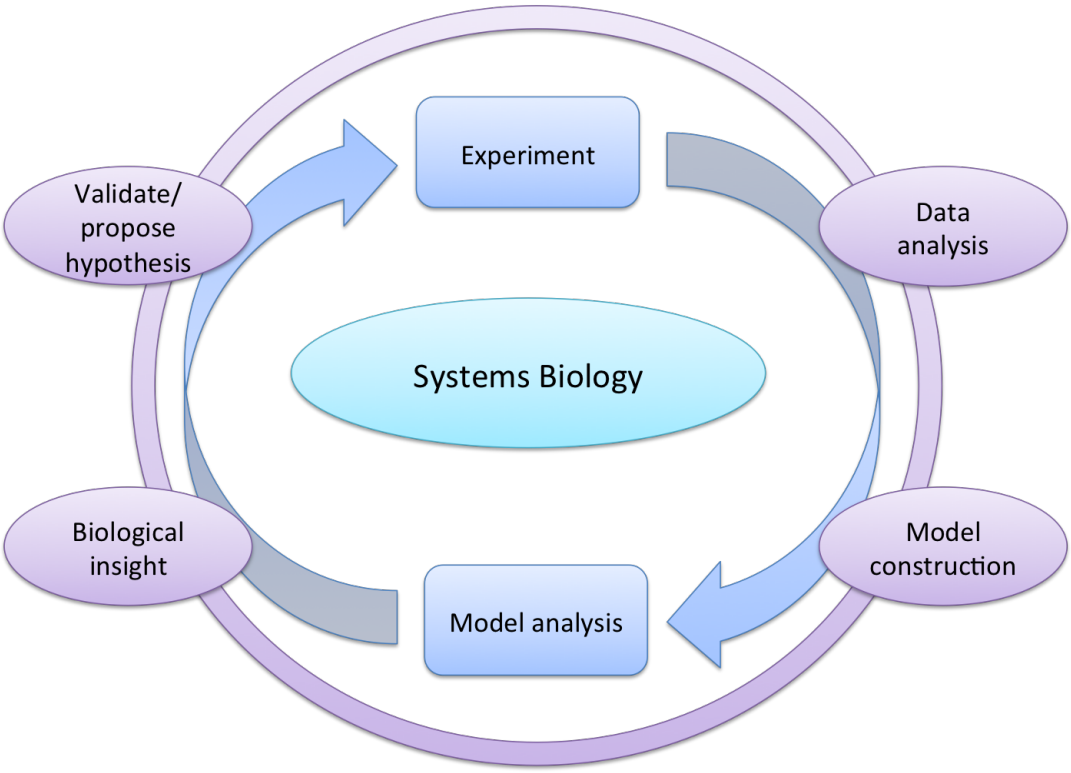

Figure 1.4: Systems Biology - an iterative cycle of data collection (experiment), data analysis, model construction, model analysis/refinement combined with biological insight to validate or propose a hypothesis, which can be used to inform further experiments.

1.10 Thesis contribution

Throughout the course of this thesis project, I have contributed to data acquisition and undertaken all of the statistical analysis. I have become familiar with the difficulties associated with collecting these data, and gained insight into causes of systematic and random errors. During data acquisition, I contributed to the measurement of biochemical metabolites in plasma samples taken from the 24 core team members during CXE 2007. I measured four oxidative stress metabolites via assay-kits, 14 diabetes and metabolism-related metabolites using xMAP technology, and plasma osmolality. I also helped with sea-level testing for the follow-up study Xtreme Alps, where I gained first-hand experience in the issues regarding CPEX testing and blood sampling.

I have performed exploratory data analysis to gain a better understanding of the main patterns seen in the dataset, and inter-individual differences seen for physiological measurements and biochemical metabolites. I have undertaken variable selection, and performed several versions of linear and multiple linear regression analysis on the dataset, to produce a series of models, capable of predicting a physiological measurement at a certain altitude, or a change in a specific physiological metabolite between two altitudes. These models can now be used to inform further analysis of the trekker plasma samples, and be used to inform the CXE follow-up study in 2013.

Chapter 2

Materials and Methods

This section details the *biochemical experiments* and *statistical analyses* that were carried out at The University of Warwick as part of my thesis project. All detailed experiments were carried out on plasma samples collected during CXE 2007, which were kept stored at -80^{o}C until use.

In addition to the data collection detailed in this section, further experiments were carried out on aliquots of the same plasma samples by members of the Feelisch laboratory at The University of Warwick. Physiological data were also collected during CXE 2007, and these data have been combined into one master dataset, denoted the *core dataset*. Physiological data are also available for a further 190 *trekker* individuals. A third dataset, denoted the *diary data* is an additional dataset collected for the 24 core team members, consisting of a small number of daily physiological measurements collected throughout the expedition. Collectively, these data were used in the statistical analysis carried out as part of my thesis project.

2.1 Xtreme Alps

In 2010, a CXE follow-up study was carried out, denoted *Xtreme Alps*. During this study, I contributed to the collection of sea-level physiological data and assisted with blood sample collection. Here, I gained first-hand experience in how the VO_2max exercise performance tests were carried out, and any problems associated with these tests. One such problem is haemolysis, where red blood cells are ruptured during blood collection, and their contents are released into the plasma. Occurrences of such events were recorded to help account for any anomalous measurements when analysing the plasma samples at a later date.

2.2 Biochemical analyses

This section details the biochemical experiments carried out as part of my thesis project. It includes information about specific methods used, as well as some background information on the metabolites being assessed.

2.2.1 Measurement of 8-iso-Prostaglandin $F_{2\alpha}$

Total 8-iso-Prostaglandin $F_{2\alpha}$ (8-isoPGF$_{2\alpha}$) levels were quantified in the 24 core team member's plasma samples by using a direct 8-isoPGF$_{2\alpha}$ Enzyme Competitive Immunoassay Kit from Assay Designs (catalogue number 901-091), according to the supplier's recommendations. 100μl plasma samples were hydrolysed by incubation with 25μl of 10 N NaOH at 45^{o}C for 2 hours. The reaction mixture was cooled on ice for 5 minutes and neutralised with 25μl of 12 N HCl, then centrifuged in a microcentrifuge for 5 minutes. The clear neutralised supernatant was then transferred to a new microcentrifuge tube, and 50μl of sample was used for the 8-isoPGF$_{2\alpha}$ assay.

The samples were incubated with the 8-isoPGF$_{2\alpha}$ antibody for 18 hours at 4^{o}C in a 96-well plate. After incubation, the contents of the wells were emptied and washed with wash buffer. Wash buffer was removed from the wells and the colour was developed by incubation with 200μl of p-nitrophenyl phosphate for 45 minutes at room temperature. The reaction was stopped by the addition of 50μl of stop solution, and the plate was read at 405nm in a Molecular Devices SpectraMax M5 Microplate reader. A standard curve was generated by measuring the optical density of 160-100 000pg/ml of 8-isoPGF$_{2\alpha}$ standards that had been processed simultaneously to the samples, on the same 96-well plate.

2.2.2 Glutathione determination

2.2.2.1 Tietze glutathione recycling assay

There are several different methods for the measurement of glutathione, with the *gold standard* being the Tietze recycling assay. A plate-reader adaptation of the Tietze recycling assay protocol had previously been used in the Feelisch lab for the determination of reduced and oxidised glutathione in fresh rat tissue and plasma. This protocol was modified over several months, to enable measurement of both reduced and oxidised glutathione in frozen human plasma samples.

However, after several months of work it was still not possible to obtain consistent standard curves at low glutathione concentrations, or reliably measure

glutathione levels in human plasma samples that had been frozen. The final sensitivity for the assay was down to 0.06μM for oxidised glutathione, and 0.47μM for reduced glutathione, and was only measurable in standards, shown in Table 2.1.

The different steps taken during the refinement of the protocol are detailed in section 3.1.2.1. The final refined assay protocol is detailed below.

2.2.2.2 Glutathione assay protocol

Reagents:

- 1x buffer (0.1M) and 5x buffer (0.5M);
  - $NaPO_4$ (Sigma-Aldrich: S9638);
  - 0.5M EDTA (Gibco/Invitrogen: 15575-038);
- NADPH (Sigma: N7505);
- 10% Metaphosphoric Acid (MPA) (Sigma-Aldrich: 239275);
- 2-Vinyl Pyridine (2-VP) (Aldrich: 132292);
- Glutathione Reductase (GR);
  - Sigma (Item No. G3664-500): Glutathione reductase from Baker's yeast;
  - Roche (Ref. No. 10105678061): Glutathione reductase from yeast;
- 5,5-Dithiobis-2-nitrobenzoic acid (DTNB) (Sigma: D8130).

Standards:
GSSG (Oxidised Glutathione): 3mM stock was made by adding 0.04595g GSSG and buffer to make 25ml. Two sets of tubes were labelled (23 total), and serial dilutions were carried out as shown in Table 2.1. 100μl of each standard was added to a new tube. 100μl of cold 10% MPA was added to each tube. 20μl of 2-VP was then added to derivatise the sample, and was incubated at room temperature for 1 hour in a flow-hood. 180μl of 5X buffer was added to neutralise the MPA, and the pH was checked to make sure it was around 7. The samples were then stored on ice until use.

GSH (Reduced Glutathione): 3mM stock was made by adding 0.02305g GSH and buffer to make 25ml. Two sets of tubes were labelled (17 in total) and dilutions were carried out as shown in Table 2.1. 100μl of each reduced standard was added to a new tube. 100μl of cold 10% MPA was added to each tube. 200μl of 5X buffer was then added to neutralise the MPA, and the pH was checked to make sure it was

	Standard concentration (μM)	Dilution factor	Volume of previous solution (μL)	Volume of buffer (μL)
GSSG				
-	300	1:10	100 (3mM)	900
1	30	1:10	100 (300μM)	900
2	15	1:2	500 (30μM)	500
3	7.50	1.2	500 (15μM)	500
4	3.75	1.2	500 (7.50μM)	500
5	1.88	1:2	500 (3.75μM)	500
6	0.94	1:2	500 (1.88μM)	500
7	0.47	1:2	500 (0.94μM)	500
8	0.23	1:2	500 (0.47μM)	500
9	0.12	1:2	500 (0.23μM)	500
10	0.06	1:2	500 (0.12μM)	500
11	0	-	-	1000
GSH				
-	300	1:10	100 (3mM)	900
1	30	1:10	100 (300μM)	900
2	15	1:2	500 (30μM)	500
3	7.50	1.2	500 (15μM)	500
4	3.75	1.2	500 (7.50μM)	500
5	1.88	1:2	500 (3.75μM)	500
6	0.94	1:2	500 (1.88μM)	500
7	0.47	1:2	500 (0.94μM)	500
8	0	-	-	1000

Table 2.1: Standard dilutions for reduced glutathione (GSH) and oxidised glutathione (GSSG) for the Tietze glutathione recycling assay.

around 7. The samples were then stored on ice until use.

Preparation of plasma samples: Three sets of eppendorf tubes were labelled for each sample to be analysed; labelled MPA, oxidised (O) and reduced (R). 100μl of 10 % MPA was added to the MPA tubes, 7.5μl of 2-VP was added to the O tubes and the R tubes were left empty. 100μl of plasma sample was added to the MPA tubes and vortexed. The MPA precipitates any protein within the sample, so that it did not interfere with glutathione measurement. The tubes were then centrifuged at 10 000g for 10 minutes at 4^{o}C. 75μl of the MPA extract (supernatant) was added as quickly as possible to each of the O and R tubes (to prevent further reduction of the sample). The O tubes were left to sit on ice for 1 hour. 67.5μl of 5X buffer was added to the O tubes to neutralise the MPA. 75μl of 5X buffer was added to the R tubes, and they were placed on ice until use.

Assay: 50μl of blank (1X buffer), standards or samples were added to the wells of a 96-well microplate. 86μl buffer, 7μl of 10mM DTNB and 21μl of 5mM NADPH was then added to each well. The plate was mixed well, and incubated at 37^{o}C for 10 minutes within the microplate reader. 36μl of 15U/ml GR was added to each well, and the change in absorbance was recorded at 412nm over 5 minutes.

Calculations: Standard curves were calculated from the OD readings for the standard samples prepared, detailed in Table 2.1. The reaction rates for the samples were calculated by subtracting the appropriate 0.0 rate from each sample. The standard curves were then used to determine the concentrations for *total* and *oxidised* glutathione. *Reduced* glutathione was calculated by subtracting twice the oxidised glutathione concentration from the total glutathione value.

2.2.2.3 Glutathione fluorescence assay

Due to the poor reliability of the glutathione assay, reduced glutathione (GSH), oxidised glutathione (GSSG) and total glutathione were quantified in the 24 core team's plasma samples by using a BioVision protocol Glutathione Assay Kit (catalogue number K264-100), according to the supplier's recommendations. 60μl of plasma sample was thawed, and added to a microcentrifuge tube containing 20μl of ice cold perchloric acid (PCA). The sample was vortexed and kept on ice for 5 minutes. The sample was spun in a microcentrifuge at 13 000g at 4^{o}C, and 40μl of the supernatant was collected and transferred to a new tube. 20μl of ice cold potassium hydroxide (KOH) was added to the sample to neutralise the samples (the pH was

tested to be 5-10). The samples were incubated on ice for 5 minutes, and then spun in a microcentrifuge at 13 000g at 4^{o}C for 2 minutes. 10μl of the neutralised sample was transferred to a 96 well plate in triplicate, to measure GSH, total glutathione and GSSG separately. These samples were then prepared further as follows:

- **To detect GSH:** 80μl of assay buffer was added to each well.
- **To detect total glutathione:** 70μl of assay buffer was added to each well. 10μl of reducing agent mix was added to each well, mixed thoroughly and incubated at room temperature for 10 minutes to convert all GSSG present to GSH.
- **To detect GSSG:** 70μl of assay buffer was added to each well. 10μl of GSH quencher was added to each well, mixed well and incubated for 10 minutes at room temperature to quench all GSH present. 10μl of reducing agent mix was then added to each well to destroy the excess GSH quencher and convert the GSSG present to GSH.

10μl of o-phthalaldehyde (OPA) probe was added to each well, mixed thoroughly and incubated at room temperature for 40 minutes. Samples were then measured for fluorescence at 340/420nm in a Molecular Devices SpectraMax M5 Microplate reader. A standard curve was generated by measuring the fluorescence of 0.19 - 25μM GSH and total glutathione standards that had been processed simultaneously to the samples, on the same 96-well plate.

2.2.3 Determination of osmolality

An Advanced Model 3300 Micro-Osmometer was used to measure the osmolality of the 24 core team member's plasma samples. 20μl plasma samples were loaded into the Micro-Osmometer with the use of Advanced Ease-Eject Samplers. Each 20μl sample was aspirated into a new sample tube, and inserted into the instrument's vertical holder. The holder was pressed down, and the testing began automatically, with results being displayed after 60 seconds.

2.2.4 BioPlex analysis

A Bio-Rad BioPlex 200 system was used in conjunction with a BioPlex Pro Human Diabetes 12 + 2-Plex Panel Complete Kit (catalogue number 171-A4S01M), according to the supplier's recommendations, to measure 14 cytokines in human plasma samples (detailed in Table 2.2). Samples were prepared separately for the 12-plex

Metabolite	Description
Adipsin	Also known as Factor D, found in high levels in adipose (fat) tissue, plays a role in humoral suppression of infectious agents. The level of adipsin is elevated in obese individuals.
Adiponectin	Involved in regulating glucose levels (decreases gluconeogenesis and increases glucose uptake) as well as the breakdown of fatty acids. Exclusively secreted from adipose tissue, highly abundant in plasma.
C-Peptide	Produced in a long chain attached to insulin. When cleaved apart, C-peptide plays a role in intracellular signalling pathways, has anti-inflammatory effects as well as aiding in the repair of smooth muscle cells. It is used to distinguish between type I and type II diabetes.
GIP	Glucose-dependent insulintropic peptide is a member of the secretin family of hormones, believed to induce insulin secretion. It is induced by hyperosmolarity of glucose in the duodenum.
Ghrelin	Shown to activate the endothelial form of nitric oxide synthase. Produced by adipose tissue to induce satiation when present at high levels, it has been associated with obesity, insulin resistance and high blood pressure [Xu et al., 2008].
Glucagon	Secreted by the pancreas, it raises blood glucose levels when they fall low by causing the liver to break down stored glycogen into glucose.
GLP-1	Glucagon-like peptide-1, increases secretion of insulin, decreases glucagon secretion, increases satiety in the brain, thus decreasing food intake and promotes insulin sensitivity of cells.
IL-6	Acts as both a pro and anti-inflammatory cytokine, secreted by T-cells and macrophages to stimulate the immune response after trauma. Also released from muscle cells, and is elevated in response to muscle contraction. It has inhibitory effects on TNF-α, IL-1, IL-1ra and IL-10.
Insulin	Secreted by the pancreas in response to high blood glucose levels. It stimulates cells to take up glucose from the blood and promotes the storage of glycogen. It inhibits the release of glucagon.
Leptin	Plays a key role in energy uptake and energy expenditure, inducing appetite and metabolism. It promotes angiogenesis by increasing VEGF levels.
PAI-1	Plasminogen activator inhibitor-1, present in increased levels during obesity and metabolic syndrome.
Resistin	Secreted by immune and epithelial cells, thought to play a role in inflammation by increasing levels of IL-1, 6, 12 and TNF-α. Thought to have links to both obesity and insulin resistance.
TNF-α	Pro-inflammatory cytokine, involved in the regulation of immune cells, it can induce fever, induce apoptosis, sepsis (via IL-1 and IL-6 production), induce inflammation and inhibit viral replication and tumorigenesis.
Visfatin	Promotes vascular smooth muscle cell maturation, activates insulin receptors and has insulin-mimic effects, such as lowering blood glucose levels and increasing insulin sensitivity.

Table 2.2: A list of metabolites related to diabetes, inflammation and metabolism, measured with a BioPlex machine.

and 2-plex assays. 25μl plasma samples were diluted by adding 75μl of human-specific BioPlex sample diluent. The samples were stored on ice until use, and 50μl of sample was used for the BioPlex assays.

A 96-well filter plate was pre-wet with 100μl of BioPlex assay buffer. The buffer was then removed by vacuum filtration, and the bottom of the filter plate was dried using a lint-free paper towel. The multiplex bead working solution was vortexed for 15-20 seconds at a medium speed, and 50μl was added to each well. The buffer was then removed by vacuum filtration, and the plate was washed twice with 100μl of BioPlex wash buffer, removing the buffer by vacuum filtration after each wash. 50μl of diluted sample or standard was added to each well, and the plate was covered with sealing tape. The plate was placed on a microplate shaker and covered with aluminium foil. The shaker speed was slowly increased to 1100rpm for 30 seconds, then reduced to 300rpm, and the plate was incubated at room temperature for 30 minutes.

After incubation, the sealing tape was removed and the buffer was removed by vacuum filtration. The sample was washed 3 times with 100μl of BioPlex wash buffer. The BioPlex detection antibody working solution was vortexed gently, and 25μl was added to each well. The plate was covered with a new piece of sealing tape and placed onto a microplate shaker, and covered with aluminium foil. The shaker speed was slowly increased to 1100rpm for 30 seconds, then reduced to 300rpm, and the plate was incubated at room temperature for 30 minutes.

After incubation, the sealing tape was taken off and the buffer was removed by vacuum filtration. The sample was washed 3 times with 100μl of BioPlex wash buffer. The 1 x streptavidin-PE was vortexed vigorously and 50μl was added to each well. The plate was covered with a new piece of sealing tape and placed onto a microplate shaker, and covered with aluminium foil. The shaker speed was slowly increased to 1100rpm for 30 seconds, then reduced to 300rpm, and the plate was incubated at room temperature for 10 minutes.

After incubation, the sealing tape was removed and the buffer was removed by vacuum filtration. The sample was washed 3 times with 100μl of BioPlex wash buffer. 125μl BioPlex assay buffer was added to each well to re-suspend the beads, and the plate was then covered in sealing tape. The plate was placed on a microplate shaker at 1100rpm for 30 seconds immediately before reading plate on the BioPlex system. The sealing tape was removed before reading.

The plate was placed in a Bio-Rad BioPlex 200 system, and read using the in-built software. Concentrations (pg/ml) of different cytokines in the plasma samples were determined by using the standard curves generated in the multiplex assays.

Each standard curve was generated using eight data points, and a nonlinear least squares minimisation algorithm was used for the curve fitting by the five-parameter logistic equation and to determine the high and low limits of detection.

2.2.5 Cardiopulmonary Exercise Testing (CPX/CPET)

Cardiopulmonary Exercise Testing (CPX/CPET) is a set of measurements that look at an individual's exercise performance in terms of their cardiac and pulmonary function. The CPX test is usually performed during exercise on a static bicycle, during which the subject is attached to an ECG and respirometry machine, and has a breathing mask fitted, as shown in Figure 2.1. The mask is attached to a computer that constantly monitors oxygen consumption and carbon dioxide production throughout the testing. The subject begins to pedal against a continually increasing resistance setting on the bike, whilst ECG readings and oxygen and carbon dioxide gas samplings are continually taken. These readings are then used to calculate an individual's *lactate threshold* (LaT, the threshold at which anaerobic respiration begins to supplement aerobic respiration), and determine their cardiopulmonary function. The lactate threshold is determined by measuring lactate levels in the blood during exercise, and is the point during exercise of increasing intensity where the lactate levels in the blood rapidly begin to accumulate above normal resting levels. This indicates that the clearance of lactate can no longer keep up with lactate production, and is used to determine when anaerobic respiration begins to supplement energy production by aerobic respiration.

CPX measures the ventilation rate, the effectiveness of ventilation, oxygen use and clearance of carbon dioxide to give a clear picture of an individual's cardiopulmonary function. CPX testing was carried out during CXE 2007, to measure each individual's exercise performance during the expedition. A full list of the measurements acquired is shown in Table 2.4.

2.3 Data available

There are three main datasets available, as detailed in Table 1.2, denoted the *core dataset*, *trekker dataset*, and the *diary dataset*. The core dataset consists of physiological information collected during CXE 2007, and biochemical metabolites measured in plasma samples taken during CXE 2007, and measured by both myself and other members of the Feelisch laboratory at The University of Warwick during the course of my thesis project. This dataset contains information for 24 core team individuals only, including 65 physiological measurements and 59 biochemical

Figure 2.1: Cardiopulmonary exercise testing for Caudwell Xtreme Everest 2007, taken from the CASE website.

metabolites, at 7 different time-points, as detailed in Tables 2.3 and 2.4. These measurements collectively form the basis for all analysis undertaken in Chapters 5, 6 and 7.

The trekker dataset consists of 62 physiological measurements taken during CXE 2007, at 5 different time points. Plasma samples are available for this group of individuals, but have not yet been measured. The diary dataset consists of 16 different daily measurements for the full 78 days of the expedition, and is available for the 24 core team individuals only. The full list of these diary measurements is shown in Table 4.1, and assessed in Chapter 4.

Number	Biochemical pathway	Analyte shorthand name	Units
1	NO	Nitrite	μM
2	NO	Nitrate	μM
3	NO	Total NOx	μM
4	NO	RSNO	nM
5	NO	RNNO	nM
6	NO	Total RxNO	nM
7	NO	cGMP	pmol/ml
8	NO	proANP	nmol/L
9	NO	BNP	fmol/ml
10	NO	CNP	pmol/L
11	ROS	proCO	mmol/mg
12	ROS	8-isoPGF	ng/ml
13	ROS	HNE	mg/ml
14	ROS	GSH	μM
15	ROS	GSSG	μM
16	ROS	GSH:GSSG ratio	
17	ROS	GSH/GSSG redox	
18	Cytokines	IL-α	pg/ml
19	Cytokines	IL-β	pg/ml
20	Cytokines	IL-1ra	pg/ml
21	Cytokines	IL-4	pg/ml
22	Cytokines	IL-6	pg/ml
23	Cytokines	IL-8	pg/ml
24	Cytokines	IL-10	pg/ml
25	Cytokines	IL-12(p40)	pg/ml
26	Cytokines	IL-13	pg/ml
27	Cytokines	IL-18	pg/ml
28	Cytokines	MIF	pg/ml
29	Cytokines	Eotaxin	pg/ml
30	Cytokines	TNF-α	pg/ml
31	Cytokines	IFN-γ	pg/ml
32	Cytokines	VEGF	pg/ml
33	Cytokines	CRP	ng/ml
34	Hormones	Epinephrine	ng/ml

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Number	Biochemical pathway	Analyte shorthand name	Units
35	Hormones	Norepinephrine	ng/ml
36	Hormones	T3	pg/ml
37	Hormones	T4	pg/ml
38	Hormones	C-Peptide	pg/ml
39	Hormones	GIP	pg/ml
40	Hormones	Ghrelin	pg/ml
41	Hormones	Glucagon	pg/ml
42	Hormones	GLP-1	pg/ml
43	Hormones	Insulin	pg/ml
44	Hormones	Leptin	pg/ml
45	Hormones	PAI-1	pg/ml
46	Hormones	Resistin	pg/ml
47	Hormones	Visfatin	pg/ml
48	Hormones	Adiponectin	pg/ml
49	Hormones	Adipsin	pg/ml
50	Hormones	EPO	mlU/ml
51	Hormones	ET-1	pg/ml
52	Metabolic/Renal Markers	Glucose	mM
53	Metabolic/Renal Markers	Cystatin-C	ng/ml
54	Metabolic/Renal Markers	Creatinine	mg/dl
55	Metabolic/Renal Markers	Lactate	mM
56	Metabolic/Renal Markers	Osmolality	mOsmo/kg
57	Other	Protein content	mg/ml
58	Other	Bicarbonate	mmol/L
59	Other	HSP-70	pg/ml

Table 2.3: Full list of biochemical metabolites measured in plasma samples taken from 24 core-team individuals, at 5 different laboratories (7 different time-points) during CXE 2007.

Parameter	Additional information
Gender	
Weight	Kg
Barometric pressure	kilopascals (kPa)
Inspired O_2 partial pressure	The partial pressure (Pi) O_2 would have if it alone occupied a volume (kPa)
Ambient temperature	Degrees Celsius
Economy	Measure of oxygen efficiency expressed as rate of oxygen consumption per workload (mls O_2 per Watt)
Maximum voluntary ventilation	Maximal breathing capacity The greatest volume of gas that can be breathed per minute by voluntary effort - L/min
Forced expiratory volume in 1 second	Volume exhaled during the first second of a forced expiratory manoeuvre, started from the level of total lung capacity - L/min
Exercise ramp wattage	The workload increase /min during the CPX measured in Watts
Haemoglobin	Iron-containing oxygen transport protein in red blood cells (g/dl)
Blood O_2 content	The volume of oxygen carried within the blood - mls O_2/L
Haematocrit	The volume % of red blood cells in the blood
SBP at rest	Systolic Blood Pressure (mm Hg)
DBP at rest	Diastolic Blood Pressure (mm Hg)
SBP after exercise	(mm Hg)
DBP after exercise	(mm Hg)
The following measurements were taken at rest, at lactate threshold and at VO_2max	
O_2 consumption	The volume of oxygen being consumed by the body per minute - mls O_2/kg/min
O_2 consumption / kg	mls O_2/kg/min (normalised per kg body weight /min)
Respiratory exchange ratio	Ratio of CO_2 production to O_2 consumption. Used in VO_2max test to see when individual has reached maximal energy consumption.
Heart rate	Number of heart beats per minute (beats/min)

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Parameter	Additional information
Minute ventilation	Volume of gas inhaled/exhaled from the lungs in one minute (L/min)
CO_2 production	The difference between the volume of CO_2 exhaled from the lungs and the volume inhaled into the lungs (L/min)
Respiratory equivalent for O_2	The ratio of the volume of inspired air and the amount of oxygen consumed by the tissues
Respiratory equivalent for CO_2	The ratio of inspired air to the amount of carbon dioxide produced
O_2 pulse	Surrogate of stroke volume during exercise. Normalises O_2 consumption for heart rate, calculated from O_2 consumption and HR (ml O2/beat)
Respiratory rate	Number of breaths per minute (breaths/min)
Tidal volume	The normal volume of air displaced between normal inspiration and expiration when extra effort is not applied (L/min)
Partial pressure end tidal O_2	The partial pressure of oxygen at the end of exhalation (mm Hg)
End tidal partial pressure CO_2	The level of CO_2 released at the end of expiration (mm Hg)
O_2 saturation	Haemoglobin (Hb) saturation measured in samples obtained from arterial puncture (%)
The following measurements were available at lactate threshold and VO_2max	
Work rate	The amount of energy expended during physical exercise (Watts)
Oxygen cost	The volume of oxygen used by the body during LaT or at VO_2max. It is directly related to the energy demands of the particular activity, and affected the nature of the substrate respired. Calculated by: work rate / O_2 consumption (/kg) (Watts/mlsO_2/kg/min)

Table 2.4: Full list of physiological measurements measured for 24 individuals during CPX testing, at 5 different laboratories during CXE 2007.

2.4 Missing data

Exposure to an extreme environment, such as the conditions experienced during CXE 2007, leads to the stimulation of multiple adaptive response pathways. These pathways communicate and interact with one another, to produce a complex systems response to stress. Such responses mean that missing data values are likely within a dataset that is trying to capture the complexities of such a response. These may be due to certain samples or a specific technique not being available for measurement (due to illness or a temporary machine failure), human error during experiments or measurements being either above or below quantifiable limits.

An incomplete dataset can become a problem when it comes to further analysis. Some analyses will not work with missing values, and as biological experiments usually contain a relatively small number of samples, leaving one sample out of the whole analysis due to one missing datapoint could bias the analyses, or leave out vital information, leading to erroneous conclusions.

2.4.1 Treatment of incomplete datasets

If a dataset is missing values, there are two options available, depending on why values are missing. If a measured sample gives a value above or below the quantifiable limit of the equipment, then there is still some potentially useful information that can be gained from this datapoint. If the sample is below the detection limit of the equipment, it is very unlikely that the sample is simply *empty* of the material you are testing it for (samples are rarely completely *empty* of a particular metabolite). The simplest solution to this problem would be to impute a value half-way between zero and the lowest limit of the equipment being used. This way, some information can be gained from the sample that may be useful to the analysis.

If samples are missing, it is more difficult to accurately impute their values. Individual values can be estimated by assessing how that individual responds in comparison to all other individuals, and impute the missing value based on that individual's *average ranking* within the group. For example, if the individual ranks highly in the group for a particular metabolite, then an assumption could be made that the individual ranks similarly for all time points, and an appropriate value could be calculated from similarly-responding individuals. However, this may not be appropriate in some cases, and relies heavily on the assumption that the individual responds in a similar way compared to other individuals in the group.

There are several missing data points in both the biochemical and physiological datasets used within this project. Due to the extreme conditions of CXE 2007,

Biochemical metabolite	Detection limit of technique used	Value imputed	Units
IL-13	0.63	0.315	pg/ml
Eotaxin	1.69	0.845	pg/ml
CRP	60.00	30.000	ng/ml
Adrenaline	0.12	0.060	fmol/ml
Noradrenaline	0.08	0.040	fmol/ml
Adipsin	422.00	211.000	pg/ml
EPO	0.40	0.200	mlU/ml

Table 2.5: List of imputed values for biochemical metabolites measured in plasma samples taken during CXE 2007. All values were *below the detectable limit* of the technique used, and were imputed as half-way between the lower detection limit of the technique used and zero.

some individuals became ill during the expedition, and did not have samples taken at particular time points. A small number of individuals suffered severely from hypoxia and had to be evacuated from the mountain at higher altitudes. The biochemical measurements contained more missing values than the physiological data, mainly due to *out of range* measurements, as there is a higher level of error associated with measuring metabolites that are present in very low concentrations. Measurements taken at higher altitudes also contain more missing data points than measurements taken at lower altitudes. It is important to know *why* a particular data point is missing, as it can have an effect on the assessment of the data, especially if it is due to an individual being ill. Some metabolites are also missing for entire altitudes. Due to this, several different *versions* of the dataset have been created, each useful for answering different questions about the dataset. The biochemical metabolites that had values imputed are shown in Table 2.5.

2.4.2 How missing values were treated in each dataset

For the core dataset, physiological measurements missing only a small number of values were imputed, to restrict the amount of bias these values may have on further analysis. This was done in a systematic way, by highlighting the individual on a box and whisker plot showing the behaviour of the variable over all altitudes. This was done to compare how the individual responded at known time points compared to the rest of the group. The individual was then ranked within the group, depending on their response. If this rank was similar across altitudes, a mean rank was calculated. The missing variable was then imputed based on this rank - for example if the mean rank was 2, the values for individuals ranked 1 and 2 were added and the sum divided by two to calculate a value in-between them. If the rank varied too much

within the group, a value could not be imputed this way. This method assumes that an individual's behaviour remains relative to the way the rest of the group is behaving, and that this relationship is the same across altitudes.

The diary dataset was collected on each day of the expedition, and has many missing data values. No information has been imputed for this dataset, as too many data values are missing, and any imputation would risk biasing any analysis carried out on this dataset as it would be mainly based on estimated data points.

2.4.3 Core team datasets

Biochemical metabolites and physiological measurements that are missing some data points are still potentially valuable for analysis. The metabolites had several missing values, ranging from one complete altitude missing (24 missing data values) for all of the glutathione metabolites, to six altitudes missing data for IL-10 (approximately 144 missing data values). These metabolites can provide information on how the group are responding, but cannot be used in model building.

Due to these limitations, two separate datasets for the core team were formed. The first dataset contained only complete information (i.e. no missing data points for any variable included in the dataset), whereas the second dataset contained all measurements that included enough information to be useful (i.e. they contained at least a full altitude worth of data). The *complete* dataset was primarily used for modelling analysis, as well as any analysis that could not be performed on data with missing values. The *incomplete* dataset was primarily used for exploratory data analysis, looking at general patterns of expression within the group.

There were 10 individuals missing for all physiological measurements at Everest Base Camp Weeks 6 and 8. Two individuals were also missing plasma samples for Everest Base Camp weeks 6 and 8 as they became very ill during the expedition, and had to be moved down to a lower altitude to recover. The main focus of this project was to assess the changes that occur with increasing hypoxic exposure, therefore, only measurements from London - Everest Base Camp Week 1 were used for modelling. This was because Everest Base Camp weeks 6 and 8 showed altitude-independent changes, and were missing too much information to be used reliably for modelling purposes. A full list of the biochemical metabolites and physiological measurements available for the core dataset are shown in Tables 2.3 and 2.4.

2.4.4 Variables omitted from the analysis

Some of the biochemical metabolites and physiological measurements were missing too many values to be imputed with any accuracy, and did not appear to give any information about any changes that occurred with increasing altitude. For example, IL-1α only had three samples out of 168 that were within the detectable limits of the BioPlex assay, used to measure multiple biochemical metabolites in the plasma samples. These biochemical metabolites have been removed from all analyses, and are as follows:

- Interleukin-1α
- Interleukin-4
- Interferon-γ

2.5 Statistical analysis

This section details the statistical techniques carried out on all of the data available from CXE 2007. All work was performed on a MacBook Pro, 3.06 GHz Intel Core 2 Duo, 4 GB, Mac OS X Lion 10.7.5. All data analysis was performed by myself using the commercial software package *matlab* 7.12 (The MathWorks Inc., Natick, MA, 2012).

Initial statistical techniques were used to understand how different measurements were changing as altitude increased, as well as looking for patterns and summarising any important observations. This information was used to define a list of metabolites that were most suitable to be used as part of modelling efforts. This section details the different types of statistical analyses used in later chapters, as well as the mathematics behind each test.

One of my personal aims for my thesis project was to gain an understanding of the statistical methods and empirical modelling techniques that can be applied to biological data. Therefore, this section describes the statistical methods used in detail.

2.5.1 Box and whisker plots

Box and whisker plots are a simple yet convenient way of summarising one or more groups of data in a graphical form, and allowing the visual analysis of the symmetry of data around each median and the variability within the dataset [McGill, 1978]. Here the data are summarised as a 'box' with the lower edge of the box showing

the lower 25th percentile and the upper edge of the box showing the upper 75th percentile. The median is shown as a red line bisecting the box, and the interquartile range is the difference between the 25th and 75th percentiles. The 'whiskers' of the plot show the smallest and largest values not considered to be an outlier, with an outlier defined as being more than 1.5 times the interquartile range above the 75th percentile, or below the 25th percentile [McGill et al., 1978], and were plotted individually as a red cross ('+').

Box and whisker plots show the differences and similarities between samples of data without making any assumptions of the underlying statistical distributions, and are therefore non-parametric. Box and whisker plots include more useful information than a barchart or histogram, and can act as a visual summary of the data, and include the median value, not the mean, as the median is less influenced by extreme values, and useful for skewed distributions. The variability in the data is shown by the interquartile range, which is a more stable summary than the total range, as, like the median, it is less prone to skewing due to extreme values.

The *boxplot* function was implemented in *matlab* to produce box and whisker plots for all of the different datasets. Data for the core team members was plotted in several places, both together, and split into lab staff and climber groups, to allow comparisons between the two different groups. Box and whisker plots of the diary data allowed the visual analysis of the day-to-day variability of the different physiological measurements available. The box and whisker plots presented for the core dataset show log-transformed data, as there were some metabolites that contained high outlying values that skewed the plots and made them difficult to interpret. All box and whisker plots for the core dataset were presented after being log-transformed, to maintain consistency across the figures. Box and whisker plots for the diary dataset were presented as raw data, as log-transformation was not necessary, and the raw data was more informative for this particular dataset.

2.5.2 Subplots

Here, a subplot divides a figure into equal segments, with each segment showing one individual's response, rather than giving an average of all responses. The *subplot* function was implemented in matlab to produce subplot graphs of all 24 individual responses for a particular physiological measurement or biochemical metabolite as altitude increases, to analyse the inter-individual variability seen for that particular measurement.

2.5.3 *F*-test for assessment of equal variances

The F-test assesses whether the variances of two samples are equal, so that the null hypothesis H_0 = *the samples come from populations with the same variance*, is compared to the alternative hypothesis H_1 = *the samples come from populations with different variances*. This test is highly sensitive to non-normality, and any departures from normality can render the results of the F-test invalid. This is especially true for *heavy-tailed* or *light-tailed* distributions, where the probability of getting high values is larger or smaller than is expected for a normal distribution. For heavy-tailed distributions, the associated p-value is smaller than the actual significance value, resulting in false-positives. The opposite is true for light-tailed distributions, with the associated p-value being much larger than the actual significance level, resulting in the F-test being less likely to detect true differences. However, it is difficult to assess the assumption of normality, unless the sample sizes are very large. When the test assumptions are met, the F-test is the most powerful test available, however, in the case of non-normality, a non-parametric test may be more suitable. For the F-test the sample variances are calculated as follows:

$$S_x^2 = \frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2, \tag{2.1}$$

$$S_y^2 = \frac{1}{m-1}\sum_{i=1}^{m}(y_i - \bar{y})^2, \tag{2.2}$$

where $\bar{x}$ and $\bar{y}$ are the sample means, n is the number of individuals for sample x, and m is the number of individuals for sample y. The test statistic is then as follows:

$$F = \frac{S_x^2}{S_y^2}, \tag{2.3}$$

and has an F-distribution with *n-1* and *m-1* degrees of freedom if the null hypothesis is true.

The *vartest2* function was implemented in *matlab* for both the diary and core datasets. This was done in part to inform the subsequent analysis of variance and t-tests to be performed on the data, and to assess whether the variances of groups changed between altitudes. A two-tailed F-test was performed to assess whether the two sample variances were significantly different from one another. It

was not of interest at this stage to assess which sample had the higher variance, just that a difference in the variance existed so that an appropriate form of the t-test could be used. There are alternative ways to test multiple variances at the same time, which may be more appropriate than performing multiple pair-wise analyses, however, these tests have lower power compared to the F-test.

2.5.4 Two-sample Student *t*-test

The Student t-test assesses whether the means of two random, normally distributed populations are equal [Student, 1908], so that H_0 = *the samples have the same mean*, is compared to the alternative hypothesis H_1 = *the samples have different means*. The standard form of the t-test assumes that the variances of the two populations are equal, and that they are both drawn from populations assumed to be normally distributed. The t statistic to test whether two means are different was calculated as follows:

$$t = \frac{\bar{x} - \bar{y}}{SD_{xy}\sqrt{\frac{2}{n}}} \tag{2.4}$$

where, if the sample sizes and variances are equal:

$$SD_{xy} = \sqrt{\frac{1}{2}(S_x^2 + S_y^2)}, \tag{2.5}$$

where S_x^2 and S_y^2 are the sample variances (as shown in equations 2.1 and 2.2), SD_{xy} is the pooled standard deviation, and $\bar{x}$ and $\bar{y}$ are the sample means. The denominator of the t statistic is the standard error of the difference between the two means.

If the F-test resulted in a rejection of the null hypothesis (i.e. the sample variances are not equal), then Welch's t-test was used. The t statistic is now calculated as:

$$t = \frac{\bar{x} - \bar{y}}{sd_{xy}}, \tag{2.6}$$

where

$$sd_{xy} = \sqrt{\frac{s_x^2}{n} + \frac{s_y^2}{m}}. \tag{2.7}$$

Here s^2 is the unbiased estimator of the variance of the two samples, and sd_{xy} is not a pooled variance. For significance testing, an approximate Student's t-distribution was used, with the number of degrees of freedom given by Satterthwaite's approximation [Satterthwaite, 1946]:

$$d.f. = \frac{\left(\frac{s_x^2}{n} + \frac{s_y^2}{m}\right)^2}{\frac{\left(\frac{s_x^2}{n}\right)^2}{(n-1)} + \frac{\left(\frac{s_y^2}{m}\right)^2}{m-1}}. \tag{2.8}$$

The *ttest2* function was implemented in *matlab*, to compare the group mean for the lab staff against the group mean for the climbers on each day of the expedition for both the core and diary datasets, at the 5% significance level. The function was used to assess whether the lab staff and climber groups responded differently to one another during the expedition, in a statistically significant manner. However, as the two group sizes were very small, there may not have been enough power in this test to reject the null hypothesis.

2.5.5 Paired Student *t*-test

The paired Student t-test is a variation of the t-test, which assesses whether the mean difference between two responses for the same individual is zero. For example, taking a measurement in a patient before and after treatment, to see if the treatment has had a significant effect on the variable measured. The test statistic is as follows:

$$t = \frac{\bar{x} - \mu}{\frac{s}{\sqrt{n}}}, \tag{2.9}$$

where $\bar{x}$ is the sample mean, $\mu = 0$ (or m) is the hypothesised population mean, s is the sample standard deviation and n is the sample size. Under the null hypothesis the test statistic will follow Student's t-distribution with n-1 degrees of freedom.

The *ttest* function was implemented in *matlab* after an initial positive analysis

of variance (described in Section 2.5.6) between group means over all altitudes, to identify exactly which groups were significantly different from one another. This function was used for both the *diary dataset* and the *core dataset*.

2.5.6 Analysis of variance

The analysis of variance (ANOVA) is a generic statistical technique that assesses how the mean value of a variable is affected by the classification of the data according to different sources of variation. The one-way ANOVA is a generalisation of the two-sample t-test, appropriate for any number of groups, and is equivalent to the two-sample t-test when assessing two groups.

If the p=0.05 (5%) level of significance is consistently accepted, then a wrong conclusion will be drawn on average once in every 20 tests performed, known as Type I error (rejecting the H_0 when it should be accepted, i.e. a false positive). If we then perform multiple t-tests across a range of different treatments, than the probability of drawing at least one false conclusion is greatly increased. This probability could be reduced by lowering the significance level to p=0.01 (1%), however, this increases the risk of making a Type II error (failing to reject the H_0, i.e. a false negative). Analysis of variance (ANOVA) overcomes these problems by comparing any number of sample means within a single test.

The null hypothesis for ANOVA is: H_0 = *the samples are drawn from normally distributed populations with equal means and variances*, compared to the alternative hypothesis: H_1 = *the samples are drawn from populations with different means.*

The test statistic can be calculated as follows:

$$F = \frac{TreatMS}{ResMS}, \tag{2.10}$$

where:

$$TreatMS = \frac{n \sum_{j=1}^{m} (\bar{x}_j - \bar{x})^2}{m-1}, \tag{2.11}$$

$$ResMS = \frac{\sum_{j=1}^{m} \sum_{i=1}^{n} (x_{ji} - \bar{x}_j)^2}{m(n-1)}, \tag{2.12}$$

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Treatment	(m-1)	$n\sum(\bar{x}_j - \bar{x})^2$	TreatSS / (m-1)	TreatMS / ResMS
Residual	m(n-1)	$\sum\sum(x_{ji} - \bar{x}_j)^2$	ResSS / (m(n-1))	
Total	n m - 1	$\sum\sum(x_{ji} - \bar{x})^2$		

Table 2.6: One-way analysis of variance table, where TreatSS refers to the treatment sum of squares, TreatMS refers to the treatment mean square, ResSS refers to the residual sum of squares and ResMS refers to the residual mean square. To complete the test, the *variance ratio* was compared with tabulated critical values of the F-distribution to test the null hypothesis for the ANOVA.

The treatment mean square (TreatMS) is calculated by dividing the treatment sum of squares (the sum of squared deviations of the treatment (sample) means multiplied by the number of observation per sample) by its degrees of freedom (m-1). The residual mean square (ResMS) is calculated by dividing the residual sum of squares (the sum of squared deviations of the individual observations about their respective sample means) by its degrees of freedom (m(n-1)).

The treatment sum of squares and residual sum of squares add up to the total sum of squares, which is the sum of the squared deviations of the individual observations about the overall mean. These values allow the construction of an ANOVA table, which summarises all of the different components of variance, assuming equal replication, such as that shown in Table 2.6. To complete the test, the *variance ratio* was compared with tabulated critical values of the F-distribution to test the null hypothesis for the ANOVA.

2.5.6.1 Assumptions in ANOVA

- The residuals (observations minus the treatment mean) should be approximately normally distributed;
- The residuals should have a common variance, which is not related to the treatment or magnitude of the mean response;
- The observations are independent.

A two-way ANOVA is a generalisation of the paired t-test, which allows the assessment of more than two treatments. This test looks at the grouping of experimental units into *blocks*, with the same number of experimental units per block. We can use ANOVA to calculate the treatment sum of squares, the block sum of

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Blocks	(b-1)	$t\sum(\bar{x}_i - \bar{x})^2$	BlkSS / (b-1)	BlkMS / ResMS
Treatment	(t-1)	$b\sum(\bar{x}_j - \bar{x})^2$	TreatSS / (t-1)	TreatMS / ResMS
Residual	(b-1)(t-1)	by subtraction	ResSS / ((b-1)(t-1))	
Total	b t - 1	$\sum\sum(x_{ji} - \bar{x})^2$		

Table 2.7: Two-way analysis of variance table without interactions, where BlkSS refers to the block sum of squares, BlkMS refers to the block mean square, TreatSS refers to the treatment sum of squares, TreatMS refers to the treatment mean square, ResSS refers to the residual sum of squares and ResMS refers to the residual mean square. To complete the test, the *variance ratio* was compared with tabulated critical values of the F-distribution to test the null hypothesis H_0 = *there is no difference between blocks or treatments.*

squares (the sum of squared deviations of the block means about the overall mean) and the total sum of squares. The residual sum of squares is usually calculated by subtraction as follows:

Residual SS = Total SS - (Block SS + Treatment SS)

The mean squares (variance) can be calculated by dividing the sum of squares by their respective degrees of freedom, and are used to test for differences between treatments of blocks in the same way as the one-way ANOVA, such as that shown in Table 2.7. To complete the test, the *variance ratio* was compared with tabulated critical values of the F-distribution to test the null hypothesis H_0 = *there is no difference between blocks or treatments.*

The *anovan* function was implemented in *matlab* for both the diary and core datasets, to assess the main effects of altitude and individual on each of the measured biochemical metabolites and physiological measurements. As there are no repeated measures available for the core dataset, we cannot assess the interactions between individual and altitude in a formal way, therefore a two-way ANOVA without interactions was performed. If the null hypothesis was rejected, then t-tests were performed between adjacent altitudes, to assess exactly which altitudes were significantly different from one another.

2.5.7 Correlations

Linear correlation looks at quantifying the linear association between pairs of variables. The correlation is described as *positive* if 'large' values of both variables tend to occur together. The correlation is described as *negative* if 'large' values of one variable tend to occur with 'small' value of the other variable. If the correlated observations lie close to a straight line the correlation is said to be *high*, and if the points are more widely scattered about that line the correlation is said to be *low*.

The Pearson product-moment correlation coefficient (also known as *Pearson's r*) is a measure of the *strength* of the linear association seen between the two variables [Pearson, 1920]. The correlation coefficient is defined as:

$$r = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{[\sum_{i=1}^{n}(x_i - \bar{x})^2][\sum_{i=1}^{n}(y_i - \bar{y})^2]}}, \tag{2.13}$$

where n is the number of observations of variables x and y, and $\bar{x}$ and $\bar{y}$ are the means of x and y, respectively.

The *scatter* and *corrcoef* functions were implemented in *matlab* to create scatter plots and calculate the correlation coefficient r and the p-value for each pair of variables. The p-value is the probability of getting a correlation as large as the observed value by random chance, when the true correlation is zero. If the p-value was less than 0.05, then the correlation between x and y was significantly different from zero. To aid interpretation, *weak* correlations are defined here as having a correlation coefficient between either -0.4 to -0.59 or 0.4 to 0.59, *medium* strength correlations have a correlation coefficient between either -0.6 and -0.79 or 0.79 and 0.6, and *strong* correlations have a correlation coefficient between either -0.8 to -1.0 or 0.8 to 1.0. The r value and p-value were used to test the null hypothesis H_0 = *there is no association between the observations of variables x and y*, against the alternative hypothesis H_1 = *there is an association between variables x and y.* A p-value less than 0.05, coupled with a high r value resulted in a rejection of the null hypothesis.

Correlations were calculated within different datasets for several reasons. Correlations were produced for the diary data to identify any redundancy in the dataset, and assess any interesting associations between variables. Correlations were produced during explanatory data analysis to identify potential associations between different variables. Correlations between biochemical metabolites were produced for variable selection for modelling, to test for any redundancy within the biochemical metabolites. If any were identified, then potentially only one of these variables

would be used in modelling efforts. Finally, correlations of residuals produced from modelling and explanatory variables used in the model allowed the assessment of whether there was any underlying structure to the residuals, and whether or not the different explanatory variables were appropriate to be included in the model.

2.5.8 Smoothing

Smoothing was used to remove some of the noise inherent within a biological system, to allow the visual comparison of different individual responses over time. Smoothing helps to pick out patterns that may be difficult to see in noisy datasets, and can be used to cope with day-to-day variability.

The *smooth* algorithm was implemented in *matlab* on the *diary data* only, for each individual for each measurement. This was done to look at the overall response for each individual over the entire expedition, whilst removing some of the noise associated with the measurement. Each individual's response was smoothed using a 3-day running mean algorithm. The algorithm calculates the unweighted mean of the previous, current and next data points, and creates a new data point. The algorithm calculates successive values, and for each calculation a new value is added into the sum and an old value drops out, smoothing each value in turn.

2.5.9 Simple Linear Regression

Simple linear regression fits a linear relationship between one dependent variable and one independent variable. The linear regression model was fitted using the *least squares fit* approach, which minimises the sum of squared vertical deviations of the observed points around the fitted line, as shown in Figure 2.2.

2.5.9.1 ANOVA in regression

In order to assess whether the fitted regression line represents a true relationship (i.e. is the underlying slope different from zero?) the analysis of variance (ANOVA) for the fitted regression line was calculated. In order to construct the ANOVA, the sum of squares explained by the regression, and residual sum of squares were determined. These were then converted to variances (MS) by dividing by the respective degrees of freedom. To complete the test, the regression variance was divided by the residual variance to obtain an F-value, which was compared with tabulated critical values of the F-distribution.

The residual sum of squares (denoted *ResSS*) is the sum of the squared differences between each observed point and the fitted line. This can be calculated

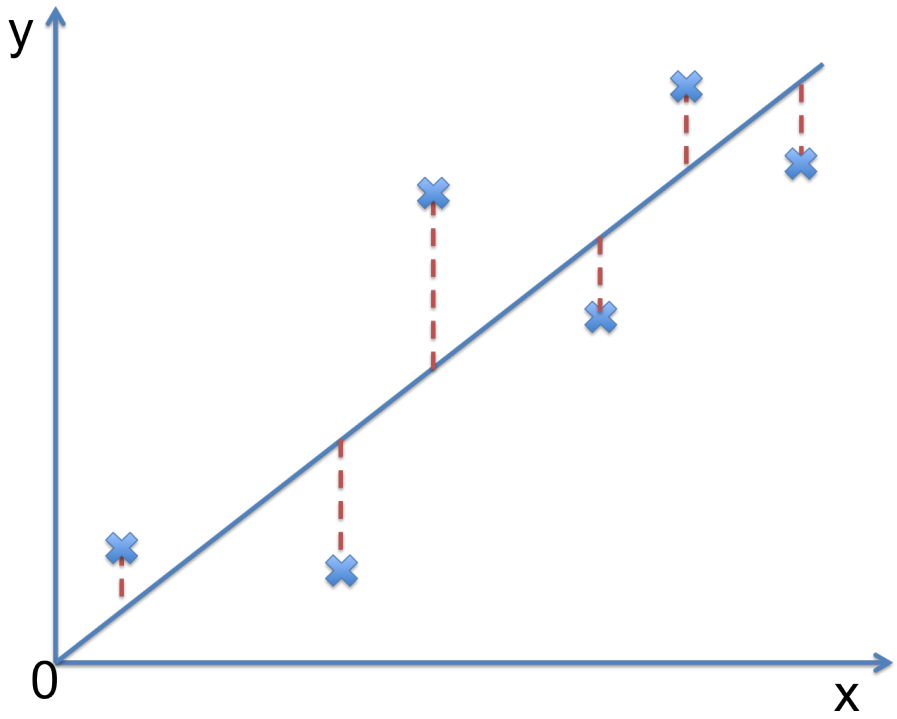


Figure 2.2: An example of a least squares fit, where a linear line is fitted through points by minimising the squared difference between the line and the observed data point (i.e. minimising the squared lengths of the red dashed lines).

by the following:

$$ResSS = \sum_{i=1}^{n}(y_i - (a + bx_i))^2, \tag{2.14}$$

summed over all of i, where a and b are the intercept and the slope of the fitted line, respectively. Parameters a and b are shown by:

$$b = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sum_{i=1}^{n}(x_i - \bar{x})^2}, \tag{2.15}$$

$$a = \frac{\sum_{i=1}^{n} y_i - b\sum_{i=1}^{n} x_i}{n} = \bar{y} - b\bar{x} \tag{2.16}$$

where $\bar{x}$ and $\bar{y}$ are the sample means, n is the number of observations of y. The numerator in equation 2.15 is the corrected sum of products of x and y (denoted S_{xy}), and the denominator is the corrected sum of squares of x, denoted S_{xx}. These can be written as:

$$S_{xy} = \sum_{i=1}^{n}(x_i y_i) - n\bar{x}\bar{y}, \tag{2.17}$$

$$S_{xx} = \sum_{i=1}^{n}(x_i^2) - n\bar{x}^2. \tag{2.18}$$

The regression sum of squares (denoted $RegSS$) is calculated as follows:

$$RegSS = \frac{(S_{xy})^2}{S_{xx}}. \tag{2.19}$$

The regression and residual variances are calculated as follows:

$$RegMS = \frac{RegSS}{Regdf}, \tag{2.20}$$

$$ResMS = \frac{ResSS}{Resdf}, \tag{2.21}$$

where the degrees of freedom for the regression (denoted *Regdf*), is one as a straight line model is being used. Therefore, the RegSS = RegMS for this case. The test statistic is calculated as follows:

$$F = \frac{RegMS}{ResMS}. \tag{2.22}$$

Additional summary statistics were calculated to assess the fit of the regression line to the data, as follows:

2.5.9.2 Lack of fit mean square (*LOFMS*)

The sum of squared deviations between the observed mean at each altitude and the fitted line, divided by the lack of fit degrees of freedom (summed across all observations), denoted as:

$$LOFMS = \frac{\sum_{i=1}^{n}(\hat{y}_i - \bar{y}_i)^2}{LOFdf}, \tag{2.23}$$

where *LOFdf* is the lack of fit degrees of freedom (which, in this case, is two less than the number of altitudes).

2.5.9.3 Pure error mean squares (*ErrorMS*)

The sum of squared deviations of individual observations about the mean for each altitude, summed over all altitudes, divided by the degrees of freedom, denoted:

$$ErrorSS = \sum_{i=1}^{n}(y_i - \hat{y}_i)^2, \tag{2.24}$$

$$ErrorMS = \frac{ErrorSS}{\sum_{i=1}^{n} N_A - 1} \tag{2.25}$$

where N_A is the means square degrees of freedom, which in this case is one less than the number of observations at altitude A.

2.5.9.4 R^2 value

The coefficient of determination, or R^2 value is a measure of how much of the variability in a data set is described by a fitted model. For example, a linear regression model with an R^2 value of 0.8 is describing 80% of the variability seen in the dataset. The R^2 value is calculated by dividing the error mean square by the total mean square and subtracting this ratio from one, as follows:

$$TotalMS = \frac{\sum_{i=1}^{n}(y_i - \bar{y})^2}{Totaldf}, \tag{2.26}$$

where $Totaldf$ is the total degrees of freedom (sample size - 1).

$$R^2 = 1 - \frac{ErrorMS}{TotalMS}. \tag{2.27}$$

The closer the final value is to one (i.e. the smaller the ErrorMS is), the better the model fits the data.

2.5.9.5 Assumptions for simple linear regression

- There is a linear relationship between the dependent and independent variable;
- The sample is representative of the population as a whole;
- The sample is normally distributed;
- The error is a random variable, where the mean is zero, and is conditional on the explanatory variables;
- The errors are uncorrelated;
- The variance of the error is constant across observations.

2.5.10 Simple linear regression uses

Simple linear regression analysis was used at several points during my thesis project. Initially, it was used on the *diary data* to assess whether each physiological measurement changed in a linear way with altitude. Simple linear regression analysis was used in exploratory data analysis to see if any metabolites showed an overall linear change during the expedition. Simple linear regression was also used as an initial modelling technique, to see if any of the dependent variables could be described as a linear function of any of the explanatory variables, as described in Section 6.2. Finally, simple linear regression was also used in the final stages of model refinement to assess the linear dependence between explanatory variable coefficient values used in a model formed by multiple linear regression, and some form of hypoxia measure. This information was used to produce a generalised model over several altitude changes, instead of having one model per altitude difference (for example the difference between all altitudes and London, not just Kathmandu and London).

The following summary statistics are available for the analysis of the diary data only, due to multiple measures being available at each altitude, and summarise the information available from the regression and ANOVA.

2.5.10.1 Goodness of fit

The *goodness of fit* (GOF) statistic was compared to an F-distribution to assess whether the regression line was significantly different from zero. The GOF statistic was calculated using the RegMS (calculated in equation 2.20) and the LOFMS (calculated in equation 2.23) as follows:

$$GOF = \frac{RegMS}{LOFMS} \tag{2.28}$$

2.5.10.2 Lack of fit

The *lack of fit* (LOF) statistic was compared to an F-distribution to formally test the goodness of fit of the regression line to the data. The LOF statistic was calculated by dividing the LOFMS (calculated in equation 2.23) by the ErrorMS (calculated in equation 2.25) as follows:

$$LOF = \frac{LOFMS}{ErrorMS} \tag{2.29}$$

If the lack of fit is *large* compared to the error term, then the means deviate more from the fitted line than is to be expected, and indicates a poor fit to the line. If the lack of fit is *small* compared to the error term, then this indicates that the mean is close to the fitted line, however, there is scatter within points around the mean.

2.5.10.3 Coefficient of Variation (C_V)

The coefficient of variation is the square root of the error mean square (the scatter of points around the mean value calculated for repeat y observations at a single value for x), divided by the mean, expressed as:

$$C_V = \frac{errorSD}{\bar{y}}, \tag{2.30}$$

where *errorSD* is the error standard deviation (square root of the *ErrorMS*, calculated in equation 2.25), and $\bar{y}$ is the variable mean. The C_V is a relative measure of the variability associated within a variable, and can be compared between variables. The C_V was calculated for the diary data, to assess whether particular physiological measurements showed high or low variability day-to-day at the same altitude, and to assess the *reliability* of measurements taken on a particular day.

2.5.11 Multiple Linear Regression

Simple linear regression assesses whether the dependent variable y can be described by one explanatory variable. Multiple linear regression is an extension of simple linear regression, where the dependent variable is potentially described by a linear combination of several explanatory variables. Explanatory variables can be combined, so that the response of one explanatory variable may depend on another. These *combination* terms are calculated by multiplying together two or more explanatory variables, as follows:

1. Simple linear regression; $y = a + bx$
2. Multiple linear regression; $y = a + bx_1 + cx_2 + dx_1x_2$,

where x_1 and x_2 are two separate explanatory variables, and x_1x_2 is the combination term. As the number of potential explanatory variables increases, it becomes more difficult to fit them all and assess the best model fit. In this case, the best model would be the one that best summarises the observed data available. Stepwise

regression techniques enable the selection of the best model for the observed data, whilst minimising the amount of time required to assess the fit of each model.

The *stepwisefit* algorithm was implemented in *matlab* for the two different stages of modelling undertaken during my thesis. In the exploratory modelling, MLR was used to describe one physiological variable from a selection of potential explanatory variables, over all altitudes. The second *targeted* form of modelling looked at using MLR to describe the percentage change seen in oxygen consumption or work rate with either absolute values at a low altitude of a selection of biochemical metabolites, or the percentage difference in biochemical metabolites at low altitudes. MLR was used to form initial models and refine models for the exploratory modelling, and form initial models that were refined separately for the targeted modelling.

The *stepwisefit* algorithm uses a systematic method for adding and removing terms from a multilinear model, based on their statistical significance in a regression. The method is a combination of forward selection and backward elimination techniques, beginning with an initial model, which is compared to the explanatory power of incrementally larger and smaller models. At each step of the algorithm, the p-value of an F-statistic is calculated and used to compare two models, one including a potential term and one without it. If the term is not currently contained in the model, the null hypothesis is that the extra term would have a coefficient of zero if it were added to the model. If there is sufficient evidence from the F-statistic and p-values produced, then the null hypothesis is rejected and the term is added to the model. Alternatively, if a term is currently in the model, the null hypothesis is again that the coefficient value is zero. If the null hypothesis is not rejected, then the term is removed from the model. This is discussed in more detail below.

The response y to predictor x is a system of linear equations:

$$y = bx, \tag{2.31}$$

where b is a matrix of coefficient estimates.

Fitting the initial model: The vector of coefficient estimators b is computed via a least-squares method using QR decomposition. It is possible to express x, an m-by-n matrix, as the product of Q, an m-by-m orthogonal matrix, and R, an m-by-n upper triangular matrix. I.e.

$$x = QR. \tag{2.32}$$

Define a matrix Q_B:

$$Q_B = Q^T y. \tag{2.33}$$

Multiple both sides of equation 2.31 by Q^T:

$$Q^T y = Q^T bx. \tag{2.34}$$

Substitute in for x:

$$Q^T y = Q^T bQR. \tag{2.35}$$

As Q is orthogonal:

$$QQ^T = I, \tag{2.36}$$

where I is the identity matrix. This removes the necessity to calculate an inverse matrix to solve the set of linear equations and speeds up the calculation. Hence it is possible to express:

$$Q^T y = bR, \tag{2.37}$$

and b can be found from:

$$b = Q_B/R. \tag{2.38}$$

Calculating the *p*-values: Following the initial model fit, explanatory variables are tested to determine whether or not they are included in the final model. As previously mentioned, the null hypothesis is that the variable would have a coefficient of zero if it were included in the model, and the user specifies the entry and exit tolerances in the form of p-values. These are calculated as follows:

1. Determine the standard error (SE):

$$SE = ErrorMS\sqrt{R/I}^2. \tag{2.39}$$

2. Calculate the critical t-value:

$$t_{crit} = b/SE. \tag{2.40}$$

3. Look up the p-value using the *matlab* function *tcdf*, which returns a probability for given values of t and degrees of freedom v using the equation shown below for the t-cumulative distribution function:

$$p = 2\int_{-\infty}^{-|t_{crit}|} \frac{\Gamma(\frac{v+1}{2})}{\Gamma(\frac{v}{2})} \frac{1}{\sqrt{v\pi}} \frac{1}{(1+\frac{t^2}{v})^{\frac{v+1}{2}}} dt, \tag{2.41}$$

where Γ is the gamma distribution.

The method for stepwise regression is then as follows:

1. Define all explanatory variables and combination terms;
2. Fit the initial model;
3. If any terms that are not already in the model have a p-value < 0.05 (i.e. it is unlikely that the term would have a coefficient of zero if added to the model), then add the term with the smallest p-value, and repeat the process. Else, go to step 4;
4. If any terms in the model have a p-value > 0.1 (i.e. it is unlikely that the term will not have a coefficient of zero), remove the one with the largest p-value and go to step 3. Else, end.

This method may build different models depending on the initial model formed, and the order in which terms are added and removed from the model. The algorithm terminates when no single step will improve the model. However, there is no guarantee that a different initial model or a different sequence of steps to add and remove terms will not produce a better fit. Therefore, stepwise fit models are locally optimal, but not globally optimal. For this analysis, the initial model began with no terms in the model, and added terms into the model based on the associated p-value.

2.5.11.1 Assumptions in multiple linear regression

- The dependent variable response is linearly dependent on one or more explanatory variables;
- The sample is representative of the population as a whole;
- The sample is normally distributed;
- The error is a random variable, where the mean is zero, and is conditional on the explanatory variables;
- The errors are uncorrelated;
- The variance of the error is constant across observations.

2.5.12 Adjusted R^2 value

The adjusted R^2 value modifies the R^2 value to take into account the number of explanatory variables used within a model, with penalties for larger numbers of explanatory variables. This is especially relevant when looking at multiple linear regression. The adjusted R^2 value will always be less than or equal to the R^2 value, and is defined as:

$$\bar{R}^2 = 1 - \frac{ErrorSS}{TotalSS} . \frac{Totaldf}{Errordf} = 1 - \frac{ErrorMS}{TotalMS}, \tag{2.42}$$

where $Totaldf$ is the total degrees of freedom (sample size - 1), and $Errordf$ is the degrees of freedom for the error [(sample size - number of explanatory variables in the model) - 1].

The adjusted R^2 value was calculated between observed data values and predicted data values for all models formed by multiple linear regression. This provided a measure for how much of the variability in the observed data was being explained by the model, adjusted for the number of explanatory variables in the model. These measures allowed the non-formal comparison of different models, to see whether or not model refinement improved the final fit seen between observed and predicted data. In most cases models could not be formally compared, as they were mostly based on slightly different datasets. For example, if a model were refined by removing one datapoint, the new model would be based on a slightly different dataset, and could no longer be formally compared to the original model.

Chapter 3

Biochemical Experimental Results

This section details the results from the biochemical experiments I carried out at The University of Warwick as part of my thesis project. It contains information on the different experimental techniques as well as some of the key results obtained by each technique. The purpose of this work was to collect a large set of biological data for later modelling efforts, and to familiarise myself with different experimental techniques, so that I could understand any problems that arose as a result of data collection. The following techniques were all carried out by myself, unless stated otherwise.

3.1 Oxidative stress

There is currently no agreement in the literature as to which measure of oxidative stress is the most valuable or significant. Therefore, three independent metabolites associated with oxidative stress were measured during this study, to gain a clear picture about how the individuals were responding during the course of the expedition. These were 8-iso-Prostaglandin $F_{2\alpha}$ (8-isoPGF$_{2\alpha}$), 4-hydroxy-2-nonenal (HNE) [Zarkovic, 2003] and glutathione levels. 8-isoPGF$_{2\alpha}$ and glutathione were measured as part of my thesis project, and their measurement is detailed below. This information was combined with the results for HNE, generated by another member of the Feelisch group, to gain an overview of how the level of oxidative stress changed in the individuals during the expedition.

3.1.1 8-iso-Prostaglandin $F_{2\alpha}$

Isoprostanes are a class of prostanoids (a group of lipid compounds derived enzymatically from fatty acids) that are generated as a result of the attack of arachidonic acid by free radicals during oxidative stress. 8-isoPGF$_{2\alpha}$ is a secondary peroxidation end product, and 8-isoPGF$_{2\alpha}$ levels have been shown to be a good indicator of oxidative stress in humans, compared to other markers [Morrow et al., 1992].

3.1.1.1 Immunoassay

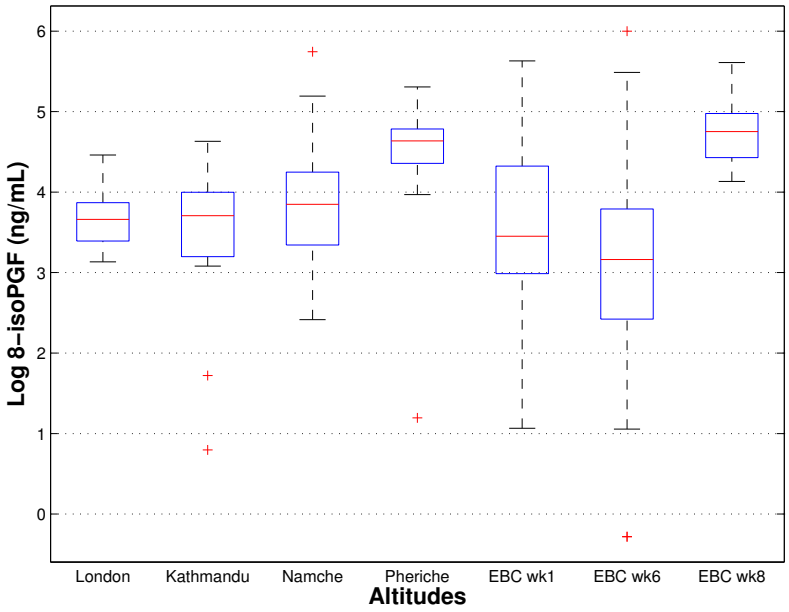
A direct 8-iso-Prostaglandin F$_{2\alpha}$ Enzyme Competitive Immunoassay Kit (Assay Designs catalogue number 901-091) was used to measure 8-isoPGF$_{2\alpha}$ levels in each of the 24 core team member's plasma samples, as detailed in Section 2.2.1. The kit uses a polyclonal antibody to bind, in a competitive manner, to the 8-isoPGF$_{2\alpha}$ available in the sample, or with an exogenous 8-isoPGF$_{2\alpha}$ with an alkaline phosphatase molecule covalently attached.

After a simultaneous incubation, the excess reagents were washed away, including any excess phosphatase. A p-nitrophenylphosphate substrate was then added, which is a chromogenic substrate that can be cleaved by a phosphatase to produce p-nitrophenol, which is yellow in colour, as shown in equation 3.1. After incubation, the substrate was washed away, and the amount of yellow product formed was measured in a microplate reader at 405nm. The amount of yellow colour produced is *inversely proportional* to the concentration of free 8-isoPGF$_{2\alpha}$ in the sample. This is because the stronger the yellow colour generated, the less 8-isoPGF$_{2\alpha}$ was present in the sample to compete with the 8-isoPGF$_{2\alpha}$ coupled to the phosphatase.

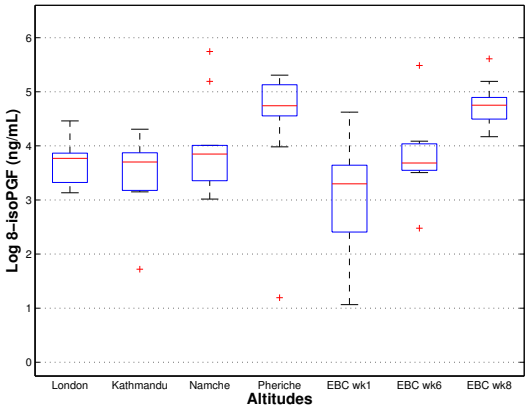
$$p-nitrophenylphosphate \xrightarrow{\text{Phosphatase}} p-nitrophenol(yellow) \tag{3.1}$$

3.1.1.2 Results

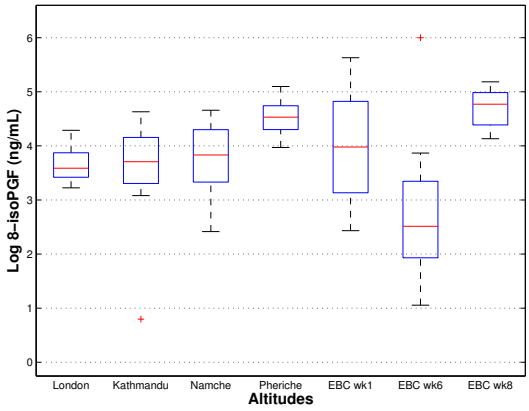
Figure 3.1 shows the results for the 8-isoPGF immunoassay. Figure 3.1a shows all 24 core team individuals plotted over 7 time points. Figures 3.1b and 3.1c show the results for the 10 lab staff and 14 climber individuals, respectively. 8-isoPGF$_{2\alpha}$ show a slight increase for all individuals between London and Namche, with the climbers having a slightly wider range of values than the lab staff. There is an increase in 8-isoPGF$_{2\alpha}$ levels between Namche and Pheriche for all individuals. There is then a decrease in 8-isoPGF$_{2\alpha}$ levels between Pheriche and Everest Base Camp week 1



(a) All individuals



(b) Lab Staff



(c) Climbers

Figure 3.1: Box and whisker plots showing how 8-isoPGF$_{2\alpha}$ levels change in 24 core team individuals during CXE 2007, showing log transformed values for (a) all individuals, (b) for 10 lab staff individuals and (c) for 14 climber individuals. Both lab staff and climber groups show similar responses up to Everest Base Camp Week 1, with differences between how the lab staff and climber groups respond between Everest Base Camp weeks 6-8.

for all groups, with the lab staff showing a larger decrease, shown in Figure 3.1b. The lab staff show an increase in 8-isoPGF$_{2\alpha}$ levels between Everest Base Camp weeks 1-6, whereas the climbers show a decrease. This may be due to the difference in ascent profile between the two groups here, shown in Figure 1.1, where climbers ascended higher than lab staff individuals. However, the climber levels would be expected to increase here, and not decrease. This may be due to the climber group having more experience at altitude than the lab staff group, and showing better anti-oxidant responses at high altitudes compared to the lab staff group.

3.1.2 Glutathione determination

Reduced glutathione (GSH) is a tripeptide (γ-glutamylcysteinylglycine) that contains a free thiol (SH) group. GSH acts as an antioxidant, providing reducing equivalents for the glutathione peroxidase catalysed reaction with harmful substances, such as hydrogen peroxide to water, as shown in Figure 1.3. This reaction forms a disulphide bond between two GSH molecules, forming oxidised glutathione (GSSG), whilst simultaneously oxidising β-nicotinamide adenine dinucleotide phosphate (β-NADPH) to NADP$^+$. Any GSSG formed is readily converted back into GSH by the enzyme glutathione reductase, which is also induced during oxidative stress [Haddad and Harb, 2005; Rossi et al., 2002].

In healthy cells more than 95% of the total glutathione in the cell is in the form of GSH and less than 5% is GSSG. If the cell is exposed to oxidative stress, GSSG will accumulate and the ratio of GSH to GSSG will decrease. Therefore, the quantification of GSH and GSSG can be used as an indicator of oxidative stress in cells and tissues [Rahman et al., 2006].

3.1.2.1 The Tietze glutathione recycling assay

The Tietze assay is based on the glutathione recycling system by 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, also known as Ellman's reagent) and glutathione reductase [Tietze, 1968; Rossi et al., 2002]. DTNB reacts with GSH to produce GSSG and the chromophoric compound 2-nitro-5-thiobenzoic acid (TNB), which is yellow. The GSSG formed is reduced back to GSH by glutathione reductase with the help of the cofactor NADPH, as shown in Figure 3.2. Any new GSH formed is free to react with DTNB to form more TNB. Therefore, the total glutathione concentration of a sample can be determined by measuring the absorbance of the mixture at 412 nm, which indicates how much TNB has been produced. This recycling system significantly increases the sensitivity of glutathione detection compared to non-recycling

methods [Tietze, 1968].

2-vinylpyridine (2-VP) is an alkylating agent that reacts with the thiol (SH) group on GSH. 2-VP can be added to the mixture to derivatise any GSH present, removing it from the reaction and allowing the quantification of GSSG present within the sample [Griffith, 1980]. The amount of GSH can then be calculated from subtracting twice the oxidised glutathione concentration from the total glutathione concentration.

A working glutathione assay protocol had previously been used in the Feelisch lab for the determination of reduced and oxidised glutathione in fresh rat tissue and plasma. My aim was to modify the protocol to allow the determination of both reduced and oxidised glutathione levels in frozen human plasma samples, taken from the CXE core team. The glutathione levels in human plasma are lower than those found in rat plasma, therefore the sensitivity of the protocol had to be increased. It was also not known how storing the samples at -80^{o}C may affect the levels detected.

Over a period of several months, the protocol was modified to improve reliability and sensitivity. The original protocol was improved to allow the detection of significantly lower quantities of glutathione than were possible in the first attempts. The changes incorporated included:

- Lowering the dilutions made to the samples;
- Using a different supplier for the main enzyme. Initially an enzyme from Sigma was used, and this was then replaced with an enzyme from Roche. The specific activity of the two enzymes was comparable;
- Including a missed neutralisation step;
- Changing the dilution of the enzyme;
- Using fresh reagents every day;
- Increasing the temperature and length of the incubation period;
- Spiking the samples with a known concentration of oxidised and reduced glutathione.

Despite all of these iterations, it was still not possible to obtain consistent standard curves at low glutathione concentrations, or reliably measure glutathione levels in human plasma samples that had been kept frozen for an extended period of time. The refined version of this lab assay is detailed in Section 2.2.2.1.

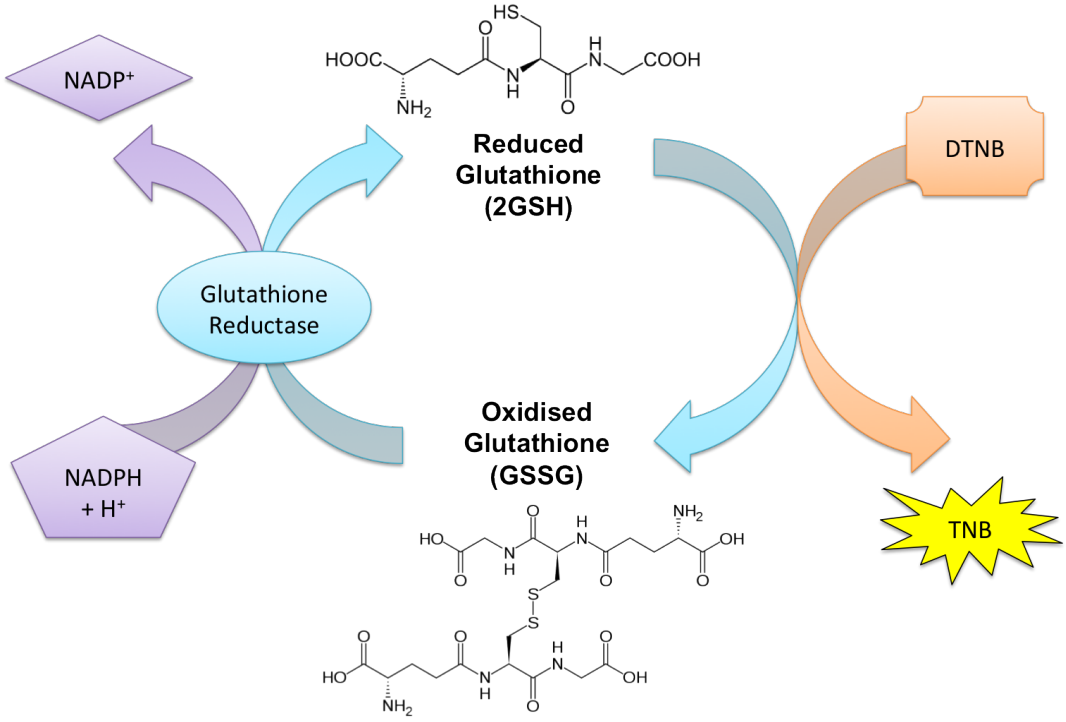


Figure 3.2: DTNB reaction with reduced glutathione (GSH) to form yellow TNB. The rate of the formation of yellow product is directly proportional to the amount of GSH present in the sample.

3.1.2.2 Glutathione fluorescence assay

Due to the poor reliability of the Tietze recycling assay, in the end a commercial Glutathione Assay Kit (BioVision, catalogue number K264-100) was used to quantify both reduced and total glutathione levels within the plasma samples, as described in Section 2.2.2.3. Oxidised glutathione levels were then calculated from these measurements. Several other commercial glutathione kits were considered, however, they all specifically stated that *"GSSG in normal resting plasma is at or below the lowest level of detection for the assay."*

The BioVision assay uses a molecule called o-phthalaldehyde (OPA), which reacts selectively with reduced thiols such as GSH to generate fluorescence, allowing the quantification of GSH within the sample. The addition of a reducing agent converts all of the GSSG present into GSH, and the addition of OPA allows the specific quantification of the total glutathione in the sample.

GSSG can either be calculated by subtracting GSH from total glutathione levels, or be measured specifically by adding a quencher to remove the GSH present, and then adding a reducing agent to destroy the quencher and convert the GSSG present to GSH for quantification by OPA.

3.1.2.3 Results

The results for the measurement of the different glutathione components are shown in Figure 3.3. The lack of results for London is due to all of the London measurements being out of range, even for this technique. Given that levels were measurable for all other altitudes, this result came as a surprise, and may have been the result of an issue with the plate or user error. It was not possible to re-run this particular plate due to the limited supply of plasma samples available. London levels of GSH and the GSH:GSSG ratio would be expected to be higher than those seen for Kathmandu. London GSSG levels would be expected to be lower than those seen for Kathmandu, and total glutathione would be expected to be similar to that seen for Kathmandu.

GSH levels show a slight decrease between Kathmandu and Namche, and GSSG levels show an increase, shown in Figures 3.3a and 3.3b, respectively. As the individuals are exposed to hypoxia, GSH present in the plasma would react with ROS producing GSSG, therefore GSH levels are expected to decrease, and GSSG levels are expected to increase [Chang et al., 1989]. The resulting GSH : GSSG ratio shows a decrease between Kathmandu and Namche, shown in Figure 3.3d, indicating an initial increase in oxidative stress.

GSH and total GSH/GSSG levels then show a steady increase up to Everest Base Camp, coupled with a decrease in GSSG levels at these altitudes. This is most likely due to an overall increase in glutathione production, which is inducible during oxidative stress. In addition, glutathione reductase is increased [Haddad and Harb, 2005; Rossi et al., 2002], and would convert the excess GSSG back to GSH, resulting in the increase in the GSH:GSSG ratio seen in Figure 3.3d.

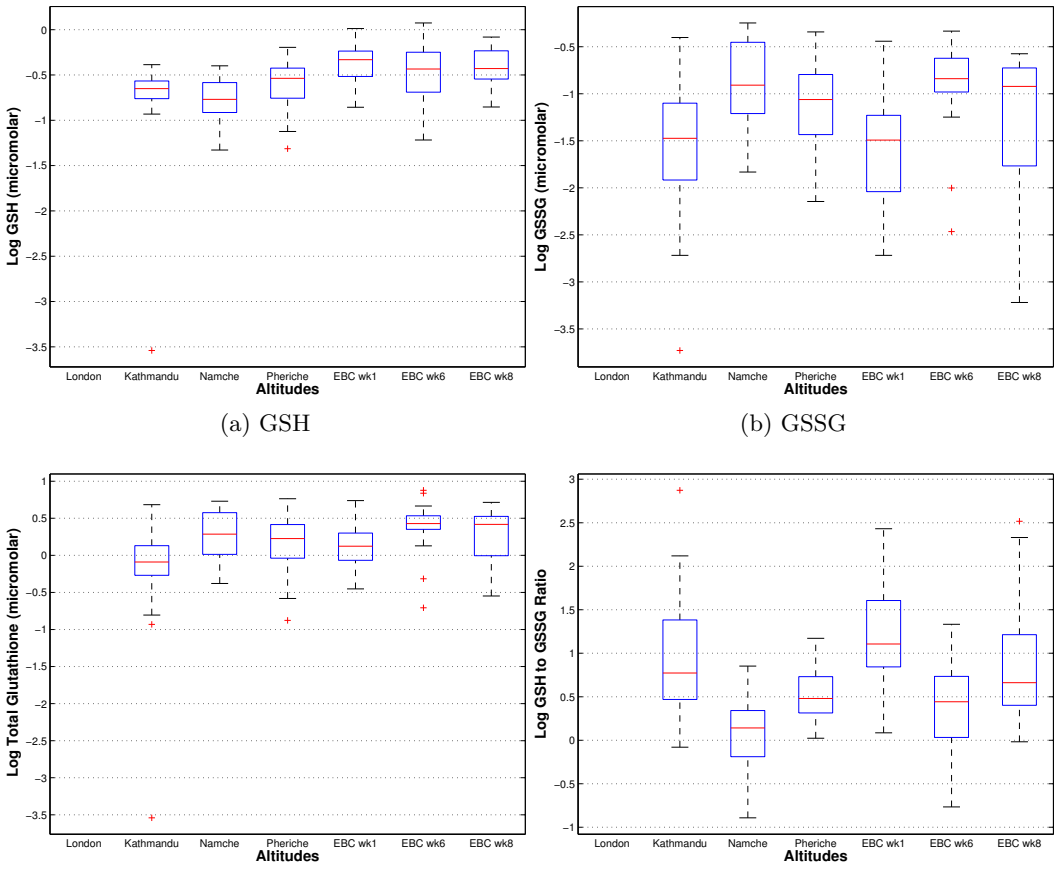
A further increase in oxidative stress is seen between Everest Base Camp weeks 1-6, shown by a decrease in GSH and the GSH:GSSG ratio, and an increase in GSSG. This shows an altitude-independent change, and shows the response to prolonged exposure to extreme altitude. This may also show the limit at which the individuals can regulate oxidative stress levels.

3.1.3 Oxidative stress summary

Overall, there was a general increase in oxidative stress seen during CXE 2007. This was shown by an overall increase in 8-isoPGF$_{2\alpha}$ levels shown in Figure 3.1a, an overall increase in HNE shown in Figure 3.4a and a decrease in the ratio of reduced to oxidised glutathione, shown in Figure 3.3d. However, this increase is not constant across all altitudes, and the metabolite levels peak at different altitudes. These differences may be due to variations in the chemical reactivity and biochemical pathways that lead to the formation of these different products. A general increase in oxidative stress was expected during the expedition; as individuals were exposed to higher levels of hypoxia, ROS levels would increase, producing more ROS-products such as 8-isoPGF$_{2\alpha}$ and HNE, and antioxidants such as GSH would be consumed [Morrow and Roberts, 1999, 2002]. None of the measures of oxidative stress showed a significant difference between lab staff and climber groups, shown by an acceptance of the null hypothesis for a t-test performed between the two groups, at a 5% significance level. However, this may be due to the sample sizes being too small to have the sufficient power to reject the null hypothesis at the 5% level (10 lab staff individuals and 14 climber individuals), and some group differences are apparent for 8-isoPGF$_{2\alpha}$ levels in Figure 3.1.

3.2 Osmolality

Osmolality is a measure of the number of osmoles of solute per kilogram of solvent (osmol/kg). The number of osmoles is the number of moles of a chemical compound that contribute to a solution's osmotic pressure, which is the pressure which must be applied to a solution to prevent the inward flow of water across a semi-permeable



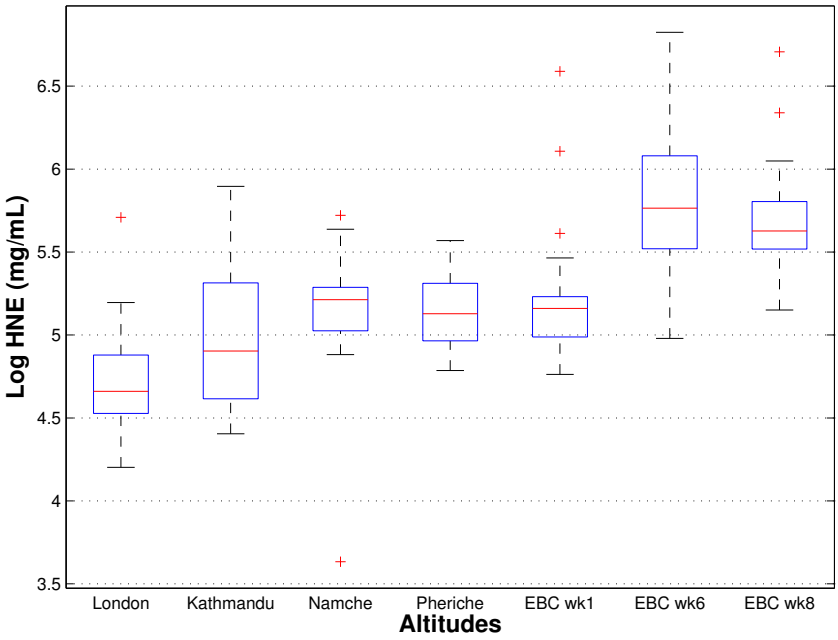
(a) GSH

(b) GSSG

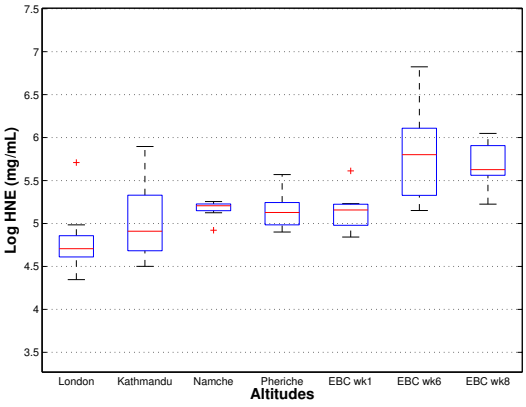
(c) Total Glutathione

(d) GSH : GSSG Ratio

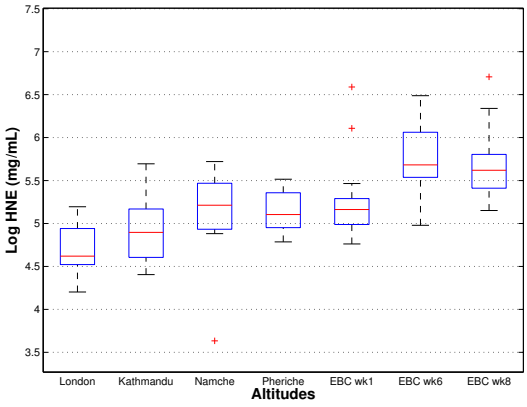
Figure 3.3: Box and whisker plots showing how log-transformed glutathione levels change in 24 core team individuals during CXE 2007. (a) shows reduced glutathione (GSH), (b) shows oxidised glutathione (GSSG), (c) shows total glutathione and (d) shows the GSH : GSSG ratio of the plasma.



(a) All individuals, log transformed values



(b) Lab Staff, log transformed values



(c) Climbers, log transformed values

Figure 3.4: Box and whisker plots showing how log-transformed values from hydroxynonenal (HNE) levels changed in 24 core team individuals during CXE 2007. (a) shows all individuals, with a general increase during the expedition. (b) and (c) show no real difference in the response seen for the sub-groups lab staff (b) or climbers (c).

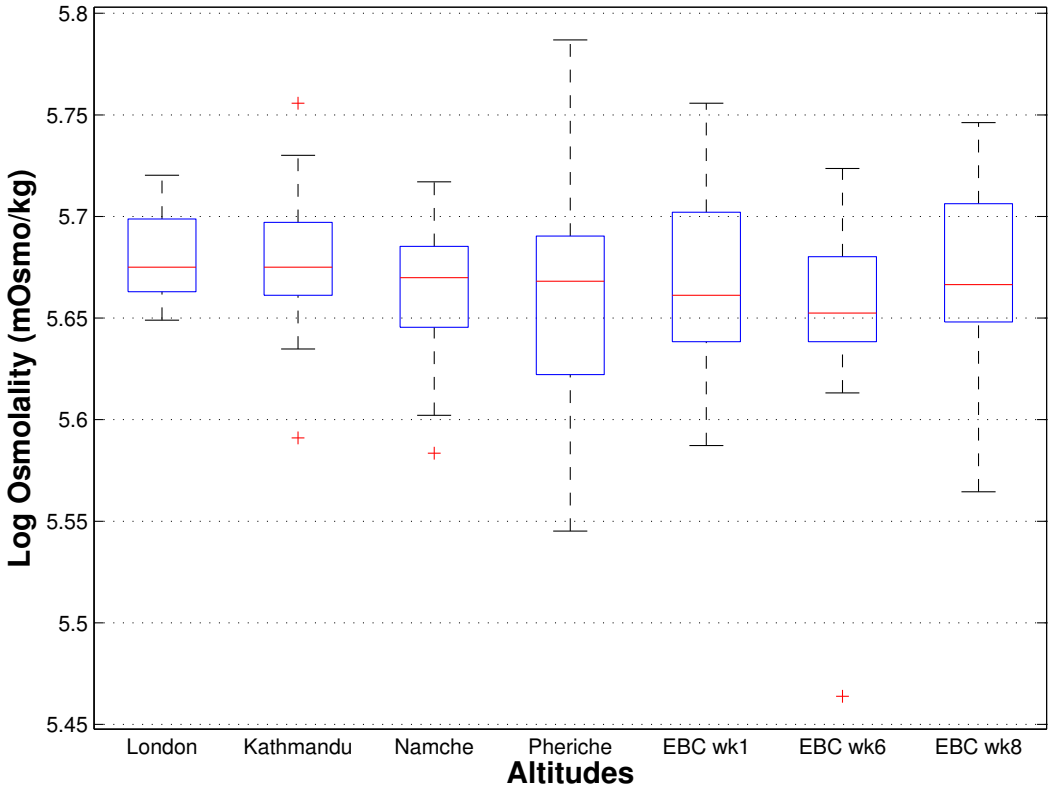


Figure 3.5: A box and whisker plot showing the change in log-transformed plasma osmolality of 24 core team members during CXE 2007. This measure was done as a control, to see whether changes in other metabolites measured in the plasma were *real changes* or due to a change in plasma density, due to fluid shifts or increased diuresis (urine output). There was no significant change in plasma osmolality during CXE 2007, assessed by the acceptance of the null hypothesis for the ANOVA at the 5% confidence level, as described in Section 2.5.6.

membrane. An Advanced Model 3300 Micro-Osmometer was used to measure the osmolality of the 24 core team member's plasma samples, detailed in Section 2.2.3. Plasma osmolality was determined by using freezing point depression (FPD) osmometry. Adding another solute to an initial solution lowers the freezing point of the mixture. For example, seawater remains liquid at temperatures below 0^oC (the freezing point of pure water at atmospheric pressure) due to its salt content. Osmometry measures the total molar concentration of dissolved solids in any solution, as the freezing point of a solution is depressed in direct relation to the solute concentration.

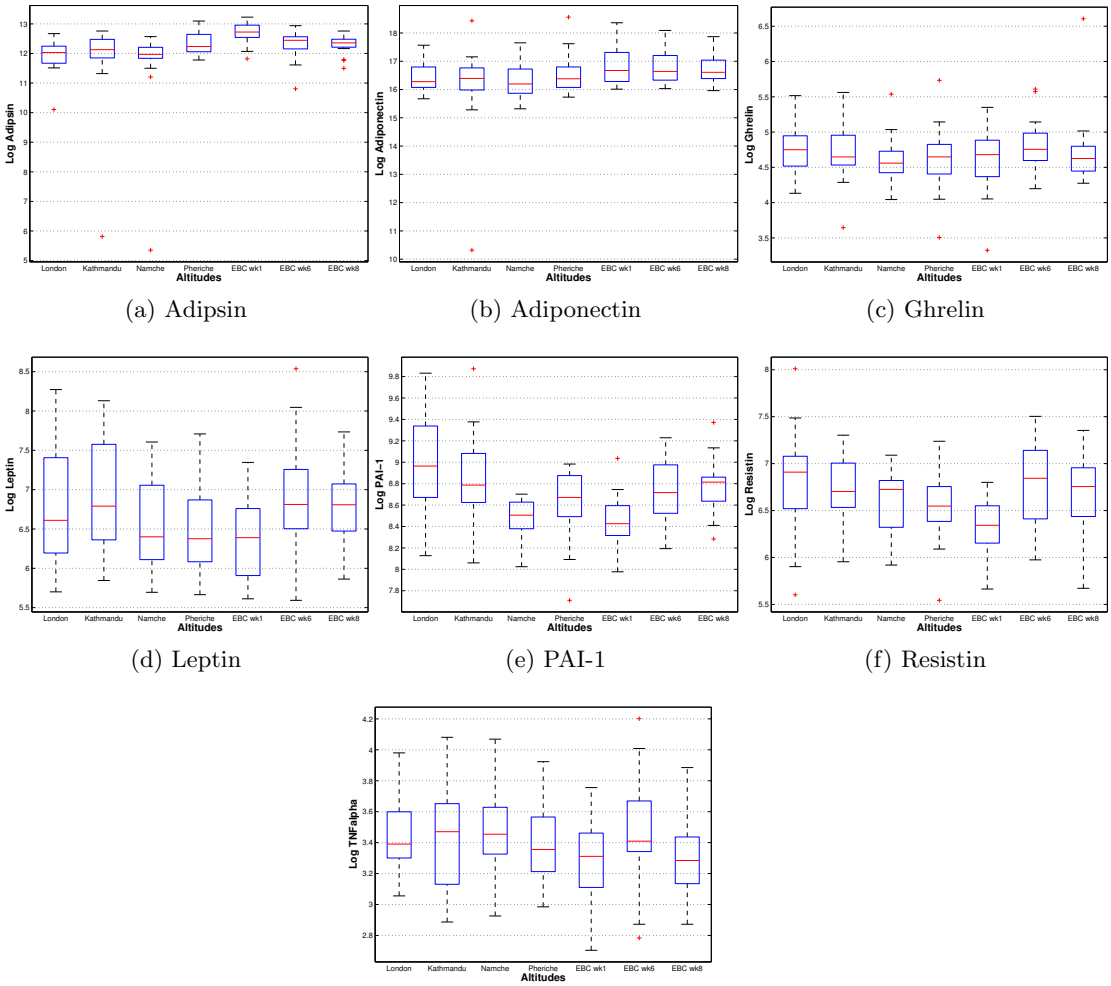
3.2.1 Results

During acute exposure to high altitude, urine output generally increases (known as diuresis) to concentrate the plasma, and increase the blood's oxygen carrying capacity. The body's fluid also shifts from the intravascular space to the interstitial and intracellular spaces, to decrease the blood plasma within hours of exposure to hypoxia [McArdle et al., 2007]. Therefore, a general increase in plasma osmolality was expected during the expedition. Figure 3.5 shows the osmolality levels for the core team's plasma samples during CXE 2007. The figure shows no change in plasma osmolality during the expedition, confirmed by a rejection of the null hypothesis for the ANOVA at the 5% confidence level. As CXE 2007 was a strictly-controlled expedition, all participants were kept well-hydrated during the entire expedition [CASE, 2012]. This would have compensated for any increased urine output, and explain why plasma osmolality stayed constant throughout CXE 2007.

3.3 BioPlex analysis

The Bio-Rad BioPlex system is based on Luminex xMAP technology, which allows the *multiplexing* of up to 100 different multiple ELISA-type assays to be performed within a single sample. Each assay is performed on the surface of a $5.6\mu m$ polystyrene bead. The beads are filled with different ratios of green and red fluorescent dye, resulting in a possible array of 100 distinct colours. Each set of beads can be combined with a different capture molecule or antibody, which can then be mixed with different samples in a microplate to detect the presence of specific antigens.

Detection follows a sandwich immunoassay method, where the beads are incubated with sample, and a fluorescently-labelled reporter tag is added to bind specifically to the sample of interest. The beads are drawn into the BioPlex machine and passed through the machine in a single file, where two lasers excite the beads

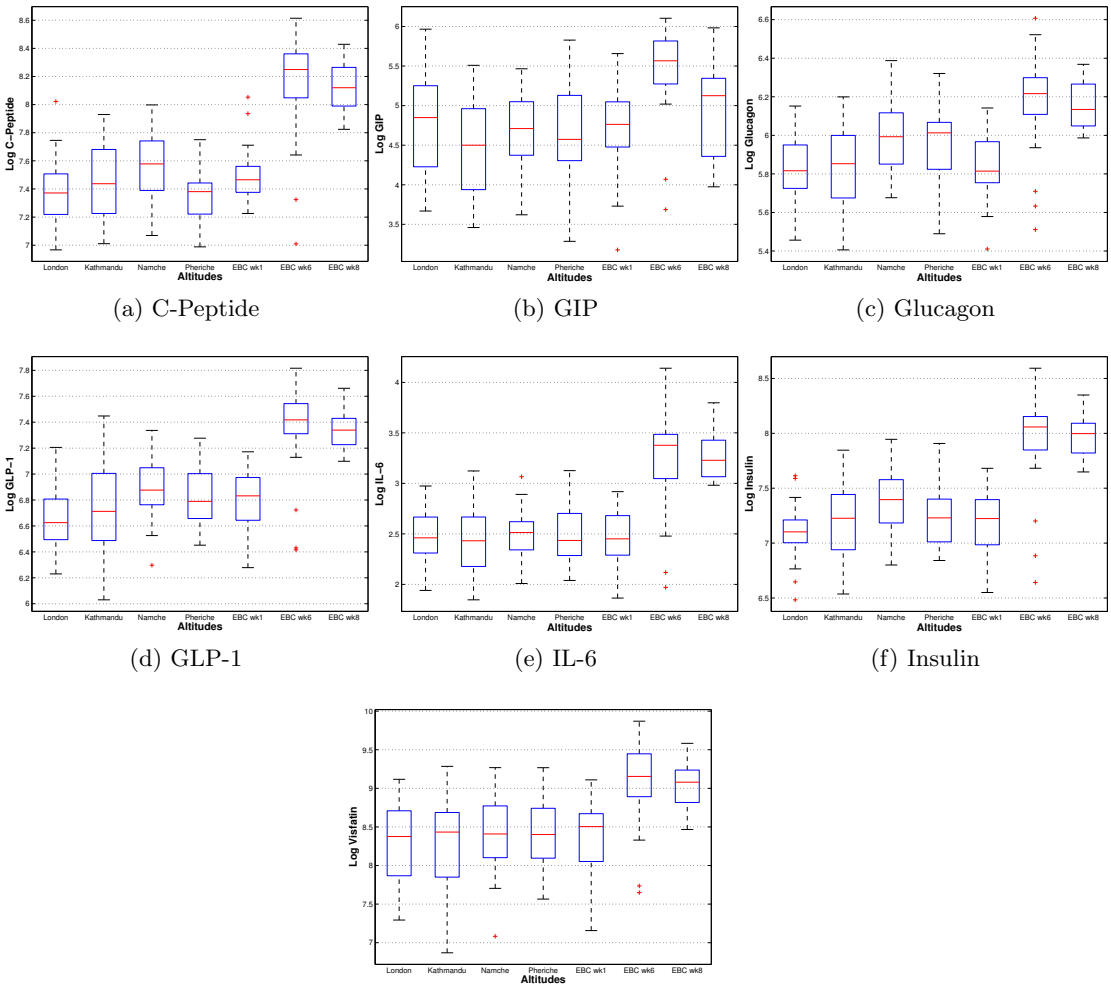


(a) Adipsin (b) Adiponectin (c) Ghrelin

(d) Leptin (e) PAI-1 (f) Resistin

(g) TNF-α

Figure 3.6: Log-transformed box and whisker plots showing 7 diabetes-related biochemical metabolites measured in 24 individuals during CXE 2007. All of the metabolites apart from ghrelin (shown in (c)) show differences between altitudes, shown by a rejection of the null for the ANOVA at the 5% level (as described in Section 2.5.6, and shown in Table 3.1).



(a) C-Peptide (b) GIP (c) Glucagon

(d) GLP-1 (e) IL-6 (f) Insulin

(g) Visfatin

Figure 3.7: Log-transformed box and whisker plots showing 7 diabetes-related biochemical metabolites measured in 24 individuals during CXE 2007. Only C-peptide, glucagon, GLP-1 and insulin show changes between altitudes, indicated by a rejection of the null for the ANOVA at the 5% level (as described in Section 2.5.6, and shown in Table 3.1). However, all of these metabolites show altitude-independent differences between Everest Base Camp weeks 1-8, which were shown to be significant by a rejection of the null for the ANOVA at the 5% level.

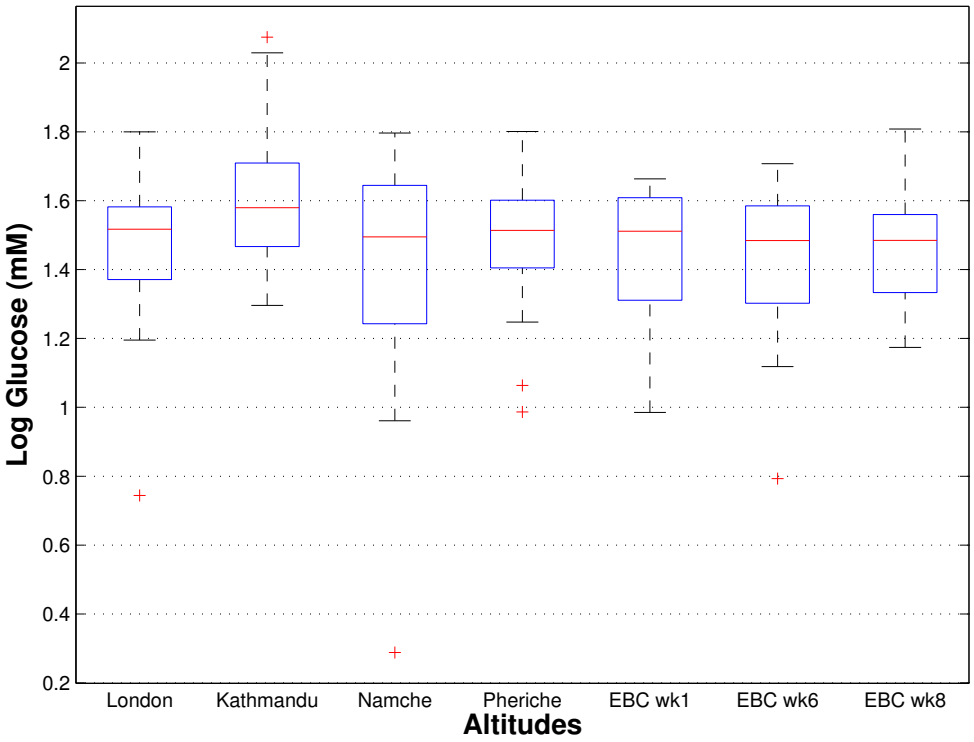


Figure 3.8: Box and whisker plot showing log-transformed glucose levels in 24 individuals during CXE 2007. Glucose levels show a significant change between London - Everest Base Camp week 1, shown by a rejection of the null hypothesis for an ANOVA at the 5% level (shown in Table 3.1). However, there is no significant change between Everest Base Camp weeks 1-8, as shown by an acceptance of the null for an ANOVA at the 5% level.

individually. The classification laser excites the dyes in each bead, identifying the bead's spectral address. The second laser excites the reporter molecule bound to the antigen of interest, allowing the quantification of the captured analyte. The signals are read simultaneously for each bead in a high-throughput manner, to allow the fast quantification of several molecules of interest in one sample.

3.3.1 Results

The BioPlex system was used to measure a total of 14 different metabolites linked to diabetes, obesity and metabolism, as described in Table 2.2. These metabolites were measured in each of the core team member's plasma samples, as detailed in Section 2.2.4, and the results are shown in Figures 3.6 and 3.7. Two separate 2-way ANOVAs (as described in Section 2.5.6, looking for the effect of altitude and individual, without interaction) were carried out here; one for results between London and Everest Base Camp (EBC) week 1, and the other for EBC weeks 1, 6 and 8. The first set of analyses tested for altitude-dependent changes, as there was a change in altitude between each of the samples. The second set of analyses tested for altitude-independent changes, as the samples were all taken at the same altitude (Everest Base Camp, 5300m).

Figure 3.6 shows responses for adipsin, adiponectin, ghrelin, leptin, PAI-1, resistin and TNF-α. Ghrelin does not show a change between altitudes, or between the three time-points at Base Camp, with significance levels for the ANOVA of 0.17 and 0.54 for London to EBC week 1 and EBC weeks 1-8, respectively (shown in Table 3.1). However, ghrelin does show differences between individuals for both of these sets of data, with significance values of $1.9\text{x}10^{-22}$ for London to EBC week 1 and 0.0015 for EBC weeks 1-8. Adiponectin does not show a difference between individuals between EBC week 1-8, with a significance value of 0.076. All other metabolites show differences between individuals and altitudes for both London to EBC week 1 and EBC weeks 1-8, indicating both hypoxia-dependent changes (London - EBC week 1) and hypoxia independent changes (EBC weeks 1-8) are occurring in these metabolites, and that there are differences between individuals.

Figures 3.6a and 3.6b show the results for adipsin and adiponectin, respectively. Both of these metabolites show low outlying values that *skew* the box and whisker plot, even when log-transformed, and make it difficult to see the general pattern during the expedition. The low outlier seen for Kathmandu for both adipsin and adiponectin is for the same individual, denoted X18, indicating that this individual is showing a particularly low response for these metabolites at that altitude. The low outlier for adipsin at Namche is for a different individual, denoted X14.

Figure 3.7 shows the responses for C-peptide, GIP, glucagon, GLP-1, IL-6, insulin and visfatin. Only C-peptide (Figure 3.7a), glucagon (Figure 3.7c), GLP-1 (Figure 3.7d) and insulin (Figure 3.7f) show changes between altitudes between London and EBC week 1. This was indicated by a rejection of the ANOVA null hypothesis at the 5% level, with significance levels of 0.0024, 0.00000037, 0.00045 and 0.00001, respectively (shown in Table 3.1). However, all of the metabolites show altitude-independent changes between EBC weeks 1-8, shown by a rejection of the ANOVA null at 5%, shown in Table 3.1. The increase seen between EBC weeks 1-8 is seen for both lab staff and climber groups in all cases, even though the two groups were exposed to different conditions during these time points, as shown in Figure 1.1. It is also worth considering that the time between EBC 1-6 is in terms of weeks and not days, as the earlier measurements were.

Figure 3.8 shows the change in glucose levels during the expedition. Glucose levels show a change between London and EBC week 1, indicated by a significance value of 0.0015, shown in Table 3.1. Interestingly, glucose does not show a change between EBC weeks 1-8, as shown by significance value of 0.8. Many of the metabolites reported in Figure 3.7 that show an increase between EBC weeks 1-6 play a role in glucose metabolism, including C-Peptide (Figure 3.7a), GIP (Figure 3.7b), glucagon (Figure 3.7c), GLP-1 (Figure 3.7d) and insulin (Figure 3.7f).

Glucose metabolism is strictly controlled, which would explain why it only shows a small change between London and EBC week 1, and does not show a change in-between EBC weeks 1-8. The sharp increase seen for many of the glucose-related metabolites between EBC weeks 1-6 suggests some form of breakdown in these pathways during prolonged exposure to extreme hypoxia. It may even suggest some form of hypoxia-induced insulin-resistance, and requires further investigation to gain an understanding of what is happening at extreme altitude. Additional plasma samples are available at intermediate time points between Everest Base Camp weeks 1-8, however, there was not sufficient time to measure these additional samples during my thesis project. The measurement of these samples would help gain a better understanding of what happens to glucose metabolism during prolonged exposure to extreme hypoxia.

Metabolite	London to Everest Base Camp week 1				Everest Base Camp weeks 1 - 8			
	Altitude		Individual		Time-point		Individual	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Adiponectin (pg/mL)	6.75	• 8.3E-05	13.94	• 6.4E-21	2.73	7.6E-02	6.62	• 5.8E-08
Adipsin (pg/mL)	28.31	• 2.5E-15	3.89	• 1.7E-06	35.62	• 7.5E-10	5.44	• 9.3E-07
Ghrelin (pg/mL)	1.65	1.7E-01	15.47	• 1.9E-22	0.62	5.4E-01	2.85	• 1.5E-03
Leptin (pg/mL)	8.82	• 4.5E-06	9.89	• 3.4E-16	7.86	• 1.2E-03	4.13	• 3.0E-05
PAI-1 (pg/mL)	13.83	• 7.5E-09	1.95	• 1.4E-02	11.77	• 8.4E-05	1.76	5.5E-02
Resistin (pg/mL)	9.25	• 2.5E-06	4.39	• 1.8E-07	11.85	• 8.0E-05	2.50	• 4.7E-03
TNFalpha (pg/mL)	3.50	• 1.1E-02	8.38	• 4.1E-14	4.28	• 2.0E-02	2.29	• 9.3E-03
C-Peptide (pg/mL)	4.46	• 2.4E-03	2.61	• 6.4E-04	44.89	• 3.0E-11	1.53	1.1E-01
GIP (pg/mL)	1.42	2.3E-01	1.95	• 1.4E-02	21.73	• 3.0E-07	3.27	• 3.9E-04
Glucagon (pg/mL)	10.70	• 3.7E-07	7.27	• 1.9E-12	25.24	• 5.6E-08	1.64	7.9E-02
GLP-1 (pg/mL)	5.59	• 4.5E-04	8.64	• 1.7E-14	38.64	• 2.5E-10	1.52	1.2E-01
Insulin (pg/mL)	8.21	• 1.0E-05	8.30	• 5.3E-14	32.54	• 2.5E-09	1.43	1.5E-01
IL-6 (pg/mL)	0.30	8.7E-01	6.73	• 1.4E-11	47.02	• 1.5E-11	1.67	7.3E-02
Visfatin (pg/mL)	0.50	7.4E-01	9.98	• 2.6E-16	24.58	• 7.6E-08	2.38	• 6.9E-03
Glucose (mM)	4.78	• 1.5E-03	3.18	• 4.4E-05	0.23	8.0E-01	0.74	7.8E-01

Table 3.1: 2-way ANOVA results assessing the effect of altitude and individual, performed between 5 altitudes (London to Everest Base Camp week 1), representing altitude-dependent changes, and separately on the three time-points at Everest Base Camp (Everest Base Camp weeks 1, 6 and 8), representing altitude-independent changes. p-values marked with a dot (•) indicate a rejection of the null hypothesis at the 5% level, indicating a difference between means, as described in Section 2.5.6.

Chapter 4

Diary Data

The *diary data* is an additional dataset of simple physiological measurements collected for the 24 core team members. It consists of daily measurements taken during the expedition, and shows the day-to-day variation of each physiological measurement within the dataset. The analysis presented in this chapter aims to assess the precision and reproducibility of these measurements during the ascent, to inform the analysis of the main core dataset. Similar physiological measurements are available in the main dataset, therefore, the analysis performed on the diary data can be used to highlight any measures that may potentially be useful for further analysis in the core dataset.

The diary data contains information collected on blood pressure, oxygen saturation, heart rate and respiratory rate. For each of these four main physiological measurements, there are two measured variables and two derivations available. The blood pressure measurements are Systolic and Diastolic blood pressure, as well as the ratio of and difference between these two biological measurements. For oxygen saturation, heart rate and respiratory rate, measurements were taken at rest and after a 2-minute step exercise test, and the ratio of and difference between these two biological measurements were also calculated. A full description of all 16 physiological measurements contained in the diary data, and the shorthand names that will be used throughout the rest of the analysis, can be found in Table 4.1.

The 2-minute step test exposes an individual to a small amount of physical stress on each day. The difference between and ratio of measurements at *rest* and *after exercise* show an individual's response to a defined amount of stress at different altitudes, and can be used as a measure of how well that individual is adapting to the environmental change. It was important to ascertain which of the four derivations for each physiological measurement was the most consistent, useful or biologically

relevant, as there may be redundancy within the measurements. This dataset allows an assessment of the validity of the single-point measurements collected in the main core study dataset, by analysing whether changes between altitudes seen for a particular measurement are within the normal day-to-day variability, or larger, indicating a true response to altitude.

This dataset can also be used to detect different *patterns* of adaptation between individuals. For example, individuals may show no significant change in response to a change in altitude, a steady change in response to a change in altitude, or an erratic or extreme response to a change in altitude. Comparing the responses seen at different altitudes allows the analysis of how individuals respond to changes in altitude, considering both short-term responses (to changes in altitude) and long-term adaptation.

Data is available for a maximum of 78 days for each individual, however, all individuals have at least one day where measurements were not collected. This may be due to an individual being ill or otherwise unavailable for testing on a particular day. Data is available for an average of 73 days for lab staff members and 63 days for climber individuals, as defined in Section 1.3. This difference is mainly due to the climber team attempting to summit between days 60-70 (shown in Figure 1.1), and measurements not being taken at extreme high altitudes.

4.1 Aims

The diary data has been used to assess the following questions:

1. Do the physiological measurements change with altitude?
2. Which of the physiological measurements are the most reliable?
3. Is there redundancy among these physiological measurements?
4. Is there evidence of different levels or types of adaptation?
5. Which of the physiological measurements are potentially the most suitable for further analysis?

4.1.1 Lab staff vs. climber group

In conducting the analyses of the diary data, the dataset was initially split into two groups, one containing the records for the 10 lab staff and the other containing the records for the 14 climbers, as detailed in Section 1.3. The climber group was a

Parameter Full Name	Abbreviation	Parameter Description	Units
O_2 Saturation at Rest	O_2Sat_{Rest}	The saturation of haemoglobin with oxygen, as measured by pulse oximetry (at rest)	%
O_2 Saturation after Exercise	O_2Sat_{Ex}	The saturation of haemoglobin with oxygen, as measured by pulse oximetry (after a 2-minute step exercise test)	%
O_2 Saturation Difference	ΔO_2Sat	The difference between oxygen saturation levels at rest and oxygen saturation levels after a 2-minute step exercise test (O_2Sat_{Ex}- O_2Sat_{Rest})	%
O_2 Saturation Ratio	O_2Sat_{Ratio}	The ratio of blood oxygen saturation levels at rest : blood oxygen saturation levels after a 2-minute step exercise test (O_2Sat_{Rest}: O_2Sat_{Ex})	Dimensionless
Heart Rate at Rest	HR_{Rest}	The number of heart beats in one minute (at rest)	Beats/minute
Heart Rate after Exercise	HR_{Ex}	The number of heart beats in one minute (after a 2-minute step exercise test)	Beats/minute
Heart Rate Difference	ΔHR	The difference between heart rate at rest and heart rate after a 2-minute step exercise test (HR_{Ex} - HR_{Rest})	Beats/minute
Heart Rate Ratio	HR_{Ratio}	The ratio of heart rate at rest : heart rate after a 2-minute step exercise test (HR_{Rest} : HR_{Ex})	Dimensionless
Respiratory Rate at Rest	RR_{Rest}	The number of breaths taken in one minute (at rest)	Breaths/minute
Respiratory Rate after Exercise	RR_{Ex}	The number of breaths taken in one minute (after a 2-minute step exercise test)	Breaths/minute
Respiratory Rate Difference	ΔRR	The difference between respiratory rate at rest and respiratory rate after a 2-minute step exercise test (RR_{Ex} - RR_{Rest})	Breaths/minute
Respiratory Rate Ratio	RR_{Ratio}	The ratio of respiratory rate at rest : respiratory rate after a 2-minute step exercise test (RR_{Rest} : RR_{Ex})	Dimensionless
Systolic Blood Pressure	SBP	The maximum blood pressure exerted by circulating blood upon the walls of the blood vessels	mmHg
Diastolic Blood Pressure	DBP	The minimum blood pressure exerted by circulating blood upon the walls of the blood vessels	mmHg
Pulse Pressure	PP	The difference between Systolic Blood Pressure and Diastolic Blood Pressure (SBP - DBP)	mmHg
Blood Pressure Ratio	BP_{Ratio}	The ratio of Systolic Blood Pressure to Diastolic Blood Pressure (SBP : DBP)	Dimensionless

Table 4.1: A full description of the measurements within the diary dataset

self-selected group with more experience and exposure to high altitude than the lab staff group. The two groups had very different ascent profiles between days 37-71, as shown in Figure 1.1, and were therefore expected to respond differently during these times.

ANOVA was performed to assess for differences between the means of the lab staff and climber groups during the entire expedition, as described in Section 2.5.6. The results for these tests did not show a significant difference between the two groups for any of the physiological measurements at the 5% level, up to 5300m. However, due to such small sample sizes at extreme altitude (down to one or two individuals, and on some days no individuals, at altitudes above 5300m), it was not possible to obtain meaningful results between the two groups at altitudes above 5300m. Therefore, the main analyses were conducted on all 24 core team members to improve the power of subsequent tests, due to the larger sample size. Lab staff and climber groups were still visually assessed separately for some variables, mainly to split the group into smaller subgroups to allow for easier visual analysis of trends or patterns.

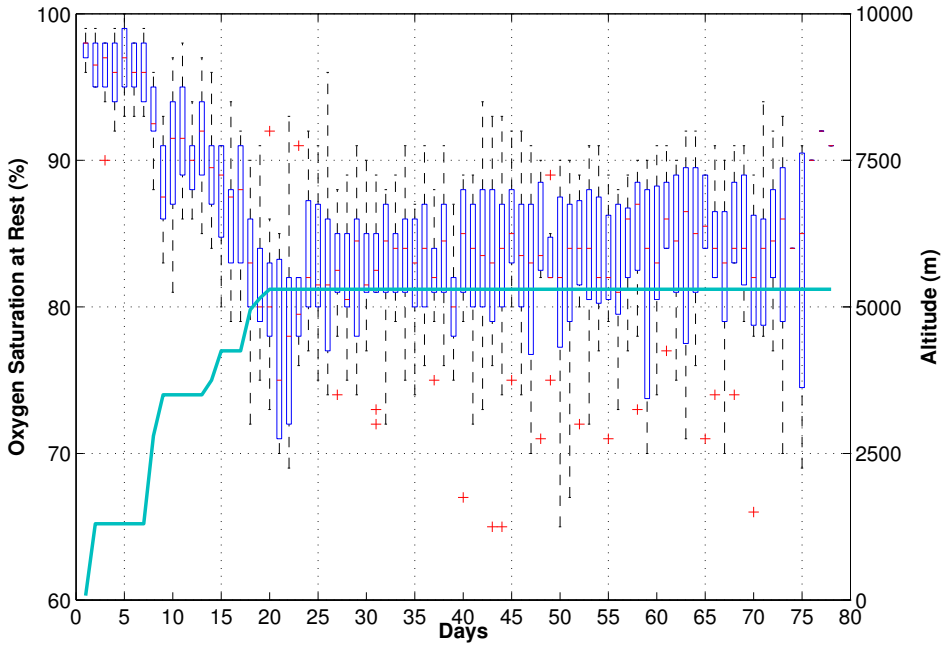
4.2 Results

Several different statistical analyses have been performed on the diary dataset, in order to address the questions posed in section 4.1. A summary of the results is described in the following sections.

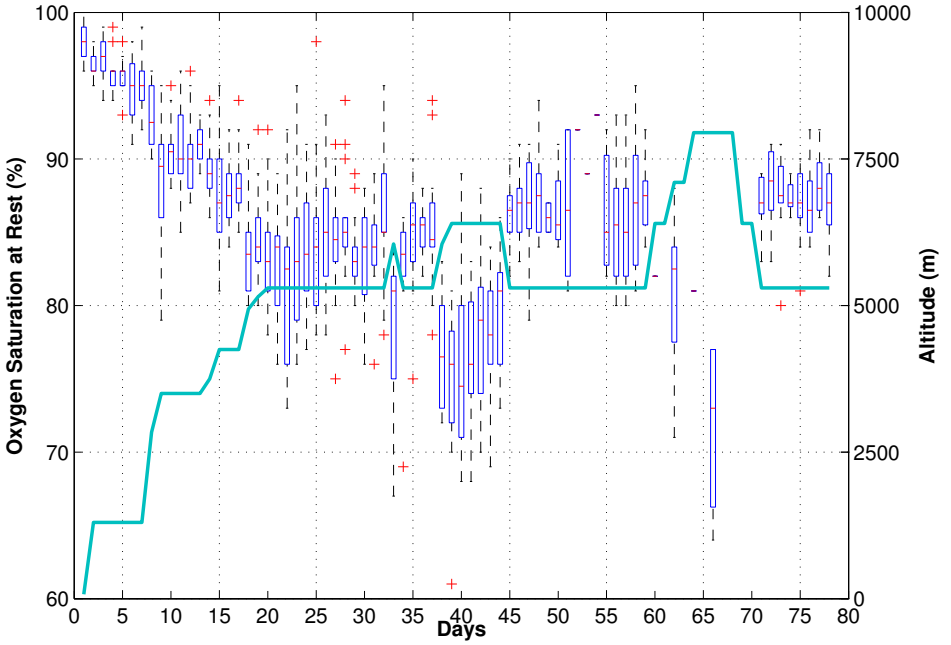
4.2.1 Do the measurements change with altitude?

The aim of this analysis was to determine whether each of the physiological measurements changed significantly during the expedition. Measurements that did not change significantly would not be useful for predictive modelling purposes, as some change is required in both the dependent and independent variable to enable the prediction of one with the other.

Box and whisker plots were produced, as described in Section 2.5.1, to visually assess how each of the physiological measurements changed during the expedition. Figures 4.1 and 4.2 show how oxygen saturation at rest and diastolic blood pressure changed in both lab staff and climber groups during CXE 2007. The ascent profile is marked on both of these figures, to enable the comparison of altitude increases to changes in the physiological measurements. Figure 4.1 shows a clear negative correlation between oxygen saturation and altitude for both the lab staff (Figure 4.1a) and climber (Figure 4.1b) groups. The change in oxygen saturation

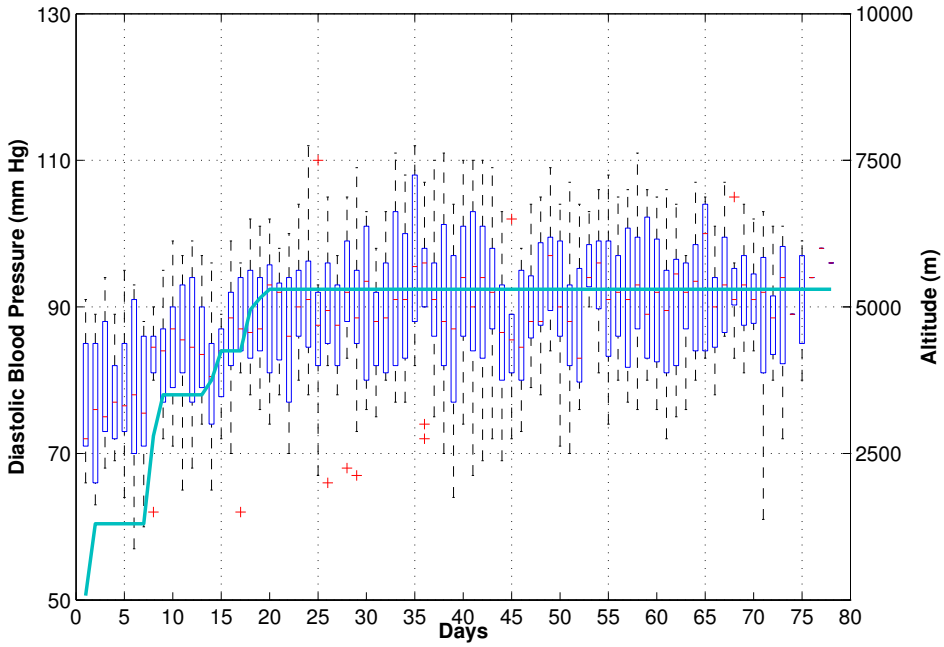


(a) Oxygen saturation at rest for 10 lab staff individuals.

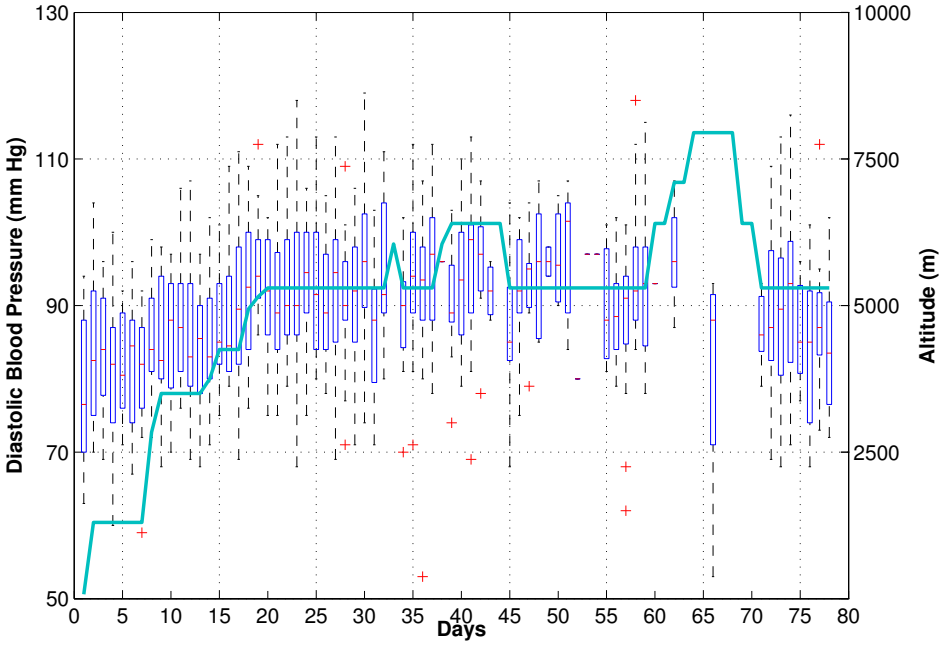


(b) Oxygen saturation at rest for 14 climber individuals

Figure 4.1: Box and whisker plots showing oxygen saturation at rest overlaid with the ascent profiles for the lab staff and climber groups during CXE 2007. There is a clear increase in variation within both groups as altitude increases, shown by an increase in the interquartile range (length of the box). Both (a) and (b) show a clear negative correlation with the shape of the box and whisker plot response.



(a) Diastolic blood pressure at rest for 10 lab staff individuals.

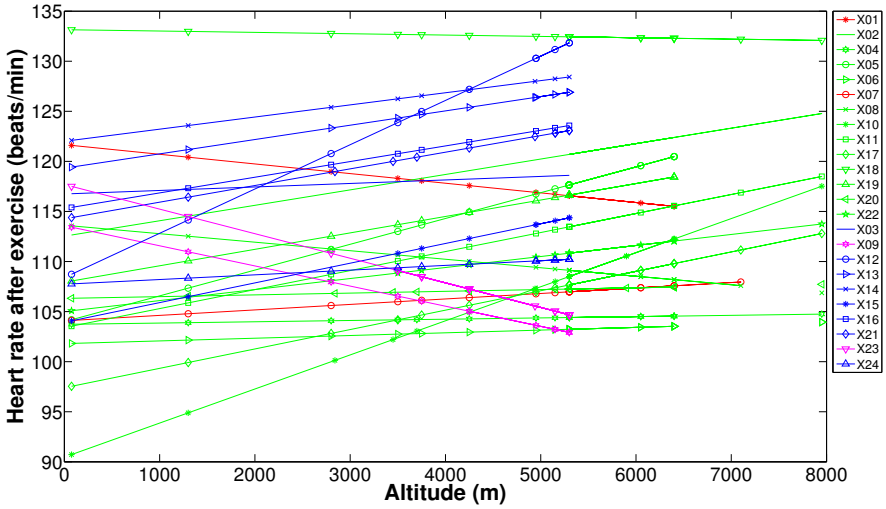


(b) Diastolic blood pressure at rest for 14 climber individuals

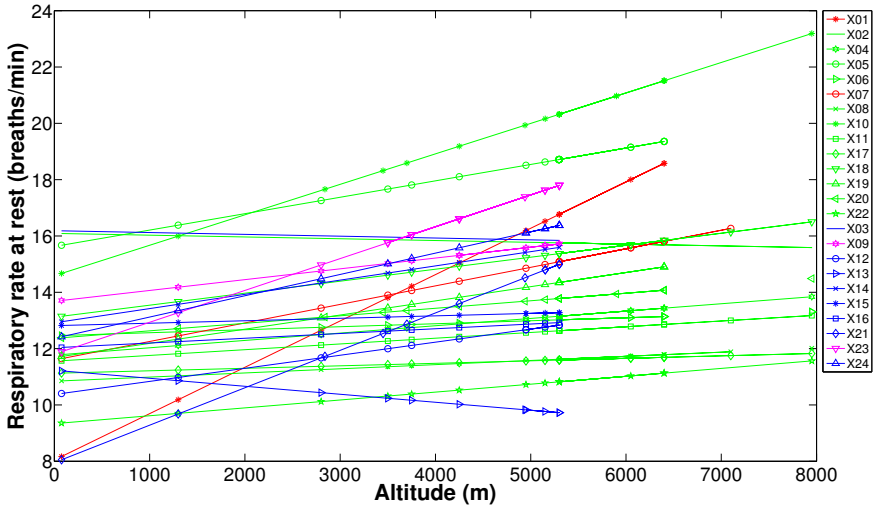
Figure 4.2: Box and whisker plots showing diastolic blood pressure (DBP) at rest overlaid with the ascent profiles for the lab staff and climber groups during CXE 2007. There is an increase in DBP until day 20, for both groups, and some variability in the DBP after day 20, which is clearest for the lab staff in (a), where the altitude remained constant.

Measurement	Slope	Intercept	C_V	*GOF*	*GOF* df	*LOF*	*LOF* df	ANOVA (altitude)		ANOVA (individual)	
								F-value	*p*-value	*F*-value	*p*-value
O_2Sat_{Ex}	-0.00408	98.91	0.061	* 1064.99	1, 11	§1.96	11, 1408	318.93	•2.1E-65	52.04	•9.7E-169
O_2Sat_{Rest}	-0.00323	100.72	0.053	* 261.75	1, 12	§5.96	12, 1475	233.50	•2.1E-65	54.18	•1.3E-176
O_2Sat_{Ratio}	0.00001	1.01	0.052	* 26.34	1, 11	§6.36	11, 1407	22.18	•2.1E-45	17.09	•6.3E-60
ΔO_2Sat	-0.00088	-1.64	0.772	* 17.39	1, 11	§6.98	11, 1407	17.96	•2.1E-36	14.06	•1.4E-48
HR_{Ex}	0.00112	109.83	0.135	* 7.79	1, 11	§1.96	11, 1382	2.96	•4.4E-04	24.46	•8.0E-86
HR_{Rest}	0.00234	62.71	0.168	* 23.42	1, 12	§4.77	12, 1483	21.93	•2.7E-48	54.25	•4.8E-177
HR_{Ratio}	0.00002	0.58	0.200	* 7.82	1, 11	§5.14	11, 1382	8.93	•1.1E-16	19.99	•2.7E-70
ΔHR	-0.00126	47.21	0.424	2.46	1, 11	§6.17	11, 1386	7.19	•7.3E-13	16.23	•1.2E-56
RR_{Ex}	0.00184	13.62	0.216	* 48.23	1, 11	§9.23	11, 1409	85.17	•1.0E-156	50.81	•1.5E-165
RR_{Rest}	0.00042	12.22	0.245	* 8.51	1, 12	§5.37	12, 1477	14.62	•2.4E-31	46.76	•1.9E-156
RR_{Ratio}	-0.00005	0.86	0.223	* 246.77	1, 11	1.15	11, 1405	30.54	•1.4E-62	13.78	•1.5E-47
ΔRR	0.00148	1.15	0.656	* 64.10	1, 11	§3.77	11, 1409	28.71	•6.8E-59	12.33	•5.0E-42
SBP	0.00203	124.60	0.110	* 26.49	1, 9	§2.15	9, 1419	19.81	•1.5E-34	112.69	•4.7E-298
DBP	0.00269	76.47	0.106	* 73.61	1, 9	§3.30	9, 1418	60.43	•8.0E-102	85.16	•4.6E-246
PP	-0.00065	48.07	0.235	* 7.67	1, 9	1.46	9, 1419	4.37	•4.6E-06	61.64	•1.7E-193
BP_{Ratio}	-0.00002	1.63	0.084	* 50.66	1, 9	§2.24	9, 1418	21.11	•6.5E-37	41.97	•2.8E-141

Table 4.2: A summary of the regression analysis and ANOVA performed for all of the diary data measurements. The C_V is calculated from the regression (as described in Section 2.5.10.3), and shows the day-to-day variability of the measurement. The slope is for the fitted regression line. The goodness of fit (*GOF*) statistic was compared to an *F*-distribution with degrees of freedom as defined in the table, to test the hypothesis H_0=*the slope is not different from zero*, as described in Section 2.5.10.1. *GOF* statistics that were determined to be significant are marked with an asterisk (*). The lack of fit (*LOF*) statistic was compared to an *F*-distribution with degrees of freedom as defined in the table, as described in Section 2.5.10.2. This was done to test the null hypothesis H_0= *the regression line fits the data well.* *LOF* statistics that were determined to be significant are marked with §. The two-way ANOVA was performed to look at the effects of altitude (H_0 = *there is no difference in the means at different altitudes*) and individual (H_0 = *there is no difference in the means for different individuals*). The null hypotheses were rejected in all cases at the 5% level (shown by a dot (•) by each significant *p*-value), indicating differences between altitudes and between individuals.

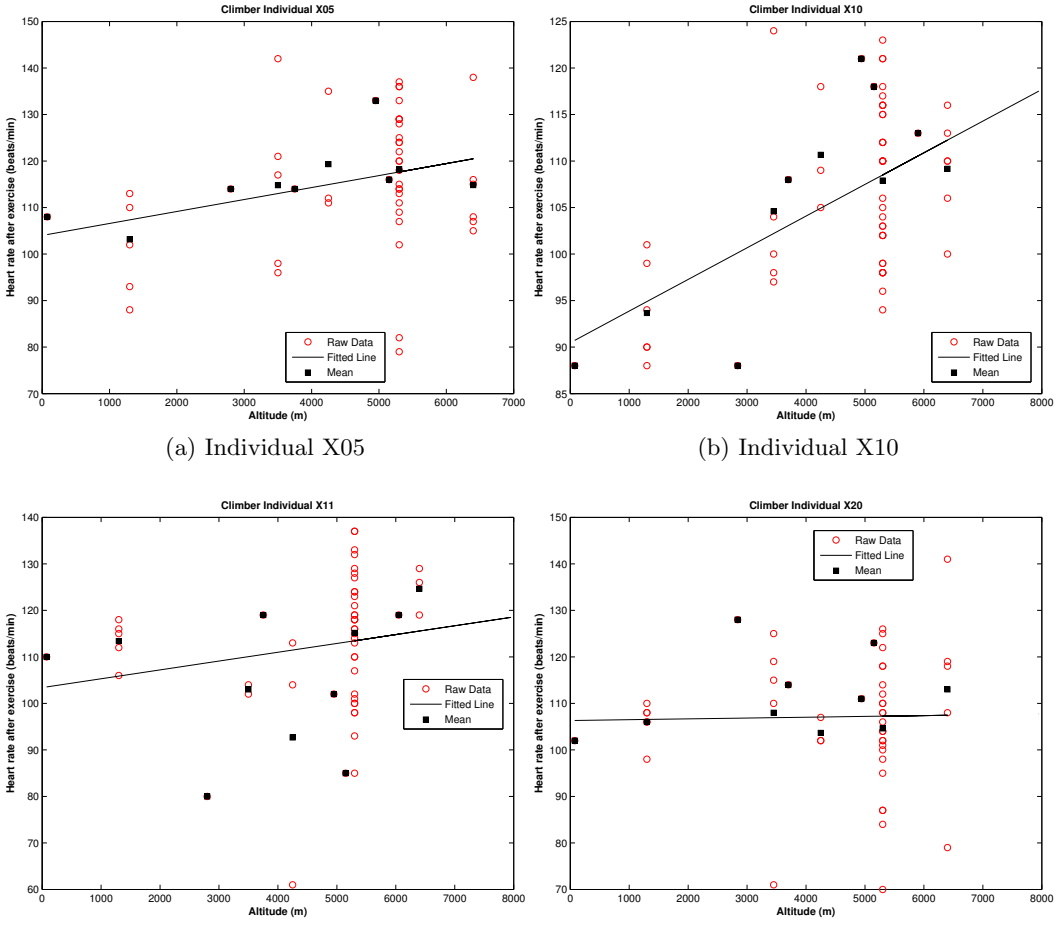


(a) Regression of heart rate after exercise over altitude for 24 individuals.



(b) Regression of respiratory rate at rest over altitude for 24 individuals.

Figure 4.3: Linear regression analysis of (a) heart rate after exercise against altitude and (b) respiratory rate at rest against altitude, for 24 individuals. Climber individuals are marked in green and red, lab staff individuals are marked in blue and magenta. Individuals X01 and X07 were climber individuals who became ill, and are labelled in red. Individual X23 is a lab staff member who became ill, and is labelled in magenta. These measurements are shown because they demonstrate the differences seen between fitted individual responses. Individual regression plots are shown in Figures 4.4 and 4.5.



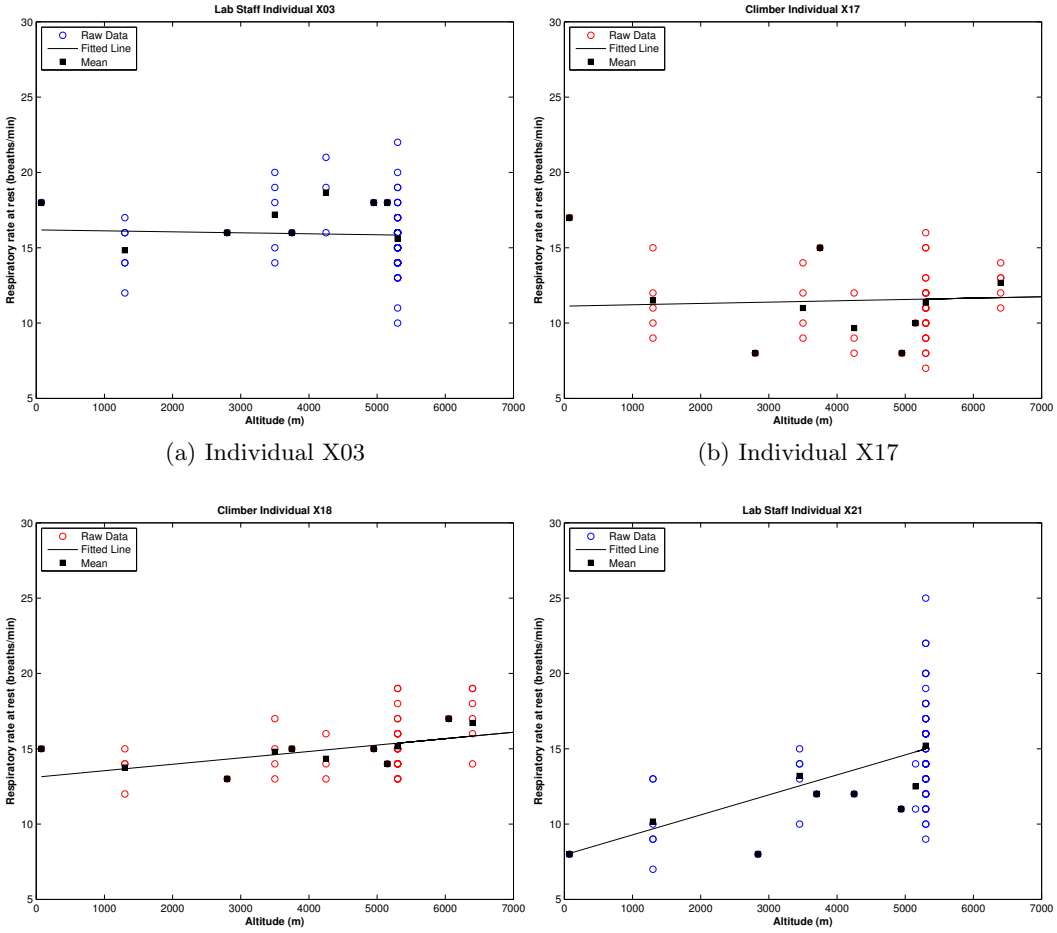
(a) Individual X05

(b) Individual X10

(c) Individual X11

(d) Individual X20

Figure 4.4: Linear regression analysis of heart rate after exercise (HR_{Ex}) against altitude for 4 individuals. The statistic values are shown in Table 4.3 for each fit. (a) shows the regression for individual X05, who shows a significant GOF statistic of 10.19, but a non-significant p-value of 0.611. This indicates that the line is not significantly different from zero, but that the response is linear, also shown by a low LOF statistic of 0.40, which shows that the means (shown as black squares) fit closely to the line. (b) shows the results for individual X10, who shows a slope significantly different from zero (shown by a significant p-value of 0.002 and significant GOF statistic value of 21.08), with means that deviate slightly from the regression line (shown by a LOF statistic value of 1.16). (c) shows the results for individual X11, who shows a significant p-value of 0.03, but a non-significant GOF statistic of 0.97. These results indicate that there are differences between means, but that the response seen is not linear, which is also shown by a high LOF statistic of 2.32, indicating that there is scatter around each of the means. (d) shows the results for individual X20, who shows a fitted regression line with a slope not significantly different from zero (shown by a non-significant p-value of 0.794 and a non-signficiant GOF statistic of 0.03), and a large amount of scatter of points around each mean (shown by a low LOF statistic of 0.67).



(a) Individual X03

(b) Individual X17

(c) Individual X18

(d) Individual X21

Figure 4.5: Linear regression analysis of respiratory rate at rest (RR_{rest}) against altitude for 4 individuals. The statistic values are shown in Table 4.4 for each fit. (a) shows the results for individual X03, who shows a fitted regression line that is not significant from zero (shown by a non-significant GOF statistic of 0.06 and a non-significant p-value of 0.176), with means (black squares) that deviate from the fitted line (shown by a high LOF statistic of 1.70). (b) shows the response for individual X17, who shows a line that appears to be not significantly different from zero (shown by a non-significant GOF statistic of 0.08), however the p-value of the F-value is significant at 0.01. This indicates that there does appear to be a difference between the means, but that the response is non-linear, which is evident by a very high LOF statistic of 3.04, indicating that the means deviate from the fitted line. (c) shows the response for individual X18, showing a significant GOF statistic of 11.10, but a non-significant p-value. This indicates that the line is not significantly different from zero, but that the response is linear, also shown by a low LOF statistic of 0.63, which shows that the means fit closely to the line. (d) shows the response for individual X21, who shows a fitted line significantly different from zero (shown by significant GOF statistic of 30.11 and a significant p-value of 0.002), with some scatter around each mean (black square, shown by a low LOF statistic of 0.73).

Individual	Slope	Intercept	C_V	*GOF*	*GOF* df	*LOF*	*LOF* df	F-value	p-value
X01	-0.0010	121.67	0.125	0.25	1, 8	§2.21	8, 43	2.03	0.059
X02	0.0015	112.52	0.142	1.20	1, 9	0.91	9, 44	0.93	0.519
X03	0.0004	116.75	0.077	0.23	1, 7	0.80	7, 59	0.72	0.674
X04	0.0001	103.73	0.095	0.03	1, 8	0.87	8, 49	0.77	0.641
X05	0.0026	103.99	0.125	* 10.19	1, 8	0.40	8, 40	0.81	0.611
X06	0.0003	101.80	0.088	0.04	1, 9	§2.64	9, 42	2.39	• 0.024
X07	0.0005	104.08	0.061	0.60	1, 8	1.52	8, 45	1.45	0.195
X08	-0.0009	113.63	0.130	0.87	1, 9	0.50	9, 40	0.49	0.885
X09	-0.0020	113.59	0.053	* 13.62	1, 7	1.13	7, 52	2.92	• 0.009
X10	0.0034	90.37	0.075	* 21.08	1, 9	1.16	9, 48	3.49	• 0.002
X11	0.0019	103.40	0.118	0.97	1, 9	§2.32	9, 40	2.31	• 0.030
X12	0.0044	108.40	0.104	* 33.53	1, 7	0.39	7, 57	1.96	0.068
X13	0.0014	119.33	0.066	3.25	1, 7	1.10	7, 61	1.42	0.208
X14	0.0012	122.00	0.160	0.63	1, 7	0.67	7, 60	0.64	0.738
X15	0.0020	103.93	0.113	3.06	1, 6	0.92	6, 60	1.19	0.325
X16	0.0016	115.29	0.083	2.90	1, 7	1.00	7, 62	1.24	0.292
X17	0.0019	97.39	0.117	1.79	1, 8	1.72	8, 41	1.87	0.085
X18	-0.0001	133.15	0.068	0.01	1, 8	§2.24	8, 40	1.99	0.066
X19	0.0016	107.93	0.112	2.53	1, 8	0.83	8, 40	0.97	0.477
X20	0.0002	106.32	0.137	0.03	1, 8	0.67	8, 38	0.59	0.794
X21	0.0017	114.29	0.177	0.65	1, 6	1.09	6, 61	1.04	0.415
X22	0.0011	105.00	0.098	4.81	1, 9	0.29	9, 46	0.40	0.941
X23	-0.0028	118.94	0.100	4.84	1, 7	1.18	7, 35	1.75	0.121
X24	0.0005	107.73	0.154	0.07	1, 7	1.25	7, 58	1.10	0.376
All Individuals	0.0011	109.83	0.135	* 7.79	1, 9	1.96	9, 62	2.439	• 0.0009

Table 4.3: A summary of the results for the individual regression analyses and two-way ANOVA tests performed for heart rate after a 2-minute exercise test against altitude, from the diary data. The results for the regression analysis performed on the group as a whole are shown at the bottom of the table. The slope and intercept values are for the fitted regression line. The C_V value is a measure of the day-to-day variability, as described in Section 2.5.10.3. *GOF* and *LOF* are the *goodness of fit* and *lack of fit* statistics, as described in Sections 2.5.10.1 and 2.5.10.2. These statistics were compared to an F-distribution with degrees of freedom as defined in the table. A significant *GOF* statistic indicates that the slope is significantly different from zero, and is marked with an asterisk (*). A significant *LOF* statistic indicates that the means deviate from the fitted regression line, and is marked with the symbol §. The F-value and p-value are both for the ANOVA performed for each individual, with values marked with a dot (•) indicating a rejection of the null hypothesis at the 5% level, indicating that the mean response is changing as altitude changes.

Individual	Slope	Intercept	C_V	*GOF*	*GOF* df	*LOF*	*LOF* df	F-value	p-value
X01	0.00165	8.05	0.204	* 11.90	1, 9	§3.08	9, 50	6.44	• 0.000003
X02	-0.00006	16.09	0.198	0.12	1, 9	0.44	9, 48	0.41	0.937609
X03	-0.00007	16.19	0.136	0.06	1, 7	1.70	7, 61	1.50	0.175641
X04	0.00026	11.77	0.184	0.85	1, 9	1.89	9, 49	1.86	0.074007
X05	0.00058	15.63	0.171	2.62	1, 9	1.73	9, 40	2.01	0.057877
X06	0.00011	12.46	0.120	0.18	1, 10	§3.57	10, 47	3.30	• 0.002047
X07	0.00066	11.60	0.153	* 5.46	1, 9	§2.14	9, 47	3.09	• 0.004206
X08	0.00015	10.84	0.253	0.36	1, 11	0.96	11, 41	0.91	0.542797
X09	0.00038	13.68	0.120	3.75	1, 7	1.35	7, 52	1.81	0.096549
X10	0.00108	14.59	0.208	* 6.58	1, 10	1.53	10, 48	2.30	• 0.023351
X11	0.00020	11.56	0.200	0.14	1, 10	§6.82	10, 48	6.28	• 0.000003
X12	0.00046	10.37	0.163	3.75	1, 7	1.65	7, 59	2.21	• 0.039128
X13	-0.00028	11.23	0.262	1.16	1, 7	1.27	7, 65	1.29	0.262145
X14	0.00051	12.92	0.178	* 6.61	1, 7	0.64	7, 62	1.08	0.388181
X15	0.00009	12.82	0.131	0.15	1, 6	2.07	6, 64	1.82	0.099237
X16	0.00017	12.03	0.134	0.76	1, 7	1.55	7, 63	1.50	0.174198
X17	0.00009	11.12	0.189	0.08	1, 9	§3.04	9, 45	2.76	• 0.009656
X18	0.00043	13.12	0.113	*11.10	1, 9	0.63	9, 44	1.27	0.273964
X19	0.00050	11.68	0.158	* 5.74	1, 8	1.19	8, 42	1.82	0.092492
X20	0.00027	12.36	0.174	0.95	1, 9	1.84	9, 43	1.83	0.084499
X21	0.00133	7.93	0.222	* 30.11	1, 7	0.73	7, 64	3.39	• 0.002622
X22	0.00028	9.34	0.164	2.71	1, 9	1.27	9, 46	1.49	0.174540
X23	0.00123	11.43	0.198	* 16.58	1, 7	0.94	7, 52	2.77	• 0.012431
X24	0.00076	12.36	0.156	* 8.98	1, 7	1.26	7, 64	2.52	• 0.019088
All Individuals	0.00042	12.22	0.25	* 8.51	1, 11	§5.37	11, 65	6.34	$2.5\text{x}10^{-15}$

Table 4.4: A summary of the results for the individual regression analysis and two-way ANOVA tests performed for respiratory rate at rest against altitude, from the diary data. The results for the regression analysis performed on the group as a whole is shown at the bottom of the table. The slope and intercept values are for the fitted regression line. The C_V value is a measure of the day-to-day variability, as described in Section 2.5.10.3. *GOF* and *LOF* are the *goodness of fit* and *lack of fit* statistics, as described in Sections 2.5.10.1 and 2.5.10.2. These statistics were compared to an F-distribution with degrees of freedom as defined in the table. A significant *GOF* statistic indicates that the slope is significantly different from zero, and is marked with an asterisk (*). A significant *LOF* statistic indicates that the means deviate from the fitted regression line, and is marked with the symbol §. The F-value and p-value are both for the ANOVA performed for each individual, with values marked with a dot (•) indicating a rejection of the null hypothesis at the 5% level, indicating that the mean response is changing as altitude changes.

was identified to be statistically significant during the expedition by an analysis of variance, at the 5% significance level, as described in Section 2.5.6. This confirms that the level of hypoxia that the individuals were exposed to during the expedition increased as altitude increased, which was as expected. Figure 4.2 shows a positive correlation between altitude increase and increases in diastolic blood pressure, which was also confirmed to be a statistically significant change by analysis of variance at the 5% significance level.

Table 4.2 shows the results for the two-way ANOVA performed to assess the main effects of altitude and individual on each of the physiological measurements. The table shows that the null hypothesis for the ANOVA was rejected for all physiological measurements at the 5% level for both altitude and individual, indicating that the average (mean) response by the group changed significantly at some point during the expedition for all of the measurements, with respect to both altitude and individual.

A simple linear regression analysis was also performed for each physiological measurement against altitude. This was done for both the group as a whole, to assess how the group was responding during the expedition, and for individuals within each group, to assess how each individual was responding during the expedition. The regression was performed to assess whether the change seen in each physiological measurement could be described by a linear fit, and to assess how well this line described the data. The results could also be used to assess whether each physiological measurement changed significantly with altitude, shown by a regression line with a slope significantly different from zero. Table 4.2 shows the results for the group regression analysis performed between each physiological measurement and altitude. The heart rate difference measurement was the only physiological measurement that did not show a regression line slope that was significantly different from zero, with a non-significant GOF statistic of 2.46, as described in Section 2.5.10.1.

Figure 4.3a and Table 4.3 show the individual regression results for all individuals for heart rate after exercise, with the results for the group regression at the bottom of Table 4.3. The group response shows a slope significantly different from zero, however, Table 4.3 shows that only four individuals have a regression slope significantly different from zero shown by significant GOF statistics, labelled in the table with an asterisk (*). Figure 4.4 shows the linear regression of heart rate after exercise against altitude for four individuals, and demonstrates the differences in response seen between individuals.

Figure 4.3b and Table 4.4 show the individual regression results for respiratory rate at rest, with the results for the group regression at the bottom of Table 4.4.

The overall group regression shows a slope significantly different from zero, with a significant value for the *GOF* statistic (labelled with an asterisk (*)). The individual responses for this measurement show some variability, with both positive and negative slopes. Figure 4.5 shows the linear regression of respiratory rate at rest against altitude for four individuals. This figure shows the difference in response seen between individuals. These results indicate that there is a lot of variability in how individuals are responding, and in some circumstances, this may mean that a group summary is not capturing the inter-individual differences seen within a group. In some cases, the group as a whole may be showing a response, however, this may be due to only a small number of individuals within that group, and may not be representative of the group as a whole.

4.2.1.1 Summary

- ANOVA showed a rejection of the null hypothesis for both altitude and individual at the 5% level for all physiological measurements, indicating that all of the measurements show a significant change at some point during the expedition;
- Simple linear regression analysis against altitude showed that the heart rate difference measurement was the only physiological measurement that did not show a slope significantly different from zero;
- Analysis of individual regression results show that even though the group as a whole may show a regression slope significantly different from zero, all individuals within that group may not. Therefore, analysis of both the individual regressions and group regression is required to fully understand what is happening in the group overall;
- Individual regression and ANOVA analyses show that for each of the physiological measurements, at least one individual shows a significant change during the expedition, highlighting that there are differences in how individuals are responding to altitude.

4.2.2 Which measurements are the most *reliable*?

The aim of this analysis was to assess the *precision* and *reproducibility* of each of the measurements within the diary dataset. Box and whisker plots were used to visually assess the variability during the expedition. Figure 4.1 shows that the variability for resting oxygen saturation increases as altitude increases for both lab staff and

Parameter Name	Coefficient of Variation from Linear Regression and ANOVA
O_2Sat_{Ratio}	0.052
O_2Sat_{Rest}	0.053
O_2Sat_{Ex}	0.061
BP_{Ratio}	0.084
DBP	0.106
SBP	0.110
HR_{Ex}	0.135
HR_{Rest}	0.168
HR_{Ratio}	0.200
RR_{Ex}	0.216
RR_{Ratio}	0.223
PP	0.235
RR_{Rest}	0.245
ΔHR	0.424
ΔRR	0.656
ΔO_2Sat	0.772

Table 4.5: A summary of all diary data measurements, ranked in increasing order of the C_V value, obtained from the regression, as described in Section 2.5.10.3. Low C_V values indicated low day-to-day variability, i.e. measurements taken on subsequent days will be very similar to one another.

climber groups, indicated by a larger interquartile range (IQR, shown by the length of the box) at higher altitudes. For example, day 1 in Figure 4.1b has an IQR of 2%, whereas day 40 has an IQR of 9%.

Figure 4.2 shows the variability for diastolic blood pressure stays roughly the same for both lab staff and climber groups at around 15-20 mmHg. Figure 4.2a shows variability in the group response for the lab staff after day 20, even though altitude remained constant. Long-term adaptation to hypoxia would expect to show a consistency over time, whereas the variability shown in Figure 4.2a is more difficult to explain.

The coefficient of variation (C_V) was calculated from the regression analysis for each physiological measurement in Table 4.1, as described in Section 2.5.10.3. The C_V can be used as a measure of the overall day-to-day variability within each measurement, and is used here as a measure of how reproducible a particular measure is, with a low C_V value indicating that two measurements taken at the same altitude will be very similar to one another. This analysis is also important for the assessment of the core dataset, which only contains single-point measures for all physiological and biochemical measurements. Therefore, the results from this analysis can be used as an indicator of how *reliable* a single measure is for each

altitude.

Table 4.5 shows a ranked summary of the C_V values for each measurement. Standard deviations must always be assessed in the context of the dataset, however, the C_V is independent of the unit in which the measurement has been taken, and is therefore dimensionless. This allows the formal comparison of the C_V value across different datasets, and is very useful for the comparison of different physiological measurements within the diary data. Table 4.5 shows that oxygen saturation measurements for rest, exercise and ratio have the lowest day-to-day variability for any of the physiological measurements, indicated by the lowest C_V values of 0.052, 0.053 and 0.061, respectively. Respiratory rate measurements show the highest day-to-day variability, with the highest C_V values of 0.216, 0.223 and 0.245 for exercise, ratio and resting values, respectively. This is likely to be due to the difficulty in taking respiratory rate measurements, as it is very difficult to see the movement of the chest when the subject is wearing several layers, especially in poor light conditions. Difference measurements showed the highest C_V values within each measurement. This is to be expected, as it is combining the error associated with two different measurements.

4.2.2.1 Summary

- The measurements showing the lowest relative day-to-day variability were considered the most *reliable* measurements. These were oxygen saturation ratio, oxygen saturation at rest and oxygen saturation after exercise, with C_V values of 0.052, 0.053 and 0.061, respectively. This indicates that single-point measurements should be suitable for oxygen saturation in the core dataset, as the measurements show low day-to-day variability for measurements taken on different days at the same altitude;

- The measurements with the highest day-to-day variability were respiratory rate at rest, after exercise and their ratio, with C_V values of 0.216, 0.223 and 0.245, respectively. This indicates that these values varied a lot between measurements at the same altitude, and may not be suitable for use in further analysis and modelling. However, this variability may be due to difficulties in taking those particular measurements;

- This analysis highlights the issue of the *reliability* of single-point measures for the core dataset.

4.2.3 Is there any redundancy within these measurements?

As there are multiple derivations of each physiological measurement available, this analysis looks at any *redundancy* within these measurements, i.e are any of the measurements showing the same response? If measurements are showing the same information, then only the measurements that are the most statistically and biologically informative would be used for further analysis to help save the time taken to carry out any further analysis.

Ideally, we want measurements that show a change with altitude and have low day-to-day variability. Correlations were calculated and scatter plots produced between all pairs of physiological measurements, to assess how similar the responses were for each measurement, as described in Section 2.5.7. If two physiological measurements show a strong correlation, as determined by a high correlation coefficient and significant p-value, then potentially only one would be needed for further analysis.

The difference and ratio measurements showed high levels of correlation with one another, with correlation coefficients of 0.868 for blood pressure, -0.893 for respiratory rate, -0.942 for heart rate and -0.989 for oxygen saturation, shown in Table 4.6. These high correlation values were expected, as the difference and ratio measurements are both slightly different ways of showing how the rest and exercise measurements are related to one another. The ratio measurements consistently showed a lower C_V than the difference measurements, as shown in Table 4.2, indicating a lower day-to-day variability for the ratio measurement compared to the difference measurement.

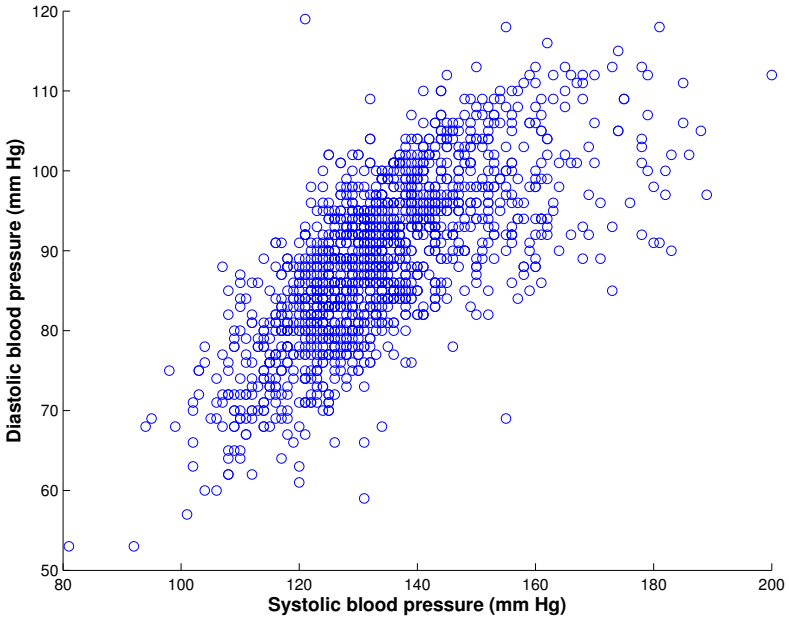
Although the ratio measurement is the most statistically reliable, the difference measurement is more commonly used in the biological and medical environment. For example, blood pressure is always expressed as *pulse pressure* (systolic blood pressure - diastolic blood pressure). A difference measure is easy to interpret, and removes the scale of the measurement. For example, if an individual has a very low blood pressure, the difference would not take this into account, and only provide the absolute difference between the values. A ratio may be misleading here, because if the blood pressure is low, then the resulting ratio may also be low. For example, a ratio value of 2 can be obtained by a 4:2 ratio, or by a 8:4 ratio, whereas the difference values for these ratios would be different. Which measure to use depends on the information required, and which method would best display this information.

Oxygen saturations at rest and after exercise show a strong significant positive correlation with a correlation coefficient of 0.824 and a p-value that was too small to be calculated by Excel (shown as $\bowtie$), indicating that they are showing a

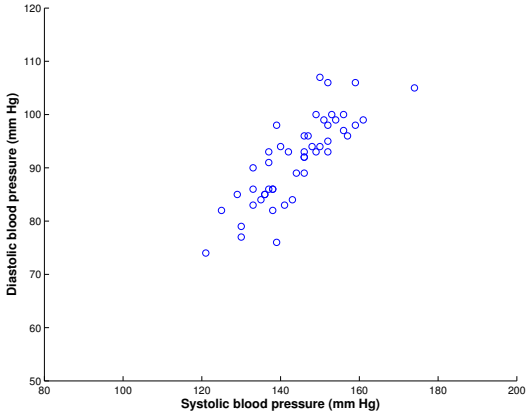
very similar response to altitude. Resting oxygen saturation levels show a lower C_V of 0.053 than oxygen saturations after exercise, which shows a C_V of 0.061. However, oxygen saturations after exercise show a larger change in response to altitude, shown by a steeper slope of -0.0041 compared to -0.0032 for resting oxygen saturation. Oxygen saturation after exercise also shows a slight difference between individual responses, whereas oxygen saturation at rest does not, suggesting this may be the most informative oxygen saturation to use for further analysis.

Respiratory rate at rest and respiratory rate after exercise show a weak positive correlation with a correlation coefficient of 0.505 and a p-value of $9.4\text{x}10^{-93}$. Systolic and diastolic blood pressure show a strong significant positive correlation, with a correlation coefficient of 0.709 and p-value of $1.9\text{x}10^{-218}$, shown in Figure 4.6. Both of these measurements show different information, and are most often used in a clinical environment together (as pulse pressure), therefore, both values would be potentially informative in further analysis, rather than using one measurement alone.

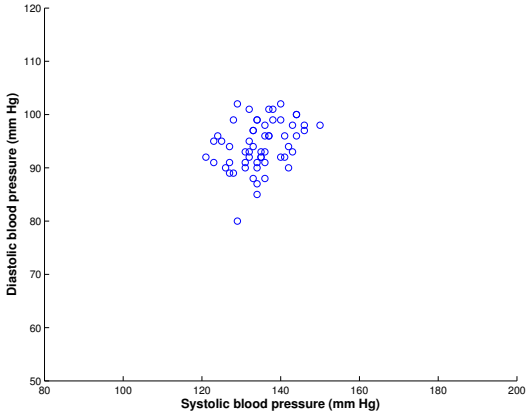
Correlations between unrelated measurements are weaker than those seen between related measurements, as shown in Table 4.6, and was to be expected, as they are measuring different physiological responses. Figure 4.7a shows a significant weak negative correlation between oxygen saturation at rest and respiratory rate after exercise for the whole core group, with a correlation coefficient of -0.346 and a p-value of $3.1\text{x}10^{-41}$. However, individuals within the group show different relationships, for example individual X18 shows a strong significant negative correlation in Figure 4.7b, with a correlation coefficient of -0.710 and a p-value of 0.034, whilst individual X08 shows no correlation in Figure 4.7c with a correlation coefficient of -0.210 and a p-value of 0.140. However, this low r-value and high p-value may be due to 4 high values for respiratory rate at high oxygen saturation levels. This correlation shows one of the adaptive responses that occurs during exposure to hypoxia; as the oxygen saturation level of the blood drops, the respiratory rate increases to try and increase the blood oxygen saturation. These individual correlations provide information on differences between individuals, and can be used to assess whether individuals are showing similar relationships between variables. Correlations between unrelated measurements that have a correlation coefficient above 0.392 are summarised in Table 4.6.



(a) Scatter plot showing the correlation between systolic blood pressure and diastolic blood pressure for 24 individuals over 78 days. r=0.709, p=1.9x10^{-218}.

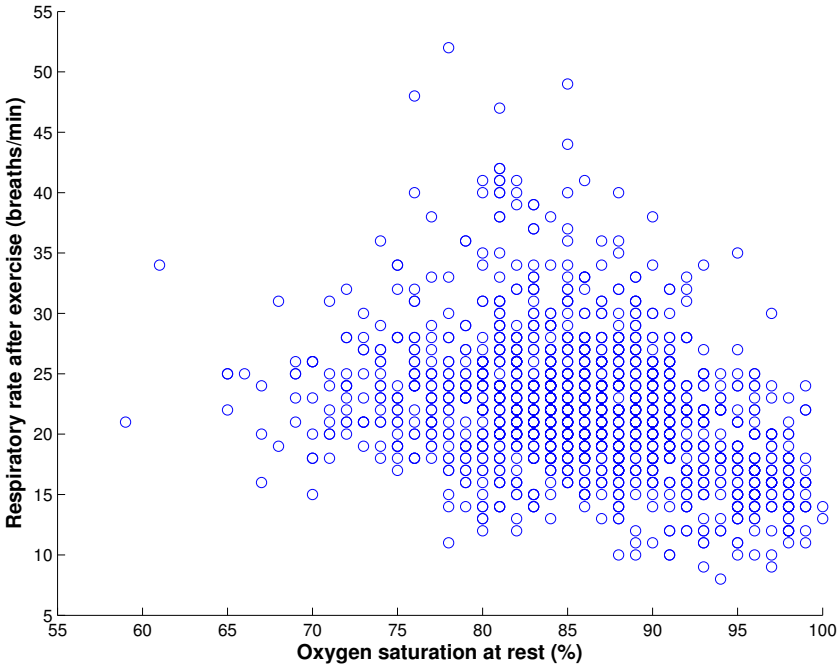


(b) Individual X19, r = 0.824, p = 3.7x10^{-13}.

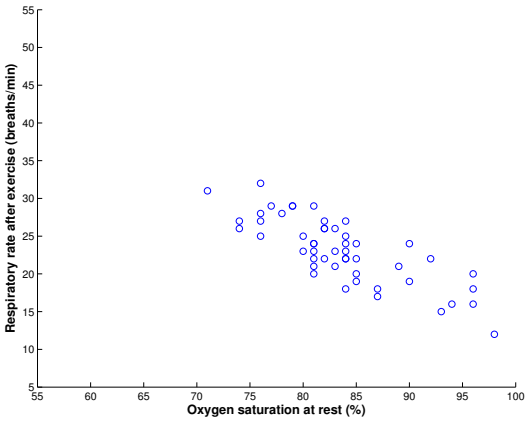


(c) Individual X04, r=0.335, p=0.010.

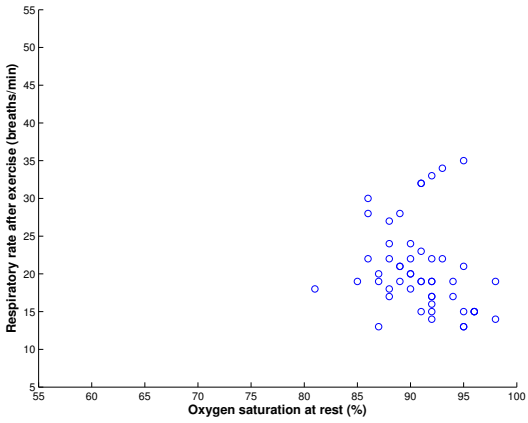
Figure 4.6: Correlation between systolic blood pressure and diastolic blood pressure. The p-value is the probability of getting a correlation as large as the observed value by random chance, when the true correlation is zero, as described in Section 2.5.7. p-values below 0.05 indicate that the correlation between x and y was significant. (a) shows a strong significant correlation for all 24 individuals, with an r-value of 0.709 and a p-value of 1.9x10^{-218}. (b) shows a strong significant correlation for individual X19, with an r-value of 0.824 and a p-value of 3.7x10^{-13}. (c) shows a very weak but significant correlation in individual X04, with an r-value of 0.335 and a p-value of 0.010.



(a) Correlation between oxygen saturation at rest and respiratory rate after exercise, for all individuals over 78 days. r= -0.346, p=3.1x10^{-41}.



(b) Individual X18, r= -0.710, p=3.4x10^{-12}.



(c) Individual X08, r= -0.210, p=0.140.

Figure 4.7: Correlations between resting oxygen saturations and respiratory rate after a 2 minute step-exercise test. The p-value is the probability of getting a correlation as large as the observed value by random chance, when the true correlation is zero, as described in Section 2.5.7. p-values below 0.05 indicate that the correlation between x and y was significant. (a) shows a weak but significant correlation in all 24 individuals, with an r-value of -0.346 and a p-value of 3.1x10^{-41}. (b) shows a strong significant correlation for individual X18, with an r-value of -0.710 and a p-value of 3.4x10^{-12}. (c) shows a very weak correlation for individual X08, with an r-value of -0.210 and a p-value of 0.140. However, this low r-value and high p-value may be due to 4 extreme values in the top right of the graph.

A - Related			
Parameter x	Parameter y	r-value	p-value
ΔO_2Sat	O_2Sat_{Ratio}	-0.989***	• ⋈
ΔHR	HR_{Ratio}	-0.942***	• ⋈
ΔRR	RR_{Ratio}	-0.893***	• ⋈
PP	BP_{Ratio}	0.868***	• ⋈
O_2Sat_{Rest}	O_2Sat_{Ex}	0.824***	• ⋈
SBP	PP	0.725**	•8.9E-234
RR_{Ex}	ΔRR	0.713**	•2.2E-221
SBP	DBP	0.709**	•1.9E-218
HR_{Ex}	ΔHR	0.703**	•8.2E-209
HR_{Rest}	HR_{Ratio}	0.682**	•2.6E-109
O_2Sat_{Ex}	O_2Sat_{Ratio}	-0.580*	•1.9E-128
RR_{Rest}	RR_{Ex}	0.505*	•9.4E-93
RR_{Ex}	RR_{Ratio}	-0.503*	•7.3E-92
O_2Sat_{Ex}	ΔO_2Sat	0.486*	•4.0E-85
HR_{Ex}	HR_{Ratio}	-0.483*	•2.5E-82
HR_{Rest}	ΔHR	-0.465*	•5.1E-76
DBP	BP_{Ratio}	-0.442*	•1.9E-69

B - Unrelated				
		Individual	Group	
Parameter x	Parameter y	Number r >0.39	r-value	p-value
O_2Sat_{Rest}	RR_{Ex}	19	-0.346	•3.1E-41
O_2Sat_{Ex}	RR_{Ex}	19	-0.361	•4.0E-45
O_2Sat_{Ex}	DBP	14	-0.249	•5.9E-21
O_2Sat_{Ex}	ΔRR	13	-0.318	•8.4E-35
O_2Sat_{Rest}	ΔRR	11	-0.296	•4.7E-11
O_2Sat_{Rest}	RR_{Ratio}	10	0.319	•6.7E-35
O_2Sat_{Ex}	SBP	9	-0.174	•7.0E-11
O_2Sat_{Rest}	HR_{Rest}	7	-0.288	•6.3E-30
HR Rest	RR_{Ex}	7	0.142	•6.8E-8
O_2Sat_{Ex}	HR_{Rest}	5	-0.235	•2.4E-19

Table 4.6: A summary of the correlations between measurements of the diary data, as defined in Table 4.1. The p-value is the probability of getting a correlation as large as the observed value by random chance, when the true correlation is zero. Measurements marked with a dot (•) show a p-value less than 0.05, indicating that the correlation between x and y was significant. p-values marked as ⋈ were too small to be calculated by Excel, but greater than zero. Weak correlations have an r-value between either -0.4 to -0.59 or 0.4 to 0.59 and are marked with an asterisk (*). Medium strength correlations have an r-value between either -0.6 and -0.79 or 0.69 and 0.79, and are marked with a double asterisk (**). Strong correlations have an r-value between either -0.8 to -1.0 or 0.8 to 1.0, and are marked with a triple asterisk (***). Correlations between unrelated measurements show the number of individuals who show a weak correlation or stronger (>0.39). The r-value and p-value are for the correlation for the entire group.

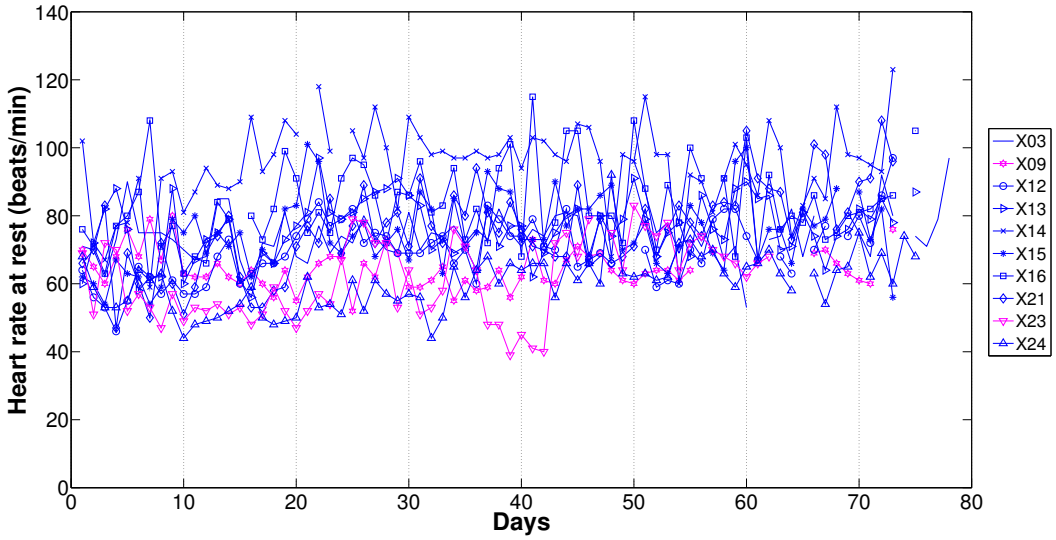
4.2.3.1 Summary

- All of the difference measurements show strong correlations with the corresponding ratio measurement;
- The C_V values for the ratio measurement show lower day-to-day variability compared to the difference measurement, indicating it may be the more reliable of the two measurements. However, the difference measurement may be more biologically useful, as it may be easier to interpret;
- Other *related* measurements tend to correlate strongly with one another, however, not strongly enough to justify the removal of one for the other, as they may both provide useful information on their own or in combination. There seems to be some amount of redundancy, but not enough to remove any of the measurements from further analysis;
- Some weak correlations are present between *unrelated* measurements. These can be used to confirm that known adaptive responses to hypoxia are occurring, to assess relationships between variables, and to look for differences between individuals (by assessing individual correlations of variables).

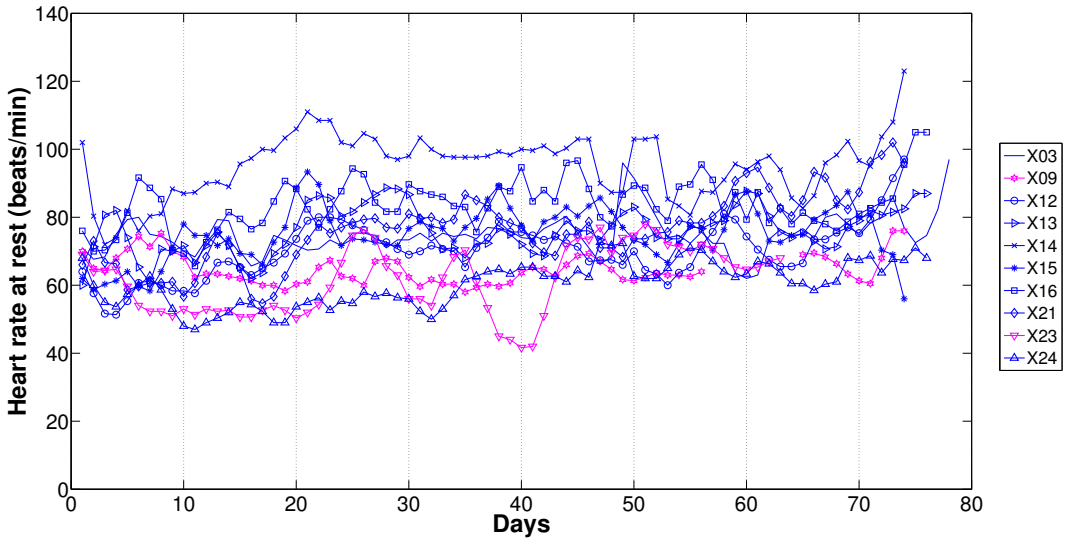
4.2.4 Is there evidence of different levels of adaptation?

The most important characteristic of measurements used in predictive modelling is the ability to distinguish between different *types* of behaviour. A *smoothing* algorithm was implemented, as described in Section 2.5.8, to allow the visual analysis of different individual responses during the expedition. These figures were used to visually identify individuals who were showing different responses compared to the other individuals within the group. Figures 4.8 and 4.9 show the raw and smoothed values for heart rate at rest and diastolic blood pressure, respectively. After smoothing, individual responses are much easier to identify. For example, individual X14 shows a very high response in Figure 4.8b, which is difficult to identify in Figure 4.8a. Figure 4.9b shows diastolic blood pressure after rest, where it is easy to identify individual X17 who shows a very low response, and individual X10 who shows a very high response, compared to Figure 4.9a.

For each individual, a simple linear regression analysis was carried out for each physiological measurement against altitude. This was done to assess whether the response seen for each of the physiological measurements against altitude could be described with a linear fit, and to assess any differences in the fit between individuals. Figures 4.3a and 4.3b show the fitted regression lines for all individuals for

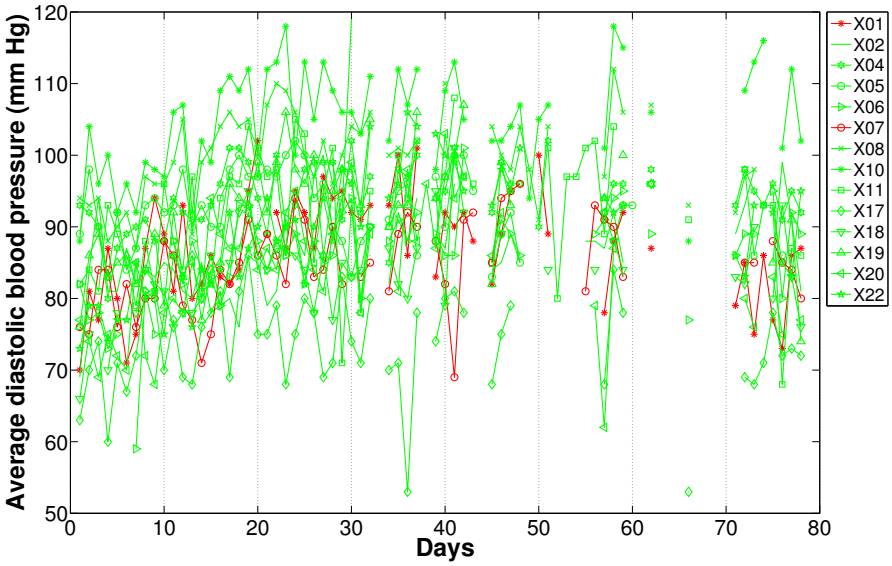


(a) Heart rate at rest for 10 lab staff individuals (raw data)

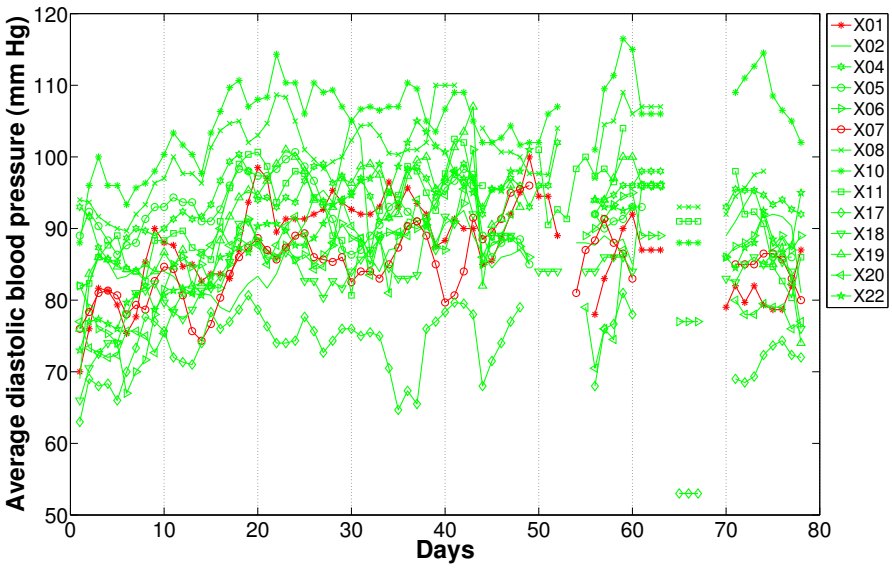


(b) Heart rate at rest for 10 lab staff individuals (smoothed)

Figure 4.8: Heart rate at rest for 10 lab staff individuals. The figures show the raw data plotted for each individual, and *smoothed* data plotted, respectively. Here, (b) shows much clearer responses, allowing the identification of individuals such as X14, who shows a very high response compared to other individuals.

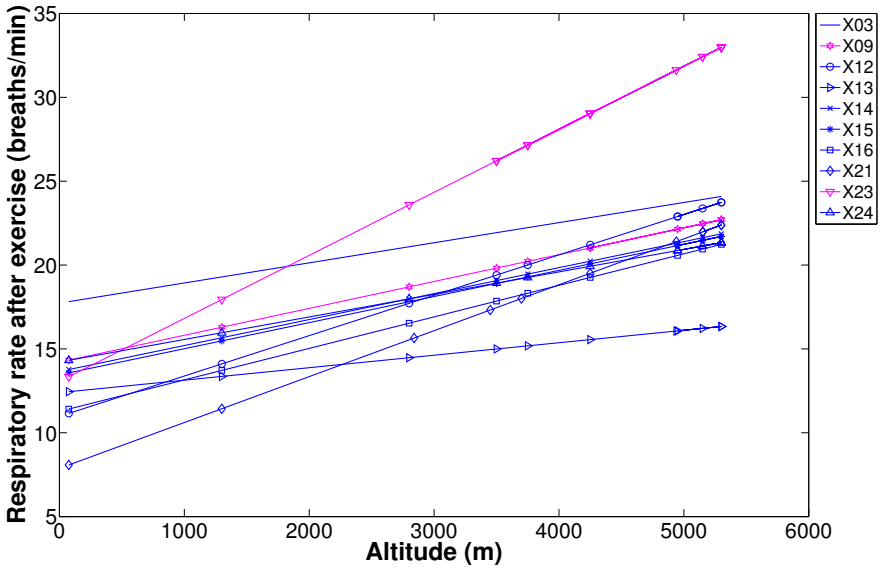


(a) Diastolic blood pressure at rest for 14 climber individuals (raw data)

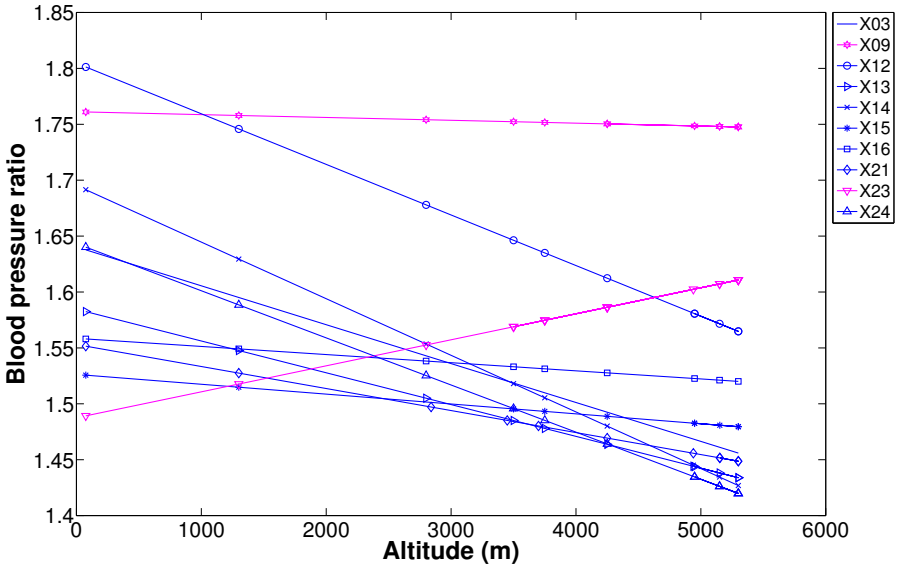


(b) Diastolic blood pressure at rest for 14 climber individuals (smoothed)

Figure 4.9: Diastolic blood pressure for 14 climber individuals. The figures show the raw data plotted for each individual, and *smoothed* data plotted, respectively. Here, (b) shows much clearer responses, allowing the identification of individuals such as X17, who shows a very low response compared to other individuals.

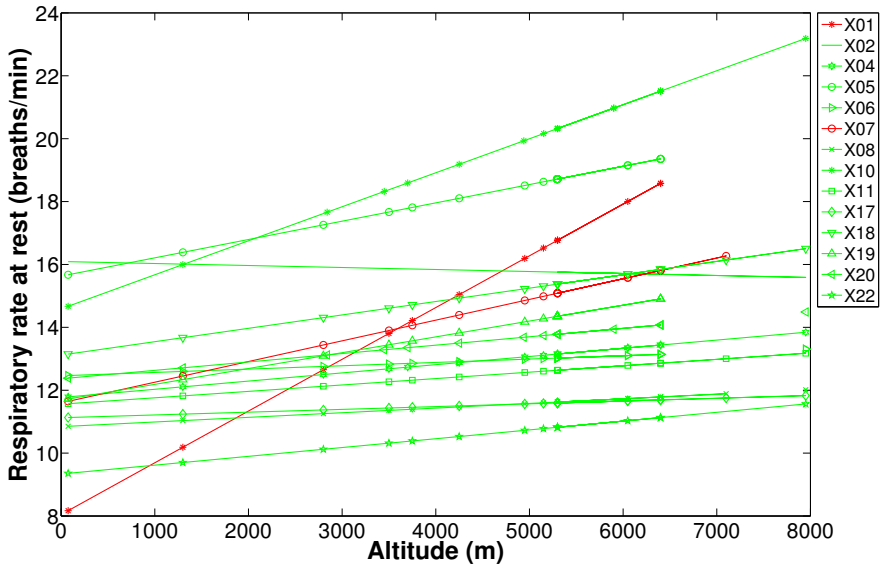


(a) Individual regressions of respiratory rate after exercise against altitude, in 10 lab staff individuals.

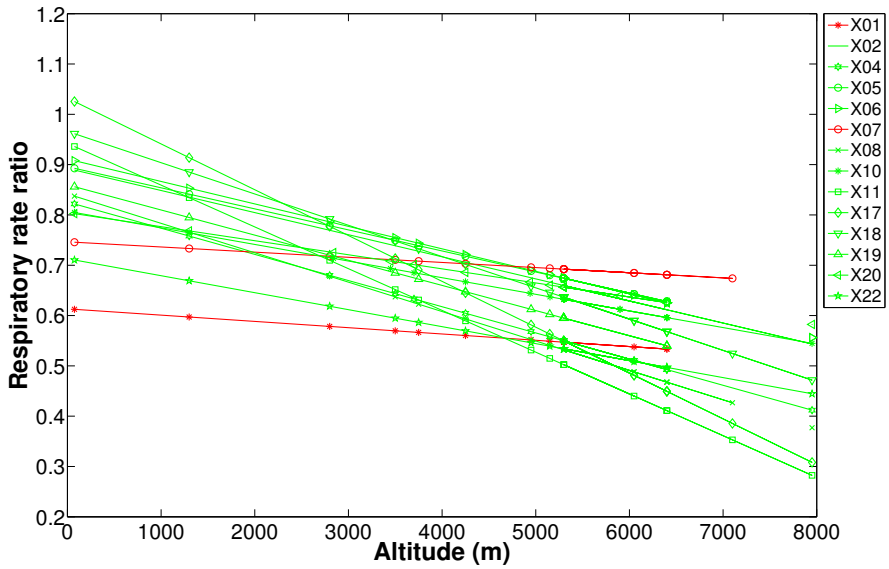


(b) Individual regressions of blood pressure ratio (SBP:DBP) against altitude, in 10 lab staff individuals.

Figure 4.10: Simple linear regression analyses for (a) respiratory rate after exercise against altitude and (b) blood pressure ratio against altitude, for 10 lab staff individuals. Individuals X09 and X23 became ill during the expedition, and are both marked in magenta. Both figures show individual X23 as having a linear response that is different compared to other individuals within the group for these measurements, whereas only (b) shows a response that is different for individual X09.



(a) Individual regressions of respiratory rate at rest against altitude, in 14 climber individuals.



(b) Individual regressions of respiratory rate ratio against altitude, in 14 climber individuals.

Figure 4.11: Simple linear regression analyses for (a) respiratory rate at rest against altitude and (b) respiratory rate ratio against altitude, for 14 climber individuals. Individuals X01 and X07 became ill during the expedition, and are both marked in red. Both figures show individuals X01 and X07 having a linear response that is different compared to others within the group for these measurements.

heart rate after exercise and respiratory rate at rest, respectively. These responses are also summarised in Tables 4.3 and 4.4. Both of these measurements show differences in how individuals are responding to altitude, indicated by positive and negative slopes for individual regression lines. However, only 4 and 10 individuals show slopes that are significantly different from zero for heart rate after exercise and respiratory rate at rest, respectively, shown in Tables 4.3 and 4.4 as having a significant *GOF* statistic (marked with an asterisk (*)) and significance value (marked with a dot (•)).

Figures 4.4 and 4.5 show four individual responses for heart rate after exercise and respiratory rate at rest, respectively. These figures show differences in response between individuals, as well as differences in the strength of the linear fit. The summary statistics in Tables 4.3 and 4.4 can be used to assess the linear fit for each individual, by comparing the slope, C_V, goodness of fit statistic, lack of fit statistic and F-value.

Individual's fitted regression figures can also be used to assess whether any individuals were showing responses very different from others within their group. To assess this, individuals who became ill during the expedition were highlighted in each figure (magenta for lab staff individuals, or red for climber individuals). This aided in visually identifying these specific individuals. Figures 4.10 and 4.11 show instances where ill individuals do seem to be responding differently to others within their group. This information can be used to inform future modelling analysis, and identifies measurements that may be potentially useful for discriminating between individuals who struggled during CXE 2007, and those who did not.

4.2.4.1 Ill individuals

Individuals X01, X07, X09 and X23 all became ill at different points during the expedition. It was hoped that these particular individuals would show a response that was *different* to other individuals within the group, to help account for why they became ill and others did not. There were several measurements that showed differences in how these particular individuals were responding.

Figure 4.3a and Table 4.3 show the simple linear regression results for heart rate after exercise against altitude. This particular measurement shows differences for how individuals X01, X09 and X23 are responding, compared to others within the total core group. Figure 4.3a shows that these three individuals have *negative slopes*, compared to most of the other individuals, who show positive slopes. The slope for individual X09 is the only slope that is significantly different from zero, shown in Table 4.3 as having a significant *GOF* statistic of 13.62 (marked with an

asterisk (*)) and a significance value of 0.009 (marked with a dot (•)). The *GOF* statistic and significance value for individual X01 and X23 are 0.25 and 0.059, and 4.84 and 0.121, respectively.

Figure 4.3b and Table 4.7 show the simple linear regression results for respiratory rate at rest against altitude. This particular measurement shows steep positive slopes for individuals X01, X07 and X23, shown as 0.00165, 0.00066 and 0.00123 in Table 4.4, respectively. All of these individuals show slopes that are significantly different from zero, with individual X01 showing *GOF* and significance values of 11.90 and 0.000003, individual X07 showing *GOF* and significance values of 5.46 and 0.004, and individual X23 showing *GOF* and significance values of 16.58 and 0.012. Individual X10 also shows a steep slope that is comparable to the individuals who became ill. The steep slope for individual X01 is also shown in Figure 4.11a.

Other respiratory rate measurements also show ill individuals as having a response that is different from others within the group. Figures 4.10a and 4.12b and Table 4.7 show the fitted regression lines for respiratory rate after exercise against altitude. Individuals X01 and X23 both show slopes that are steeper than other individuals, with slope values of 0.003 and 0.004, respectively. All ill individuals slow slopes that are significantly different from zero, with significant *GOF* values (marked with an asterisk (*)), and significance values (marked with a dot (•)) in Table 4.7. Figures 4.11b and 4.12c, and Table 4.7 show the fitted regression lines for respiratory rate ratio against altitude. Individuals X01 and X07 both show slopes that are not significantly different from zero, whereas other individuals show steep negative slopes.

Figures 4.10b and 4.12a and Table 4.7 show the fitted regression lines for blood pressure ratio against altitude. Individual X09 shows a high intercept value of 1.76, with a slope that does not change significantly with altitude. Individual X23 shows a *positive* response here, whereas all other individuals show negative responses over altitude. The fitted regression line for individual X23 shows a significance value of 0.04, however, the *GOF* statistic is non-significant at 3.33. This may indicate that there are differences between the means at each altitude for individual X23, but the response may not be linear, as indicated by a significant *LOF* statistic of 2.24. All of these measurements could be useful in modelling attempts, to allow the discrimination between individuals who became ill, and those who did not.

4.2.4.2 Summary

- There are differences seen between individuals for all measurements, however, the differences seen between individuals are clearer for some measurements

than others;

- Smoothed responses and individual regression analyses against altitude can both be used to assess how individuals responded during the entire expedition, and identify individuals who are responding in a different way to others within the group;

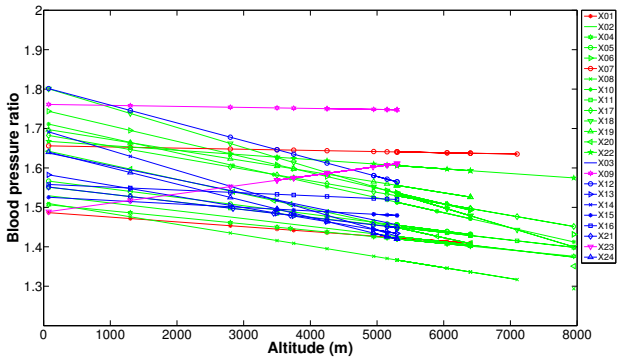
- Several of the physiological measurements showed differences in how *ill individuals* were responding compared to other individuals within the group. These measurements were BP_{Ratio}, HR_{Ex}, RR_{Ex}, RR_{Ratio}, RR_{Rest} and ΔRR. These measurements may be useful in the next stages of modelling for discriminating between individuals who became ill and those who did not.

4.3 Which parameters are most suitable for use in modelling?

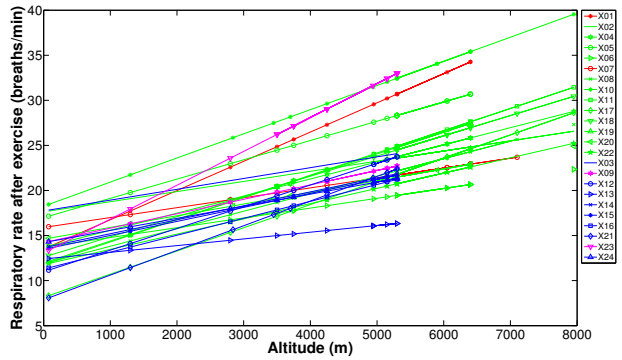
The analyses carried out on the diary data have been used to determine which of the measurements available are the most informative for further analysis, by looking at whether individual's responses change with increasing altitude, how stable each measurement is day-to-day and whether and they can be used to differentiate between different individuals, especially individuals who became ill.

Table 4.8 shows a final *ranked summary* of the diary data measurements, indicating which would be most useful for further analysis and modelling. The top section of this table contains BP_{Ratio}, HR_{Ex}, RR_{Ex}, RR_{Ratio}, RR_{Rest} and ΔRR. These measurements are ranked in order of increasing C_V value, as a measure of the relative day-to-day reliability of each measurement. Whilst these measurements do not show the best reliability out of all of the measurements assessed, these measurements all show differences in how individuals are responding (indicated by differences in slopes and intercepts for fitted regression lines), and differences in how *ill individuals* are responding, compared to other individuals within the group, as described in Section 4.2.4.1.

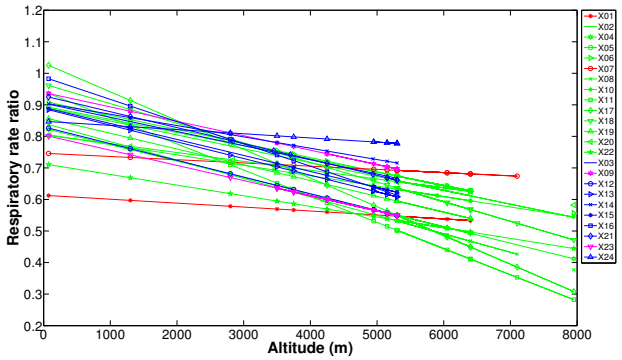
The second group of 6 measurements are HR_{Rest}, SBP, DBP, PP, O_2Sat_{Ratio} and ΔO_2Sat. These measurements are also ranked in order of increasing C_V value, as a measure of their reliability. Each of these measurements show differences in how individuals are responding, but do not show differences in how ill individuals are responding. These measurements were deemed the next most *useful* group of measurements to use in further analysis/modelling.



(a) Individual regressions of blood pressure ratio at rest against altitude, in 24 core individuals.



(b) Individual regressions of respiratory rate after exercise against altitude, in 24 core individuals.



(c) Individual regressions of respiratory rate ratio against altitude, in 24 core individuals.

Figure 4.12: Simple linear regression analyses for (a) blood pressure ratio against altitude, (b) respiratory rate after exercise against altitude and (c) respiratory rate ratio against altitude, for 24 core team individuals. Climber individuals X01, and X07 became ill during the expedition, and are both marked in red. Lab staff individuals X09 and X23 also became ill during the expedition, and are marked in magenta.

Individual	Slope	Intercept	C_V	*GOF*	*GOF* df	*LOF*	*LOF* df	*F*-value	*p*-value
$\mathbf{RR}_{Ex}$									
X01	0.003244	13.50	0.166	* 27.95	1, 8	2.21	8, 44	§8.84	• 0.0000002
X07	0.001097	15.90	0.139	* 26.17	1, 8	0.71	8, 45	§2.70	• 0.0131970
X09	0.001602	14.21	0.137	* 130.39	1, 7	0.27	7, 52	§4.57	• 0.0002923
X23	0.004075	12.01	0.179	* 22.57	1, 7	2.56	7, 35	§9.48	• 0.0000007
$\mathbf{RR}_{Ratio}$									
X01	-0.000012	0.61	0.225	0.94	1, 8	1.39	8, 44	1.38	0.2275220
X07	-0.000010	0.75	0.181	1.18	1, 8	0.73	8, 45	0.74	0.6688670
X09	-0.000046	0.94	0.154	* 52.23	1, 7	0.36	7, 51	§2.70	• 0.0147495
X23	-0.000050	0.81	0.215	* 24.04	1, 7	0.49	7, 35	1.92	0.0883433
$\mathbf{BP}_{Ratio}$									
X01	-0.000012	1.49	0.068	2.10	1, 8	0.97	8, 47	1.08	0.3915860
X07	-0.000003	1.66	0.073	0.04	1, 8	1.85	8, 45	1.66	0.1283250
X09	-0.000003	1.76	0.077	0.05	1, 7	0.80	7, 53	0.71	0.6833970
X23	0.000025	1.48	0.066	3.33	1, 7	1.74	7, 45	§2.24	• 0.0416432

Table 4.7: A summary of the results for the simple linear regression regression analyses performed for multiple physiological measurements against altitude, for the individuals who became ill, denoted X01, X07, X09 and X23. The slope and intercept values are for the fitted regression line. The C_V value is a measure of the day-to-day variability, as described in Section 2.5.10.3. *GOF* and *LOF* are the *goodness of fit* and *lack of fit* statistics, as described in Sections 2.5.10.1 and 2.5.10.2. These statistics were compared to an *F*-distribution with degrees of freedom as defined in the table. A significant *GOF* statistic indicates that the slope is significantly different from zero, and is marked with an asterisk (*). A significant *LOF* statistic indicates that the means deviate from the fitted regression line, and is marked with the symbol (§). The *F*-value and *p*-value are both for the ANOVA performed for each individual, with values marked with a dot (•) indicating a rejection of the null hypothesis at the 5% level, indicating that the mean response is changing as altitude changes.

The final four measurements are O_2Sat_{Rest}, O_2Sat_{Ex}, HR_{Ratio} and ΔHR. Whilst these measurements show some of the lowest C_V values for any of the diary data measurements, they either do not show differences in how individuals are responding (O_2Sat_{Rest}, O_2Sat_{Ex} and HR_{Ratio}), or they do not show a slope significantly different from zero (ΔHR). However, measurements that do not show a change with altitude could still be useful as explanatory variables, as long as they show differences between individual responses.

The next stage in this analysis will be to repeat these and other statistical analyses on the main core dataset, and to identify other physiological measurements that may be useful indicators of how well an individual is adapting to altitude, as well as identifying which of the biochemical metabolites may be good predictors of acclimatisation. Any variables identified in this way will then be used in further modelling processes.

Parameter Name	Slope	C_V	*GOF*	Differences?	Ill individuals?
BP_{Ratio}	-0.00002	0.084	* 50.66	§Yes	• Yes
HR_{Ex}	0.00112	0.135	* 7.79	§Yes	• Yes
RR_{Ex}	0.00184	0.216	* 48.23	§Yes	• Yes
RR_{Ratio}	-0.00005	0.223	* 246.77	§Yes	• Yes
RR_{Rest}	0.00042	0.245	* 8.51	§Yes	• Yes
ΔRR	0.00148	0.656	* 64.10	§Yes	• Yes
HR_{Rest}	0.00234	0.168	* 23.42	§Yes	No
SBP	0.00203	0.110	* 26.49	§Yes	No
DBP	0.00269	0.106	* 73.61	§Yes	No
PP	-0.00065	0.235	* 7.67	§Yes	No
O_2Sat_{Ratio}	0.00001	0.052	* 26.34	§Yes	No
ΔO_2Sat	-0.00088	0.772	* 17.39	§Yes	No
O_2Sat_{Rest}	-0.00323	0.053	* 261.75	No	No
O_2Sat_{Ex}	-0.00408	0.061	* 1064.99	No	No
HR_{Ratio}	0.00002	0.200	* 7.82	No	No
ΔHR	-0.00126	0.424	2.46	§Yes	No

Table 4.8: A summary of all diary data measurements, ranked in decreasing order of potential *usefulness* in further analysis/modelling. *Slope* is the slope for the fitted regression line for all 24 individuals. C_V is the coefficient of variation calculated from the regression, as described in Section 2.5.10.3. *GOF* is the goodness of fit statistic, as described in Section 2.5.10.1. Values marked with an asterisk (*) show slopes that are significantly different from zero. *Differences?* indicates whether there are differences in how individuals are responding, such as differences in slope and intercept values for the fitted regression lines. *Ill individuals?* indicates whether the particular measurement is showing differences in how ill individuals are responding, as detailed in Section 4.2.4.1.
The first 6 measurements show a slope significantly different from zero, differences in how individuals are responding and differences in how ill individuals are responding. These are ranked in increasing order of C_V value, as a measure of *reliability*. These measurements are deemed the most likely to be of use in further analysis/modelling. The second 6 measurements show differences in how individuals are responding, but not specifically for ill individuals. These measurements are also ranked in order of increasing C_V value, and are deemed the next group of measurements to be of most use in the next stage of analysis/modelling. The final four measurements are deemed the least useful for modelling, as they show no differences in how individuals are responding, or do not show a slope that is significantly different from zero.

Chapter 5

Exploratory Data Analysis

Exploratory data analysis (EDA) is a hypothesis-generating approach to analysing data, which avoids any unnecessary bias that may be present when undergoing confirmatory data analysis. The aims of EDA are to maximise the users insight into the dataset, to undercover any underlying structure to the data, to clarify important variables, to detect outliers and anomalies and to test the validity of initial assumptions. EDA utilises various graphical techniques to allow the user to explore the data with an *open-mind*. Graphical representations allow fast and easy identifications of any patterns or outliers in the data, whilst allowing the user the ability to gain new and unexpected insight into the dataset.

5.1 Which data are available?

Data is available for two groups of individuals, comprising a group of 24 *core team* members and a larger group of 190 *trekker* individuals, as detailed in Table 1.2. The full lists of biochemical metabolites and physiological measurements available for the core team are detailed in Tables 2.3 and 2.4. A majority of the trekker samples have yet to be analysed up to this date. Data is available at London (75m), Kathmandu (1300m), Namche (3500m), Pheriche (4250m) and Everest Base Camp Weeks 1, 6 and 8 (5300m), as shown in Figure 1.1.

One important point to take into consideration is that these analyses are almost exclusively based on the results of a small self-selected group of 24 *core team* members, consisting of doctors and scientists who each had a certain level of fitness and mountaineering experience. As these 24 are a highly selected group, they may not be truly representative of the population as a whole. EDA was used here to generate hypotheses from the biochemical data for the 24 core team members, which

may be validated at a later date once the 190 trekker plasma samples have been measured.

It is also important to note that the 24 core team members did not simply rest at Everest Base Camp for the period over which samples were taken. 14 individuals attempted to summit after EBC week 1, and their ascent profile is shown in red in Figure 1.1. Individuals who ascended to altitudes higher than Base Camp would have been subjected to much higher levels of oxidative stress than the individuals who stayed at Base Camp. However, the main focus of this study was to assess the differences in individual responses to an identical ascent profile (i.e. the same amount of hypoxic exposure). Therefore, most analyses were performed for data collected on 5 occasions from London to Everest Base Camp week 1 only.

5.2 Aims

The aims of this preliminary analysis were to:

- Visually summarise all of the available data and identify the main patterns of hypoxic response seen in the physiological measurements and biochemical metabolites;
- Identify physiological measurements and biochemical metabolites that are changing in a similar way across individuals as altitude increases;
- Identify any outliers and *quality check* the data before further analysis and modelling;
- Assess and rank the physiological measurements in order of suitability as a proxy for hypoxia adaptation, based on their statistical suitability, and whether they are of biological interest;
- Assess and rank the biochemical metabolites in order of suitability as potential predictors of the physiological measurements, based on their statistical suitability and whether they are of biological interest.

5.3 Data analysis techniques

Several different operations have been performed on the dataset, comprising:

1. Plotting box and whisker plots for all physiological measurements and biochemical metabolites to assess how each measurement changes in response to

increasing altitude, and the variability of each measurement across altitudes and between individuals;

2. Plotting all individual responses for a particular measurement on one figure or *subplot*, to show the different responses seen and assess inter-individual variability in response;

3. Performing simple linear regression analyses against altitude to assess how each biochemical metabolite and physiological measurement changes with increasing altitude, and to see whether a linear fit is appropriate to describe the relationship between the two;

4. A two-way Analysis of Variance (ANOVA) without interactions for each physiological measurement and biochemical metabolite to assess the effect of altitude and individual on the response.

This section will look at the different data analysis techniques used, and summarise interesting results from each technique.

5.3.1 Box and whisker plots

The *boxplot* function was implemented in *matlab*, as described in Section 2.5.1, to produce box and whisker plot graphs of all 124 physiological measurements and biochemical metabolites for the core group, and 63 physiological measurements for the trekker group. Each plot shows a summary of all individuals over five increasing altitudes.

5.3.1.1 Results

The box and whisker plots produced for the core data show several different *patterns* of response during CXE 2007. Figures 5.1 and 5.2 show examples of different patterns of response seen for the biochemical metabolites and physiological measurements, respectively. Table 9.2 (located in the appendix) shows the full results for the interquartile ranges (IQR) for each variable for each altitude, as a measure of the variability. Table 5.1 shows a summary of the results for a 2-way ANOVA performed for these particular variables. A full list of the ANOVA results for all biochemical metabolites and physiological measurements is shown in Table 9.1, in the appendix.

Figures 5.1a and Figure 5.1b show an overall increase for both nitrite and cGMP levels during the expedition. This was expected, as they are known to play

a key role in hypoxia adaptation (as described in Section 1.4.1.1). The increase of nitrite levels between Kathmandu and Pheriche may be due to the large increase in altitude between these two time-points, as shown in Figure 1.1. cGMP only shows an increase between London and Namche, which may be due to only initial adaptive responses involving the increase of cGMP. Figure 5.1c shows an initial decrease of RSNO levels between London and Kathmandu, and then an increase between Kathmandu and Everest Base Camp week 1. The reasons for this particular pattern are less clear, but may be due to lower NO availability to form RSNOs at Kathmandu [Rassaf et al., 2002]. Figure 5.1d shows that osmolality shows no change during the expedition, and is described in more detail in Section 3.2. All of these changes were tested for their significance by a two-way ANOVA without interactions, to assess the effect of altitude and individual, and the ANOVA results for these particular metabolites are shown in Table 5.1.

Figures 5.2a and 5.2b show a general decrease for both oxygen saturation at rest and work rate at VO_2max, which is to be expected. Oxygen saturation is known to decrease during hypoxia, and is used later in the analysis as a proxy for *hypoxia exposure*. Work rate is a measure of the body's ability to utilise oxygen, and is also known to decrease in response to decreasing oxygen availability [McArdle et al., 2007].

Figure 5.2c shows a general increase for haematocrit levels, which is to be expected. Haematocrit is the volume percentage of red blood cells in whole blood. One of the main adaptations to hypoxia is an increase in the blood's oxygen carrying capacity [McArdle et al., 2007]. This is achieved by increased urine output, shifting of body fluid and increased red blood cell production, as described in Sections 1.2.3 and 3.2, which leads to an increase in haematocrit. Figure 5.2d shows that resting heart rate only shows an increase between Pheriche and Everest Base Camp week 1. A much earlier increase was expected, as increasing heart rate is an early response to hypoxia, as described in Section 1.2.3. However, the late increase may be due to the high fitness level of the 24 core individuals, and only increases when they are exposed to very high levels of hypoxic stress.

Some of the figures show an increase in variability during the expedition, shown by an increase in the interquartile range (IQR, length of the box). For example, Figures 5.1d and 5.2a and Table 9.2 show an increase in the IQR for both osmolality (from 0.036 to 0.064), and oxygen saturation at rest (from 0.020 to 0.100) between London and Everest Base Camp week 1. This is to be expected, due to various adaptive responses taking effect. The more stress an individual is exposed to, the stronger the expected response to that stress, which accentuates any inter-

	ANOVA (altitude)		ANOVA (individual)	
Variable	F-value	p-value	F-value	p-value
Nitrite (mM)	22.07	• 8.6E-13	2.25	• 3.5E-03
RSNO (nM)	18.01	• 5.9E-11	2.04	• 9.1E-03
cGMP (pmol/mL)	9.21	• 2.7E-06	2.65	• 5.4E-04
Osmolality (mOsmo/kg)	1.57	1.9E-01	1.48	9.8E-02
Oxygen saturation at rest	146.30	• 5.2E-38	3.97	• 1.5E-06
Work rate at VO_2max	239.68	• 9.3E-47	56.48	• 2.5E-43
Haematocrit	57.79	• 2.7E-24	5.60	• 1.1E-09
Resting heart rate	24.58	• 1.0E-13	13.88	• 1.8E-20

Table 5.1: 2-way ANOVA results for a selection of biochemical metabolites and physiological measurements, looking at the effects of altitude and individual, without interaction. Significance levels marked with a dot (•) indicate that the null hypothesis has been rejected at the 5% level.

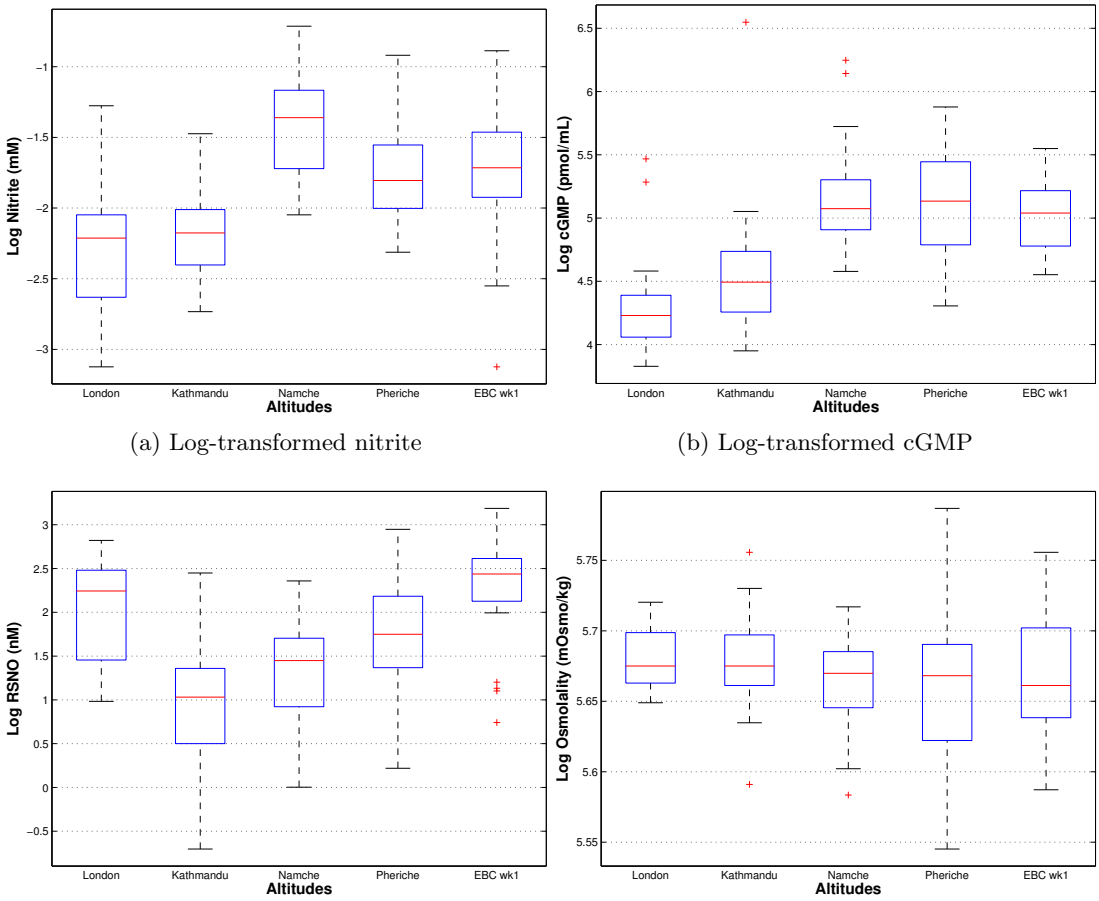
individual variability seen in those response readouts. The change in variability may have an impact on the power of the ANOVA carried out.

Some variables show similar variability during the expedition, shown by similar IQR values for each altitude. Figures 5.1a, 5.1b, 5.2b, 5.2c and 5.2d show box and whisker plots for nitrite, cGMP, work rate at VO_2max, haematocrit and resting heart rate, respectively. These figures and Table 9.2 show that the IQR is similar across altitudes for these particular variables. A small number of variables show a decrease in variability during the expedition, shown by a decrease in the IQR. For example, Figure 5.1c and Table 9.2 shows that the IQR for RSNO decreases from 1.026 for London to 0.488 for Everest Base Camp week 1. The reason for this decrease is less clear.

5.3.1.2 Treatment of potential outliers

Here, outliers are defined as data points that are more than 1.5 times the interquartile range either above the 75th percentile, or below the 25th percentile [McGill et al., 1978], and are labelled as a red '+' on the box and whisker plots. Outliers may be due to instrument or calculation error, or they may show an individual-specific response, resulting in an unusually high or low observation. They may also indicate illness of an individual at the time the sample was taken. If a datapoint is labelled as a potential outlier, it needs to be checked as it can have a great impact on the overall outcome of any analysis performed on the data. Any potentially outlying values were double-checked in the original dataset, to confirm the value was correct.

Potential outliers for the physiological data in the smaller core group can be compared to data from the larger trekker group to identify the position of the



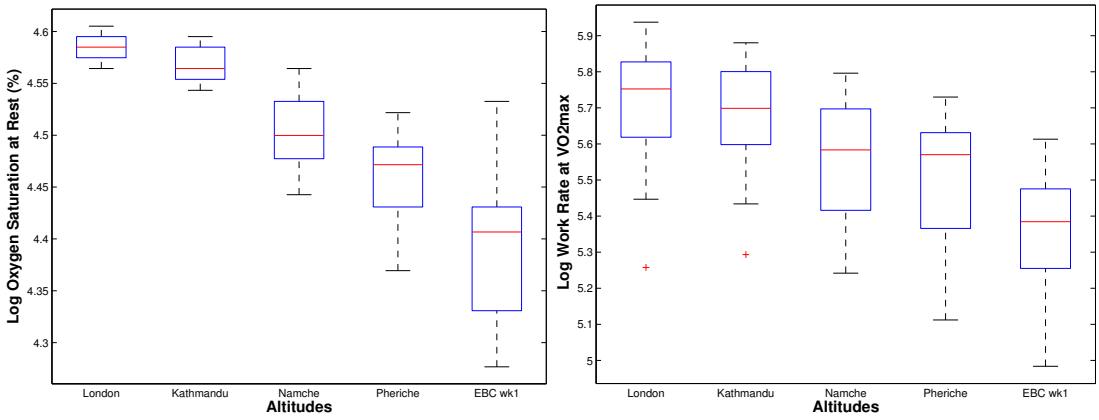
(a) Log-transformed nitrite

(b) Log-transformed cGMP

(c) Log-transformed RSNO

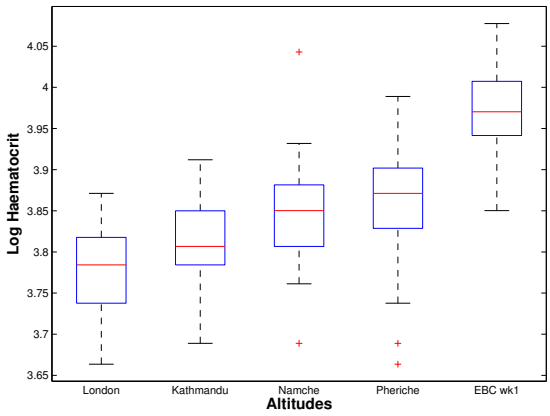
(d) Log-transformed osmolality

Figure 5.1: Examples of different patterns of response seen in several biochemical metabolites in 24 individuals during CXE 2007. (a) shows nitrite, (b) shows cGMP, (c) shows RSNO and (d) shows osmolality. (a) shows an increase between Kathmandu and Namche, (b) shows a general increase until Namche and then a levelling-off, (c) shows an initial decrease between London and Kathmandu, followed by an increase, and (d) shows no change during the expedition. All of these changes were confirmed with a 2-way ANOVA without interactions, as shown in Table 5.1.

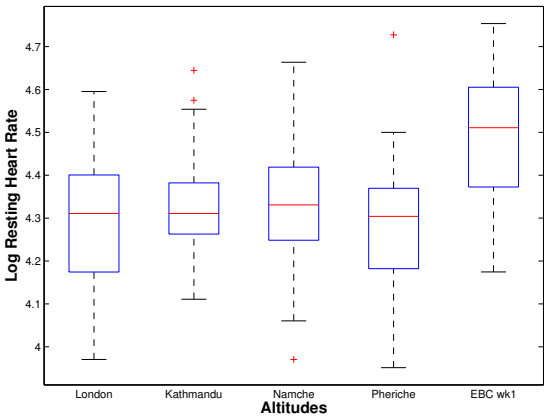


(a) Log-transformed oxygen saturation at rest

(b) Log-transformed work rate at VO_2max



(c) Log-transformed haematocrit



(d) Log-transformed resting heart rate

Figure 5.2: Examples of different patterns of response seen in several physiological measurements in 24 individuals during CXE 2007. (a) shows oxygen saturation at rest, (b) shows work rate at VO_2max, (c) shows haemoatocrit and (d) shows resting heart rate. (a) and (b) show a general decrease during the expedition, (c) shows a general increase and (d) shows an increase between Pheriche and Everest Base Camp week 1. All of these changes were confirmed with a 2-way ANOVA without interactions, as shown in Table 5.1.

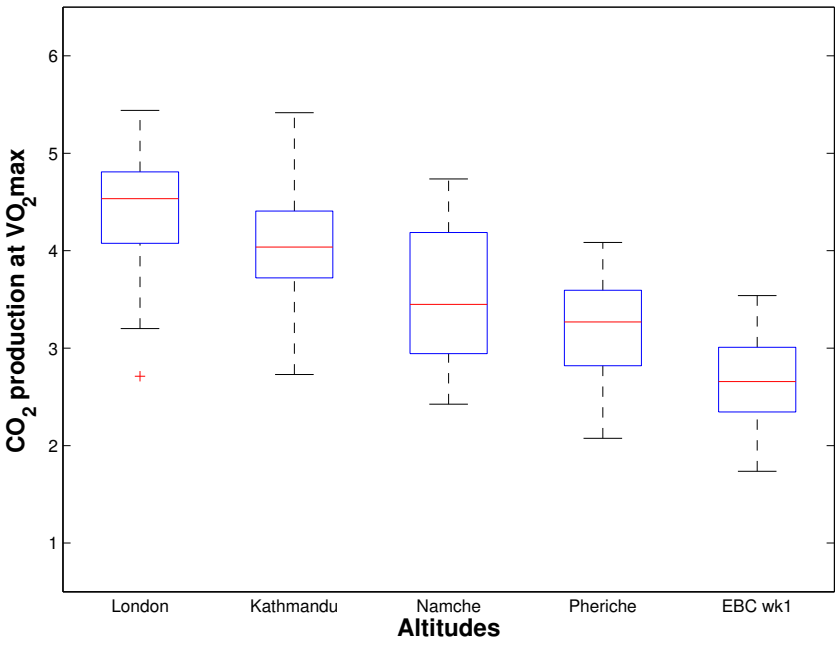
outlier in a larger sample size. Figure 5.3 shows an observation at London that appears to be a potential outlier within the 24 individuals, but is not for the 190 individuals. This emphasises that whilst a sample size of 24 is suitable to show the general population response, a larger sample size gives more information about individual responses, especially when highlighting individuals who are responding differently to everyone else.

5.3.2 Subplots

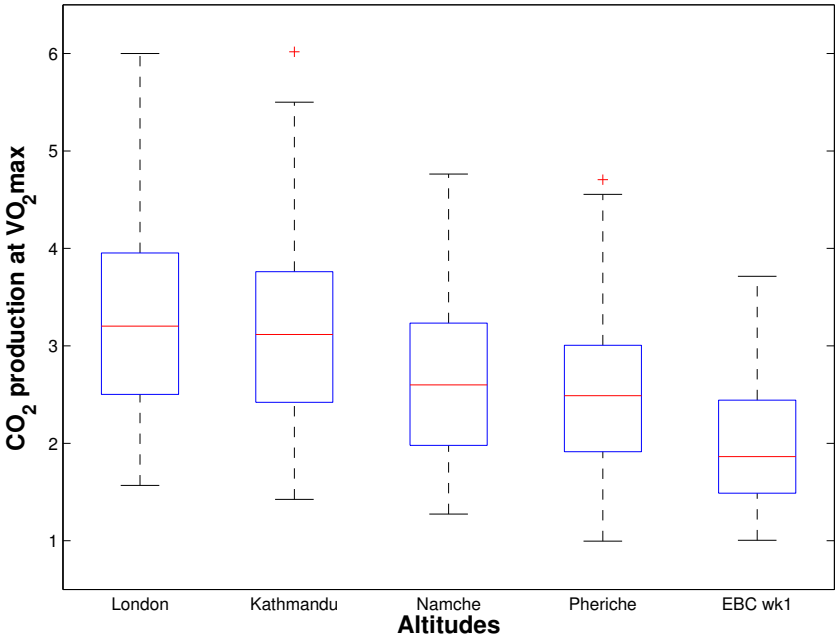
Subplots allow a swift visual representation of several individual's responses to be compared and analysed for trends, and clearly show individuals who show a response vastly different from others. Subplots also highlight potential outliers and indicate the individual to whom the outlier belongs, to aid in further analysis. The *subplot* function was implemented in *matlab*, as described in Section 2.5.2, to produce figures showing all individual responses for all biochemical metabolites and physiological measurements. Males were labelled in cyan (lab staff) and blue (climbers) and females in magenta (lab staff) and red (climbers), to look for any obvious gender differences, or differences between lab staff and climber groups. Individuals who have data points labelled as asterisks (*) became ill during the expedition (individuals X01, X07, X09 and X23).

5.3.2.1 Results

The subplot figures indicate that most of the physiological measurements show low inter-individual variability (i.e. individuals show a similar response over altitude), whereas the biochemical metabolites tend to show a higher level of inter-individual variability in their response. Figure 5.5 shows subplots for haemoglobin and work rate at VO_2max, which both show low variability between individuals. In comparison, Figure 5.4 shows subplots for nitrate and IL-8, which both show much higher variability between individuals compared to Figure 5.5. This is to be expected, as the biochemical metabolites are showing the response of multiple stress-response pathways, which cross-over and communicate with one another, or may just contain more *noisy* data. This creates a very complex pattern of responses, which can vary greatly between individuals. Analysis of the biochemical metabolites in the remaining 190 trekker individual's plasma samples will be vital for further assessing the inter-individual variability seen for the biochemical metabolites.

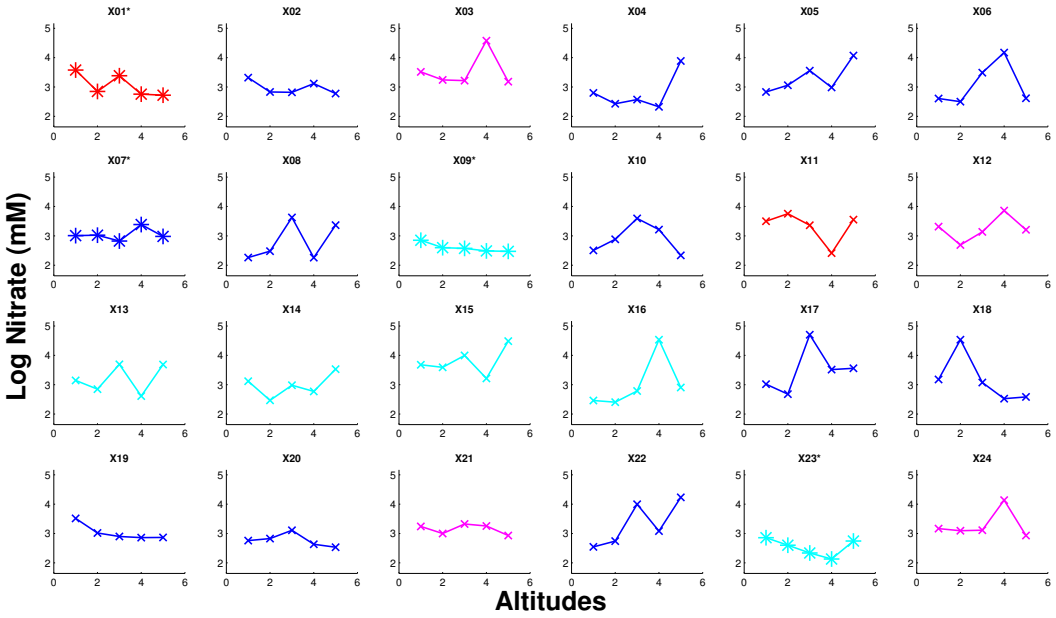


(a) CO_2 production at VO_2max for 24 core team individuals.

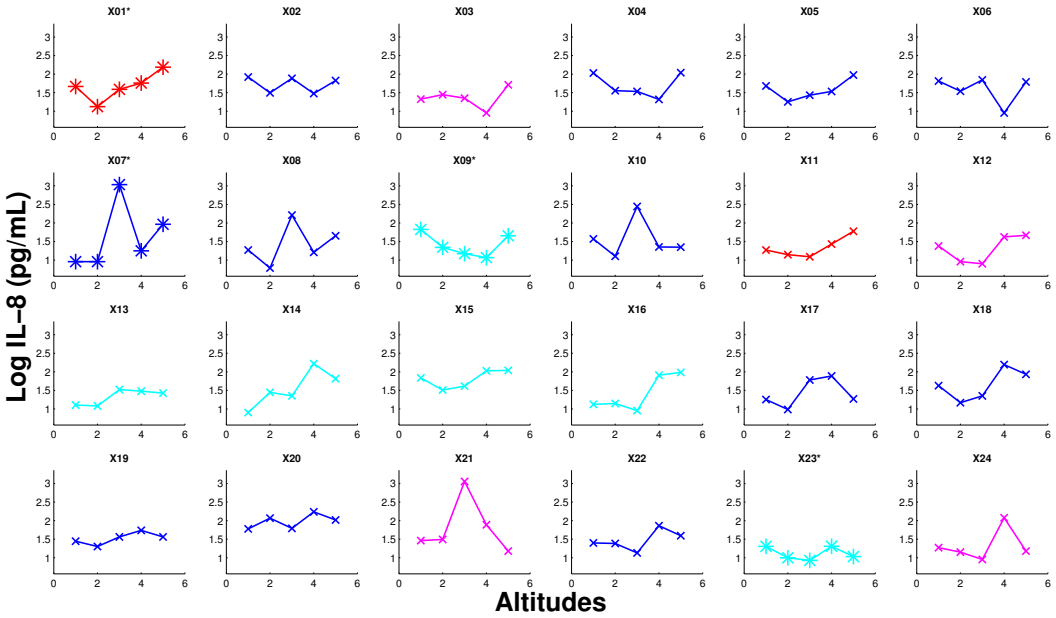


(b) CO_2 production at VO_2max for 190 individuals.

Figure 5.3: box and whisker plots showing carbon dioxide production at VO_2max for (a) 24 core team individuals and (b) 190 trekker individuals. (a) shows generally a higher CO_2 production compared to (b), and both figures show a general decrease over altitude. (a) shows an outlier for London, which becomes part of the whisker for (b). The whiskers for (b) are also much larger compared to (a). This figure shows the importance of the size of a sample when looking at potential *outlying* points.

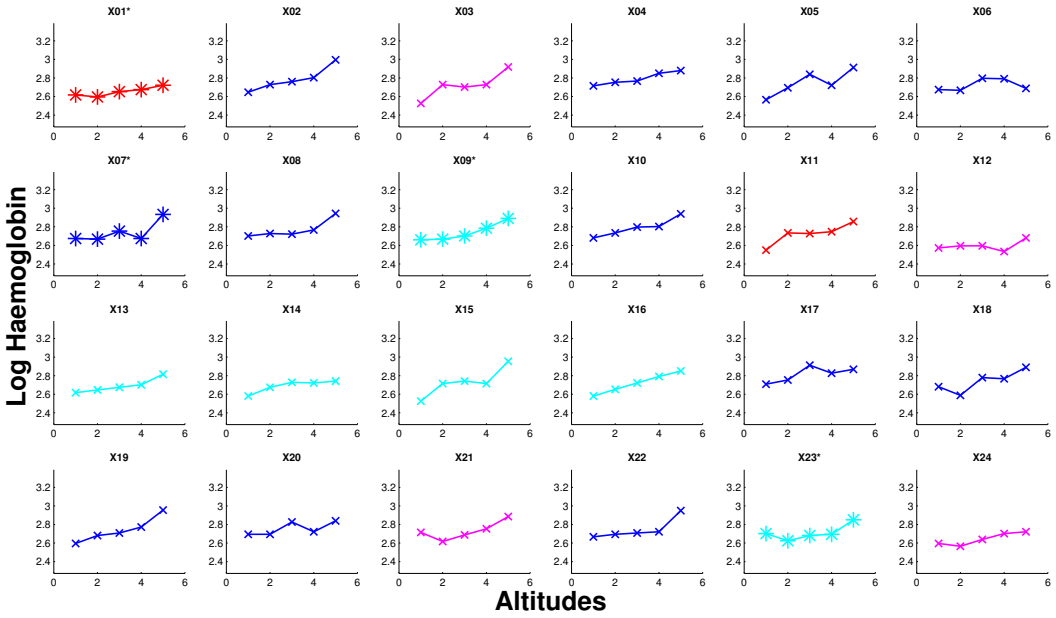


(a) Subplot of log-transformed nitrate levels in 24 individuals during CXE 2007

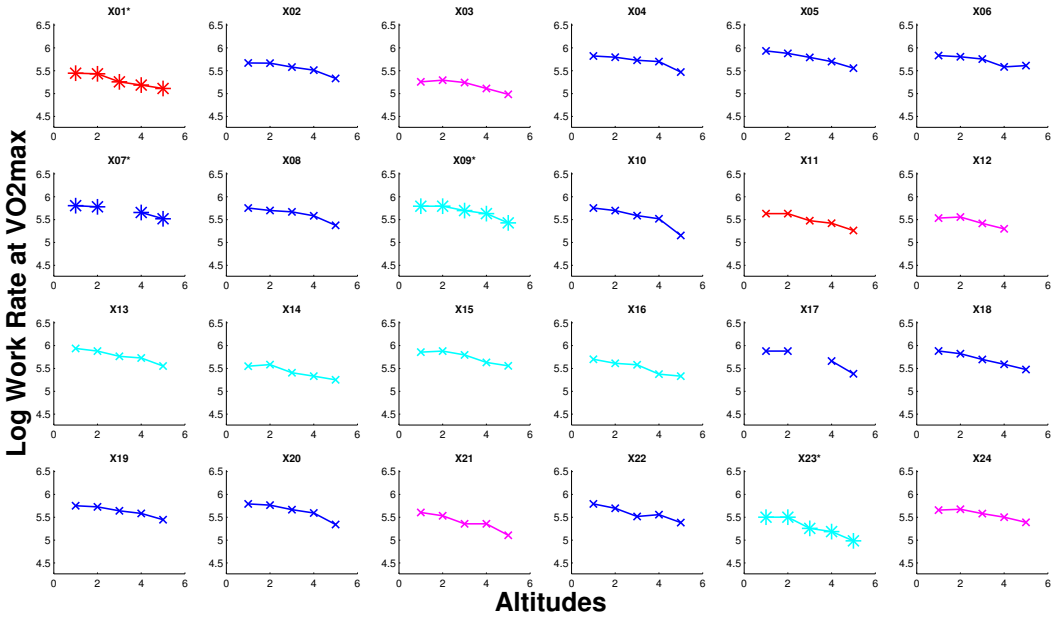


(b) Subplot of log-transformed IL-8 in 24 individuals during CXE 2007

Figure 5.4: Subplots showing how log-transformed nitrate levels (a) and IL-8 (b) change during CXE 2007 in 24 individuals. The horizontal axis shows the increase in altitude, with 1-5 representing London, Kathmandu, Namche, Pheriche and Everest Base Camp. Females are labelled in magenta (lab staff) and red (climbers) and males are labelled in cyan (lab staff) and blue (climbers). The labels above each graph represent that individual's denotative. Missing data points are due to observations not being available in the dataset. Both figures show relatively high inter-individual variability compared to physiological measurements (shown in Figure 5.5).



(a) Subplot of log-transformed haemoglobin levels in 24 individuals during CXE 2007



(b) Subplot of log-transformed work rate at VO_2max in 24 individuals during CXE 2007

Figure 5.5: Subplots showing how log-transformed haemoglobin levels (a) and work rate at VO_2max (b) change during CXE 2007 in 24 individuals. The horizontal axis shows the increase in altitude, with 1-5 representing London, Kathmandu, Namche, Pheriche and Everest Base Camp. Females are labelled in magenta (lab staff) and red (climbers) and males are labelled in cyan (lab staff) and blue (climbers). The labels above each graph represent that individual's denotative. Missing data points are due to observations not being available in the dataset. Both measurements show relatively low inter-individual variability compared to biochemical metabolites (shown in Figure 5.4).

5.4 Variable selection

Frequently, experiments generate a large amount of data, which can be used to create hypothesis-generating models, capable of predicting the output of further experiments. In the absence of any prior biological knowledge, many, if not all of the possible variables may be used in initial models to reduce possible model bias (approximation error). However, a complex model that encompasses many insignificant variables may have less predictive power, and the results may be difficult to interpret. In this case, a simpler model with fewer variables may be more appropriate. Variable selection is key to solving this problem, and looks at selecting the variables with the most predictive power over the dependent variable. Automatic methods such as step-wise selection or elimination allow the swift analysis of large numbers of potential variables. However, they can be computationally expensive and may not take into consideration any prior biological knowledge. The main question here is *which* variables to choose, and the main challenge is *how* to choose them. The methods in this section look at selection methods for both the physiological measurements (dependent variable) and biochemical metabolites (explanatory variables) of interest for modelling methods.

5.4.1 Variable selection criteria

Suitable dependent and explanatory variables for modelling were selected based on the following criteria, which were assessed with a statistical approach:

1. A significant change during the expedition. This was assessed via an ANOVA and by assessing fitted regression lines against altitude;
2. Variability in response between individuals, to allow the distinction between how individuals are responding. This was assessed by analysing fitted regression lines against altitude;
3. Ideally, low day-to-day variability, shown by a low C_V value (as described in Section 2.5.10.3). Diary data was used here for some measurements, as described in Chapter 4;
4. Ideally, a difference between lab staff and climbers, and ill individuals and individuals who did not become ill. Diary data was also used here for some measurements.

Physiological measurements and biochemical metabolites were assessed for their suitability for use in modelling by calculating several summary statistics, and

combining this with prior biological knowledge. Variables that measured very similar components were compared via correlations to see if both were needed for modelling purposes. Binary information such as gender was removed from the analysis, as well as any variables missing a large amount of data, so that any models constructed would be as accurate and complete as possible.

5.4.2 Physiological measurement selection

For each individual, a simple linear regression (SLR) model was fitted for each physiological measurement against altitude, as described in Section 2.5.9. Fitting a SLR model against altitude allowed the assessment of whether the measurement showed a linear response with increasing altitude, and whether there were differences between individuals for the response. The fitted lines were then summarised across all individuals for each measurement, by calculating the mean and standard deviation of the slopes, intercepts and R^2 values. The mean and standard deviation for the R^2 values were used as indicators of how well a SLR described the relationship between the physiological measurement and altitude, across all individuals. The intercept was used as an indicator of the calculated physiological measurement value when hypoxia exposure was zero, and the slope mean and standard deviation were used as indicators of how much variability there was between individuals for the response seen. If any measurements overlapped with the diary data, then the information gained during Chapter 4 was used to inform the analysis of the corresponding measurement in the core data.

Several physiological measurements were highlighted by members of the CXE team as being of *high biological interest*. Indicators of *hypoxaemia* (low partial pressure/content of oxygen within the blood) show how well an individual is loading haemoglobin with oxygen and transporting it in the blood. Hypoxaemia indicators include haemoglobin, oxygen saturation at rest and oxygen content. *Oxygen consumption* is an analogue of how well the body utilises oxygen, and can be an indicator of how well an individual is adapting to low oxygen levels. Parameters of interest that show oxygen consumption are work rate at LaT and VO_2max and oxygen consumption at rest, LaT and VO_2max. *Oxygen efficiency* is a measure of how well oxygen is being used by the body, and is measured by looking at oxygen economy.

For the SLR analysis, a high standard deviation of the slope coupled with a low standard deviation for the intercept and high R^2 was preferred. This indicated that there was a good linear relationship between the physiological measurement and altitude (shown by a high R^2 value), and that there was a difference in how

individuals were responding to the exposure to hypoxia (shown by differences between individual's fitted slopes). If any measurements did not change significantly over altitude, as determined by an ANOVA, or did not show differences between individual responses, then they were not used in further modelling efforts. The resulting ranked physiological measurement list is shown in Table 5.2. This was constructed by looking at the summary statistics, and highlighting metabolites of specific biological interest with an asterisk (*).

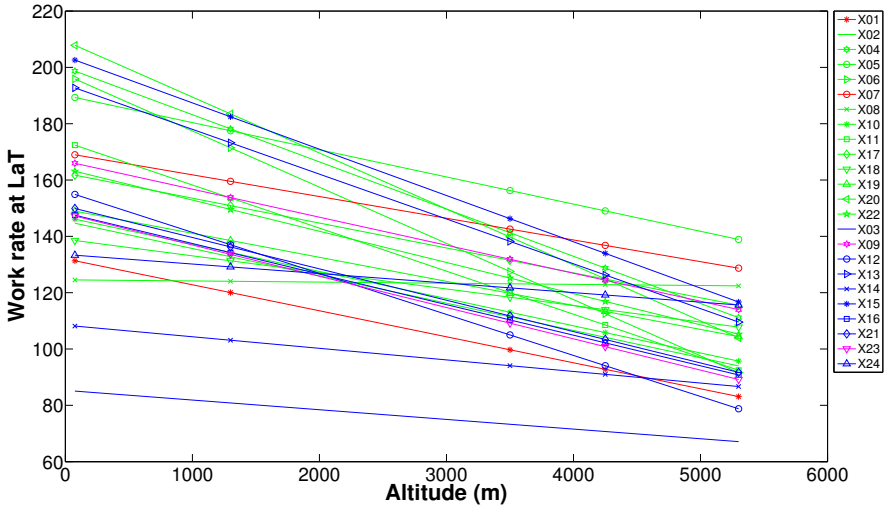
Figures 5.6a and 5.6b show examples of the fitted regression models for the physiological measurements against altitude, for all individuals. Figure 5.6a shows the results for work rate at LaT and Figure 5.6b shows the results for heart rate at VO_2max. Both of these measurements are ranked highly as potential dependent variables for modelling in Table 5.2, due to high mean R^2 values for the fitted lines, and a high standard deviation for the slopes.

Work rate at LaT is also a measurement of particular biological interest, as it is a measure of oxygen consumption. Heart rate after exercise was identified in the diary data as showing responses for ill individuals that were different to other individuals within the group. Figure 4.3a shows the simple linear regression results for heart rate after exercise against altitude for the diary data. Heart rate after exercise and heart rate at VO_2max are not identical measurements, but both show the heart rate after exercise. Interestingly, the diary data measurement shows a general increase in heart rate for most individuals, with ill individuals showing a decrease. However, the core data shows a general decrease for most individuals. This may be due to differences in what the measurements are actually showing, and may limit how useful the diary data information is for analysing the core dataset.

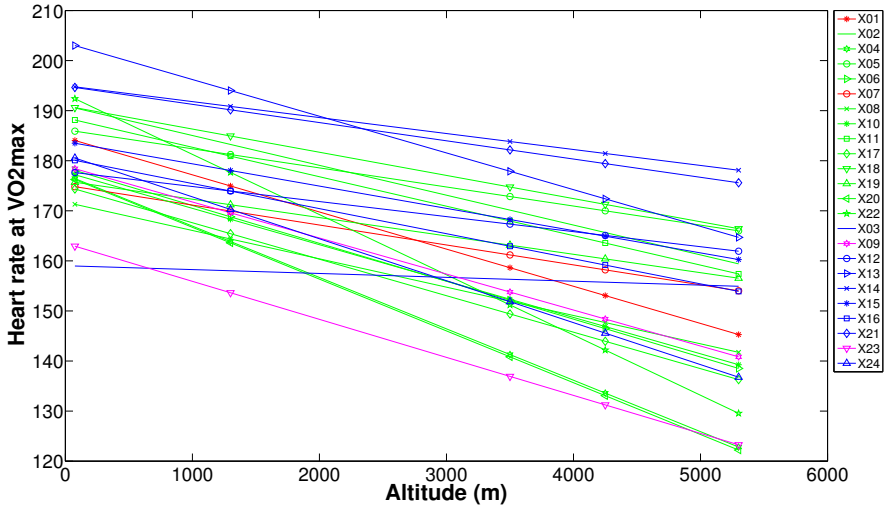
The top group of measurements in Table 5.2 are all measurements of biological interest, and are ranked by decreasing standard deviation of the slope (to show difference in how individuals are responding). The second group includes measurements identified from the diary data as showing differences in how ill individuals were responding, as described in Section 4.2.4.1. The measurements are also ranked by decreasing standard deviation of the slope. The final group of measurements have been identified purely by their statistical summaries, and are also ranked by decreasing standard deviation of the slope.

5.4.3 Biochemical metabolite selection

Explanatory variables are variables that are expected to have some influence over a dependent variable of interest. The biochemical metabolites were used as potential explanatory variables, with the aim of finding a combination of biochemical metabo-



(a) Work rate at VO_2 max regressed against altitude



(b) Heart rate at VO_2max regressed against altitude

Figure 5.6: Examples of simple linear regression analyses carried out for the physiological measurements against altitude. (a) shows work rate at LaT against altitude, and (b) shows heart rate at VO_2max against altitude. Both figures show separate fitted regression lines for 24 individuals. These measurements are both ranked highly as potential dependent variables for modelling in Table 5.2, due to a high mean R^2 value for the fitted lines (indicating that the model is a good fit to the data) and differences between individual slopes (shown by a high slope standard deviation in Table 5.2), indicating differences between how individuals are responding. Lab staff individuals are in blue, with ill individuals in magenta, and climber individuals are in green, with ill individuals in red.

Parameter name	Slope mean	Slope SD	Intercept mean	Intercept SD	R^2 mean	R^2 SD
∗Work rate at LaT	-0.010561	0.00513	158.27	30.90	0.729	0.284
∗Work rate at VO_2max	-0.017348	0.00397	317.00	47.86	0.882	0.067
∗Oxygen saturation at rest	-0.003058	0.00096	99.43	1.11	0.903	0.073
∗Oxygen consumption at LaT (kg)	-0.001556	0.00088	26.69	4.63	0.749	0.221
∗Economy	-0.000222	0.00023	10.88	0.90	0.488	0.329
∗Haemoglobin	0.000584	0.00022	13.72	0.67	0.671	0.198
∗Resting oxygen consumption (kg)	0.000080	0.00014	5.24	0.82	0.309	0.296
•Heart rate at LaT	-0.003113	0.00333	132.11	15.40	0.515	0.347
•Heart rate at VO_2max	-0.006097	0.00265	181.72	10.16	0.782	0.192
•Respiratory rate at VO_2max	0.001186	0.00178	45.98	10.27	0.435	0.293
•Resting respiratory rate	0.000492	0.00052	17.16	2.83	0.297	0.268
Maximum voluntary ventilation	0.006373	0.00383	168.24	27.95	0.598	0.311
Minute ventilation at LaT	0.002077	0.00230	44.65	10.41	0.518	0.340
Diastolic blood pressure at rest	0.001240	0.00211	78.58	9.13	0.331	0.278
Resting heart rate	0.001802	0.00208	72.80	10.91	0.338	0.290
Resting minute ventilation	0.001085	0.00064	11.60	1.84	0.547	0.274
Resting oxygen pulse	-0.000054	0.00021	5.60	1.05	0.376	0.307
Respiratory exchange ratio at VO_2max	-0.000014	0.00002	1.22	0.06	0.304	0.288
Respiratory exchange ratio at LaT	-0.000008	0.00001	0.89	0.05	0.371	0.308

Table 5.2: A ranked list of physiological measurements for use as dependent variables in the modelling analysis. The top group are all measurements of specific biological interest (labelled with an asterisk (*)), and are ranked by decreasing standard deviation of the slope (to show difference in how individuals are responding). The second group were highlighted during analysis of the diary data, for ill individuals showing responses that were different to other individuals within the group (labelled with a dot (•), as described in Section 4.2.4.1. These measurements are also ranked by decreasing standard deviation of the slope. The final group have high mean R^2 values for the fitted regression lines, indicating a good fit to the model, and are ranked by decreasing standard deviation of the slope.

lites that would be able to predict a value or change in a particular physiological measurement of interest. To ensure only the most suitable biochemical metabolites were used as potential explanatory variables in modelling, all of the biochemical metabolites were assessed for several characteristics. They had to show a significant change between altitudes during the expedition, show differences in how individuals were responding and only be missing a small number of data points.

Several biochemical metabolites were highlighted as being of *high biological interest*. These included members of the NO and ROS pathways, as described in Sections 1.4.1.1 and 1.4.2. These particular biochemical metabolites are known to play a role in hypoxia adaptation, and it was thought that these may play an important role in the modelling process.

Data was available for a total of 59 biochemical metabolites, and 13 metabolites were removed from modelling for several reasons. proANP, BNP and CNP are known to influence cGMP levels, and were originally measured to determine whether any change seen in cGMP levels was due to changes in these three metabolites (a non-hypoxic response) or the action of nitric oxide on soluble guanylate cyclase (a hypoxic response). These metabolites were only measured as a control for cGMP, and were removed from the modelling process. Protein carbonyls are an important measure of oxidative stress within the body. Protein carbonyls were measured with a protein ELISA plate, which showed high variability between plates measuring samples from the same altitude (a 75-80% decrease was seen between two plates). Therefore, the data was considered spurious and removed from further analysis.

Nine biochemical metabolites were removed due to a lack of data points. These metabolites included all four glutathione measurements, due to a lack of data points at London levels (the vital measurement used for modelling and comparative analysis). Other metabolites removed due to a lack of data points included IL-1α, IL-1β, IL-4, IL-10 and interferon-γ. Explanatory variables must also show differences between individual responses. This was analysed by performing a linear regression between each of the biochemical metabolites against altitude, and looking for differences in individuals slope values. All biochemical metabolites showed differences in slopes, intercepts and gradients for fitted regression lines, indicating differences in how individuals were responding. This may also be caused by noise within the dataset, and is an important point to take into consideration in the next stage of analysis.

A final list of the 46 biochemical metabolites suitable for initial modelling efforts is shown in Table 5.3, with metabolites of specific biological interest marked with an asterisk (*).

Metabolite	Slope Mean	Slope SD	Intercept Mean	Intercept SD	R^2 Mean	R^2 SD
*Nitrite	0.00002	0.00002	0.12	0.04	0.350	0.24
*Nitrate	0.00170	0.00392	21.57	10.83	0.261	0.21
*Total NOx	0.00172	0.00393	21.68	10.83	0.261	0.21
*RSNO	0.00053	0.00111	5.51	2.41	0.284	0.27
*RNNO	0.00003	0.00129	11.16	4.32	0.229	0.23
*Total RXNO	0.00056	0.00213	16.67	5.73	0.221	0.21
*cGMP	0.01782	0.01617	93.02	83.05	0.450	0.26
*8-isoPGF	0.00787	0.00851	40.14	20.86	0.303	0.28
*HNE	0.00190	0.00691	36.70	42.56	0.451	0.28
IL-1ra	-0.00047	0.00392	39.53	17.40	0.198	0.22
IL-6	0.00004	0.00067	12.08	3.31	0.259	0.27
IL-8	0.00034	0.00036	4.11	1.24	0.243	0.21
IL-12(p70)	0.00031	0.00094	7.24	7.48	0.222	0.22
IL-13	0.00017	0.00018	1.46	0.65	0.327	0.24
IL-18	-0.00546	0.00767	116.20	56.31	0.405	0.33
MIF	0.53250	0.69174	5905.25	1545.77	0.472	0.31
Eotaxin	0.00063	0.00691	76.23	42.42	0.290	0.25
TNFalpha	-0.00067	0.00167	33.01	9.54	0.304	0.28
VEGF	0.00869	0.01189	81.63	55.27	0.359	0.31
CRP	0.60843	1.21997	2964.17	4907.02	0.221	0.21
Adrenaline	0.00001	0.00001	0.06	0.03	0.187	0.20
Noradrenaline	0.00001	0.00004	0.14	0.05	0.249	0.32
T3	0.21402	0.75427	3318.68	1849.76	0.418	0.25
T4	2.84280	14.31760	92453.90	50772.00	0.357	0.27
C-Peptide	0.02274	0.07773	1708.52	355.25	0.221	0.25
GIP	-0.00129	0.01541	127.21	64.79	0.248	0.23
Ghrelin	-0.00196	0.00519	118.59	40.48	0.257	0.25
Glucagon	0.00609	0.01080	350.53	59.04	0.211	0.21
GLP-1	0.02609	0.04214	837.20	252.68	0.348	0.25
Insulin	0.03196	0.07273	1334.43	396.88	0.246	0.18
Leptin	-0.11242	0.15481	1254.98	972.25	0.450	0.31
PAI-1	-0.78621	0.69800	8644.11	3286.20	0.530	0.29
Resistin	-0.07318	0.08187	1018.25	442.48	0.417	0.32
Visfatin	0.07488	0.37754	4456.18	2351.47	0.269	0.23
Adiponectin	1535.64000	2071.05000	14453600.00	11682700.00	0.228	0.20
Adipsin	27.54690	17.51600	147056.00	61527.00	0.362	0.22
EPO	0.00274	0.00417	15.16	8.57	0.418	0.32
ET-1	-0.00047	0.00167	25.77	32.33	0.212	0.24
Glucose	-0.00007	0.00018	4.70	0.84	0.224	0.25
Cystatin C	0.14954	0.11037	961.04	189.07	0.549	0.31
Creatinine	0.00001	0.00002	0.82	0.12	0.344	0.30
Lactate	0.00012	0.00015	1.37	0.42	0.355	0.31
Osmolality	-0.00098	0.00187	293.03	5.23	0.248	0.18
ProteinContent	-0.00042	0.00126	82.94	5.28	0.176	0.19
Bicarbonate	-0.00123	0.00056	24.67	2.22	0.506	0.28
HSP-70	-0.00004	0.00105	3.79	8.06	0.254	0.24

Table 5.3: A list of biochemical metabolites for use as explanatory variables in the modelling analysis. Parameters marked with an asterisk ($*$) are of specific biological interest.

5.5 Summary

- These analyses are based on a relatively small self-selected group of 24 individuals. The results from these analyses can be verified at a later date when the 190 trekker plasma samples are measured, and will provide more information on a *population response*;

- Not all of the physiological measurements and biochemical metabolites available were suitable for modelling purposes, and several statistical analyses were combined with biological information to create two lists of variables suitable for modelling (one for physiological measurements (dependent variables), and one for biochemical metabolites (explanatory variables));

- Simple linear regression analysis against altitude allowed the assessment of how physiological measurements and biochemical metabolites were changing with increasing hypoxic exposure. This was used as a basis for the selection of variables for modelling;

- Several physiological measurements were selected as potential indicators of hypoxia adaptation. These measurements showed a change during the expedition (assessed via an ANOVA) and showed variability between individual responses (shown by variability in the slope for fitted regression lines against altitude). Measurements of particular biological interest comprise of indicators of hypoxaemia, oxygen consumption and oxygen efficiency, and are marked in Table 5.2 with an asterisk (*);

- A list of 46 biochemical metabolites has been finalised for use as potential explanatory variables in further modelling analysis. These metabolites show a change during the expedition, and contain enough data to be potentially useful for modelling. Biochemical metabolites of particular biological interest were from the NO and ROS pathways, and are marked in Table 5.3 with an asterisk (*).

Chapter 6

Model Exploration

This chapter describes the initial modelling efforts undertaken to produce a number of models, capable of predicting the response seen in one dependent variable (physiological measurement) using a combination of one or more explanatory variables (biochemical metabolites). This initial stage of modelling is *exploratory* in nature, and several physiological measurements were used as potential dependent variables, as identified in Chapter 5. The overall suitability of each physiological measurement as a dependent variable was assessed by looking at the strength of the resulting predictions from the model formed, and the biological relevance of the variable (i.e. has it been highlighted as a measurement of particular biological interest, as described in Section 5.4.2).

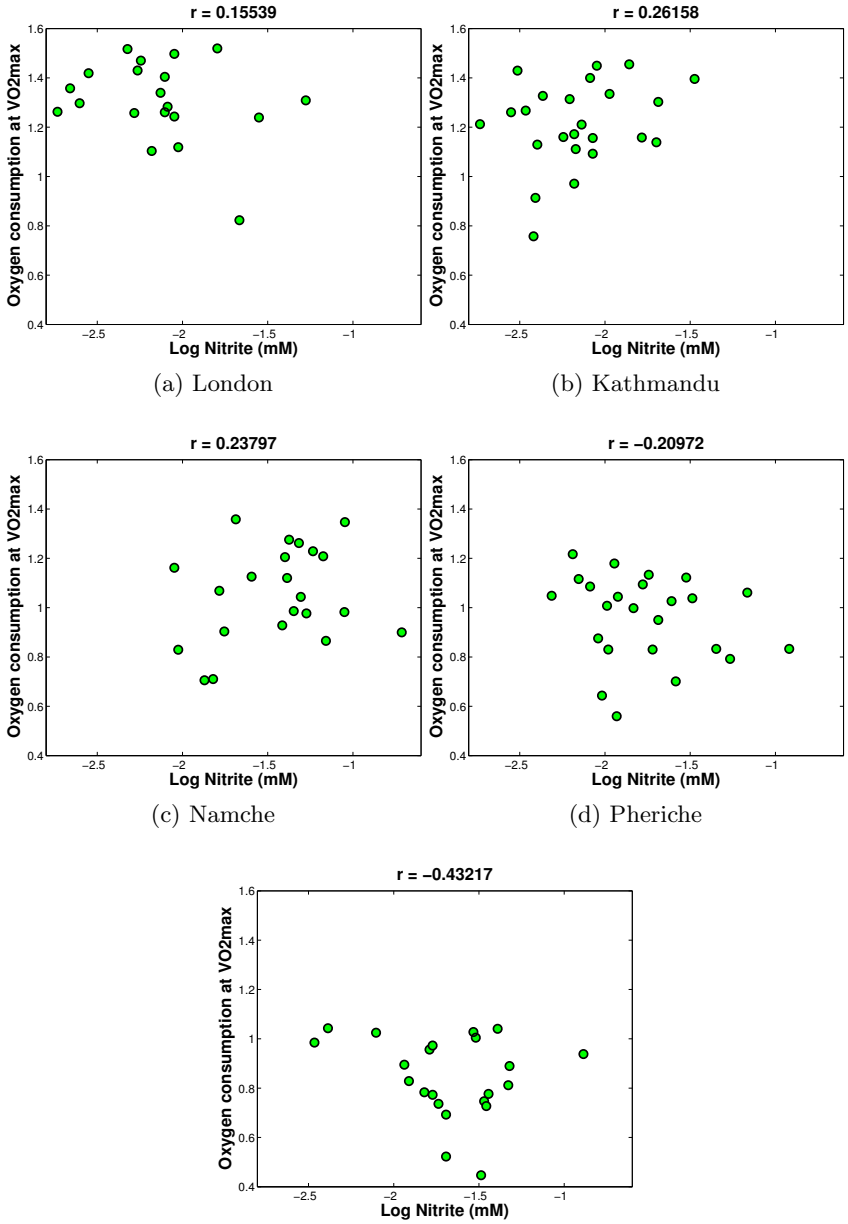
6.1 Aims

The main aims of this stage of model building were to:

- Identify possible linear relationships between physiological measurements (dependent variable) and a combination of biochemical metabolites (explanatory variables);
- Identify physiological measurements that are predictable by a combination of one or more biochemical metabolites.

6.2 Simple linear regression

Simple linear regression (SLR) looks at the linear relationship between one dependent variable and one explanatory variable, as described in Section 2.5.9. This initial



(a) London (b) Kathmandu

(c) Namche (d) Pheriche

(e) Everest Base Camp

Figure 6.1: Nitrite correlated against oxygen consumption at VO_2max, for each altitude, separately. Each plot shows the two variables correlated for that altitude. The r-values are the correlation coefficients between the two variables. Whilst none of these correlations are strong, these figures show a *change* in the relationship between these two metabolites during the expedition [Levett et al., 2011].

method looks for simple linear relationships between two variables, and was used to identify individual explanatory variables that may be useful for complex model building. Each of the physiological measurements listed in Table 5.2 were regressed against each of the biochemical metabolites listed in Table 5.3 (for all altitudes together).

Some initial associations were identified during the writing of a paper published in Scientific Reports in 2011, which looked at correlating NO metabolites against oxygen consumption measures separately for each altitude [Levett et al., 2011]. The paper identified several associations, for which the correlation coefficients were only significant at London (sea-level). It also identified relationships that changed over the course of the expedition. One example is shown in Figure 6.1, which shows correlations between oxygen consumption at VO_2max and nitrite. This figure shows an initial weak positive correlation value between the variables, that becomes negative by Pheriche. This highlights a problem - simple models may assume that the relationship between two variables remains the same throughout the expedition, whereas the example shown in Figure 6.1 shows that this is not always the case.

The aim of this initial analysis was to identify relationships that were consistent over all altitudes. This would be the first step towards building a predictive model, capable of looking at a variety of scenarios. In a clinical environment, it may not be clear how hypoxic a patient is when they first arrive into the ICU (i.e. which *altitude* they are equivalent to). Therefore, we want a *general* model that is able to incorporate the hypoxic status of a patient and the change in the relationships seen between variables during hypoxic exposure (such as those shown in Figure 6.1). The fitted regression lines were assessed for their fit by assessing the scatter plot between the dependent and independent variables, as well as the R^2 value, goodness of fit statistic, lack of fit statistic and F-statistic calculated for the fit, as described in Section 2.5.9.

6.2.1 Results

The simple linear regression models produced showed poor R^2 values, with a maximum R^2 value of 0.35, indicating that none of the dependent variables could be explained by one explanatory variable. Most of the regression models produced showed very poor R^2 values of less than 0.1. However, these results were expected, due to the complex nature of the system studied. These results were used to identify potential explanatory variables that may be of use in a more complex modelling method, to see if a *combination* of multiple biochemical metabolites could explain

more of the variability associated with the physiological measurements of interest.

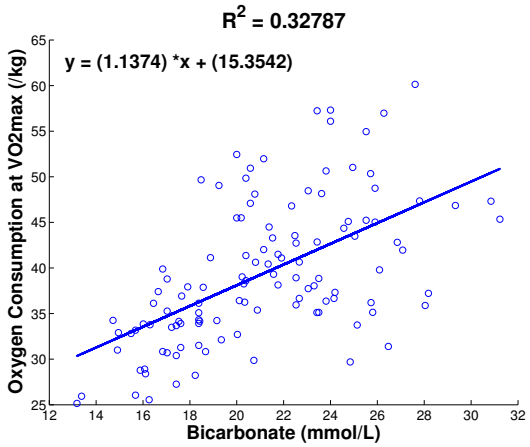
Figure 6.2 shows some of the typical results from the simple linear regression analysis performed for each physiological measurement. Figure 6.2a shows an example of one of the strongest relationships seen, for the regression of oxygen consumption at VO_2max (normalised for body weight) against bicarbonate. This figure shows an R^2 value of 0.328, indicating that bicarbonate is explaining 32.8% of the variability associated with oxygen consumption.

Figures 6.2b, 6.2c and 6.2d show the regression results for work rate at VO_2max against creatinine, work rate at LaT against adipsin and oxygen consumption at LaT against IL-6. All of these figures show no clear relationship between the dependent and independent variables, and were typical of the vast majority of the results gained from the simple linear regression analyses.

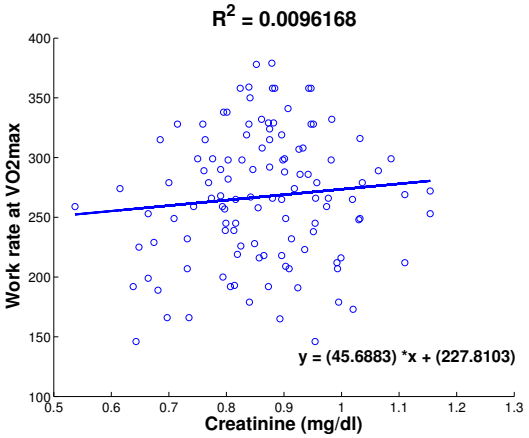
6.3 Multiple linear regression

Multiple linear regression (MLR) was chosen as the next model building method due to the number of potential explanatory variables available, shown in Table 5.3. MLR looks at the inclusion of a *combination* of terms, as described in Section 2.5.11. In some cases, additional explanatory variables were calculated as the product of original explanatory variables. The inclusion of these combination terms allows the assessment of interaction effects within the statistical model, and may highlight potentially interesting biological combinations to be assessed further. However, including all possible combination terms is computationally expensive, and was only possible when considering a relatively small subset of the biochemical metabolites as explanatory variables, and not when using the full range of biochemical metabolites available, shown in Table 5.3.

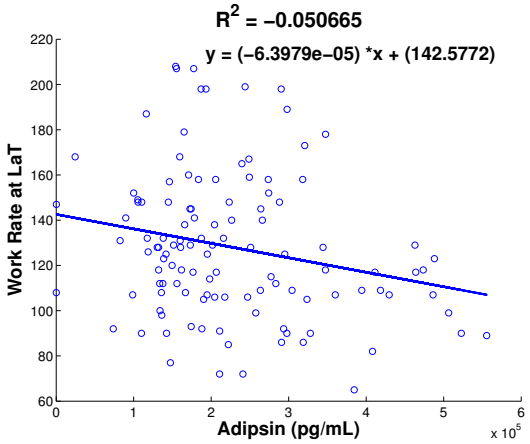
As there were a large number of potential explanatory variables available, an automated selection algorithm was chosen to assess potential relationships between each dependent variable and the multiple explanatory variables. Stepwise regression was chosen to swiftly select the best model for the observed data, whilst minimising the amount of time required to fit each model. In this case, the *best* model is the one that best describes the observed data available, measured by the calculation of an adjusted R^2 value, as described in Section 2.5.12. The *stepwisefit* algorithm was implemented in *matlab* to produce the best model for each of the physiological measurements listed in Table 5.2, as described in Section 2.5.11. All of the models fitted were formed using MLR, and as such no biological information was used to determine the inclusion or exclusion of specific explanatory variables at this stage.



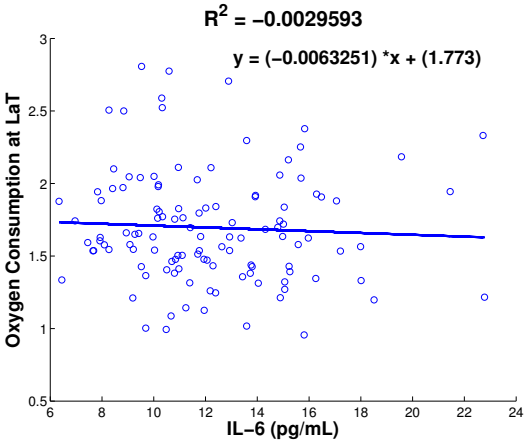
(a) O_2 consumption at VO_2max (normalised for body weight) vs. bicarbonate



(b) Work rate at VO_2max vs. creatinine



(c) Work rate at LaT vs. adipsin



(d) O_2 consumption at LaT vs. IL-6

Figure 6.2: Simple linear regression figures, regressing a physiological measurement against a biochemical metabolite. (a) shows a very weak linear relationship between oxygen consumption at VO_2max (normalised for body weight) against bicarbonate. The R^2 value for this model is 0.32787, indicating that bicarbonate is explaining 32.8% of the variability associated with the dependent variable.
(b), (c) and (d) were more typical of the regression results, showing the results for (b) work rate at VO_2max against creatinine, (c) work rate at LaT against adipsin and (d) oxygen consumption at LaT against IL-6. These figures show that the biochemical metabolites have no relationship with their respective dependent variable.

6.3.1 Assessment of model stability and fitting

All models produced were assessed to determine how well each model fitted the observed data, and examine the relationships between the dependent variable and each of the explanatory variables.

6.3.2 Uncertainty, variability and residuals

For a given model, all predicted values will exhibit some degree of *uncertainty*. This uncertainty (or error) should be random, so that predicting higher or lower than the actual response should carry the same probability (i.e. the error is symmetric). The magnitude of error should also be independent of the time when the observation occurred. The uncertainty represents any incomplete knowledge about a system, and the level of uncertainty may be reduced with more or better data and a greater understanding of the system. Error is distinct from *variability*, which is the range in values of naturally occurring parameters. This variability is inherent to the data, and cannot be reduced by increasing the amount of data, gaining better data or a greater understanding of the system.

Residuals are defined as being the difference between values fitted from a model and the observed values. Residuals can be used as an estimate of the error associated with a model, and can be used to assess the appropriateness of each parameter included in a model. Examination of the residual values allows the assessment of whether the assumptions made during the modelling process are reasonable, and whether the choice of each of the explanatory variables, or even the entire model choice, is appropriate for the intended use. The overall *pattern* of residuals should follow a *normal distribution* and be homoscedastic (i.e. show a homogeneity of variance). Any departure from these assumptions generally indicates that there is some structure to the residuals, which is not accounted for within the model.

For each model, the residual values were plotted against each explanatory variable, and against values fitted by the model, and assessed for heteroscedasticity. 95% confidence intervals (CI) for the residuals were added to each figure to aid in the assessment of the residual values. They were calculated from the residual mean square (ResMS, shown in equation 2.21) as follows:

$$95\%CI = \sqrt{ResMS}(t - value), \tag{6.1}$$

where the 5% t-value is the critical value of the t-distribution for the number of degrees of freedom for that model, calculated as n - (the number of explanatory

variables used in the model - 1).

6.3.3 Hypothesis testing

In order to determine the appropriateness of each fitted model, univariate correlations were performed between (a) the residual values and the fitted values for the model and (b) the residual values and each of the explanatory variables used in the model, as described in Section 2.5.7. If a significant correlation exists between the residuals and the fitted values, then this suggests that there is a structure to the residuals, and that the model is not describing the variability associated with the dependent variable. If a significant correlation exists between the residuals and the explanatory variable, then this suggests that the particular explanatory variable may not be suitable for use in the model, as it is not taking any of the variability out of the model.

6.3.4 Model refinement

Initial MLR models were formed for each physiological measurement, for the combined data over all altitudes. These initial models were then refined through the following steps:

1. Apply multiple linear regression for the dependent variable for the combined data against all altitudes, using a selection of biochemical metabolites as explanatory variables;

2. Produce a summary for each dependent variable, including figures showing observed vs. fitted values and the related adjusted R^2 value, residual values vs. fitted values and residual values vs. each explanatory variable used;

3. Use summary figures to assess the suitability of each of the explanatory variables in the model (assess for heteroscedasticity and outliers);

4. Remove any observations with *high leverage*, or the metabolite containing the observation with high leverage from the analysis and refit the MLR to assess the effect of removing the values on the model formed;

5. Compare initial and refined models formed, and determine the *best* model for each of the dependent variables. This may not always be the best fitting model, but may be the most reliable model, or the model that predicts a dependent variable of biological interest.

6.4 Different sets of explanatory variables

Two different subsets of the explanatory variables listed in Table 5.3 were used in multiple linear regression:

- NO metabolites only, including pairwise combination terms;
- All biochemical metabolites listed in Table 5.3, with no interactions/combinations.

The NO metabolites are labelled in Table 5.3 with an asterisk (*), and are described in detail in Sections 1.4.1 and 1.4.2. These metabolites were considered in a separate set of models because nitric oxide is known to play a role in hypoxia adaptation, and it was thought that the NO and ROS-related metabolites may interact and be good predictors of hypoxia adaptation and performance at altitude. The second set of models were created using the complete range of biochemical metabolites listed in Table 5.3.

6.4.1 Exploratory modelling - NO metabolites

All of the MLR analyses carried out using the NO metabolites alone resulted in poor models with low adjusted R^2 values ranging from 0.034 to 0.222, indicating that the models produced were not explaining the variability associated with each of the observed dependent variables. Figure 6.3 shows some of the best initial models formed for oxygen consumption at LaT (normalised for body weight, Figure 6.3a), work rate at VO_2max (Figure 6.3b), economy (Figure 6.3c) and haemoglobin (Figure 6.3d). Table 6.1 shows the *best* models produced for each of the dependent variables listed in Table 5.2, using NO metabolites and their combinations as explanatory variables, ranked in order of decreasing adjusted R^2 value. The table shows that the best model produced was for oxygen consumption at LaT (normalised for body weight), with an adjusted R^2 value of 0.222, indicating that it was explaining 22.2% of the variability associated with oxygen consumption. The worst model produced was for minute ventilation at LaT, which showed an adjusted R^2 value of 0.034, and only included one explanatory variable. The final models produced were similar to the best models produced using simple linear regression, shown in Figure 6.2. Ten of the models formed only use one explanatory variable (mostly for the lowest ranked dependent variables in Table 6.1), making the results no more informative than those gained from simple linear regression. Figure 6.3 shows some of the final models produced, with fitted values plotted against observed values. All of these figures show very poor predictability of the response.

Dependent Variable	Adjusted R^2	ResMS	Intercept	Explanatory Variable	Coefficient	p-value
Oxygen consumption at LaT (normalised)	0.222	14.791	26.48	Nitrite	-11.70340	0.01436
				8-isoPGF$_{2\alpha}$	-0.07099	0.00003
				RSNO x cGMP	-0.00114	0.01458
				RNNO x 8-isoPGF$_{2\alpha}$	0.00460	0.00060
Work rate at LaT	0.219	753.002	135.74	RNNO	1.14517	0.04964
				Nitrite x RSNO	-10.30580	0.00032
				cGMP x 8-isoPGF$_{2\alpha}$	-0.00092	0.00034
Work rate at VO_2max	0.205	2262.440	292.87	RSNO x 8-isoPGF$_{2\alpha}$	-0.03346	0.00091
				Total RXNO x cGMP	-0.00576	0.02675
Haemoglobin	0.200	2.251	15.20	RNNO	-0.10087	0.00399
				Nitrite x RSNO	0.65419	0.00003
				RNNO x HNE	0.00031	0.03548
Oxygen saturations at rest (diary)	0.160	40.729	93.93	RSNO x HNE	-0.00203	0.00018
				RNNO x 8-isoPGF$_{2\alpha}$	0.00306	0.04138
				cGMP x 8-isoPGF$_{2\alpha}$	-0.00030	0.00213
Heart rate at VO_2max	0.140	299.737	170.78	Nitrite x RSNO	-6.06334	0.00004
Resting respiratory rate	0.135	10.254	15.58	Nitrite	7.80748	0.04938
				HNE	0.01707	0.00019
				cGMP x HNE	-0.00005	0.00283
Economy	0.116	0.831	10.69	Nitrite x RSNO	-0.18029	0.02194
				Nitrite x cGMP	-0.00765	0.01445
Resting minute ventilation	0.110	14.987	12.83	Nitrite x 8-isoPGF$_{2\alpha}$	0.05442	0.03419
				Nitrite x HNE	0.04011	0.02366
Maximum voluntary ventilation	0.098	894.282	169.78	Nitrite x HNE	0.44817	0.00062
Resting oxygen pulse	0.068	1.529	5.74	RSNO x 8-isoPGF$_{2\alpha}$	-0.00068	0.00380
Heart rate at LaT	0.066	211.701	131.08	Nitrite	-49.21910	0.00437
Respiratory rate at VO_2max	0.050	98.689	48.13	RSNO x 8-isoPGF$_{2\alpha}$	0.00468	0.01204
Resting oxygen consumption (normalised)	0.044	0.585	5.20	Nitrite x HNE	0.00786	0.01707
Resting heart rate	0.043	174.346	75.18	RSNO x 8-isoPGF$_{2\alpha}$	0.00583	0.01853
Respiratory exchange ratio at VO_2max	0.039	0.007	1.21	RNNO x HNE	-0.00001	0.02359
Respiratory exchange ratio at LaT	0.037	0.003	0.88	HNE	-0.00013	0.02671
Minute ventilation at LaT	0.034	135.226	45.67	HNE	0.02942	0.03154

Table 6.1: An ordered summary of MLR results using NO metabolites and pairwise combination terms as explanatory variables. *Adjusted R^2* is the adjusted R^2 value, as described in Section 2.5.12. *ResMS* is the residual means square associated with the regression, as described in Section 2.5.6. *Coefficient* is the coefficient slope for the explanatory variable in the MLR, and the *p-value* is the significance level associated with that explanatory variable for inclusion in the model. Dependent variables are ranked in order of decreasing adjusted R^2 value.

As these models had low predictive power, higher orders of combined variables were not considered. This was because it was not considered meaningful to introduce further combinations of variables purely to reduce residual values for the fitted model without an understanding of the variable relationship or impact on the physiological measurement being assessed. From a statistical perspective, it may seem a good idea to incorporate higher order combinations of explanatory variables, however, from a biological perspective it is very difficult to interpret what these combinations of explanatory variables would actually mean.

6.4.1.1 Work rate at LaT

Table 6.1 shows that one of the most powerful models produced was for work rate at LaT. This model is shown in detail as an example of why the NO metabolites were not able to explain the variability associated with the dependent variables. Figures 6.4 and 6.5 show the summary figures produced for a MLR analysis for work rate at LaT, using NO metabolites as potential explanatory variables. Figure 6.4a shows the fitted values plotted against the observed values. The adjusted R^2 value generated is a measure of how much of the variability in the observed data is explained by the model. For this model, the adjusted R^2 value is only 0.219, indicating that the model is only describing 21.9% of the variability seen in the observed data. The model also shows systematic error, underestimating high responses and overestimating low responses. This indicates that the subset of explanatory variables used is not capturing the variability seen in the dependent variable, and that the overall model is not describing the dependent variable.

Figure 6.4b shows the residual values plotted against the fitted values for the model. This figure shows a clear heteroscedastic relationship, with variability within the residual values increasing as the fitted values increase. This indicates that there is variability in the model that is not being accounted for by the explanatory variables being used. Figures 6.5a, 6.5b and 6.5c show the residual values plotted against each of the explanatory variables used within the model. Each of these figures also suggest heteroscedasticity within the residuals. These figures indicate that the model is failing to describe the variability in the dependent variable, and therefore may not be suitable for the observed data.

There is some evidence of redundancy within the NO metabolites, shown by high r-values and significant p-values for correlations calculated between the metabolites, shown in Table 6.2. This table shows very high correlations between *related metabolites*, such as Nitrate and NOx (r-value = 1, p-value = $1.9\text{x}10^{-250}$) and RNNO and RXNO (r-value=0.925, p-value=$2.2\text{x}10^{-51}$), which is to be expected,

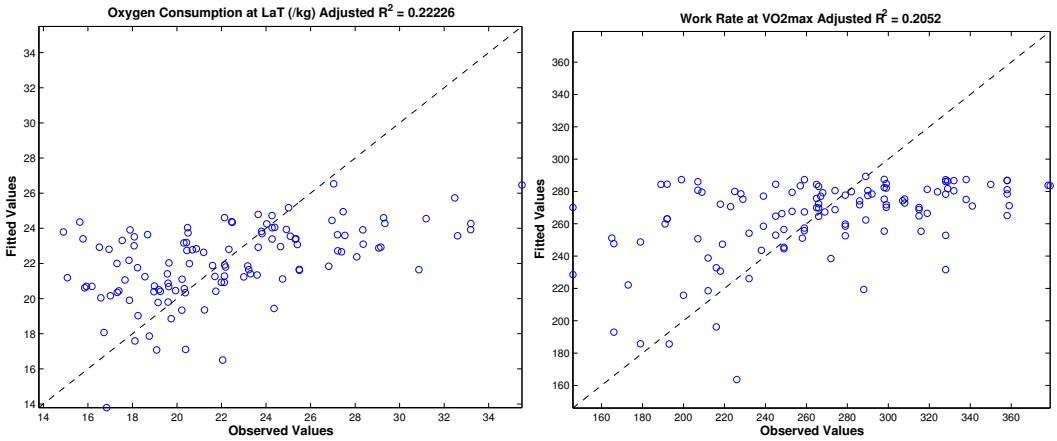
as NOx is calculated as the sum of nitrate and nitrite levels, and RXNO is the sum of RNNO and RSNO. As the NO metabolites are strongly linked with the response to hypoxia, they may be showing too narrow a response, and may not be able to explain the variability associated with non-hypoxic factors such as UV exposure or the physical effort associated with the expedition. Therefore, there may not be enough *variability* in the NO metabolite values to explain the physiological responses.

Alternatively, as NO is known to play a crucial role in several bodily functions across several organs and tissues (as described in Section 1.4.1.1), then these metabolites may be highly regulated independently of oxygen availability, to ensure the proper performance across the whole physiological oxygen gradient. There is also evidence that some of the NO metabolites do not show a significant change over altitude during the expedition. For example, nitrate shows a very low slope value for the fitted simple linear regression line against altitude, shown in Table 5.3, and an acceptance of the null hypothesis for a two-way ANOVA performed for altitudes and individuals. Therefore, the NO metabolites may not be *good enough* as explanatory variables on their own to explain the variability associated with the dependent variables of interest.

6.4.2 Exploratory modelling - All biochemical metabolites

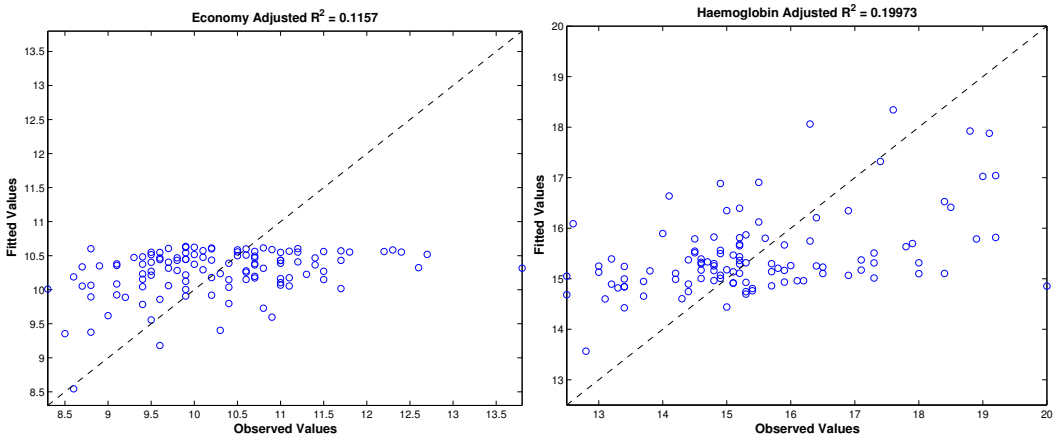
In order to try and explain as much of the variability in the dependent variables as possible, the next stage of modelling looked at utilising all of the available biochemical metabolites described in Table 5.3 as potential explanatory variables. Only linear relationships were looked at here, due to the large initial number of explanatory variables available. The initial results from this analysis were more promising, with higher adjusted R^2 values between the fitted and observed values than those seen using the NO metabolites alone. The results for the initial models produced are shown in Table 6.3, and are ranked in order of decreasing adjusted R^2 value.

A series of statistical tests was then performed on the eight models with an adjusted R^2 value above 0.4, shown in Table 6.3 as dependent variables above the line. Only initial models that had an adjusted R^2 above 0.4 were refined. 0.4 was chosen as an arbitrary *cut-off* value for model refinement, as there was not sufficient time to refine all of the models. Any refinement may include the removal of observations or explanatory variables from the model, leading to a decrease in the adjusted R^2 value. Therefore, only the strongest models were refined, to ensure the final models were meaningful and explained as much of the observed variability as possible.



(a) Oxygen consumption at LaT (normalised)

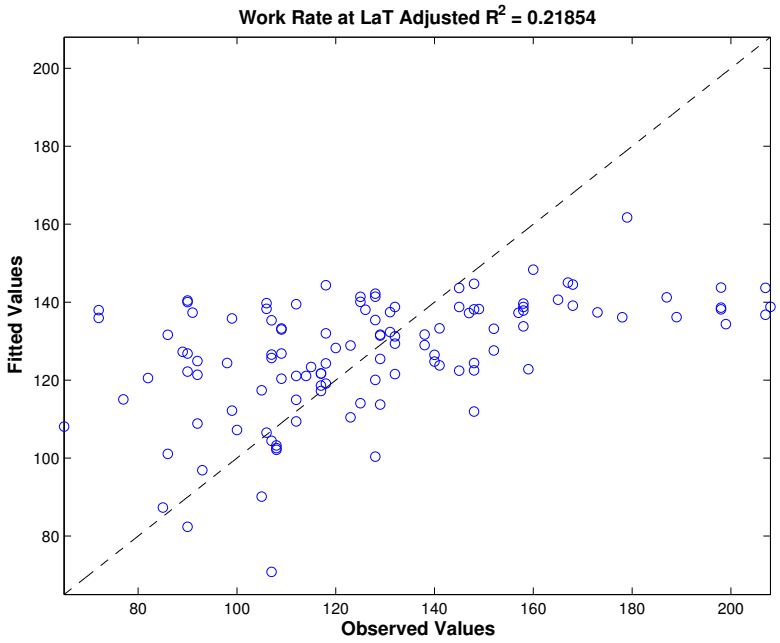
(b) Work rate at VO_2max



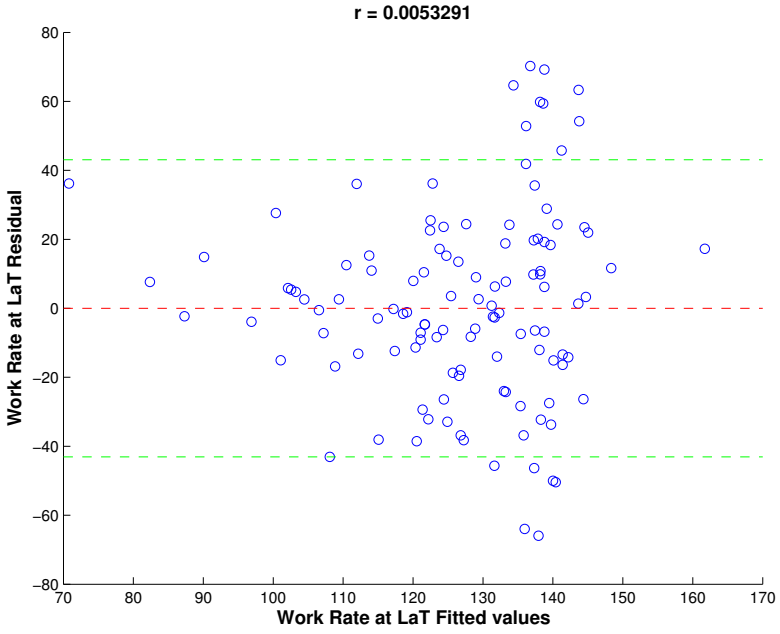
(c) Economy

(d) Haemoglobin

Figure 6.3: Examples of the *best* MLR models produced for various physiological measurements, using the NO metabolites as explanatory variables.

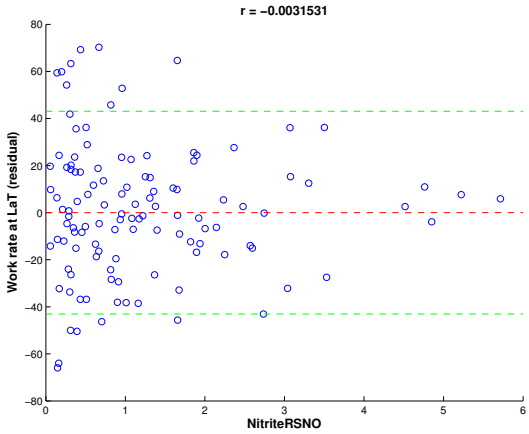


(a) Observed vs. fitted values

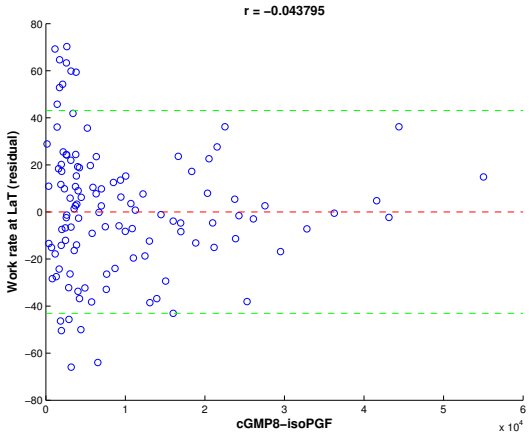


(b) Residuals vs. fitted values

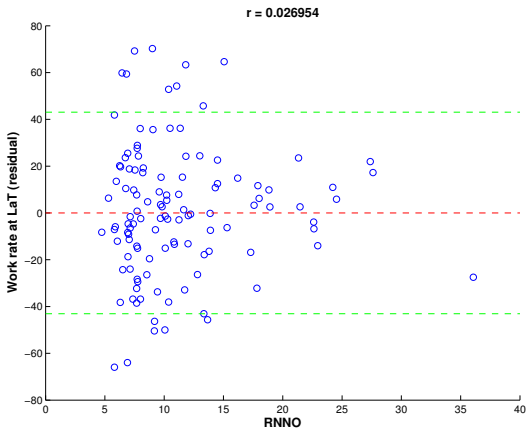
Figure 6.4: Summary figures produced for a MLR between work rate at LaT and NO metabolites (metabolites playing a role in NO and ROS pathways). (a) shows observed vs. fitted values and (b) shows the residual values for work rate at LaT (observed y - fitted $\hat{y}$) plotted against the fitted values for work rate at LaT. (b) shows a large amount of variability within the residual values when plotted against fitted values, indicating that it is not explaining the variability associated with the dependent variable. Therefore, this model is not appropriate for this dependent variable.



(a) Nitrite x RSNO



(b) cGMP x 8-isoPGF$_{2\alpha}$



(c) RNNO

Figure 6.5: Residual figures plotted against explanatory variables used in a MLR for work rate at LaT. All residuals show heteroscedasticity (higher variability associated with lower values for the explanatory variable values), indicating they may not be suitable explanatory variables for this model.

Metabolite x	Metabolite y	r-value	p-value
Nitrate	Total NOx	***1.000	• 1.9E-250
RNNO	Total RXNO	***0.925	• 2.2E-51
RSNO	Total RXNO	***0.899	• 4.2E-44
RSNO	RNNO	**0.682	• 9.4E-18
Nitrite	cGMP	0.349	• 9.2E-05
cGMP	HNE	0.343	• 2.6E-04
Nitrite	Total NOx	0.248	• 6.2E-03
Nitrite	Nitrate	0.242	• 7.7E-03
Nitrite	HNE	0.239	• 1.2E-02
cGMP	8-isoPGF$_{2\alpha}$	0.198	• 3.0E-02
Total NOx	Total RXNO	0.191	• 3.7E-02
Nitrate	Total RXNO	0.190	• 3.7E-02
Total NOx	RSNO	0.170	6.3E-02
Nitrate	RSNO	0.169	6.4E-02
Total NOx	RNNO	0.161	8.0E-02
Nitrate	RNNO	0.160	8.1E-02
Nitrite	RSNO	0.160	8.1E-02
Total NOx	cGMP	0.144	1.2E-01
Nitrate	cGMP	0.142	1.2E-01
Nitrite	Total RXNO	0.121	1.9E-01
RNNO	HNE	-0.109	2.6E-01
Nitrite	8-isoPGF$_{2\alpha}$	0.098	2.9E-01
Total RXNO	HNE	-0.090	3.5E-01
Total NOx	HNE	0.088	3.6E-01
Nitrate	HNE	0.087	3.7E-01
RNNO	cGMP	-0.085	3.6E-01
Nitrite	RNNO	0.081	3.8E-01
8-isoPGF$_{2\alpha}$	HNE	-0.073	4.5E-01
RNNO	8-isoPGF$_{2\alpha}$	-0.057	5.4E-01
Total RXNO	cGMP	-0.053	5.6E-01
Total RXNO	8-isoPGF$_{2\alpha}$	-0.047	6.1E-01
RSNO	8-isoPGF$_{2\alpha}$	-0.031	7.4E-01
RSNO	HNE	-0.029	7.6E-01
RSNO	cGMP	0.015	8.7E-01
Total NOx	8-isoPGF$_{2\alpha}$	0.009	9.2E-01
Nitrate	8-isoPGF$_{2\alpha}$	0.007	9.4E-01

Table 6.2: Correlations calculated between the NO and ROS metabolites, ranked in decreasing strength of the absolute value for the correlation coefficient (r-value). The p-value is the probability of getting a correlation as large as the observed value by random chance, when the true correlation is zero. Metabolites marked with a dot (•) show a p-value less than 0.05, indicating that the correlation between x and y was significant.
Medium strength correlations have a correlation coefficient between either -0.6 and -0.79 or 0.69 and 0.79, and are marked with a double asterisk (**). Strong correlations have a correlation coefficient between either -0.8 to -1.0 or 0.8 to 1.0, and are marked with a triple asterisk (***).

Dependent variable	Adjusted R^2	ResMS	Intercept	Explanatory variables used in the model
* Work rate at VO_2max	0.717	827.342	-273.27	Nitrite, 8-isoPGF$_{2\alpha}$, IL-1ra, MIF, VEGF, GIP, Resistin, Adiponectin, Glucose, Cystatin C, Creatinine, Osmolality, Protein Content, Bicarbonate.
* Oxygen saturation at rest	0.678	14.915	92.11	HNE, IL-18, MIF, T4, EPO, Cystatin C, Bicarbonate.
* Work rate at LaT	0.581	417.973	-86.3	Nitrite, IL-1ra, IL-12(p70), IL-18, MIF, Eotaxin, C-Peptide, Adipsin, EPO, Glucose, Cystatin C, Creatinine, Osmolality, HSP-70.
• Heart rate at VO_2max	0.566	150.763	113.44	RSNO, IL-1ra, IL-18, Ghrelin, GLP-1, Cystatin C, Protein Content, Bicarbonate.
* Haemoglobin	0.551	1.248	17.62	IL-1ra, C-Peptide, Leptin, Resistin, Adiponectin, Cystatin C, Bicarbonate.
Resting minute ventilation	0.539	7.791	-4.39	IL-1ra, TNF-α, C-Peptide, Resistin, Adiponectin, Cystatin C, Osmolality, Bicarbonate.
Resting heart rate	0.474	97.043	95.14	IL-12(p70), IL-18, MIF, Noradrenaline, Bicarbonate.
Resting oxygen pulse	0.463	0.876	-1.63	IL-6, IL-8, IL-18, TNF-α, VEGF, T4, Ghrelin, Glucagon, Leptin, Creatinine, Osmolality.
Minute ventilation at LaT	0.383	84.006	-30.45	VEGF, PAI-1, Adiponectin, EPO, Creatinine, Osmolality, Bicarbonate.
• Respiratory rate at VO_2max	0.382	61.145	55.6	MIF, T3, Resistin, Visfatin, Adiponectin.
Maximum voluntary ventilation	0.377	625.845	179.1	IL-12(p70), TNF-α, Insulin, Leptin, Glucose.
* Oxygen consumption at LaT (kg)	0.349	12.845	20.2	Nitrite, MIF, EPO, Bicarbonate.
• Resting respiratory rate	0.345	7.37	-4.5	RNNO, cGMP, HNE, IL-18, Cystatin C, Osmolality.
* Resting oxygen consumption (kg)	0.344	0.413	4.86	Nitrite, 8-isoPGF$_{2\alpha}$, IL-12(p70), Noradrenaline, Leptin, Resistin, Cystatin C, HSP-70.
Respiratory exchange ratio at VO_2max	0.292	0.005	1.2	Cystatin C, Bicarbonate.
• Heart rate at LaT	0.251	167.308	128.81	IL-18, Glucagon, Leptin, Lactate.
Respiratory exchange ratio at LaT	0.248	0.002	0.92	MIF, Eotaxin, Cystatin C.
* Economy	0.107	0.864	10.43	Nitrite, IL-1ra.
Diastolic blood pressure at rest	0.097	114.121	71.59	Eotaxin, Adrenaline.

Table 6.3: A summary of the MLR results using all biochemical metabolites as explanatory variables. *ResMS* is the residual mean square, as described in Section 2.5.6. Any models below the line had an adjusted R^2 value below 0.4, and were not refined further. This was due to the low initial adjusted R^2 value, which would only reduce when individual values or explanatory variables were removed. Dependent variables that are marked with an asterisk ($*$) are of specific biological interest. Variables marked with a dot ($\bullet$) were highlighted in the diary data as showing differences between ill individuals (described in Section 4.2.4.1). The dependent variables are ranked in descending order according to the adjusted R^2 value, which is described in Section 2.5.12.

Several figures of the residuals were produced to check the quality of the fitted models. These figures showed the residual values for the fitted response plotted against each of the explanatory variables used within the model, and the residual values plotted against the fitted values for the model. Ideally, we want to see an even scatter of residual values around zero as either the explanatory variable or the fitted values increase. Correlation values were computed for each of the plots, as described in Section 2.5.7, and the null hypothesis (H_0 = *there is no correlation between the observations of variables x and y*) was accepted for all correlations between residual and explanatory variables, and residual and fitted values. The figures were also visually assessed for heteroscedasticity, to determine whether there was any underlying structure to the residuals.

95% confidence intervals were added to the figures to assess how well the model was predicting the dependent variable. If the model is estimating the dependent variable well overall, then 95% of the total points should lie within the green intervals. Observations that lay outside of the confidence intervals were poorly fitted. Observations with high leverage that gave a residual value close to zero may skew the model by influencing the resulting regression between that explanatory variable and the dependent variable, as shown in Figure 6.6. Any observations that were thought to have high leverage were highlighted for further assessment. These observations were removed one-by-one to assess the impact of the observation on the model. It is important to note that removal of any one observation would also remove this specific observation from all other explanatory variables for that model. Therefore, if that observation was also important for the strength of the regression seen between the dependent variable and another explanatory variable, then it may have an effect here also.

For each of the dependent variables assessed, the initial model best described the observed data available, indicated by the largest adjusted R^2 value, which was to be expected, as the initial models were all based on the full dataset available. Once the model was refined and any observations with high leverage or explanatory variables were removed, then the adjusted R^2 value decreased, indicating that the removal of the values led to less of the variability within the observed data being described by the resulting model. However, the full model may be strongly influenced by the observations with high leverage included. It is also important to note that these models were fitted without any prior biological information being used to determine whether or not a particular explanatory variable was included in the model. The refinement of the model allowed the use of prior biological information, to assess whether the outlying values made *biological sense*, and should be left in

the model or omitted.

6.4.3 Results

Table 6.3 shows the initial models formed for each dependent variable by stepwise MLR, using all biochemical metabolites as possible explanatory variables. The table also shows that several of the biochemical metabolites used as explanatory variables are included in multiple models. Figures 6.7, 6.8 and 6.9 show examples of the models formed from MLR for different dependent variables, and show how well the fitted values by the model match the observed data.

Figure 6.7 shows the results for the MLR performed for heart rate at VO_2max. Figure 6.7a shows the observed values plotted against the fitted values by the model, colour-coded by altitude, and Figure 6.7b shows the residual values plotted against the fitted values by the model. Figure 6.7a shows a reasonable fit of the observed data to the fitted data, with an adjusted R^2 value of 0.566, indicating that the model is explaining 56.6% of the variability associated with the observed data. This figure also shows an even scatter of points around the dashed line, indicating that it is fitting values for all altitudes with similar levels of error.

The physiological measurement of heart rate after a 2-minute step-exercise test (HR_{Ex}) from the diary data (described in Chapter 4) is similar to the heart rate at VO_2max measurement in the core data. Both measurements show the heart rate at different altitudes after exercise, although the amount of exercise at VO_2max is much higher. Therefore, the results from the analysis of HR_{Ex} within the diary data may be used to inform the use of heart rate at VO_2max as a dependent variable, to aid in understanding the model formed.

Heart rate after exercise was highlighted in the diary data as showing differences between ill individuals, and is marked with a dot (•) in Table 6.3. The diary data showed that HR_{Ex} was a reasonably stable measurement between days, with a low C_V value of 0.135 calculated from the simple linear regression performed for the diary data measurement against altitude, shown in Table 4.2. HR_{Ex} also showed differences in how individuals were responding, shown by differences in the slopes and intercepts for simple linear regression analyses performed against altitude for each individual, identifying it as a potentially good dependent variable for modelling. The adjusted R^2 for the MLR performed for heart rate at VO_2max may be low due to the inter-individual variability being relatively high for this type of measurement, shown in Figure 4.3a and Table 4.3. Some differences in how individuals are responding are required for modelling. However, the MLR is attempting to fit a model to all individuals, therefore, the high inter-individual variability for this type

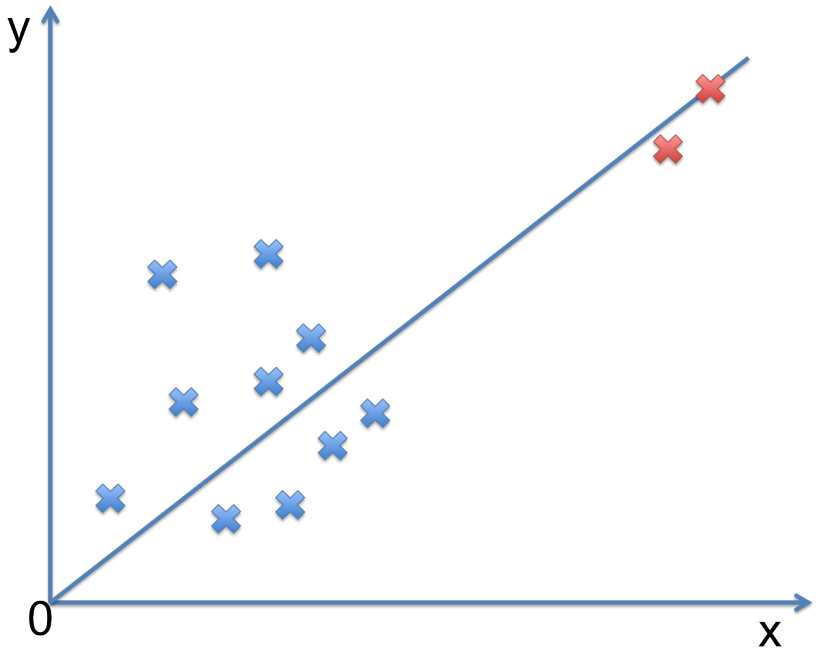
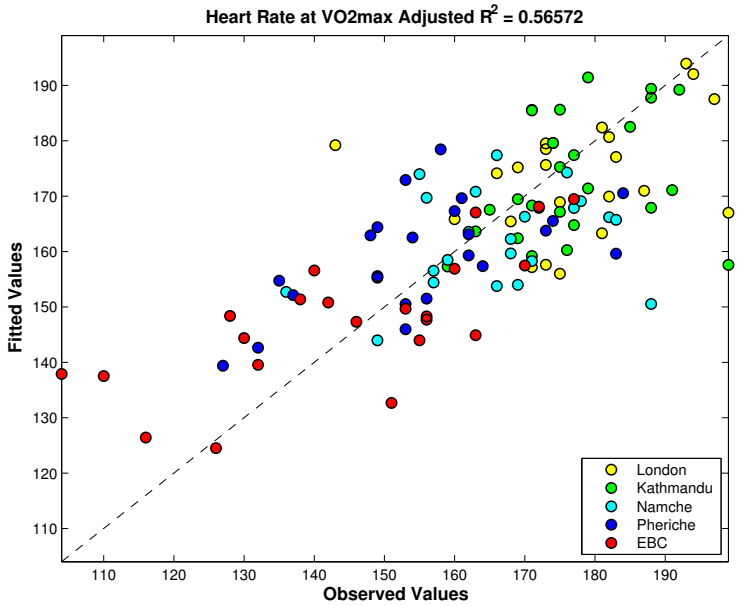
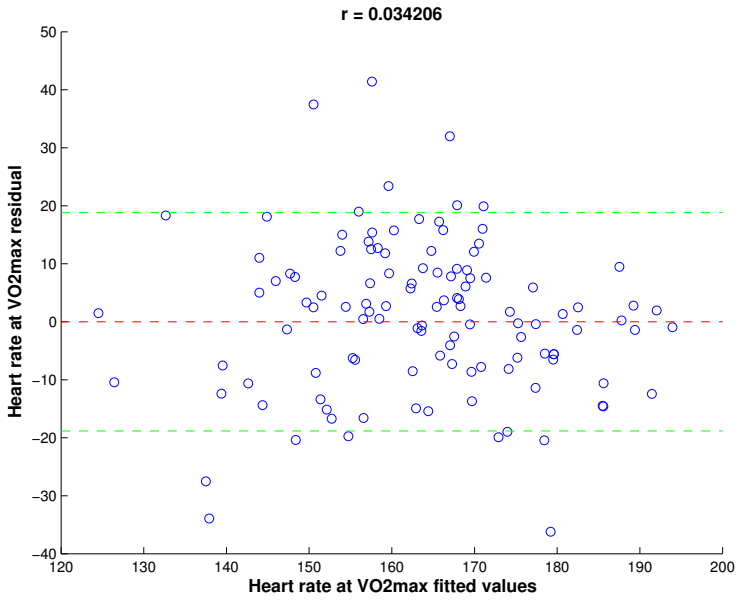


Figure 6.6: An example of a linear regression performed, where x contains two observations with *high leverage*, in red. Data points far from the mean x value are potentially more informative than those near it. If there are only a few of these points, then they can have a misleading effect on the fitted line, as shown here.

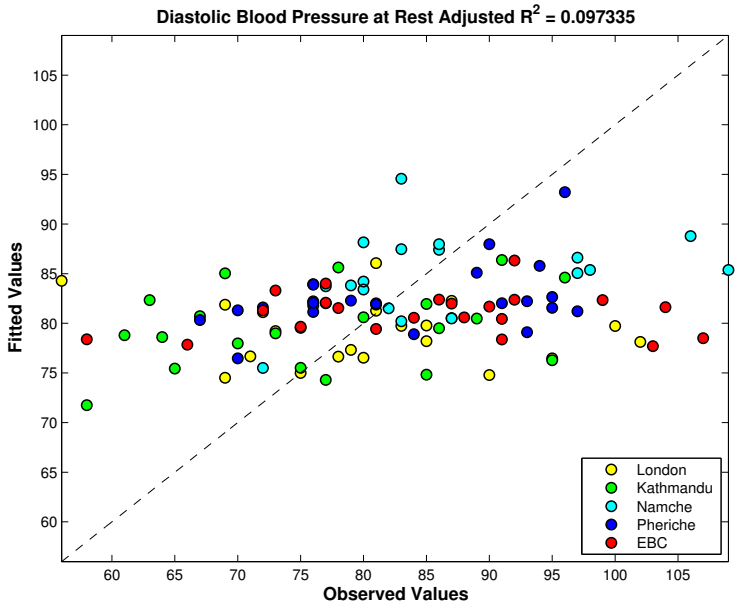


(a) Observed vs. fitted values

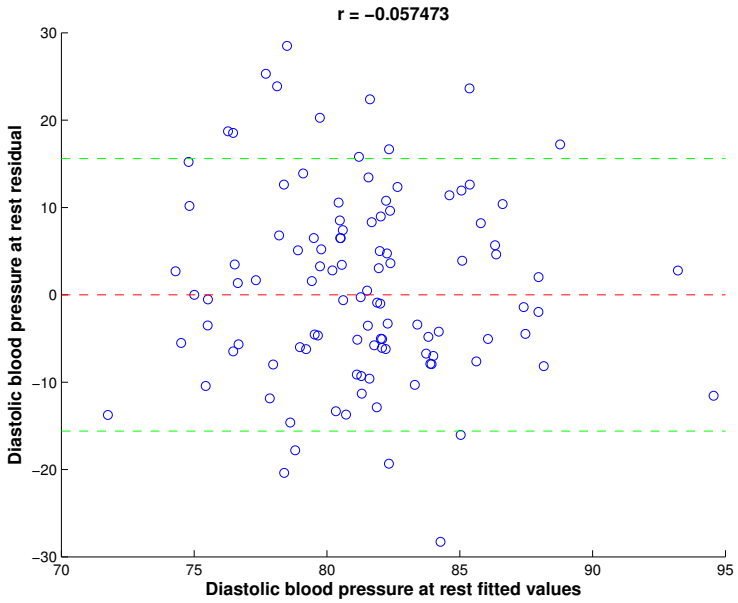


(b) Fitted values vs. residual values

Figure 6.7: Observed vs. fitted values for a multiple linear regression model predicting heart rate at VO_2max, and residuals plotted against fitted values from the model. (a) shows each altitude colour-coded, and shows relatively even scatter around the line, indicating that the model is fitting high and low values with equal probability.



(a) Observed vs. fitted values



(b) Fitted values vs. residual values

Figure 6.8: Observed vs. fitted values for a multiple linear regression model predicting diastolic blood pressure at rest, and residuals plotted against fitted values from the model.

of measurement may make it more difficult to reliably predict a *population response* using MLR.

Figure 6.8 shows the results for the MLR performed for diastolic blood pressure (DBP). Figure 6.8a shows the observed values plotted against the fitted values from the model, and Figure 6.8b shows the residual values plotted against the fitted values from the model. Figure 6.8a shows no relationship between the fitted data and observed data, with an adjusted R^2 value of 0.097. Analysis of DBP in the diary data (shown in Chapter 4) indicated that DBP seemed a good potential dependent variable. DBP showed good day-to-day consistency, shown by a low C_V calculated from a simple linear regression of DBP against altitude. DBP also showed a significant change against altitude and between individuals, tested by a two-way ANOVA for altitude and individual. The reason for the poor prediction from the MLR is unclear, but may be due to the lack of linear relationships being present with any of the explanatory variables, as only two explanatory variables were included in the MLR, but even these do not appear to be predictive.

6.4.3.1 Work rate at VO_2max

Table 6.3 shows that similar biochemical metabolites were used in several of the MLR models formed. These included biochemical metabolites that were highlighted as containing observations with *high leverage*, that may be having a high impact on the resulting relationships within the MLR. The model produced for work rate at VO_2max contained a high number of explanatory variables, including several that were contained in other models and contained observations with high leverage (listed in Table 6.4). Therefore, the model for work rate at VO_2max is detailed here as an example of the refinement process, and to determine whether any of the explanatory variables highlighted may be highly influential on the MLR models formed.

Work rate at VO_2max was identified as a variable of biological interest in Section 5.4.2, as it is a measure of how well the body is using oxygen to do work (i.e. oxygen utilisation vs. consumption). Work rate is known to decrease during exposure to hypoxia, and how much it changes can be used as an indication of how well an individual is adapting to the hypoxic conditions. An individual who shows a low reduction is considered to have adapted to that change better than an individual who has a larger reduction in work rate. This is because a low reduction in work rate indicates that an individual is still able to perform a similar amount of work with less oxygen available.

MLR was performed on work rate at VO_2max using all of the biochemical metabolites listed in Table 5.3 as potential explanatory variables. The initial MLR

produced a model with an adjusted R^2 value of 0.717 - the highest adjusted R^2 value for any initial model formed, as shown in Table 6.3. This model was chosen for refinement due to the high adjusted R^2 value associated with the initial model and the fact that the variable is of biological interest. This model also contained a relatively large number of explanatory variables, and was assessed further to see if a reduction in the number of explanatory variables used would yield similar results to the initial modelling, or whether all of the explanatory variables were required to produce a reasonable model with a high adjusted R^2 value, indicating that it is explaining a good proportion of the variability associated with the dependent variable.

Figures 6.9 and 6.10a show the observed vs. fitted values for the MLR performed between work rate at VO_2max, using all biochemical metabolites listed in Table 5.3 as potential explanatory variables. Figure 6.9 shows a colour-coded version of Figure 6.10a, labelling each altitude in a different colour. The figures show a high adjusted R^2 value of 0.717, indicating that the model is describing 71.7% of the variability seen within the observed values for work rate at VO_2max. Figure 6.9 shows relatively even scatter around the dashed line (which shows where a perfect 1:1 relationship would lie between the fitted and observed values), indicating that the model is fitting high and low values equally well. The model also seems to be fitting values for each altitude equally well, shown by even scatter for each colour above and below the dashed line. Figure 6.10b shows the residual values plotted against the fitted values from the model, and shows reasonable homogeneity of variance (i.e. no underlying structure or pattern to the residual values from the model). This indicates that the assumptions for the model are being met. The top row of Table 6.5 shows the explanatory metabolites included in this model, as well as their associated coefficients and significance levels for use in the model.

Figures 6.11 and 6.12 show the residual values for work rate at VO_2max plotted against each of the explanatory variables included in the model. The figures shown in Figure 6.11 show explanatory variables that were deemed to be suitable for use within the model without further assessment. They showed no discernible patterns or heteroscedasticity. This indicates that these explanatory variables show true relationships with the dependent variable, and are suitable for use within the model.

There were some explanatory variables within Figure 6.11 that showed potential outlying observations, however, they were not deemed to need further assessment, as they were not considered to be observations with high leverage. The outlying value seen for MIF (shown in Figure 6.11h) is also associated with a large

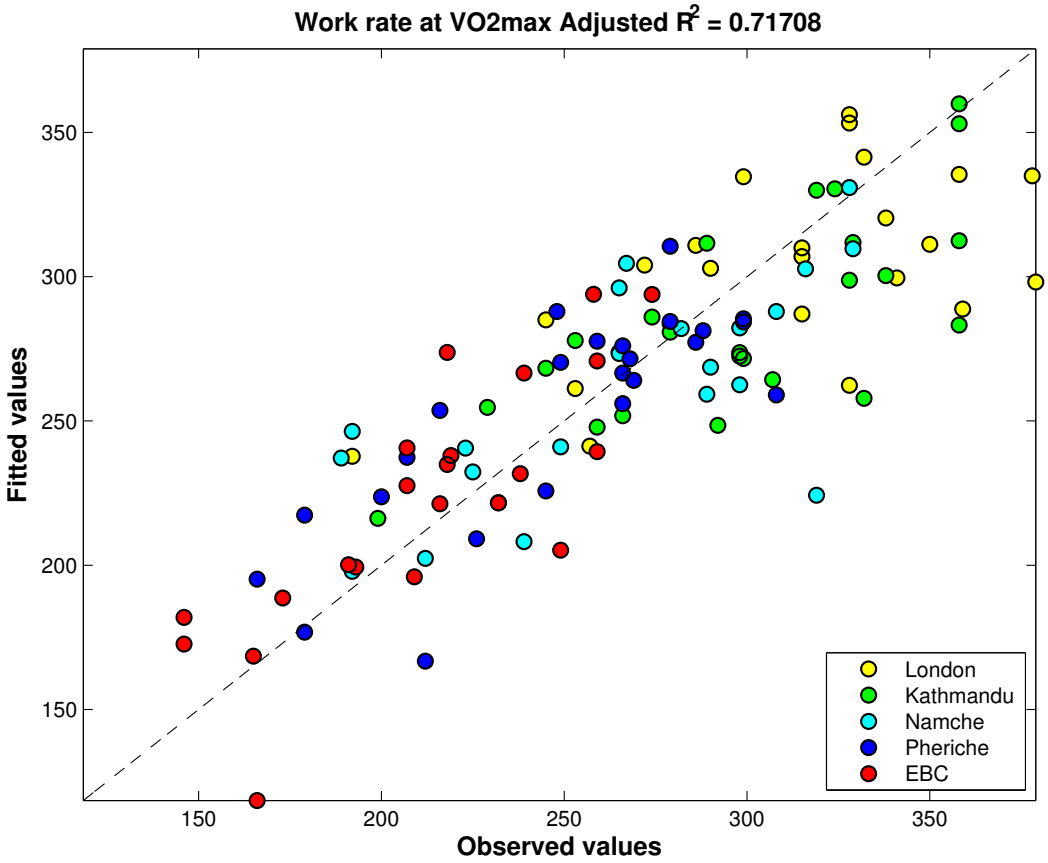
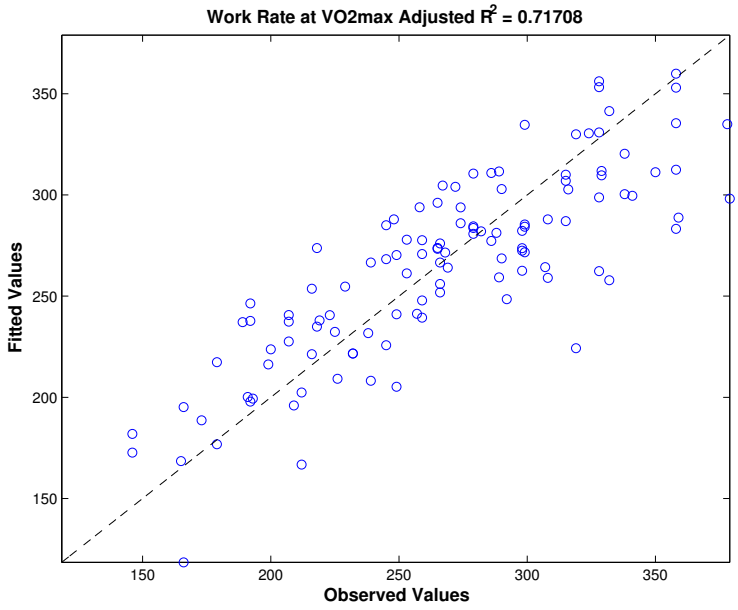
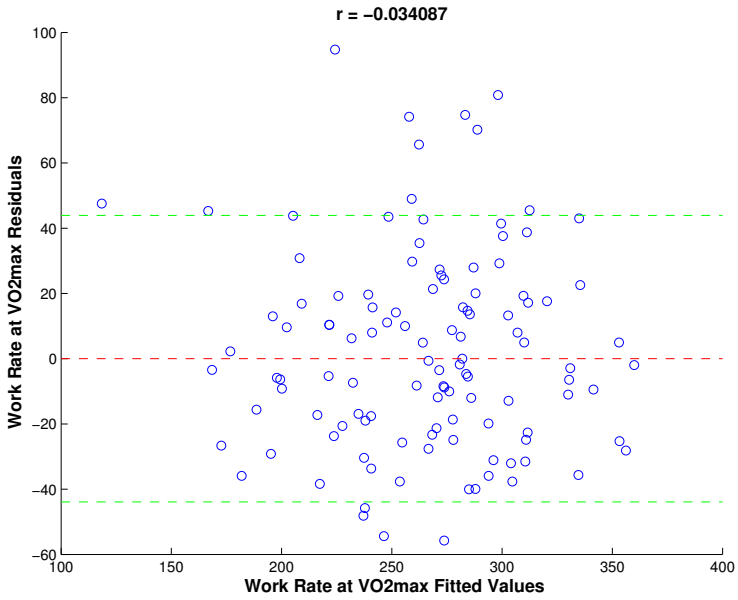


Figure 6.9: Observed vs. fitted values for a multiple linear regression model predicting work rate at VO_2max, and residuals plotted against fitted values from the model. The figure is colour-coded by altitude, with London values in yellow, Kathmandu values in green, Namche values in cyan, Pheriche values in blue and Everest Base Camp values in red. The figure shows relatively even scatter around the line, indicating the model is fitting high and low responses equally well.

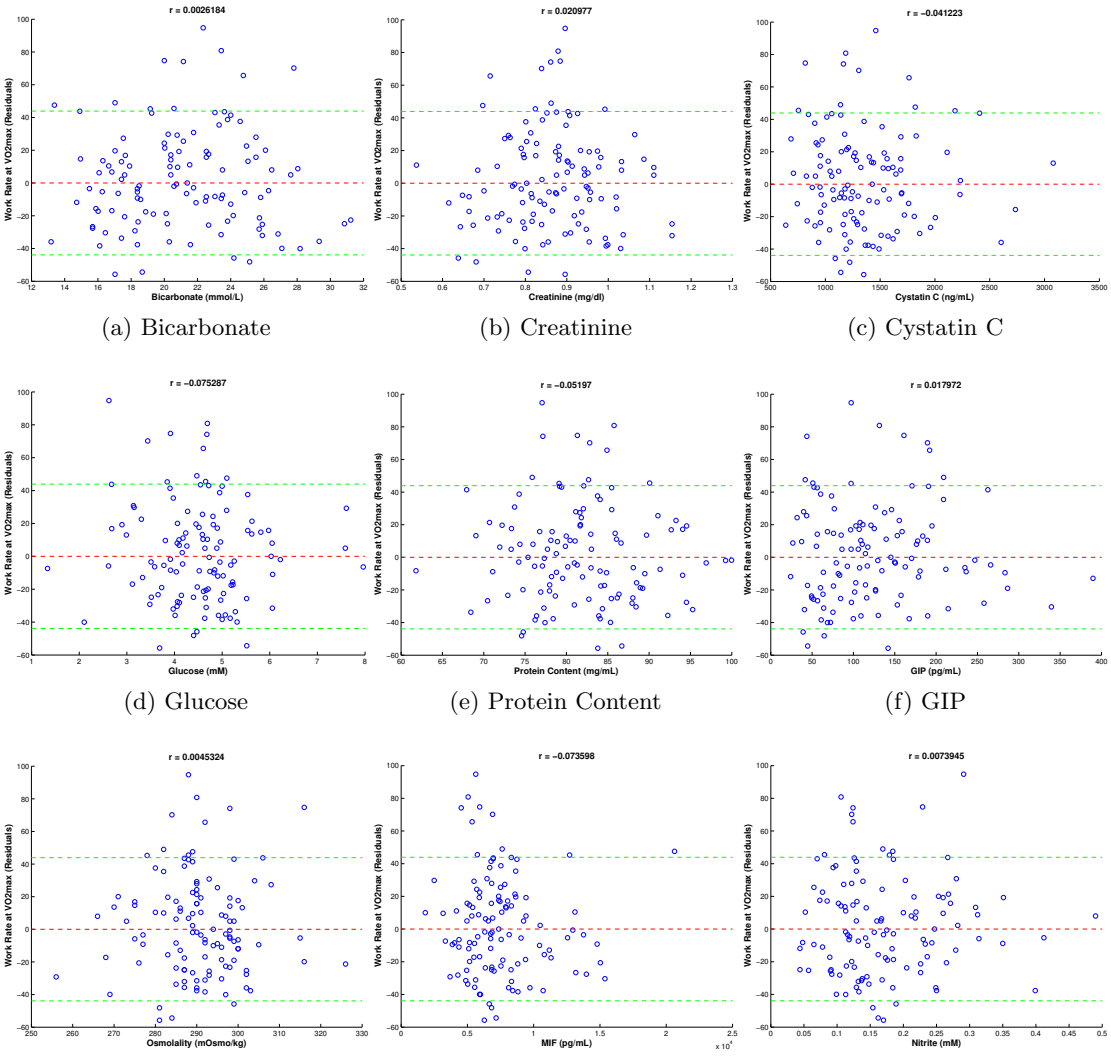


(a) Observed vs. fitted values



(b) Fitted values vs. residual values

Figure 6.10: Observed vs. fitted values for a multiple linear regression model predicting work rate at VO_2max, and residuals plotted against fitted values from the model.



(a) Bicarbonate (b) Creatinine (c) Cystatin C

(d) Glucose (e) Protein Content (f) GIP

(g) Osmolality (h) MIF (i) Nitrite

Figure 6.11: Figure 1 of 2 showing residuals for work rate at VO_2max plotted against each explanatory variable used in a multiple linear regression. None of these residuals show any discernible pattern, or require further assessment.

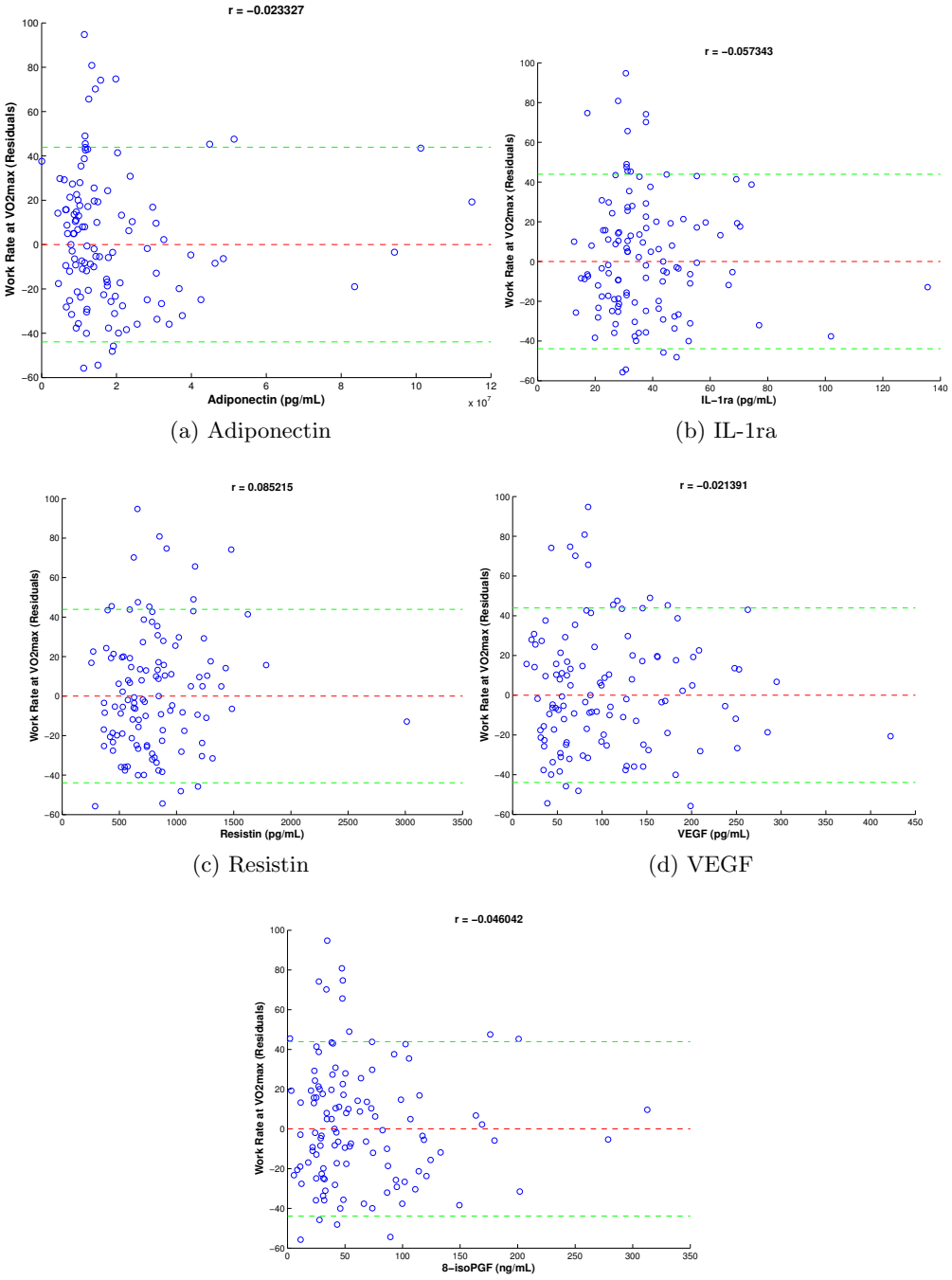


Figure 6.12: Figure 2 of 2 showing residuals for work rate at VO_2max plotted against each explanatory variable used in a multiple linear regression. None of these residuals show any heteroscedasticity, however, some potentially outlying values were cause for concern due to their correlation with low value residuals. This indicates that these values may be having a biasing effect on the regression between the dependent variable and the explanatory variable in question. Therefore, all of these values were assessed further to ascertain their impact on the model formed.

Explanatory variable	Individual	Altitude
Adiponectin	X24	Kathmandu
	X24	Pheriche
	X21	EBC week 1
	X24	EBC week 1
IL-1ra	X02	London
Resistin	X02	London
VEGF	X16	EBC week 1
8-isoPGF$_{2\alpha}$	X21	Namche
	X08	EBC week 1

Table 6.4: A summary of observations considered to have *high leverage* in MLR.

residual value, indicating that this point is not having a large effect on the model, and is badly predicted by the model overall. Low residual may also be *balanced out* by other values. For example, Figure 6.11i shows low residual values for nitrite that are both above and below the zero residual line in red, balancing each other out.

Figure 6.12 shows plots of explanatory variables that require further investigation. Although these figures all show a good homoscedastic spread of points, there are individual points that require further assessment, as they have the potential to be observations with high leverage. These explanatory variables were also used in several of the other models shown in Table 6.3, therefore, it is important to determine whether these points are suitable for use in further modelling efforts.

All of the explanatory variables shown in Figure 6.12 have observations with high leverage values associated with low residual values from the model, indicating that they may be having a strong influence on the resulting regression between the dependent variable and that particular explanatory variable. Each of these points is listed in Table 6.4. Three of the high leverage adiponectin values are associated with individual X24, indicating that this individual may have unusually high values for this particular metabolite. The other observations with high leverage are distributed well between individuals and over different altitudes, indicating that it is not one individual or altitude that is associated with these potentially highly influential observations.

6.4.3.2 Assessment of potential outliers

It is difficult to ascertain whether the outlying values measured are truly erroneous, or a *real* response to the extreme conditions experienced during CXE 2007. The current literature on these biochemical metabolites in human samples is very poor, and mostly consists of clinical situations at sea level. The literature does not contain

information about these metabolites measured at different altitudes, and many of the metabolites are measured via a variety of different techniques, making comparisons to our values very difficult.

To assess the impact of the potential outliers shown in Figure 6.12, the observations with high leverage were removed one at a time, and the MLR was refit. Four values were removed for adiponectin, two values were removed for 8-isoPGF$_{2\alpha}$ and one value was removed for each of IL-1ra, resistin and VEGF, as detailed in Table 6.4. MLR was run separately on each of the five new datasets formed (with observations with high leverage omitted). The new models formed could not be formally compared to one another or to the initial model, as they were all based on sightly different data sets. However, the changes seen in adjusted R^2 and residual mean square values could still be assessed to see which model explained most of the variability seen in the dependent variable. Differences in the explanatory variables included and their coefficient values could also be assessed to determine the effects of removing the potentially erroneous data points.

Table 6.5 shows information for the initial and refined models formed using MLR on work rate for VO_2max. This table shows the adjusted R^2 and residual mean square values for each of the models, as well as the intercept, explanatory variables included in each model and their coefficients and significance levels for inclusion in the model, as described in Section 2.5.11. The coefficient values provided for explanatory variables not included in a particular model are the values they *would* have had if they had been included. If these variables had been included, then it is likely that the coefficient values for the other explanatory variables would change.

The initial model gave the highest adjusted R^2 value for all of the models shown in Table 6.5. This is to be expected, as it resulted from a MLR using all of the data available for analysis. Therefore, the initial model explains more of the variability seen within the observed data than any of the refined models, where observations with high leverage have been removed. The initial model also has the lowest value for the residual mean square compared to any of the refined models, which is a measure of the difference between the observed data and the model, with a small residual mean square value indicating a good fit of the model to the data. This indicates that the initial model is statistically the *best* model for the observed data available. Observations with high leverage were removed to assess how much of an impact they had on the regression, and their effects are described below.

6.4.3.3 Adiponectin

Adiponectin is a protein that is produced and secreted by adipocytes, and regulates the metabolism of lipids and glucose [Nedvídková et al., 2005]. It is known to play roles in the body's response to insulin and the inflammatory response. Low levels of adiponectin are associated with obesity and increased risks of heart attack [Ukkola and Santaniemi, 2002; Kizer et al., 2008], although this is not currently fully understood. Adiponectin is very abundant in human plasma compared to other hormones, and shows sexual dimorphism, with females showing higher levels than males.

Adiponectin levels were measured via xMAP technology, as described in Section 2.2.4. A *diabetes panel assay* was used to measure adiponectin levels, as well as several other metabolites thought to play a role in type II diabetes, listed in Table 2.2. The assay included information about cytokine levels that had been validated in real patient samples. The samples validated by the assay manufacturer (BioPlex) were taken from 10 healthy control patients and 10 patients suffering from type II diabetes. However, the validated levels were measured in *serum*, which has had clotting factors such as fibrin removed, and may result in slightly different measurements when compared to the CXE plasma samples. It is important to note that these *validated* samples were measured by BioPlex and were only measured in a very small sample size. The BioPlex validated samples and CXE samples gave results in the range of:

- BioPlex healthy validated samples: mainly ∼8,000,000 to ∼40,000,000 pg/mL, with outliers up to ∼100,000,000 pg/mL
- BioPlex type II diabetes validated samples: mainly ∼10,000,000 to ∼60,000,000 pg/mL, with outliers up to ∼90,000,000 pg/mL;
- CXE samples: mainly ∼5,000,000 to ∼50,000,000 pg/mL, with outliers down to ∼30,000 and up to ∼110,000,000 pg/mL.

This indicates that the values obtained from measurement of the CXE samples seem reasonable when compared to the BioPlex validated samples. The CXE samples have a larger range than the BioPlex validated samples, however, the CXE samples were taken from individuals exposed to an extreme environment. No information is given on the sex of the individuals for the BioPlex validated samples, which would impact the range of results obtained. Finally, serum was measured for the BioPlex validated samples compared to plasma for the CXE samples, which may give *cleaner* results with a lower variability compared to plasma samples.

The MLR analysis was run with the four observations with high leverage removed, and the results for this analysis are shown in Table 6.5. This refined model has a lower adjusted R^2 value than the initial model of 0.697 compared to 0.717, indicating that it is describing 2% less of the variability seen in the observed data than the initial model. The intercept value for the model decreases from -273.27 to -317.30 compared to the initial model, and the ResMS increases from 827.34 for the initial model to 880.22 for the refined model. This indicates that there is a larger discrepancy between the model and the observed data, compared to the initial model.

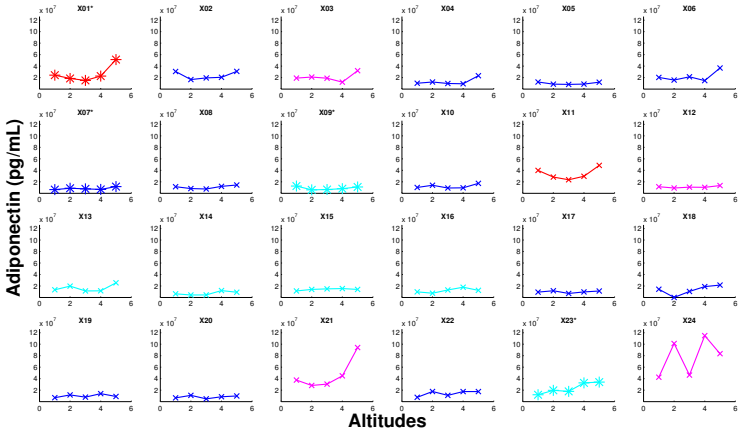
Adiponectin is still included as an explanatory variable in the refined model, although its coefficient value increases by 57.14% compared to the initial model value. The refined model no longer includes nitrite or resistin as explanatory variables in the model, with their p-values dropping to 0.074 and 0.065, respectively. Figure 6.13 shows individual responses for adiponectin, nitrite and resistin. Table 6.4 shows that the observations with high leverage removed for adiponectin were for individual X24 (Kathmandu, Pheriche and Everest Base Camp week 1) and X21 (Everest Base Camp week 1), and are also shown in Figure 6.13a. However, the corresponding data points for nitrite (shown in Figure 6.13b) and resistin (shown in Figure 6.13c) are not outlying points. Therefore, it is not clear why the removal of these points leads to the removal of nitrite and resistin as explanatory variables.

Several explanatory variable coefficient values change in the refined model, with the largest differences seen in 8-isoPGF$_{2\alpha}$, GIP and protein content, which change by $\uparrow$28.04%, $\downarrow$61.54% and $\uparrow$47.5%, respectively. These results indicate that the removal of the four outlying points has an effect on the explanatory variables included in the model, as well as their coefficient values. The refined model explained less of the variability seen in the observed data, and is a slightly worse fit to the data compared to the initial model.

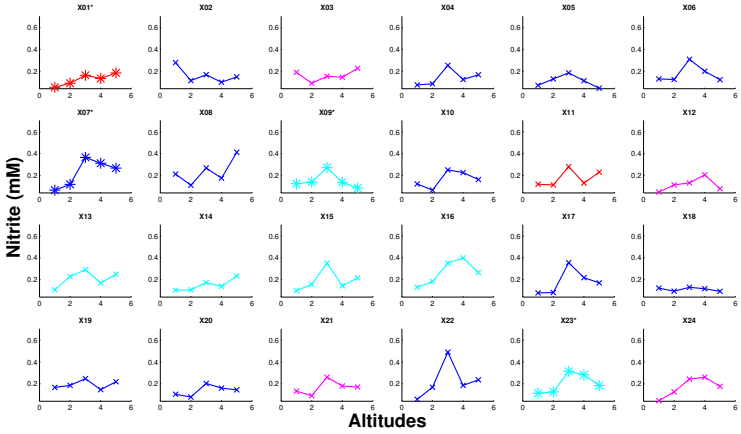
Adiponectin levels are relatively straightforward and quick to measure in a high-throughput manner along with other cytokines due to the multiplexing abilities of the xMAP technology. Combined with the biological information suggesting that similar values have been measured in human samples before, the four outlying values should not be removed, as they are likely to be reliable values for adiponectin levels in human plasma.

6.4.3.4 IL-1ra

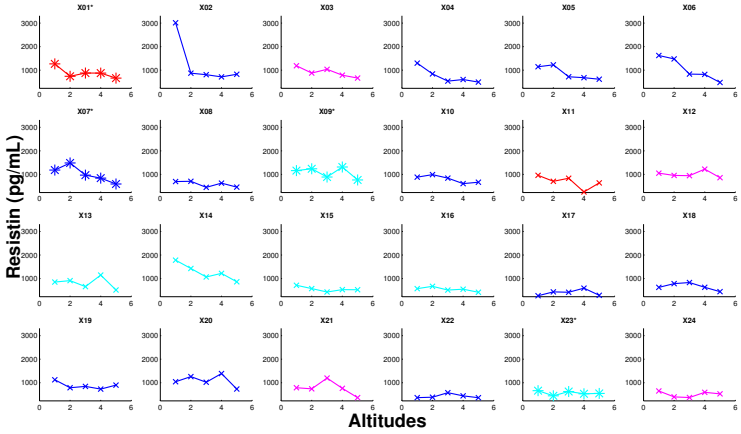
Interleukin-1 (IL-1) is a pro-inflammatory cytokine, which binds to the IL-1 receptor to promote inflammation and aid in the defence against infection [Arend et al., 1998].



(a) Adiponectin



(b) Nitrite



(c) Resistin

Figure 6.13: Subplots of all 24 individual responses during CXE 2007 for: (a) adiponectin, (b) nitrite and (c) resistin.

Interleukin 1 receptor antagonist (IL-1ra) binds to the interleukin-1 receptor in a competitive manner with IL-1, acting as an *anti-inflammatory cytokine*. The levels of IL-1ra were measured via xMAP technology, as described in Section 2.2.4

MLR was run with the highest outlying value for IL-1ra removed, and the results are shown in Table 6.5. This refined model showed a lower adjusted R^2 value and higher ResMS value compared to the initial model, of 0.683 and 933.97, respectively. There are also several differences in the explanatory variables included in the model, as well as their coefficients. 8-isoPGF$_{2\alpha}$, VEGF, GIP and resistin are no longer included in the model, and IL-13 and C-Peptide are new explanatory variables included in the refined model, but not in the initial model.

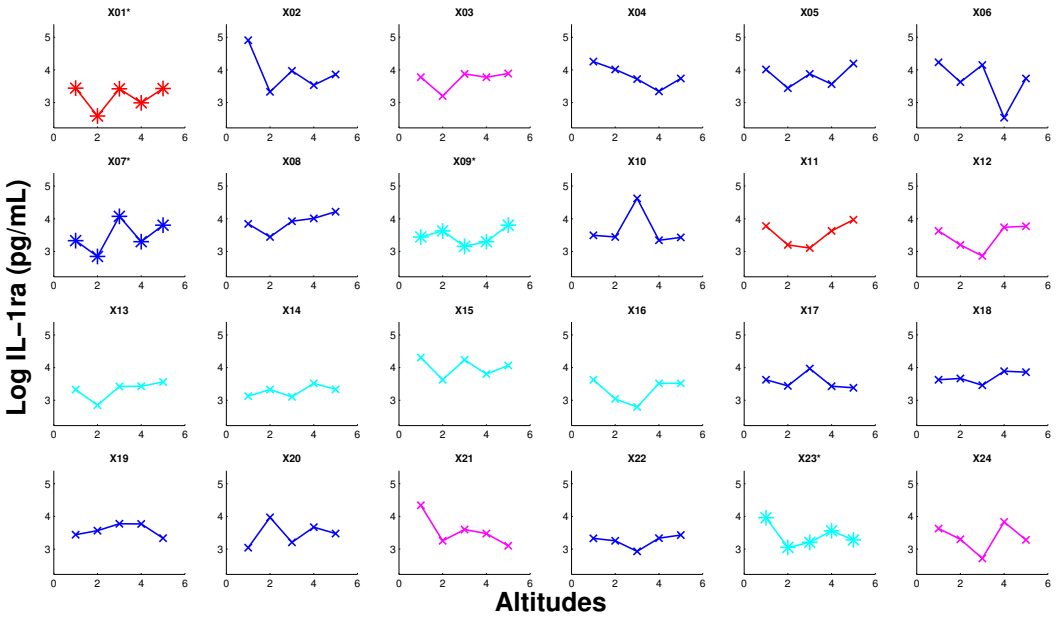
Table 6.4 shows that the observation with high leverage removed for IL-1ra was for individual X02 at London, and is shown to be a potential outlier in Figure 6.14a. This datapoint is also shown to be a potentially outlying value for GIP (shown in Figure 6.14b), resistin (shown in Figure 6.13c) and VEGF (shown in Figure 6.16a). This indicates that this datapoint may also be an observation with high leverage for the regressions between those explanatory variables and the dependent variable, and the removal of the point results in those explanatory variables being removed from the MLR, as the relationship with the dependent variable is no longer strong enough. The datapoint is not an outlying value for 8-isoPGF$_{2\alpha}$ (shown in Figure 6.15a), and it is unclear why the removal of this point would result in 8-isoPGF$_{2\alpha}$ no longer being included in the model.

6.4.3.5 Resistin

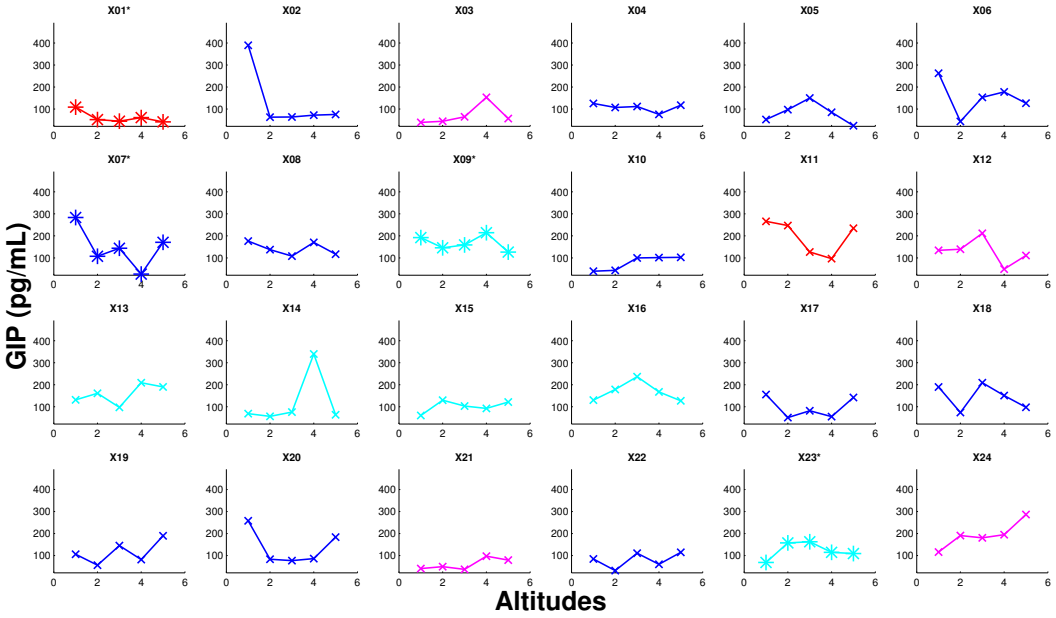
Resistin is secreted by immune and epithelial cells in humans. Resistin is thought to be the link between obesity and type II diabetes [Steppan et al., 2001], however, how resistin may link these two processes is currently unknown. Resistin levels were measured via a BioPlex system as part of the *diabetes panel*, and as such provided information on validated patient samples in a similar way to adiponectin. The validated and CXE samples gave results in the range of:

- Healthy validated samples: ∼2000 to ∼7500 pg/mL;
- Type II diabetes validated samples: ∼2000 to ∼7500 pg/mL, with an outlier at ∼14000 pg/mL;
- CXE samples: ∼250 to ∼1500 pg/mL, with an outlier at ∼3000 pg/mL.

The samples measured from CXE gave values that were much lower than the validated samples. Therefore, the outlying value measured in the CXE samples



(a) IL-1ra



(b) GIP

Figure 6.14: Subplots of all 24 individual responses during CXE 2007 for: (a) IL-1ra and (b) GIP.

may be an extreme value for this dataset, but a reasonable measured value when compared to the validated samples. Resistin levels are known to be higher in obese individuals [Steppan et al., 2001]. High levels of fitness were required to take part in CXE 2007, and this may account for the relatively low resistin values in CXE participants compared to the validated samples.

MLR was run without the highest outlying value for resistin, which was the same datapoint removed for IL-1ra, (individual X02 at London) as shown in Table 6.4. As removing one value removes the corresponding point from all other metabolites, the same refined model was produced for IL-1ra and resistin.

6.4.3.6 8-isoPGF$_{2\alpha}$

Isoprostanes are a class of prostanoids that are formed by the attack of arachidonic acid by free radicals during oxidative stress, and can be used as a marker of oxidative stress, as described in Section 3.1. MLR was run with the two highest outlying values for 8-isoPGF$_{2\alpha}$ removed, and the results are shown in Table 6.5. This refined model had the lowest adjusted R^2 and the highest ResMS value for all of the models formed, of 0.629 and 1087.69, respectively. This indicates that this refined model is the worst fitting of any formed for this dependent variable, and that the two points removed are important in the relationship between 8-isoPGF$_{2\alpha}$ and work rate at VO_2max, as well as in other relationships within the MLR.

The explanatory variables included in the model were different to those included in the initial model. IL-1ra, MIF, VEGF, resistin, osmolality and protein content were no longer included in the model, and IL-13 was included as a new explanatory variable. Table 6.4 and Figure 6.15a show the observations with high leverage for 8-isoPGF$_{2\alpha}$ were for individual X21 at Namche, and individual X08 at Everest Base Camp week 1. The first data point (for individual X21) shows a low value for MIF (shown in Figure 6.16b), and a relatively high value for resistin (shown in Figure 6.13c). The second data point (for individual X08) shows a high value for C-peptide at Everest Base Camp week 1, shown in Figure 6.16c. These points may also be observations with high leverage for these explanatory variables, indicating why they are removed from the MLR if those two points are removed. Neither of the observations with high leverage were outlying values for IL-1ra (shown in Figure 6.14a), VEGF (shown in Figure 6.16a), osmolality (shown in Figure 6.15b) or protein content (shown in Figure 6.15c), and the reason for the removal of these explanatory variables from the MLR is less clear.

The coefficient values for explanatory variables were also different from the initial model, with the largest differences seen for nitrite (↑52.96%) and 8-isoPGF$_{2\alpha}$

(↑74.77%). The intercept value changed from -273.27 for the initial model to 84.03 for the refined model, indicating that the removal of these two points is having a large effect on every aspect of the model formed, as is vital for several relationships within the MLR.

6.4.3.7 VEGF

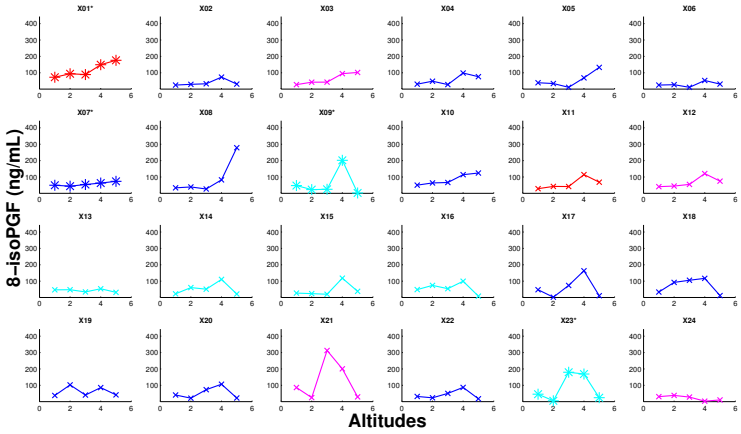
Vascular endothelial growth factor (VEGF) is a signalling protein that is produced by cells to stimulate angiogenesis and vasculogenesis. This hormone is released to increase the oxygen supply to tissues when the circulation of oxygen is not adequate, as previously mentioned in Section 1.4.1.

MLR was run with the highest outlying value for VEGF removed, and the results are shown in Table 6.5. The refined model has a lower adjusted R^2 value and a higher ResMS value compared to the initial model of 0.631 and 1076.70, respectively. This model includes the same explanatory variables as the 8-isoPGF$_{2\alpha}$ model, with similar coefficient values for the explanatory variables used. However, the value removed was not the same as either value removed for 8-isoPGF$_{2\alpha}$. The intercept is also similar to that for the 8-isoPGF$_{2\alpha}$ model, at 76.69. This indicates that the removal of this datapoint is having a similar effect on the MLR as the removal of the 8-isoPGF$_{2\alpha}$ points.

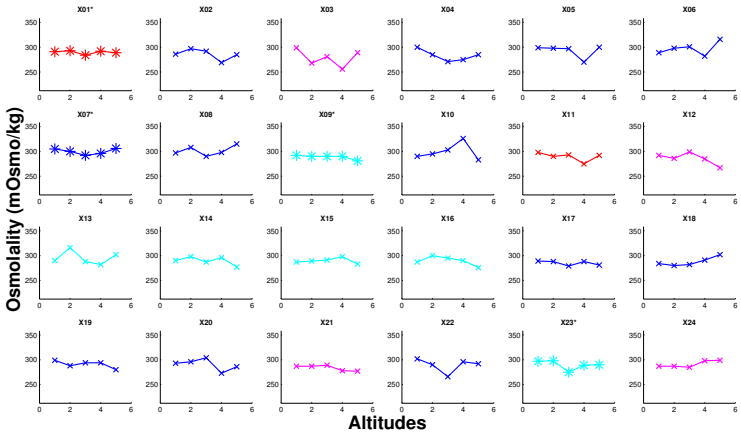
The observation with high leverage for VEGF is shown in Figure 6.16a and Table 6.4 to be for individual X16 at Everest Base Camp week 1. This particular datapoint is also shown to be an outlier for MIF (shown in Figure 6.16b), and may explain why this explanatory variable is removed from the MLR if the datapoint is removed. However, this datapoint does not seem to be an outlying value for IL-1ra (shown in Figure 6.14a), C-Peptide (shown in Figure 6.16c), resistin (shown in Figure 6.13c), osmolality (shown in Figure 6.15b) or protein content (shown in Figure 6.15c), and the reason for the removal of these particular explanatory variables from the MLR is less clear.

6.4.4 Summary

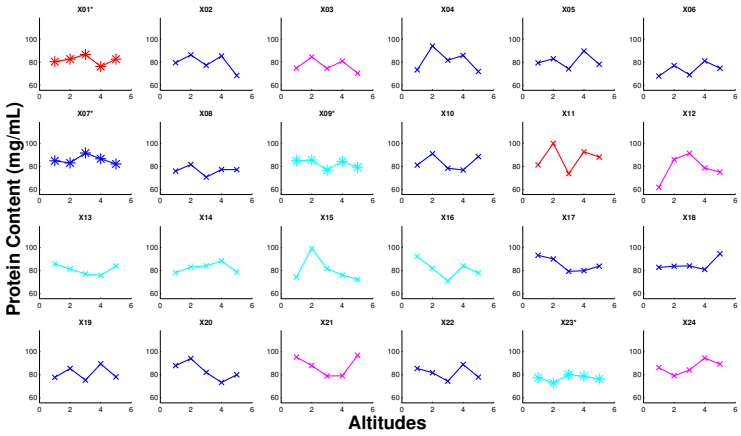
The models formed in this sectionshow that several of the explanatory variables used within the models contain observations with high leverage, which may affect the relationships in the MLR. Removal of any of these points leads to a *worse* model being formed, which explains less of the variability associated with the dependent variable compared to the initial model formed. As previously stated, the literature regarding the measurement of these biochemical metabolites in samples similar to



(a) 8-isoPGF$_{2\alpha}$

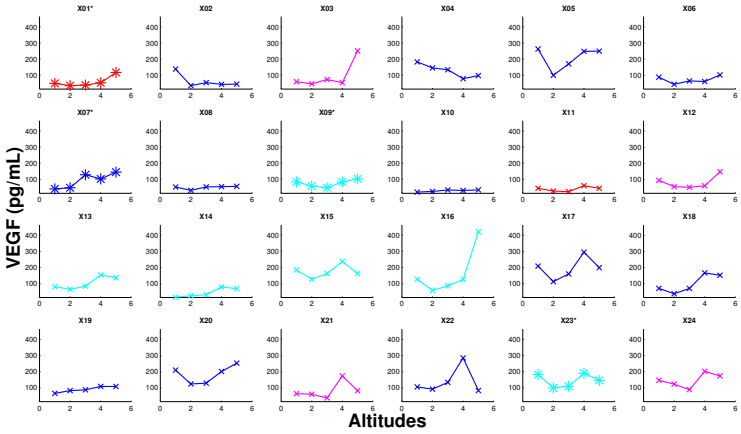


(b) Osmolality

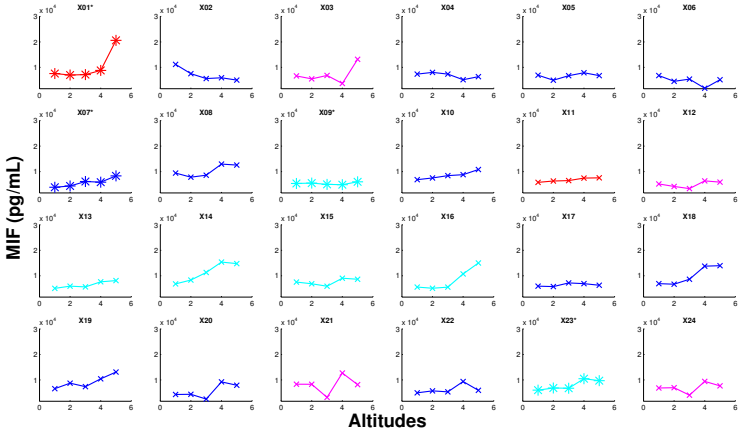


(c) Protein Content

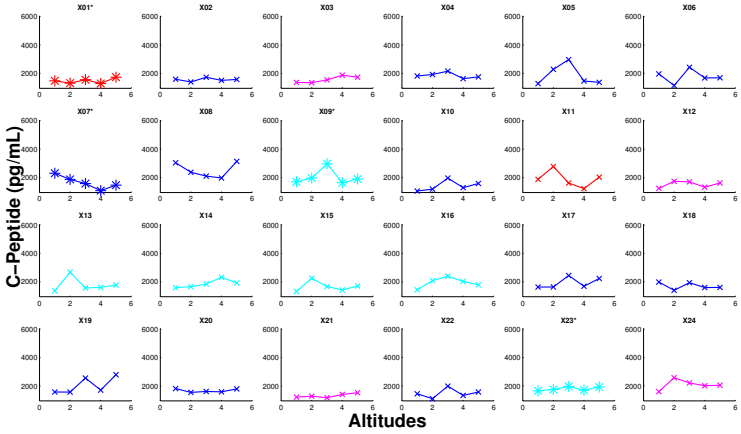
Figure 6.15: Subplots of all 24 individual responses during CXE 2007 for: (a) 8-isoPGF$_{2\alpha}$, (b) osmolality and (c) protein content.



(a) VEGF



(b) MIF



(c) C-Peptide

Figure 6.16: Subplots of all 24 individual responses during CXE 2007 for: (a) VEGF, (b) MIF and (c) C-Peptide.

the CXE samples is very poor, with most measurements involving clinical patients at sea-level. The range of techniques used to measure these different metabolites also makes the comparison with other studies problematic. Therefore, for the purposes of further modelling, it was assumed that the results obtained for each of the explanatory variables are showing *true responses* to the extreme conditions the individuals are exposed to. Measurement of the trekker samples will further verify whether this assumption is reasonable or not.

6.5 Conclusions

- The NO metabolites alone are not enough to describe the variability seen within any of the dependent variables. This may be due to there not being enough variability within the NO metabolite values to explain the physiological responses, or NO metabolite levels being kept highly regulated independently of oxygen availability;
- Linear combinations selected from all of the biochemical metabolites can be used to produce a model capable of predicting some of the physiological measurements;
- Some of the physiological measurements of interest are poorly predictable, even when all available biochemical metabolites are used as explanatory variables. This may be due to high inter-individual variability for some of the physiological measurements. The reasons for the poor predictability for some of the physiological measurements remain unclear;
- MLR on work rate at VO_2max produces the model with the highest adjusted R^2 value, explaining 71.7% of the variability seen within the observed data. This makes it a good potential dependent variable in the next stage of targeted modelling;
- The initial models formed for each dependent variable describe the largest proportion of the variability seen in the dependent variable, which is as expected;
- Any refinement of the initial models resulted in a lower adjusted R^2, a higher residual mean square value and different explanatory values and coefficients being included in the model, which is as expected. Any removal of observations lead to the removal of the corresponding observations in all other explanatory variables for that model. Therefore, if that observation was also important for the strength of the regression seen between the dependent variable and

Model		Nitrite	8-iso PGF	IL-1ra	IL-13	MIF	VEGF	C-Peptide	GIP	Resistin	Adiponectin	Glucose	Cystatin C	Creatinine	Osmolality	Protein Content	Bicarbonate
Initial																	
$\bar{R}^2$=0.717	Coef	-97.81	-0.107	0.687	0.12	-0.003	0.152	0.0073	0.168	-0.0232	-0.0000007	15.64	-0.0423	117.3	0.918	1.2	3.506
Intercept= -273.27	p-value	0.0169	0.068	0.0007	0.9806	0.01	0.0007	0.451	0.0008	0.016	0.00006	0.000002	0.0000005	0.00002	0.0021	0.0086	0.0001
ResMS= 827.34	In model?	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1
Adiponectin	Coef	-73.48	-0.137	0.584	-0.77	-0.0029	0.163	0.003	0.104	-0.0177	-0.0000011	13.33	-0.0393	101.95	0.942	1.77	3.147
$\bar{R}^2$=0.697	Change	↓33.11%	↑28.04%	↓17.64%	-116%	↓3.45%	↑7.24%	↓143.33%	↓61.54%	↓31.07%	↑57.14%	↓17.33%	↓7.63%	↓15.06%	↑2.61%	↑47.50%	↓11.41%
Intercept= -317.30	p-value	0.0744	0.023	0.0032	0.8826	0.016	0.0005	0.736	0.0338	0.065	0.00134	0.000065	0.0000058	0.0002	0.0027	0.0001	0.00067
ResMS= 880.22	In model?	0	1	1	0	1	1	0	1	0	1	1	1	1	1	1	1
IL-1ra	Coef	-127.44	-0.106	0.664	12.2	-0.0035	0.138	0.0245	0.113	-0.0179	-0.0000005	10.34	-0.0421	101.92	0.665	1.37	3.899
$\bar{R}^2$=0.683	Change	↑30.29%	↓0.94%	↓3.46%	↑10067%	↑16.67%	↓10.14%	↑235.62%	↓48.67%	↓29.61%	↓40.00%	↓51.26%	↓0.48%	↓15.09%	↓38.05%	↑14.17%	↑11.21%
Intercept= -233.91	p-value	0.0048	0.094	0.0065	0.0002	0.004	0.0605	0.002	0.1235	0.1501	0.002599	0.001799	0.00000214	0.00031	0.0355	0.0044	0.000031
ResMS= 933.97	In model?	1	0	1	1	1	0	1	0	0	1	1	1	1	1	1	1
Resistin	Coef	-127.44	-0.106	0.664	12.2	-0.0035	0.138	0.0245	0.113	-0.0179	-0.0000005	10.34	-0.0421	101.92	0.665	1.37	3.899
$\bar{R}^2$=0.683	Change	↑30.29%	↓0.94%	↓3.46%	↑10067%	↑16.67%	↓10.14%	↑235.62%	↓48.67%	↓29.61%	↓40.00%	↓51.26%	↓0.48%	↓15.09%	↓38.05%	↑14.17%	↑11.21%
Intercept= -233.91	p-value	0.0048	0.094	0.0065	0.0002	0.004	0.0605	0.002	0.1235	0.1501	0.002599	0.001799	0.00000214	0.00031	0.0355	0.0044	0.000031
ResMS= 933.97	In model?	1	0	1	1	1	0	1	0	0	1	1	1	1	1	1	1
VEGF	Coef	-119.57	-0.132	0.371	14.12	-0.0016	0.128	0.0106	0.148	-0.0141	-0.0000006	13.2	-0.0486	117.76	0.665	0.97	4.152
$\bar{R}^2$=0.631	Change	↑22.25%	↑23.36%	↓85.18%	↑11667%	↓87.50%	↓18.75%	↑45.21%	↓13.51%	↓64.54%	↓16.67%	↓18.48%	↑14.89%	↑0.39%	↓38.05%	↓23.71%	↑18.43%
Intercept= 76.69	p-value	0.0056	0.045	0.063	0.0001	0.217	0.0977	0.296	0.0053	0.158	0.00256	0.000183	0.0000004	0.00008	0.0501	0.0527	0.00002
ResMS= 1076.70	In model?	1	1	0	1	0	0	0	1	0	1	1	1	1	0	0	1
8-isoPGF$_{2\alpha}$	Coef	-149.61	-0.187	0.347	12.15	-0.0019	0.104	0.0059	0.154	-0.0138	-0.0000006	12.32	-0.0495	128.53	0.648	0.93	4.099
$\bar{R}^2$=0.629	Change	↑52.96%	↑74.77%	↓97.98%	↑10025%	↓57.89%	↓46.15%	↓23.73%	↓9.09%	↓68.12%	↓16.67%	↓26.95%	↑17.02%	↑9.57%	↓41.67%	↓29.03%	↑16.91%
Intercept= 84.03	p-value	0.0009	0.018	0.0902	0.0003	0.151	0.178	0.592	0.0039	0.177	0.00254	0.000503	0.0000003	0	0.0621	0.0634	0.00003
ResMS= 1087.69	In model?	1	1	0	1	0	0	0	1	0	1	1	1	1	0	0	1

Table 6.5: Refinement results for a MLR model for **work rate at VO_2max**, showing the effects of removing individual points from the explanatory variables available. The $\bar{R}^2$ value is the adjusted R^2 value for that particular model, *Coef* is the coefficient value for a particular explanatory variable (values provided for explanatory variables not included in a particular model are the coefficient values they *would* have had if they had been included), the p-value is used to determine which variables are included in the model, and *In model?* shows the explanatory variables included in each model.

another explanatory variable, then it may have an effect here also, reducing the strength of the model further compared to the initial model;

- The removal of observations with high leverage was performed to assess the extent of the changes that would occur with the removal of specific observations with high leverage;
- The decision to remove a data point or explanatory variable depends on the *reliability* of the values obtained for that variable. This is difficult to determine, due to a lack of information in current literature surrounding values obtained from human plasma, especially during exposure to extreme environments. Therefore, the assumption that the dataset is reliable will be used for the next stage of modelling.

6.6 Relevance to modelling approaches in critically ill patients

The results from this analysis were used as a basis for the next stage of *targeted* modelling, specifically aimed for application to a critical illness environment. The next stage of modelling focusses on dependent variables highlighted in the initial modelling stages as *predictable* with a combination of the biochemical metabolites available. This stage will also look at predicting a *change* in a dependent variable, as a predictor for how well an individual will adapt to hypoxic exposure. Explanatory variables used in this stage of analysis are expected to be used as part of the MLR in the next stage of modelling.

Chapter 7

Model Development

This chapter describes the final stage of modelling, involving the targeted MLR of the change seen in six specific physiological measurements. This chapter describes the steps undertaken during the modelling process, comprising technique, refinement and model selection.

7.1 Aims

The main aims of the final stage of modelling were to:

- Build on the exploratory modelling undertaken in the previous chapter, targeting the use of MLR on the % change seen in specific dependent variables (oxygen consumption and work rate);
- Identify which, if any, of the oxygen consumption or work rate measurements can be predicted using absolute biochemical metabolite values at one low altitude;
- Identify which, if any, of the oxygen consumption or work rate measurements can be predicted using a % change in biochemical metabolite values between two low altitudes;
- Refine the four initial models produced for one dependent variable (one for each altitude difference) to produce a *generalised* model, capable of predicting the % change seen in a dependent variable over all altitude differences;
- Produce a list of biochemical metabolites present in several models formed, for measurement in the trekker samples for model validation, and for the future XE2 study;

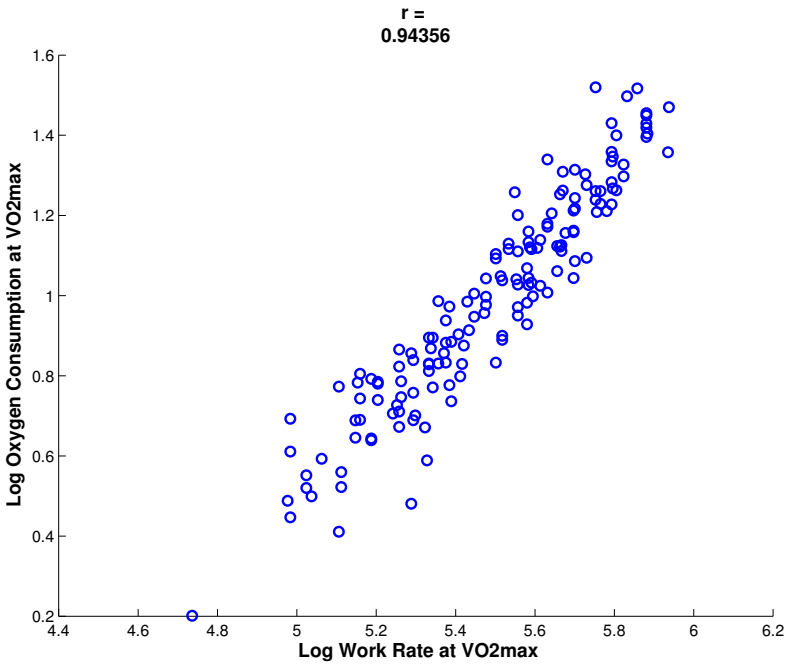
- Understand the uses and limitations of any models formed.

7.2 Targeted multiple linear regression

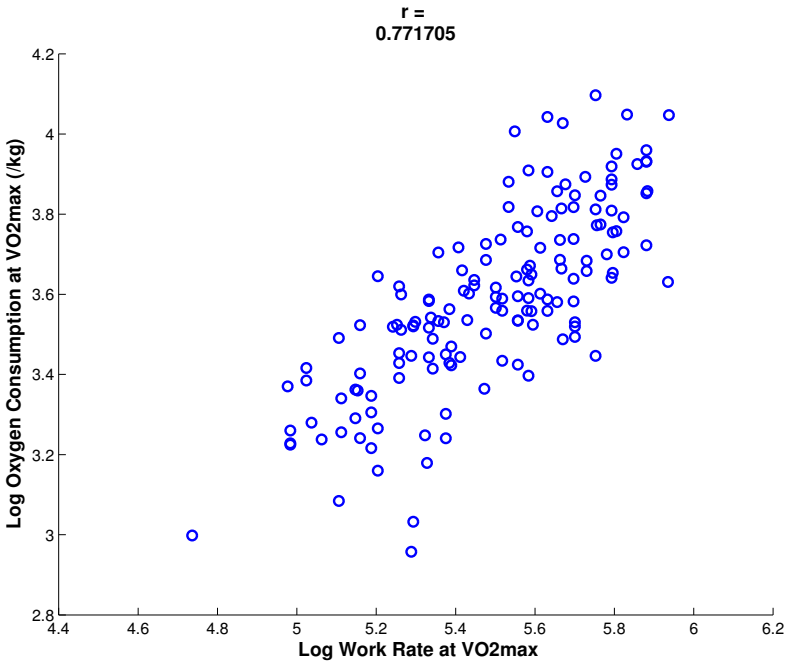
The final stage of modelling expanded on the approach used in the previous chapter, focussing on predicting a *change* in a physiological measurement (dependent variable), with a combination of biochemical metabolites (explanatory variables). Ideally, an absolute value, or change between two low altitudes would be used for the explanatory variables, in order to apply the models to a critical illness environment (the explanatory variables need to be easily measurable in critically ill patients). This stage of modelling focussed on predicting a *change* in a physiological measurement for two reasons; firstly, to see if a change would be more predictable than an absolute value, and secondly, because a change would be a more biologically relevant value to predict.

The dependent variables chosen for this stage of modelling were work rate and oxygen consumption. These measurements were chosen as they were considered the most *biologically relevant* out of all of the physiological measurements available, and therefore the most informative to be able to predict. Work rate was also identified in the previous chapter as a physiological measurement that was highly predictable by the biochemical metabolites (explanatory variables) available. Exercise is severely limited at high altitude, and this limitation has been well documented [McArdle et al., 2007]. *Oxygen consumption* is a measure of an individual's ability to transport and utilise oxygen to do work, and reflects the aerobic fitness of that individual. *Work rate* is a measure of the body's ability to perform work, and is highly correlated with oxygen consumption, as shown in Figure 7.1a. Both Oxygen consumption and work rate are known to decrease with increasing altitude, although the underlying mechanism of this is not well understood.

Individuals who adapt well to the decrease in oxygen availability will show the smallest decrease in oxygen consumption and work rate at higher altitudes, compared to London. Presumably, this is because they are able to adapt better to the lower oxygen levels available, and still transport and utilise oxygen efficiently. Therefore, the percentage loss in oxygen consumption and work rate were used in this final stage of modelling as dependent variables, as they were considered good *indicators of acclimatisation to hypoxia*. Oxygen consumption is usually normalised for body weight, which does not correlate as highly with work rate, as shown in Figure 7.1b. Therefore, different variations of these two measurements were assessed during modelling to determine which, if any, could be successfully predicted with



(a) O_2 consumption at VO_2max vs work rate at VO_2 max



(b) O_2 consumption at VO_2max / kg vs. work rate at VO_2 max

Figure 7.1: Two scatter plots, showing (a) oxygen consumption at VO_2max vs. work rate at VO_2max, and (b) oxygen consumption at VO_2max normalised for body weight (/ kg) vs. work rate at VO_2max. Both correlations are performed between log-transformed values. (a) shows a higher correlation between work rate and oxygen consumption, compared to (b) work rate and oxygen consumption normalised for body weight over all altitudes.

Variable type	Altitude / Time-point	Measurement type	Hypoxic level
Physiological	Kathmandu - London	% Difference	Low
(Dependent variable)	Namche - London	% Difference	Medium
	Pheriche - London	% Difference	High
	Everest Base Camp - London	% Difference	Very high
Biochemical	London	Absolute values	Normoxia
(Explanatory variable)	Kathmandu	Absolute values	Low
	Namche	Absolute values	Medium
	Kathmandu - London	% Difference	Low
	Namche - London	% Difference	Medium

Table 7.1: A list of different variations of dependent and explanatory variables used in targeted multiple linear regression. MLR was run for each of the dependent variable altitude differences, using one of the variations of the explanatory variables listed above. The four models produced for the dependent variable (one for each altitude difference) were then combined to create a *generalised* model, capable of predicting the dependent variable over a range of different altitudes. Absolute values at low altitude and differences between low altitudes were used for the explanatory variables to see which altitude was the most predictive, and to produce a series of models that could be applied to different situations, depending on the information available.

MLR. Using the percentage loss allowed a more generalised model to be formed, which would be more applicable to the population as a whole. The different variations of work rate and oxygen consumption used in this final stage of modelling were:

- Oxygen consumption at LaT;
- Oxygen consumption at LaT, normalised for body weight (/kg);
- Oxygen consumption at VO_2max;
- Oxygen consumption at VO_2max, normalised for body weight (/kg);
- Work rate at LaT;
- Work rate at VO_2max.

7.2.1 Explanatory variables

The full range of biochemical metabolites shown in Table 5.3 were used as potential explanatory variables for this stage of modelling. However, several variations of the biochemical metabolites were used, to try to establish the best relationship between the dependent and explanatory variables. The explanatory variables used

in modelling needed to be measurable in critical ill patients, so that any final models produced could be applied to predict how well a critically ill patient will adapt to hypoxic conditions. Therefore, only absolute measurements taken at low altitudes and the percentage differences between low altitudes were used for the explanatory variables for this stage of modelling. These included measurements taken at London (normoxic levels), Kathmandu (moderate hypoxia), Namche (substantial but not severe hypoxia) and percentage changes between these low hypoxia measurements. To measure the changes in biochemical metabolites, one would apply a small hypoxic stress to a patient and take blood samples for measurement before and after the stress was applied. This method would not threaten the patient's health and would enable the examiner to see how the individual responds to a small amount of stress. The different variations of the explanatory variables used in modelling are summarised in Table 7.1.

7.2.2 Producing a *general* model

MLR was run four times for each dependent variable listed in Section 7.2, once for each altitude difference shown in Table 7.1. These four initial MLR models were then summarised for analysis, showing the adjusted R^2 value for predicted vs. measured values, the intercept, and the explanatory variables and coefficient values fitted in each MLR model.

A generalised MLR model was then constructed by combining the four initial MLR models for each dependent variable. The coefficient values for each of the explanatory variables used in the MLR were assessed to determine whether they were similar between the four MLR models, or whether they showed an altitude-dependent change. All coefficients and the intercept value for each model were regressed against different measurements of hypoxia, in order to create one model that could predict a range of outcomes based on one set of input variables, and a hypoxia-based measure.

This was achieved by expanding the normal MLR equation of $y = ax_1+bx_2+c$ (shown in equation 7.1), so that a, b and c are all dependent on some measure of hypoxia exposure, shown in equations 7.2, 7.3 and 7.4, respectively. Therefore, each explanatory variable was regressed against hypoxia exposure in turn, and the resulting equation was substituted back into the original (shown in equation 7.5), as follows:

$$y = ax_1 + bx_2 + c; \tag{7.1}$$

$$a = [a_1(hypoxia) + a_2]; \tag{7.2}$$

$$b = [b_1(hypoxia) + b_2]; \tag{7.3}$$

$$c = [c_1(hypoxia) + c_2]; \tag{7.4}$$

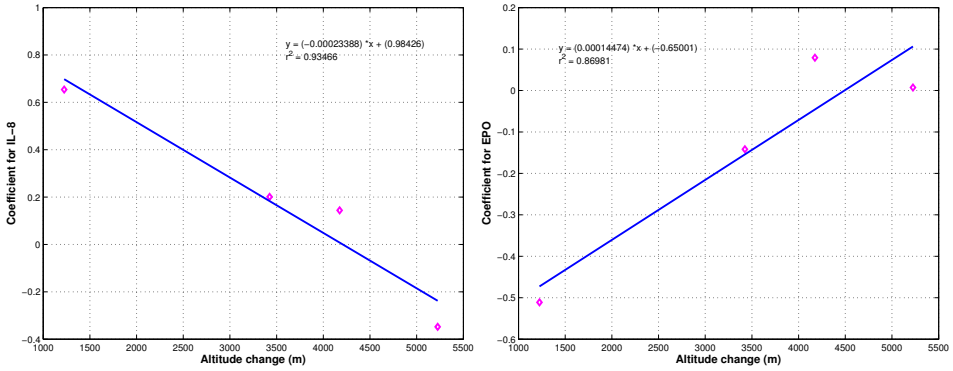
$$y = [a_1(hypoxia) + a_2]x_1 + [b_1(hypoxia) + b_2]x_2 + [c_1(hypoxia) + c_2]. \tag{7.5}$$

This was repeated using each of the five variations of the biochemical metabolites shown in Table 7.1 as explanatory variables. In summary, for each dependent variable:

1. Run multiple linear regression on each altitude difference shown in Table 7.1 (four in total), using all biochemical metabolites in Table 5.3 as explanatory variables;
2. Produce a summary of the four initial models produced, including explanatory variables used, coefficient values and intercept value;
3. Run simple linear regression analysis for each explanatory variable, looking at how the coefficient value changes over some measure of hypoxia exposure (e.g. altitude);
4. Run simple linear regression analysis for the four intercept values, assessing how the intercept value changes over some measure of hypoxia exposure;
5. Combine the four initial models into one *generalised* model, substituting the explanatory variable coefficient value for the equation gained from the regression analysis;
6. Assess the fit for the generalised model by plotting measured values against values predicted from the model, assessing residuals and calculating the goodness of fit statistic, lack of fit statistic and F-value for the fit.

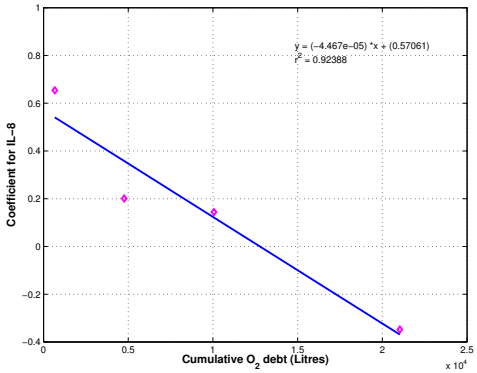
7.2.3 Finding a suitable hypoxia measure

In order to construct a generalised model, each explanatory variable used in the four initial models was assessed, to see how its coefficient value changed with increasing altitude difference. Simple linear regression analysis was performed between each explanatory variable coefficient value and a measure of *hypoxic exposure*. The measure of hypoxic exposure would be used in the final generalised model, and needed

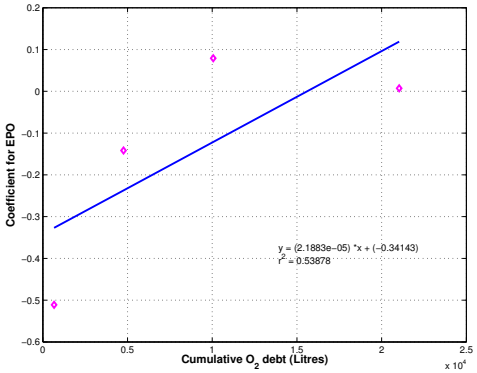


(a) IL-8 vs. altitude change (m)

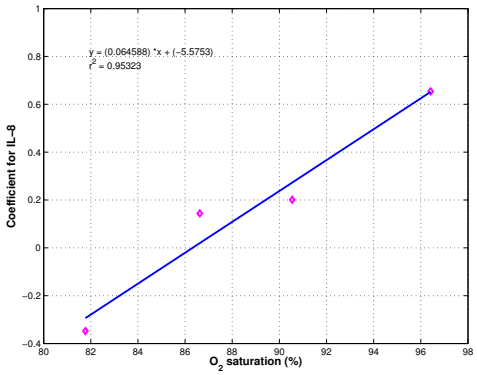
(b) EPO vs. altitude change (m)



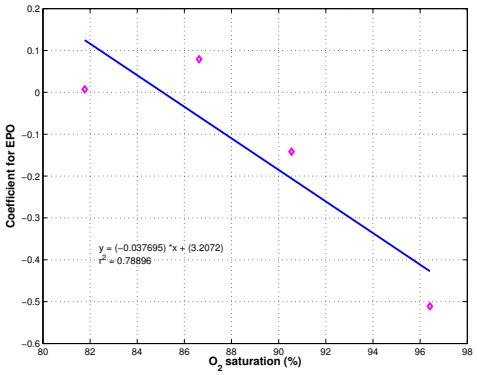
(c) IL-8 vs. cumulative O_2 debt (L)



(d) EPO vs. cumulative O_2 debt (L)



(e) IL-8 vs. O_2 saturation



(f) EPO vs. O_2 saturation

Figure 7.2: Examples of linear regression analyses carried out between coefficients for explanatory variables used in a MLR and three different measures of hypoxia exposure. (a) and (b) show coefficient values regressed against a change in altitude, (c) and (d) against cumulative oxygen debt and (e) and (f) against oxygen saturation. The final three data points are most closely clustered together for altitude, and most evenly spaced out for cumulative oxygen debt. Altitude gives the best linear relationship, whereas cumulative oxygen debt gives the weakest linear relationship. Oxygen saturation is the only measure easily taken in critically ill patients, and is a good compromise between the other two measurements.

to be measurable in critically ill patients. Altitude was initially used, and whilst this measure showed a good linear relationship with most of the coefficients (as shown in Figures 7.2a and 7.2b), it would not be a good surrogate for the hypoxic level of a critically ill patient. An even spread of data points is important for linear regression analysis, as uneven clustering of data points can have a biased impact on the regression formed, especially if the number of data points is small. The values for altitude difference showed an uneven spread of altitude points, with three values clustered at higher altitudes. This would have had a high influence on the regression analysis performed. This emphasises that the design of CXE was not ideal for performing this type of modelling analysis, and was the main limitation to the analysis that was carried out.

Oxygen debt looks at the cumulative exposure to an increasingly hypoxic environment. A detailed expedition ascent profile was constructed, which took into consideration how far the group ascended each day, any stop-overs they had, average breathing rates at each altitude both at rest and during exercise, and relative oxygen availability at different altitudes. This detailed ascent profile was then used to calculate how much oxygen had been inhaled into the lungs during different stages of the expedition. These values were then compared to the amount of oxygen an individual would have inhaled if they had stayed in London (75m above sea level) for the entire expedition. The difference between these two values, denoted *oxygen debt* could be used as a measure of *hypoxic exposure*. This measure had the most even spread of data points for all hypoxic measures assessed, as shown in Figures 7.2c and 7.2d. Whilst this is the most accurate measure of how much hypoxia an individual has been exposed to, it would be very difficult to measure in critically ill patients, especially if they were already hypoxic before entering the ICU. If a patient is hypoxic due to an inability to transport oxygen or use the oxygen once it reaches the tissues, then this measure would not capture that information.

Oxygen saturation of the blood is well documented in hypoxia studies, and is known to decrease on exposure to hypoxia, due to less oxygen being available. Oxygen saturation is also very easy to measure in a critically ill patient in a non-invasive manner, minimising disruption to the patient. However, if the patient is hypoxic due to an inability to use the oxygen being delivered to the tissues, the oxygen saturation could be high, but the individual could still be severely hypoxic at the cellular level. This is an important limitation to this study, and has to be taken into consideration when looking at the application of the models formed. The resulting regressions would be a *mirror image* of the previous two hypoxic measures, as oxygen saturation decreased during the expedition, whereas altitude and oxygen

debt increased. The four data points are more evenly spread out than they were for altitude difference, however, they are not as evenly spaced as they were for oxygen debt. Therefore, this measure is a *compromise* between the information we need to perform the regression analysis, and what can be measured in a critically ill patient. It may not be applicable in all cases, however, it is the best measure available from this dataset.

As only four data points are available for the regression analysis, any kind of fit is a *best guess*, and requires a lot of assumptions to be made (for example, for a linear fit we assume that the response remains linear in-between the altitude points available). We cannot say with certainty that a relationship is 100% linear or otherwise with only four data points, even if the R^2 value is very high - as we do not know what is happening in-between these data points. If the R^2 value is high, we can only say that a linear relationship seems appropriate from the data available. Fitting a higher order polynomial to only four points is not appropriate, as we do not have enough information to fit such a curve. It is also not meaningful to remove data points to improve the fit, as there are only four points to begin with, removing any of them would reduce the certainty of the fit further still.

7.3 Results

MLR analysis was run for six different dependent variables, as listed at the end of Section 7.2. Each MLR was run four times, once for each altitude difference shown in Table 7.1. This was also done for each variation of explanatory variables shown in Table 7.1. Summary tables were produced, detailing statistics for the four initial models produced for each dependent variable. Table 7.2 shows an example of the generic summary of the MLR results for all dependent variables, in this example using absolute values at London for the explanatory variables.

Models that had an adjusted R^2 value of 0.4 or above were marked with an asterisk (*). This was judged to be an appropriate threshold value for refinement, as the models explained 40% or more of the variability of the dependent variable. In order to produce a generalised model, each of the four initial models formed for one dependent variable needed to have an adjusted R^2 value above 0.4, in order to give the best possibility for producing a good generalised model. These dependent variables were marked with a plus ($^+$) in the summary table, as shown in Table 7.2.

One model was refined for each variation of biochemical metabolites shown in Table 7.1, to show the principle of producing a generalised model, and due to time constraints. The models refined were chosen due to high adjusted R^2 values for each

of the four initial models, and the number of metabolites used within each model (a low number was preferred, for easy measurement in critically ill patients). One model was refined for each variation of biochemical metabolites in order to produce a *range* of models that would be applicable to different situations, depending on the information available. For example, a model based on absolute London values (i.e normoxia) for the biochemical metabolites would be most appropriate for applying to an individual who is not yet hypoxic. If the individual was already slightly hypoxic, then a model based on absolute values at Kathmandu for the biochemical metabolites (i.e. moderately hypoxic, equivalent to around 1300m altitude or 96% oxygen saturation) would be more appropriate. If more than one dependent variable produced four good initial models, then the dependent variable with the highest overall adjusted R^2 values was refined, to maximise the probability of forming a good generalised model, however, this was not guaranteed. Further work could look at refining the other initial models produced, to see if they produce a generalised model with better predictability than those shown here.

7.3.1 Work rate at VO_2max vs. absolute values at London

Table 7.2 shows the summary of the results for the MLR for all dependent variables, using absolute London values of the biochemical metabolites as explanatory variables. Two dependent variables showed good adjusted R^2 values for all four initial models produced; oxygen consumption at VO_2max and work rate at VO_2max, and are marked with a plus ($^+$) in Table 7.2. Table 7.3 shows an expansion of the table for work rate at VO_2max, showing the explanatory variables included in each initial model. Both of these variables show adjusted R^2 values above 0.4, and a good number of explanatory variables used in the models. These two variables are known to correlate highly with one another, as shown in Figure 7.1a. Therefore, only work rate at VO_2max was refined, as it showed higher overall adjusted R^2 values and lower ResMS values for the initial models formed compared to oxygen consumption at VO_2max. One potential next step in this analysis would be to construct a generalised model for the oxygen consumption model, and compare it to the work rate model formed.

Table 7.3 shows the full summary for the four initial models formed for work rate at VO_2max, including explanatory variables used in each model and their associated coefficient values. Each of these explanatory variables was regressed against oxygen saturation, and the results are shown in Table 7.4. The table shows that a majority of the explanatory variables show a reasonable linear relationship with oxygen saturation. This is shown by mostly high R^2 values between the linear

Dependent variable	Adjusted R^2	ResMS	Explanatory variables
Oxygen consumption at LaT			
Kathmandu - London	* 0.978	4.21	Nitrite, RSNO, RNNO, cGMP, IL-1ra, MIF, Epinephrine, Protein Content, HSP-70
Namche - London	0	276.75	
Pheriche - London	0	189.222	
EBC - London	0	168.213	
Oxygen consumption at LaT (kg)			
Kathmandu - London	* 0.937	11.17	Nitrite, Nitrate, cGMP, IL-1ra, MIF, C-Peptide, Protein Content
Namche - London	0	285.55	
Pheriche - London	0	199.908	
EBC - London	0	207.65	
$^+$Oxygen consumption at VO2max$^+$			
Kathmandu - London	* 0.577	30.4	HNE, MIF, Noradrenaline, Resistin
Namche - London	* 0.861	13.57	Nitrite, RNNO, IL-8, T3, Leptin, Glucose, Lactate
Pheriche - London	* 0.480	29.68	Total NOx, MIF
EBC - London	* 0.411	29.75	Noradrenaline, Leptin
Oxygen consumption at VO2max (kg)			
Kathmandu - London	0	68.0301	
Namche - London	* 0.825	16.18	RNNO, IL-8, C-Peptide, GIP, Leptin
Pheriche - London	* 0.435	35.24	Nitrate, MIF
EBC - London	* 0.463	35.29	Noradrenaline, Leptin
Work rate at LaT			
Kathmandu - London	0	364.27	
Namche - London	0	259.582	
Pheriche - London	* 0.892	24.44	IL-1ra, IL-12(p70), MIF, T3
EBC - London	0	182.02	
$^+$Work rate at VO2max$^+$			
Kathmandu - London	* 0.984	0.25	Nitrate, RSNO, IL-1ra, IL-8, IL-18, VEGF, T3, Leptin, EPO, Creatinine
Namche - London	* 0.524	15.81	cGMP, Adrenaline
Pheriche - London	* 0.681	6.31	Adrenaline, GLP-1, Visfatin
EBC - London	* 0.584	14.73	8-isoPGF, Adrenaline

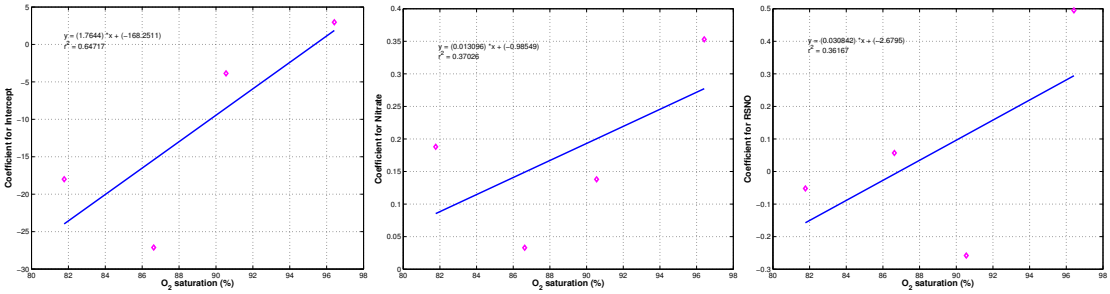
Table 7.2: A summary of the initial MLR results for each dependent variable in Table 7.1, with absolute biochemical metabolite values at London used as explanatory variables. Adjusted R^2 values above 0.4 are marked with an asterisk (*). Dependent variables where all four initial models had an adjusted R^2 value above 0.4 are suitable for model refinement, and are marked with a plus ($^+$).

4 models formed		Nitrate	RSNO	cGMP	8-isoPGF	IL-1ra	IL-8	IL-18	VEGF	Adrenaline	T3	GLP-1	Leptin	Visfatin	EPO	Creatinine
Kathmandu - London	Coefficient	0.353	0.495	0.0019	-0.009	-0.039	0.654	0.0186	0.0126	-3.976	0.00023	0.0006	-0.001060	-0.000005	-0.5111	-16.117
Adjusted R^2 = 0.984	p-value	0.00000002	0.000002	0.739	0.506	0.0002	0.001	0.0001	0.001	0.469	0.003	0.444	0.0001	0.936	0.0000001	0.0000001
Intercept = 2.97	in model?	1	1	0	0	1	1	1	1	0	1	0	1	0	1	1
Namche - London	Coefficient	0.138	-0.258	-0.0662	-0.107	0.037	0.201	0.0048	-0.0016	-71.072	0.00061	0.0008	0.000010	0.000078	-0.1418	-10.091
Adjusted R^2 = 0.524	p-value	0.225	0.350	0.003	0.195	0.308	0.785	0.770	0.912	0.018	0.141	0.874	0.993	0.865	0.425	0.345
Intercept = -3.86	in model?	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Pheriche - London	Coefficient	0.033	0.057	-0.0033	-0.072	0.016	0.144	-0.0064	-0.0105	-74.957	0.00030	0.0209	0.000070	-0.001330	0.0791	-7.781
Adjusted R^2 = 0.681	p-value	0.655	0.758	0.809	0.103	0.499	0.779	0.544	0.235	0.0004	0.230	0.0003	0.923	0.008	0.470	0.133
Intercept = -27.12	in model?	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
EBC - London	Coefficient	0.188	-0.052	0.0296	-0.271	0.024	-0.348	0.0049	-0.0029	-77.633	0.00047	-0.0008	0.000045	0.000213	0.0073	6.118
Adjusted R^2 = 0.584	p-value	0.092	0.859	0.210	0.001	0.504	0.609	0.762	0.825	0.008	0.187	0.875	0.967	0.631	0.965	0.556
Intercept = -18	in model?	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0

Table 7.3: An expansion of a section of Table 7.2, showing a summary of the MLR performed between work rate at VO_2max and biochemical metabolites as explanatory variables. The MLR was performed four times, once for each altitude difference: Kathmandu - London, Namche - London, Pheriche - London, Everest Base Camp - London. *In model?* shows whether a particular explanatory variable was included in each model or not (1 = included, 0 = not included). *Coefficient* is the coefficient value for that particular explanatory variable in the model. If the explanatory variable was not included in the model (i.e. *In model?* = 0), the associated coefficient value is the value it *would* have had, if it had been included in the model (if it had been included in the model, all other coefficient values are likely to have changed).

Explanatory variable	Slope	Intercept	R^2	F-value	df	p-value
Intercept	-1.76438	4.4388	0.647	3.67	3, 17	0.196
Nitrate	-0.01310	0.2963	0.370	1.18	3, 17	0.392
RSNO	-0.03084	0.3391	0.362	1.13	3, 17	0.399
cGMP	0.00261	-0.0331	*0.160	*0.38	3, 17	*0.601
8-isoPGF	-0.01584	0.0283	0.771	6.72	3, 17	0.122
IL-1ra	0.00380	-0.0248	0.494	1.96	3, 17	0.297
IL-8	-0.06459	0.7463	0.953	40.76	3, 17	•0.024
IL-18	-0.00112	0.0156	0.458	1.69	3, 17	0.324
VEGF	-0.00119	0.0101	0.581	2.77	3, 17	0.238
Adrenaline	-4.89147	-12.7149	0.733	5.50	3, 17	0.144
T3	0.00001	0.0003	*0.143	*0.33	3, 17	*0.622
GLP-1	0.00030	0.0027	*0.032	*0.07	3, 17	*0.820
Leptin	0.00007	-0.0009	0.686	4.36	3, 17	0.172
Visfatin	-0.00001	-0.0001	*0.013	*0.03	3, 17	*0.885
EPO	0.03769	-0.4822	0.789	7.48	3, 17	0.112
Creatinine	1.43553	-19.9378	0.894	16.93	3, 17	0.054

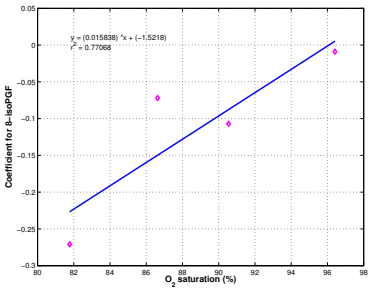
Table 7.4: Summary of the regression analysis performed between coefficients for explanatory variables from a MLR and oxygen saturation. The MLR was performed between work rate at VO_2max (dependent variable) and absolute values for biochemical metabolites at London (explanatory variables). The slope and intercept are for the fitted regression lines for each separate regression analysis. The R^2 value was calculated for the regression line, as described in Section 2.5.9.4.
The F-value and p-value were calculated from the regression, as described in Section 2.5.9. This value is calculated from a lack-of-fit sum of squares, and is not very meaningful as it is based on only four data points. The only F-test that shows a significant p-value is for IL-8 (marked with a dot (•)), which has an R^2 value of 0.953, which is the highest for any metabolite assessed. The poorly fitting regression lines for cGMP, T3, GLP-1 and visfatin are all marked with an asterisk (*). These metabolites are associated with the highest p-values, confirming that these are the poorest fitting regression lines to the data. A larger number of data points would provide greater power to reject the null hypothesis for this test.



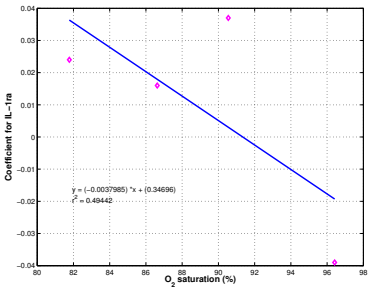
(a) Intercept

(b) Nitrate

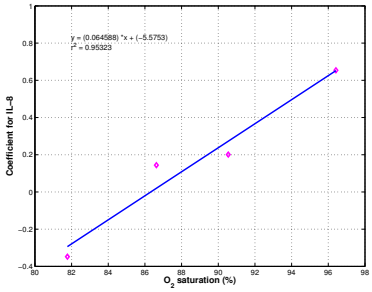
(c) RSNO



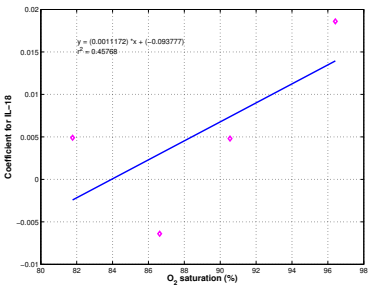
(d) 8-isoPGF



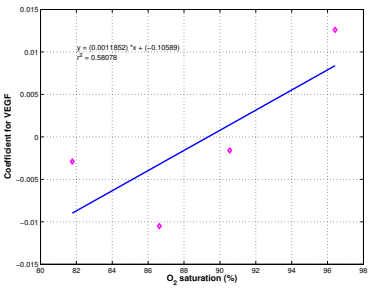
(e) IL-1ra



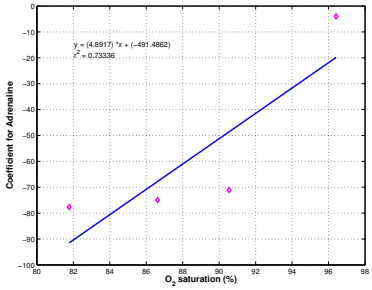
(f) IL-8



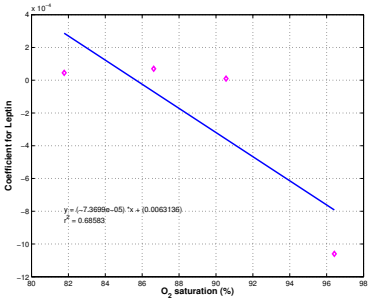
(g) IL-18



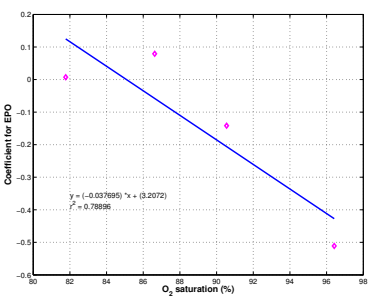
(h) VEGF



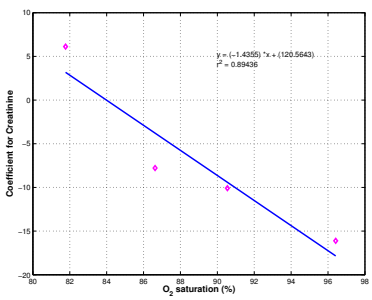
(i) Adrenaline



(j) Leptin

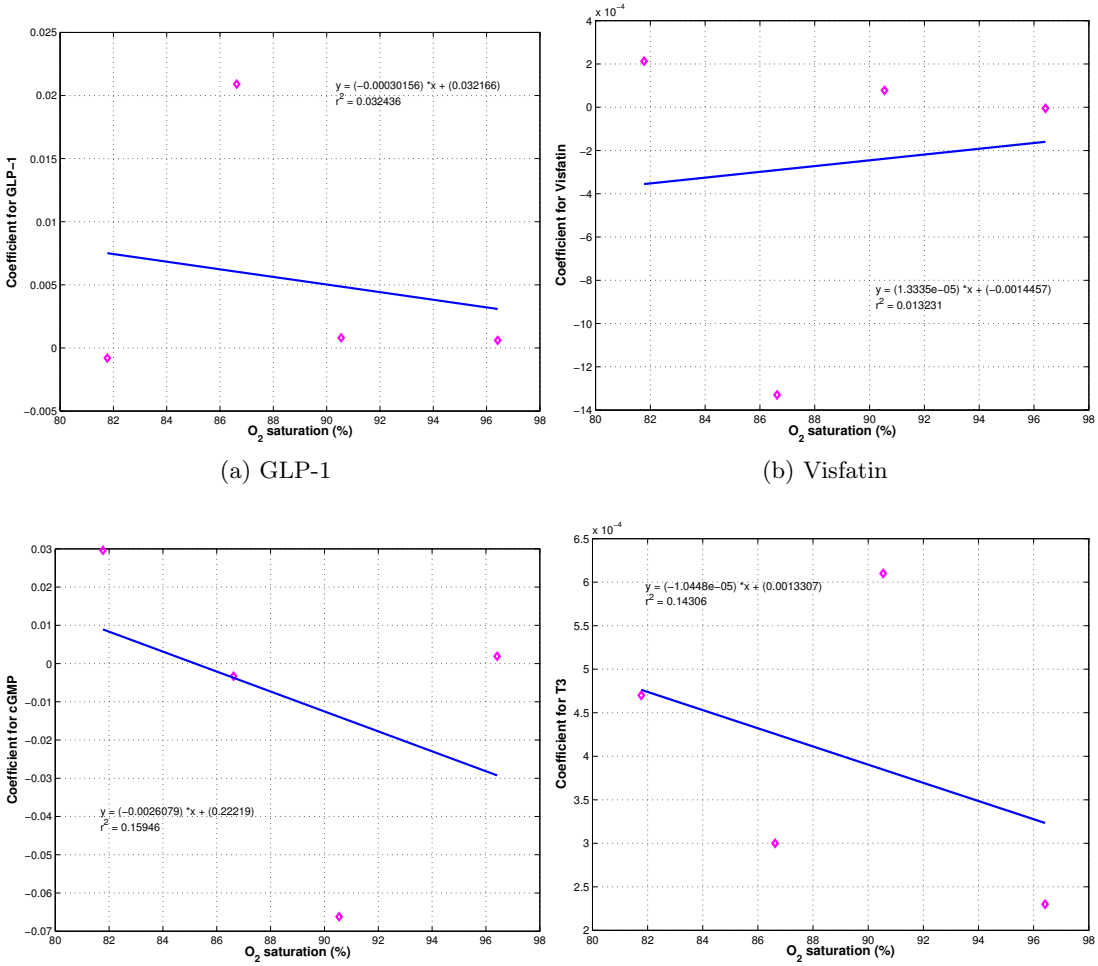


(k) EPO



(l) Creatinine

Figure 7.3: Simple linear regression analysis performed for coefficient values against oxygen saturation. All of these coefficients show a reasonable linear relationship against oxygen saturation. As there are only four points available here, we are only able to fit a *linear* line, which is obviously not suitable in some cases (for example (b), (g), (h), (i), (j) and (k) look almost curved in their response). However, a linear fit is used here as a *best estimate* for the data available.



(a) GLP-1 (b) Visfatin

(c) cGMP (d) T3

Figure 7.4: Linear regression analysis performed between coefficient values included in MLR for work rate at VO_2max and oxygen saturation. All of these coefficients showed low R^2 values for the resulting regression line and the original coefficient values. All plots show one altitude which does not follow a linear relationship, resulting in the poor resulting R^2 value. The remaining values for GLP-1 (a) and visfatin (b) show only a small change with oxygen saturation, therefore, these coefficient values were assessed to see if the use of a constant term was more appropriate than a linear dependence on oxygen saturation for the MLR for these metabolites. As cGMP (c) and T3 (d) show a change with altitude, the linear relationship against oxygen saturation was used for these metabolites in the MLR.

regression line and the coefficient values.

The F-statistic was calculated for each regression, as described in Section 2.5.3. All of these linear regression analyses are based on four data points, and more data points are needed to give the F-test the power to reject the null hypothesis. For this dataset, only IL-8 passed the F-test with a R^2 value of 0.953, a F-value of 40.76 and a p-value of 0.024 (marked with a •), showing that the model generated from the regression analysis was a good fit to the data. However, the smallest F-values and largest p-values were associated with the worst fitting regression lines, seen for cGMP, T3, GLP-1 and visfatin, indicated by the lowest R^2 values of 0.160, 0.143, 0.032 and 0.013, respectively. Therefore, the F-value and associated p-value give an indication of how well each model fits the data, but there is not enough data to definitively accept or reject the null hypothesis for the F-test.

Figures 7.3 and 7.4 show the figures produced from the simple linear regression analyses of the explanatory variable coefficients for work rate at VO_2max against oxygen saturation. All figures show the actual coefficient values in magenta, and the fitted regression line in blue. Figure 7.3 shows the regression figures where a linear fit is a *reasonable estimate* for the relationship between the explanatory variable coefficient and oxygen saturation. The R^2 values for these regression figures range from 0.362 to 0.953. The threshold R^2 value is lower here than for previous analyses due to the smaller number of data points that the regression is based on, and because the linear fits for nitrate (shown in Figure 7.3b with an R^2 value 0.370) and RSNO (shown in Figure 7.3c with an R^2 value of 0.362) both show an increase with increasing oxygen saturation, and seem a reasonable estimate of the relationship against oxygen saturation.

Figure 7.4 shows the weakest of the resulting regression fits. All four metabolites shown in Figure 7.4 show one outlying datapoint compared to the other three. However, it is not very meaningful to remove this one point, due to such few data points being available. Figures 7.4a and 7.4b show an almost constant coefficient value, apart from the one outlying datapoint. These metabolites were highlighted for further refinement at a later stage. Figures 7.4c and 7.4d show an overall decrease in coefficient value as oxygen saturation increases.

For the initial generalised model, the linear fits were substituted into the MLR for all explanatory variables. The measured values were plotted against values predicted from the model, and are shown in Figure 7.5. The adjusted R^2 value for this figure is 0.738, indicating that the generalised model is explaining 73.8% of the variability seen in the measured data. The cyan data points show the results for % difference between Kathmandu - London, the blue for Namche - London,

the magenta for Pheriche - London and the red for Everest Base Camp - London. This model shows systematic error, overestimating loss at low altitude (Kathmandu, shown in cyan) and underestimating loss at high altitude (Everest Base Camp, shown in red).

The initial model for Kathmandu-London contained 10 explanatory variables (as shown in Table 7.2), which have all changed slightly due to the regression against oxygen saturation. The overestimation of the response at this altitude may be due to the perturbation of these explanatory variables to accommodate the prediction of other altitude differences, which has led to a less accurate prediction at this particular altitude. The initial model for EBC - London only contains two explanatory variables, and the extra explanatory variables included in the generalised model from other initial models may not be appropriate for predicting the response at this particular altitude. The closest predictions are for the middle altitudes of Namche and Pheriche, shown in blue and magenta, respectively.

7.3.1.1 Further refinement

Overall, the biochemical coefficient values seemed to show a good linear relationship with oxygen saturation. However, four explanatory variables showed very poor linear relationships with oxygen saturation. These were GLP-1, visfatin, cGMP and T3, shown in Figure 7.4. All of these plots show one datapoint that is very different from the others, resulting in a poor linear fit. As the regression is only based on four data points, the removal of one point would severely affect the reliability of the resulting regression performed. The plots for cGMP and T3 show a general decrease with oxygen saturation, therefore, the linear relationship against oxygen saturation was used for these metabolites in the MLR. However, GLP-1 and visfatin could reasonably be assumed to be constant, when excluding the one outlying value. Therefore, constant values were tested for both of these metabolites, to see if the overall fit of the model could be improved.

Figure 7.6 shows observed values plotted against fitted values from six further refined MLR models. These models looked at using a constant value for GLP-1 or visfatin, or completely removing the metabolites from the MLR to assess the impact on the final model. Figures 7.6a, 7.6b and 7.6c show refined models for GLP-1, and Figures 7.6d, 7.6e and 7.6f show refined models for visfatin.

The adjusted R^2 value was used to informally compare the different models. The adjusted R^2 value increased to 0.812 when a constant value was used for GLP-1 that was based on all four data points, shown in Figure 7.6a. This indicates that using a constant value for GLP-1 helps to explain a further 7.4% of the variability

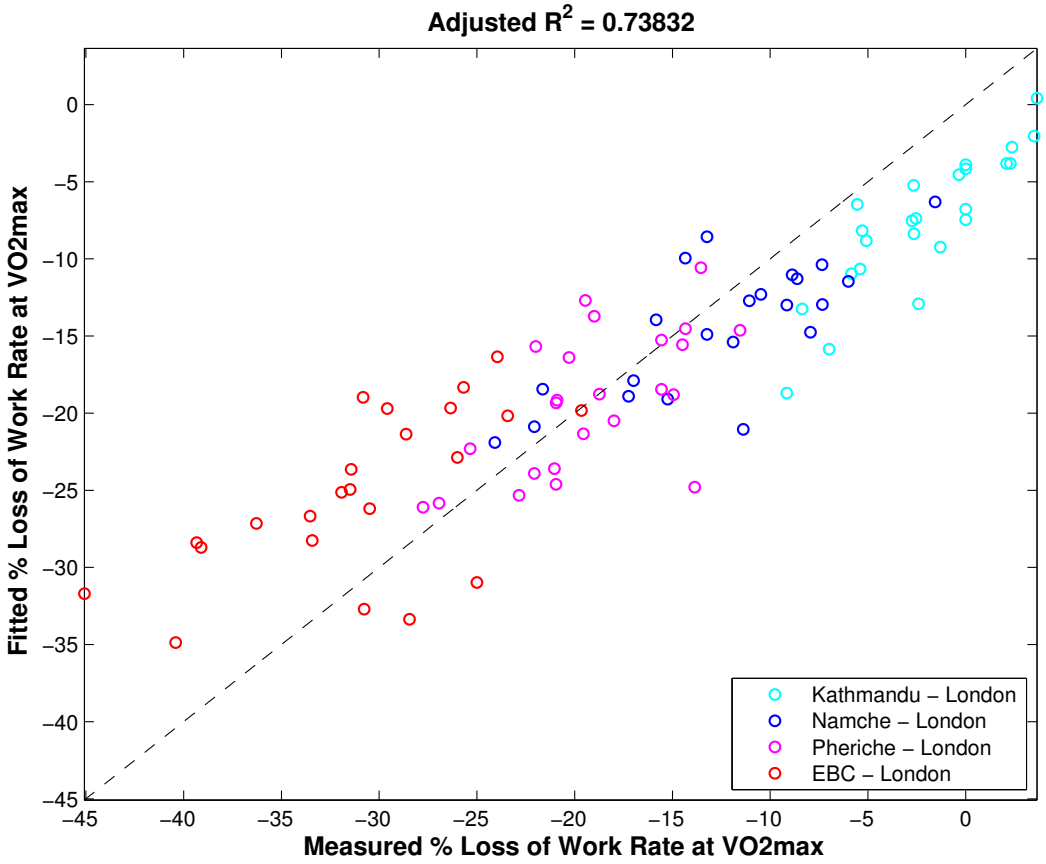


Figure 7.5: Measured values vs. values fitted from a MLR model, fitting the % loss in work rate at VO_2max seen between London and 4 increasing altitudes. Cyan shows results for Kathmandu - London (low hypoxia), blue shows Namche - London (medium hypoxia), magenta shows Pheriche - London (high hypoxia) and red shows Everest Base Camp - London (very high hypoxia). The adjusted R^2 value for this model is 0.738, indicating that it is explaining 73.8% of the variability associated with work rate at VO_2max. The figure shows that there is a higher % loss in work rate at higher altitudes, which is to be expected. This model shows systematic error, overestimating loss at low altitude (Kathmandu, shown in cyan) and underestimating loss at high altitude (Everest Base Camp, shown in red). This may be due to the generalised model being less appropriate for fitting responses at those particular altitude differences.

seen within the observed data. All other variants of the refined model resulted in a decrease in the overall model's adjusted R^2 value. Therefore, the linear equation was kept for visfatin and a constant value was used for GLP-1 in the final model.

The final refined model formed is shown in Figure 7.7, and has a adjusted R^2 value of 0.812, indicating that it is explaining 81.2% of the variability seen in work rate at VO_2max. This final model still shows some systematic error, overestimating the percentage difference between Kathmandu and London, and underestimating the percentage difference between Everest Base Camp and London, as shown in Figures 7.8a and 7.8d, respectively.

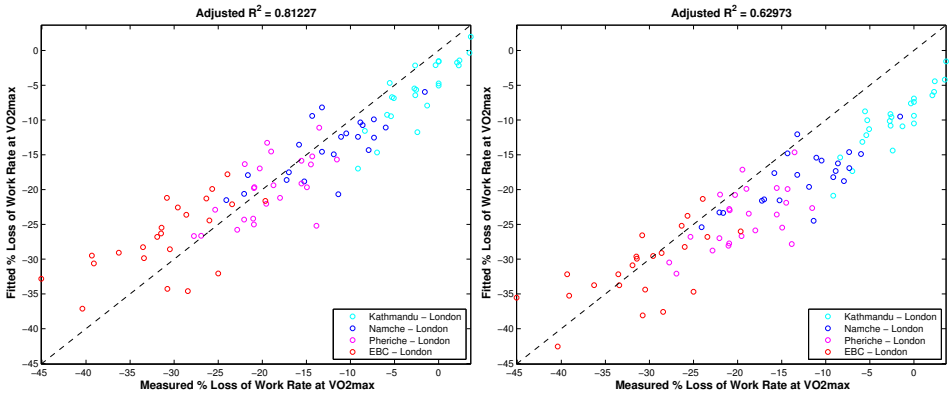
However, this appears to be less than is seen in the first refined model, shown in Figure 7.5, indicating that the refined model describes the variability seen at London and Everest Base Camp better than the initial model formed. Overall, these metabolites seem to show good predictive power over work rate at VO_2max.

7.4 Final models

The previous section detailed the refinement process for one model, created for work rate at VO_2max, using absolute values at London for the biochemical metabolites (explanatory variables). This model was refined in detail, as it was considered the most potentially useful in both a critical illness environment and for mountaineering. It would also be a good model to validate with the data for the 190 trekker individuals, and the follow-up study XE2. The next section will present a selection of *finalised models* that have already been through this refinement process, to show some of the final results of the targeted multiple linear regression modelling process.

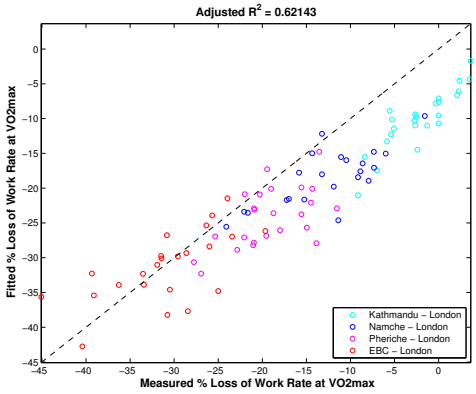
Multiple linear regression was used to form models for oxygen consumption and work rate, using biochemical metabolites as explanatory variables. All of these models were formed using the method outlined in Section 7.2.2. These models are all based on different biochemical measurements, for example absolute values at London or the percentage difference between London and Kathmandu. Two different *types* of model were formed, as summarised below:

- The *London model*, *Kathmandu model* and *Namche model*, produced using absolute values of the biochemical metabolites at London, Kathmandu or Namche as explanatory variables, to predict the percentage change in a dependent variable for several hypoxic exposures/altitudes;
- The *Kathmandu - London model* and *Namche - London model*, produced using the percentage difference of the biochemical metabolites between Kath-

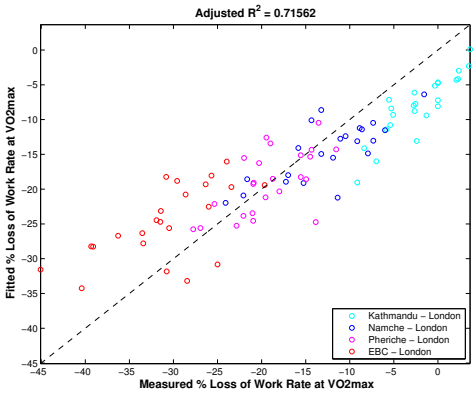


(a) Average GLP-1 (4 points)

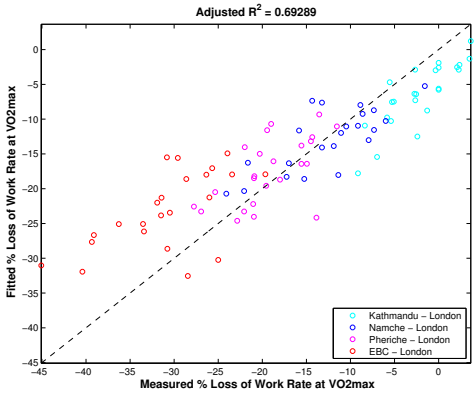
(b) Average GLP-1 (3 points)



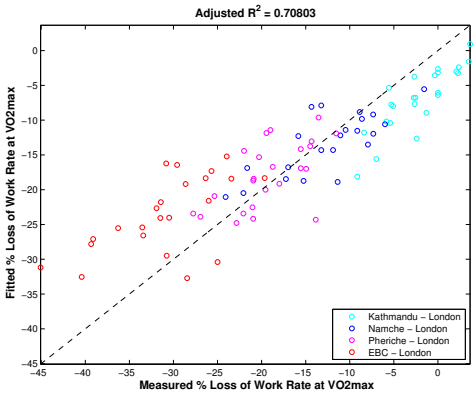
(c) GLP-1 removed



(d) Average visfatin (4 points)



(e) Average visfatin (3 points)



(f) Visfatin removed

Figure 7.6: Model refinement results after MLR on percentage loss of work rate at VO_2max. Each figure shows measured values plotted against values fitted from the model. The adjusted R^2 value associated with each figure is a measure of how well the model explains the variability seen in the observed data. The best model from this analysis is shown in (a), where a constant coefficient term is used for GLP-1 in the model instead of a term that changed over altitude, which is simply the average of the four coefficient terms acquired at each of the different altitudes.

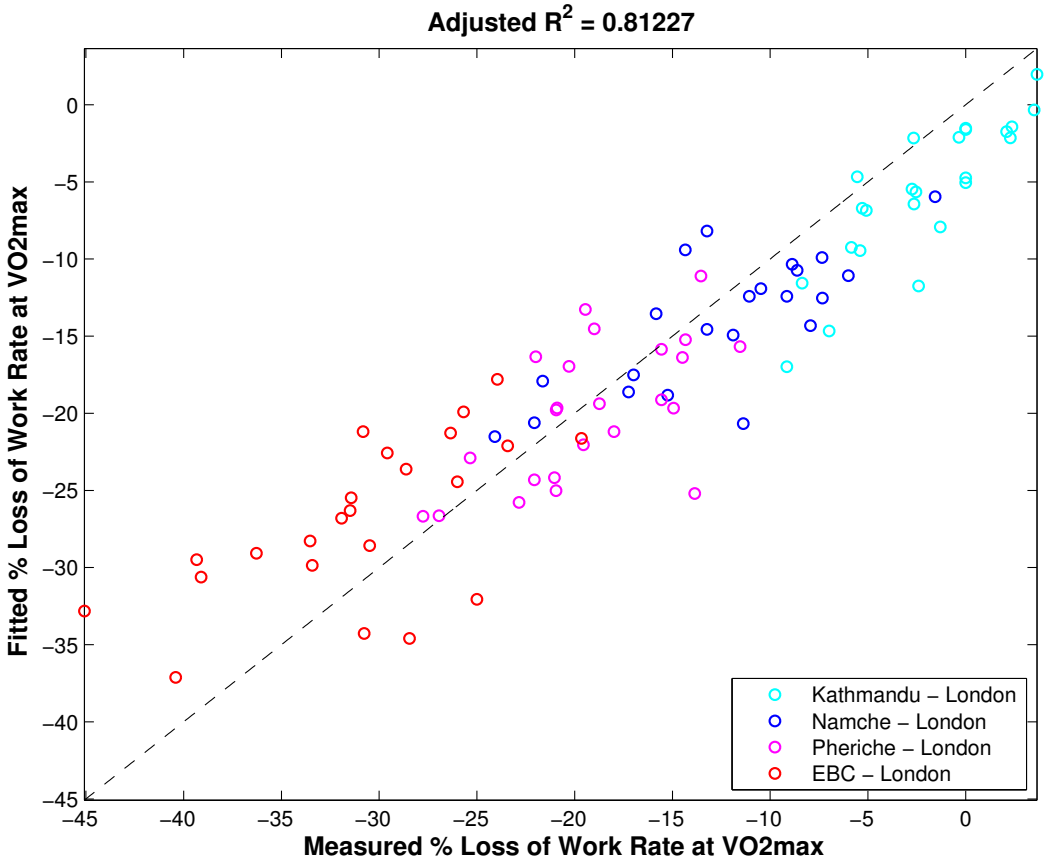
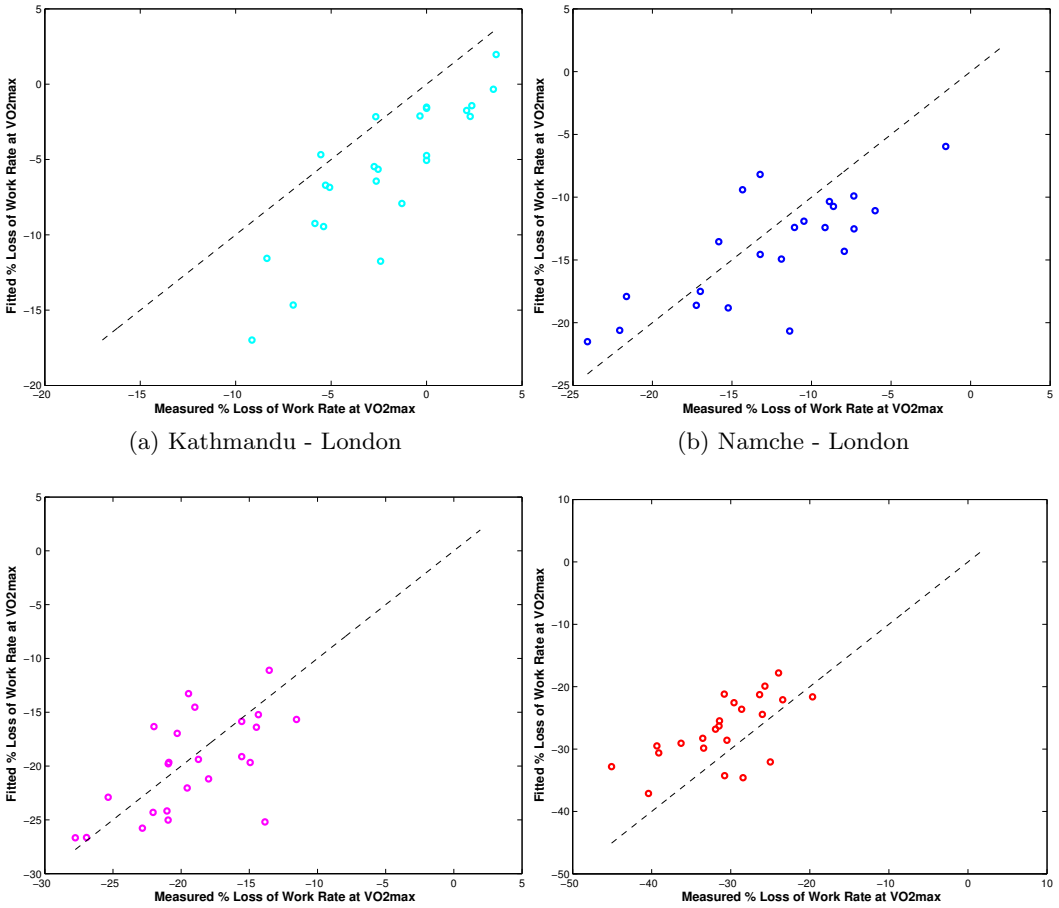


Figure 7.7: Measured values vs. values fitted for a MLR model, fitting the % loss in work rate at VO_2max seen between London and 4 increasing altitudes. Cyan shows results for Kathmandu - London (low hypoxia), blue shows Namche - London (medium hypoxia), magenta shows Pheriche - London (high hypoxia) and red shows Everest Base Camp - London (very high hypoxia). The adjusted R^2 value for this model is 0.812, indicating that it is explaining 81.2% of the variability associated with work rate at VO_2max. This model shows systematic error, overestimating loss at low altitude (Kathmandu, shown in cyan) and underestimating loss at high altitude (Everest Base Camp, shown in red). This may be due to the generalised model being less appropriate for fitting responses at those particular altitude differences.



(a) Kathmandu - London

(b) Namche - London

(c) Pheriche - London

(d) Everest Base Camp - London

Figure 7.8: Measured values vs. values fitted for a MLR model, fitting the % loss from London for work rate at VO_2max (% loss in work rate). All plots show a general good fit, however, the fitted values show systematic error. (a) shows values for Kathmandu - London are overestimated, and (d) shows values for Everest Base Camp - London are generally underestimated, however, to a lesser extent than (a). (b) and (c) show the best fits for Namche - London and Pheriche - London, respectively. This means that the model is the most accurate at fitting the response at a medium hypoxic exposure.

In terms of diagnosis, this model is more likely to *overestimate* how badly an individual will adapt to hypoxia at low hypoxic exposure (i.e. false positive, shown in (a)), and *underestimate* how badly an individual will adapt at extreme hypoxic exposures (i.e. more false negatives, shown in (d)), which could potentially be more serious in a critical illness environment, or with a climber wanting to ascend to extreme altitude. The use of a *correction term* could help to account for some of the systematic error seen for (a) and (d).

mandu and London or Namche and London as explanatory variables, to predict the percentage change in a dependent variable for several hypoxic exposures/altitudes.

This provides a number of models that can be applied to different circumstances, depending on the information available. If the aim were to predict the response of mountaineers during an ascent to high altitude, then the *London model* would be most appropriate, as a blood sample could be taken at sea level, and used to predict how that individual would adapt to different altitudes. For critically ill patients, the model applied would depend on the condition of the patient. If a patient had newly arrived at the ICU and unlikely to be hypoxic, then the *London model* would be the most appropriate. If the patient was slightly hypoxic, then the *Kathmandu model* would be more appropriate. If the patient was in a stable enough condition to be exposed to a small amount of stress, then the patient would be exposed to a low amount of hypoxia via an oxygen mask, and blood samples would be taken before and after the hypoxic exposure. These samples would then be measured for a set of biochemical metabolites, which would be used in one of the difference models, such as the *Kathmandu - London model*. The final models produced are detailed below.

7.4.1 London model

The London model was produced by performing MLR on work rate at VO_2max, using absolute values of the biochemical metabolites at London as explanatory variables. The final figure of observed vs. values fitted by the model is shown in Figure 7.7. The final adjusted R^2 value for this model was 0.812, indicating that it is explaining 81.2% of the variability within the observed data. The model uses a constant value for GLP-1, and a linear dependence on oxygen saturation for all other explanatory variables. The equation for the London model is as follows:

Work rate at VO_2max =
[Nitrate * (-0.013096 * O_2Sat + 0.2963)] +
[RSNO * (-0.030839 * O_2Sat + 0.3391)] +
[cGMP * (0.002608 * O_2Sat -0.0331)] +
[8-isoPGF * (-0.015838 * O_2Sat + 0.0283)] +
[IL-1ra * (0.003798 * O_2Sat -0.0248)] +
[IL-8 * (-0.064588 * O_2Sat + 0.7463)] +
[IL-18 * (-0.001117 * O_2Sat + 0.0156)] +

[VEGF * (-0.001185 * O_2Sat + 0.0101)] +
[Adrenaline * (-4.89147 * O_2Sat -12.7149)] +
[T3 * (0.00001 * O_2Sat + 0.0003)] +
[GLP-1 * -0.0054] +
[Leptin * (0.000074 * O_2Sat -0.0009)] +
[Visfatin * (-0.000013 * O_2Sat -0.0001)] +
[EPO * (0.037694 * O_2Sat -0.4822)] +
[Creatinine * (1.43553 * O_2Sat -19.9378)] +
(-1.76438 * O_2Sat + 4.4388)

7.4.2 Kathmandu model

The Kathmandu model was produced by performing MLR on work rate at VO_2max, using absolute values of the biochemical metabolites at Kathmandu as explanatory variables. The final figure of observed vs. values fitted by the model is shown in Figure 7.9. The adjusted R^2 value for this model was 0.857, indicating that it is explaining 85.7% of the variability associated with the observed data. The model shows a slight tendency to underestimate values, shown by a higher scatter in points above the dashed line, which shows where a perfect 1:1 relationship between observed and fitted data would lie. The model also shows the best predictions for Namche - London and Pheriche - London, with lower scatter around the dashed line for these measurements compared to Kathmandu - London and EBC - London.

This model would be a good target for further refinement, due to the high predictability (shown by a high adjusted R^2 value) and the small number of explanatory variables used in the model. The model uses a constant value for GIP, and a linear dependence on oxygen saturation for all other explanatory variables. The equation for the Kathmandu model is as follows:

Work rate at VO_2max =
[cGMP * (-0.000871621 * O_2Sat + 0.0692098)] +
[TNFalpha * (-0.00307143 * O_2Sat + 0.373616)] +
[VEGF * (0.00119403 * O_2Sat -0.113077)] +
[GIP * (-0.0030042)] +
[Adipsin * (-1.03E-06 * O_2Sat + 0.000119648)] +
[Creatinine * (-0.168058 * O_2Sat + 7.24898)] +
(2.41639 * O_2Sat -230.265)

7.4.3 Namche models

The attempts to produce a generalised model using absolute values of biochemical metabolites at Namche as explanatory variables did not yield positive results. MLR on individual altitudes produced some very strong modelling results, however, the attempts to generalise these results over all altitudes did not produce a model with strong predictive power, indicated by low and negative adjusted R^2 values for finalised models. This suggests that the models produced were not suitable for the dependent variable being predicted, over all altitudes. Therefore, two of the MLR on individual altitudes are presented, as they provide the most predictive power from the explanatory variables available. These include oxygen consumption at VO_2max normalised for body weight, predicting the percentage loss between London and Everest Base Camp, and work rate at VO_2max, predicting the percentage loss between London and Namche.

These models would have fewer uses than the generalised models formed, as they require individuals to be exposed to a high level of hypoxia to obtain values for the explanatory variables, and are only capable of predicting the response at one hypoxic level. However, they may still be useful for severely hypoxic patients who were to undergo surgery or receive a treatment that would increase their hypoxic state, to see if they will have any difficulties coping with the higher level of hypoxia.

Figure 7.10a shows the observed vs. fitted values for a MLR performed on oxygen consumption at VO_2max, normalised for body weight. The adjusted R^2 value for this model is very high at 0.998, indicating that the model is predicting 99.8% of the variability seen within the observed data. Figure 7.10b shows the results for work rate at VO_2max. The adjusted R^2 value for this model is lower at 0.691, indicating that it is explaining 69.1% of the variability of the observed data. There seems to be an even scatter of points around the dashed line for both models, indicating they are overestimating and underestimating values with the same probability.

7.4.4 Kathmandu - London model

The Kathmandu - London model was produced by performing MLR on work rate at VO_2max, using the percentage differences between London and Kathmandu (Kathmandu levels - London levels) for the biochemical metabolites as explanatory variables. The final figure of observed vs. fitted values for this model is shown in Figure 7.11. The adjusted R^2 value for this model was 0.902, indicating that it is explaining 90.2% of the variability associated with the observed data. The model

shows an even scatter of points above and below the dashed line, indicating that the model is overestimating and underestimating values with the same probability, and seems relatively equal for all altitude differences. This model has the highest adjusted R^2 value of all models produced, indicating that it is the *strongest* out of all the models produced and best fit for the observed data available.

This model would also be a good target for further refinement, due to the high predictability and the relatively small number of explanatory variables used in the model. The model uses a linear dependence on oxygen saturation for all explanatory variables. The equation for the Kathmandu - London model is as follows:

Work rate at VO_2max =
[Nitrite * (-0.00181257 * O_2Sat + 0.154603)] +
[HNE * (0.000500961 * O_2Sat -0.0860054)] +
[T3 * (-0.00164845 * O_2Sat + 0.142948)] +
[GIP * (0.00155984 * O_2Sat -0.143077)] +
[Insulin * (0.00267985 * O_2Sat -0.210178)] +
[Resistin * (0.00443372 * O_2Sat -0.444642)] +
[EPO * (-0.00259166 * O_2Sat + 0.302993)] +
[Creatinine * (-0.00549015 * O_2Sat + 0.565245)] +
(2.01976 * O_2Sat -195.07)

7.4.5 Namche - London model

The Namche - London model was produced by performing MLR on work rate at VO_2max, using the percentage difference between London and Namche for the biochemical metabolites as explanatory variables. The final figure of observed vs. fitted values for this model is shown in Figure 7.12. The model shows an adjusted R^2 value of 0.87472, indicating that it is explaining 87.472% of the variability associated with the observed data. The data shows a relatively even spread of points above and below the dashed line, indicating that the model is underestimating and overestimating values with the same probability. The model shows the least scatter around the line for the EBC - London predictions, shown in red. This indicates that the model produced the strongest predictions for the response to high levels of hypoxia. The model uses a constant term for resistin, and a linear dependence on oxygen saturation for all other explanatory variables. The equation for the Namche - London model is as follows:

Work rate at VO_2max =
[8-isoPGF * (0.00212489 * O_2Sat -0.208226)] +
[IL-1ra * (0.00679786 * O_2Sat -0.610947)] +
[IL-12(p70) * (-0.00188627 * O_2Sat + 0.199826)] +
[IL-13 * (0.000280559 * O_2Sat + 0.0438252)] +
[VEGF * (-0.00479035 * O_2Sat + 0.411325)] +
[T3 * (2.93E-05 * O_2Sat -0.0087744)] +
[GIP * (0.000328302 * O_2Sat -0.0373064)] +
[Resistin * (-0.024176051)] +
[Creatinine * (0.0105785 * O_2Sat -0.942044)] +
[Protein Content * (0.00664081 * O_2Sat -0.53097)] +
(1.64614 * O_2Sat -161.588)

7.5 Biochemical metabolites of interest

There were several biochemical metabolites that were used as explanatory variables in more than one MLR analysis. Table 7.6 shows the biochemical metabolites used as explanatory variables in all of the *generalised* models reported in this chapter. The table ranks biochemical metabolites in order of how many times they were used as an explanatory variable in MLR. This list indicates metabolites that need to be measured in the 190 trekker plasma samples in order to validate the generalised models reported in this project.

All of the generalised models formed are for work rate at VO_2max, as this was the strongest model formed for each of the different biochemical metabolite variations used as explanatory variables shown in Table 7.1. This indicates that the biochemical metabolites measured have the strongest relationship with the exercise measurement work rate at VO_2max, which is the amount of work the body can do at VO_2max, i.e. a measure of the body's maximal work output. The use of this particular physiological measurement as a diagnostic tool for hypoxic adaptation is based on the assumption that performance at altitude can be used as a *proxy* for hypoxia adaptation. This assumption formed the basis for this model building, and would need to be tested, for example during the XE2 follow-up study, to determine if this assumption is reasonable, and how accurate and useful this particular measurement is.

The biochemical metabolites included in the MLR include metabolites from a range of different biochemical pathways, including oxidative stress, metabolism and

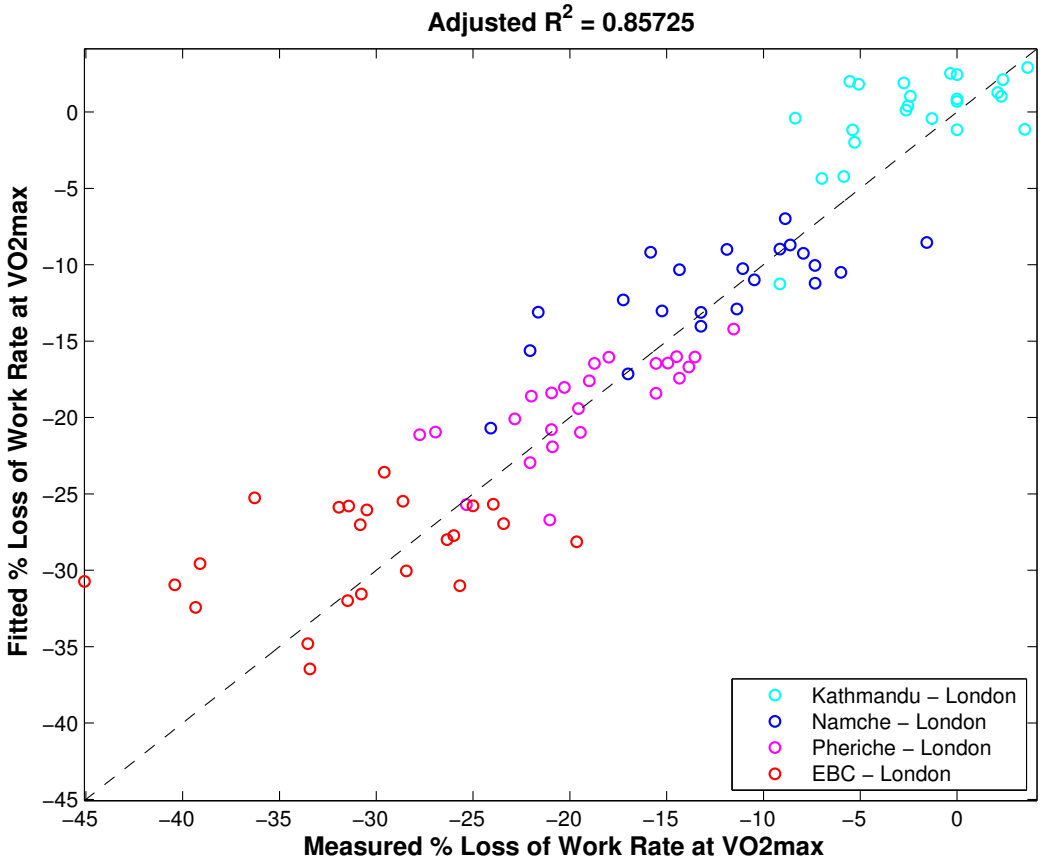
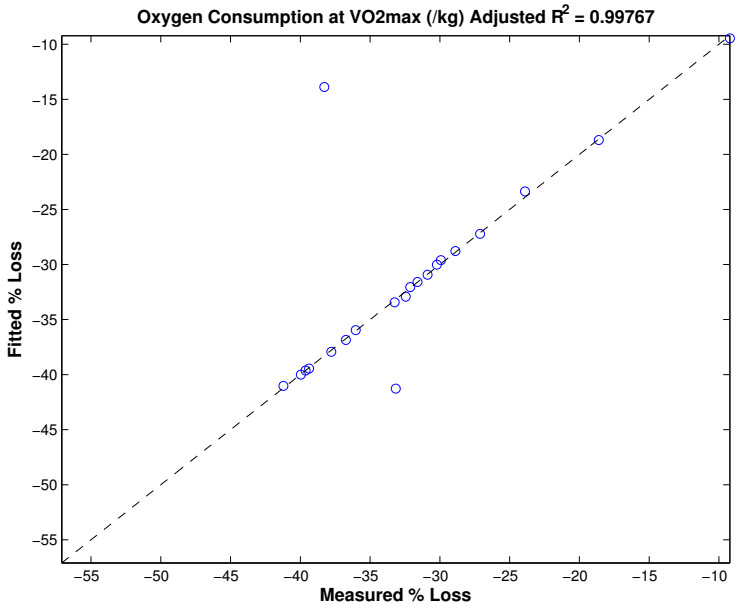
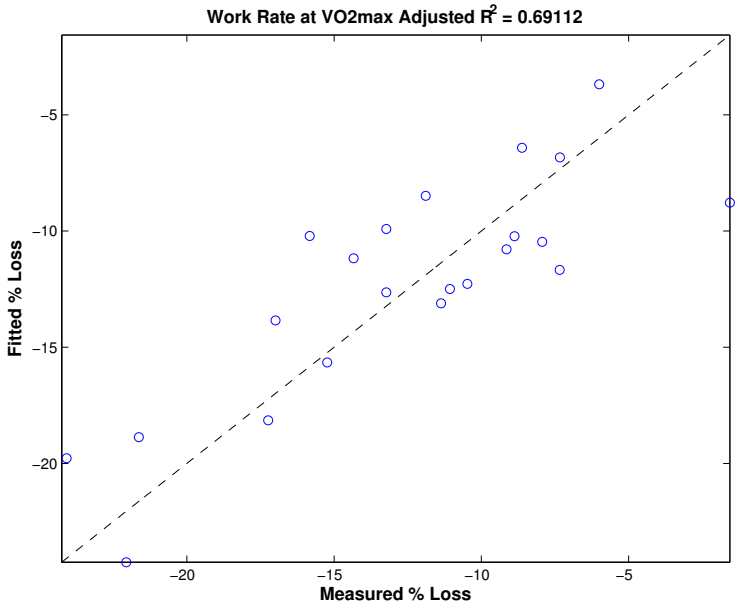


Figure 7.9: Measured vs. fitted % loss of work rate at VO_2max from London levels, calculated using a MLR with biochemical metabolites at Kathmandu as explanatory variables. The model shows a good adjusted R^2value of 0.857, indicating that it is explaining 85.7% of the variability associated with the observed data.



(a) O_2 consumption at VO_2max / kg (Everest Base Camp - London)



(b) Work rate at VO_2max (Namche - London)

Figure 7.10: Measured vs. fitted values for two MLR analyses, using absolute values at Namche for the biochemical metabolites as explanatory variables. (a) shows the results for a MLR for oxygen consumption at VO_2max, normalised for body weight (Everest Base Camp - London). This figure shows a very strong adjusted R^2 value of 0.998, indicating that it is explaining 99.8% of the variability seen in the observed data. (b) shows the results for work rate at VO_2max (Namche - London). This figure shows a lower adjusted R^2 value of 0.691, indicating that the model is explaining 69.1% of the variability associated with the observed data.

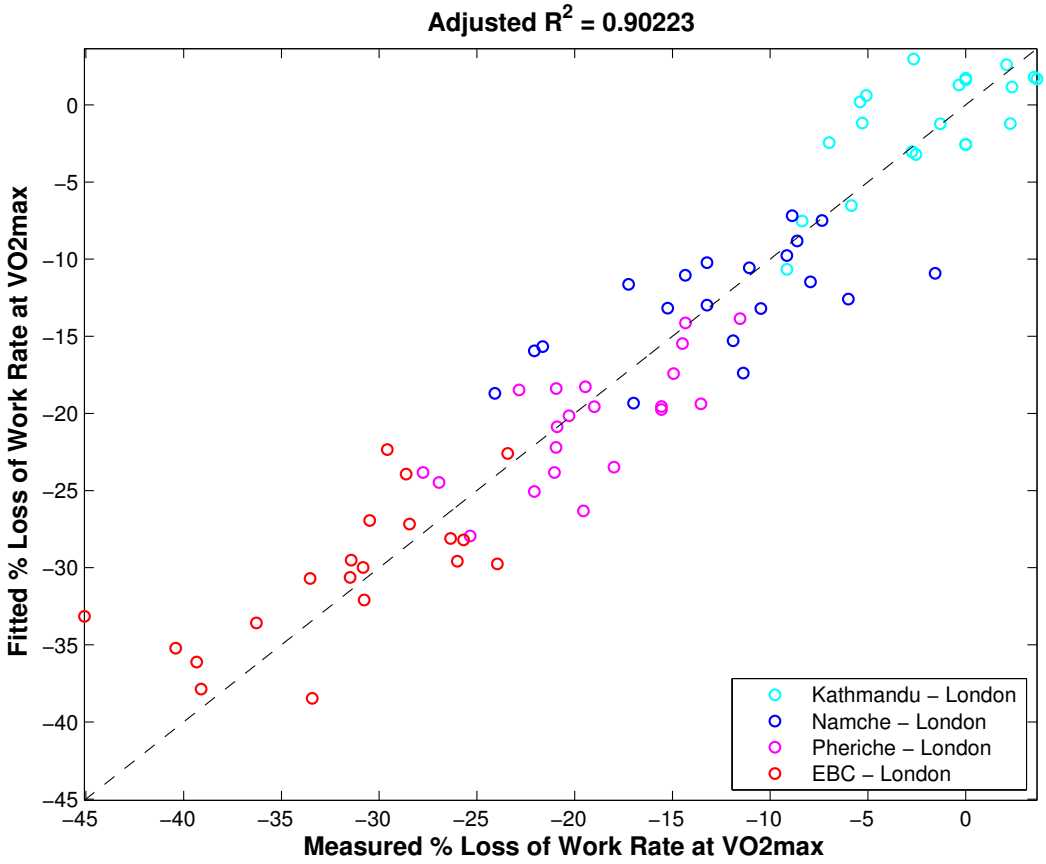


Figure 7.11: Measured vs. fitted values for a MLR for work rate at VO_2max from London levels, using the % difference in biochemical metabolites between Kathmandu and London as explanatory variables. This model shows a strong adjusted R^2 value of 0.902, indicating that the model is describing 90.2% of the variability associated with the observed data. There is an even scatter of points either side of the dashed line, indicating that the model is overestimating and underestimating values with the same probability. This is the strongest out of all of the models formed, indicated by the highest adjusted R^2 value.

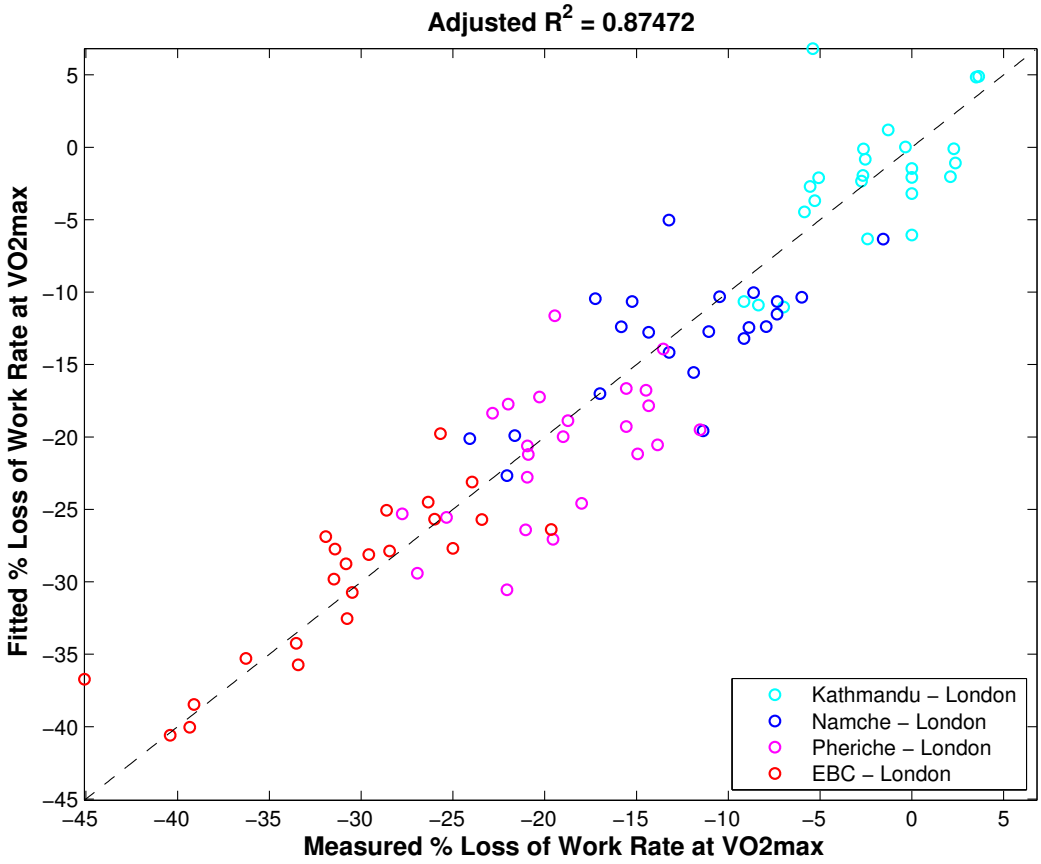


Figure 7.12: Measured vs. fitted values for a MLR for work rate at VO_2max from London levels, using the % difference in biochemical metabolites between Namche and London as explanatory variables. This model shows a good adjusted R^2 value of 0.874, indicating that it is explaining 87.4% of the variability associated with the observed data. The scatter of points around the dashed line is relatively even, indicating that the model is overestimating and underestimating values with the same probability. The model also seems to predict the largest percentage losses seen with the most accuracy, shown by the red points very close to the line at around -40% loss of work rate at VO_2max.

inflammation. In isolation, the direct relationship between each of these biochemical metabolites and work rate at VO_2max may be unclear and poor (for example the simple linear regression analysis performed for work rate at VO_2max over creatinine (shown in Figure 6.2b) shows a very poor relationship between the two variables, however, creatinine is one of the most highly-featured explanatory variables in the targeted MLR), and it is only when these metabolites are combined that they show predictive power over work rate at VO_2max. The biochemical metabolites that are used as explanatory variables in MLR on several occasions include VEGF, cGMP and EPO, which are all known to play a role in hypoxia adaptation. VEGF induces new vascular growth, to improve the blood supply in response to hypoxia, cGMP increases blood flow by acting on blood vessels to induce vascular relaxation, and EPO stimulates the production of new erythrocytes to improve the blood's oxygen carrying capacity.

Table 7.6 contains a total of 25 different biochemical metabolites required for the generalised MLR models reported. These metabolites were measured by a variety of different experimental techniques, including multiplexing assays, different ELISA kits, HPLC and a tri-iodide-based chemiluminescence assay. Some of these metabolites are relatively straightforward to measure, whereas others require substantial upfront costs for the equipment required. 15 of the metabolites were measured via different types of multiplexing assays. These are based on Luminex xMAP technology, and require a multiplexing reading machine (such as the Bio-Rad BioPlex 200) to analyse the samples. However, the actual assays are very quick and straightforward to perform, and it is possible to measure multiple biochemical metabolites in a single biological sample at the same time. The multiplexing system is a high-throughput technique, making it potentially very useful in a hospital environment after the upfront costs have been considered.

Several of the metabolites were measured via different ELISA kits, which are easy to purchase directly from the supplier, and straightforward to carry out. These assays require minimal upfront costs, if equipment such as a microplate reader is already available, and all of the non-standard solutions for the assay itself are provided with the kit. This makes these potentially the easiest out of the metabolites to replicate in a lab environment or measure in a hospital environment.

The most difficult metabolites to measure were nitrite, nitrate and RSNO. Nitrite and nitrate require a specialised HPLC machine, and nitrite and nitrate levels have only been successfully quantified in plasma samples by a handful of labs worldwide [Lauer et al., 2001; Yang et al., 2003]. RSNO was measured via a tri-iodide-based chemiluminescence assay. This again requires specialised equipment,

which may not be available in another lab or hospital environment, and would require substantial upfront costs. These samples could be sent to a specialised lab for measurement, however, the logistics of doing so, as well as a substantial wait time makes these measurements more difficult to perform for critically ill patients.

7.5.1 Model comparison

Table 7.5 shows a comparison of the four general models produced in this chapter. It summarises the biochemical measurements used as explanatory variables, the adjusted R^2 calculated between the observed data and values fitted from the model, the number of explanatory variables used in each model and whether any of these variables would be difficult to measure in another lab or hospital environment.

We want a model that will give good diagnostics, with an adjusted R^2 value close to one, a small number of explanatory variables and no metabolites that would be difficult to measure. Nitrite, nitrate and RSNO were identified in the previous section as *difficult to measure* in either another lab or a hospital environment. This is because they require substantial upfront costs for specialised equipment to measure these metabolites, as well as experience measuring them at low levels in human plasma in a high-throughput manner.

Two of the models contained metabolites that would be difficult to measure, and two did not. The two that did not were the *Kathmandu model* and the *Namche - London model*. These two models had adjusted R^2 values of 0.857 and 0.874, respectively. They contained 6 and 10 explanatory variables, respectively. Without any further analysis, these two models were considered better for application to a critical illness environment, as they would be relatively easy to measure. The *Kathmandu model* was considered the better of the two, because even though the adjusted R^2 value was slightly lower compared to the *Namche - London model*, it only contained 6 explanatory variables, compared to 10 for the other model, making it cheaper to measure the metabolites for this model. The *Kathmandu model* also only contains two metabolites that were not measured via the high-throughput multiplexing technique, whereas the *Namche - London model* contained three.

7.5.2 Further refinement

The *London* and *Kathmandu - London* models were refined, to assess the impact of removing nitrite, nitrate and RSNO from the MLR on the final model formed. Nitrite was removed from the final MLR between work rate at VO_2max and the percentage difference in biochemical metabolites between London and Kathmandu.

The resulting generalised model is shown in Figure 7.14. This model shows an adjusted R^2 value of 0.898, indicating that the model is describing 89.8% of the variability associated with the observed data. Therefore, it is only describing 0.4% less of the variability in the observed data than the original model including nitrite, shown in Figure 7.11. It does not seem necessary to include nitrite in this model, as it is relatively difficult to measure compared to other metabolites, and does not significantly improve the overall effectiveness of the model formed.

Nitrate and RSNO were removed as explanatory variables from the final MLR between work rate at VO_2max and absolute values of the biochemical metabolites. The resulting generalised model loses all predictive power, as shown in Figure 7.13. The figure shows no relationship between the observed and fitted values, indicating that nitrate and RSNO were key explanatory variables within the model. Therefore, it is not possible to use this model without these metabolites.

7.6 Conclusions

- Several models predicting oxygen consumption and work rate were formed, which showed different predictive power over the different dependent variables. For each set of biochemical metabolites (explanatory variables), one model was refined to form a *generalised model*, capable of predicting a change in a physiological measurement at several different hypoxic exposures;

- Work rate at VO_2max shows the strongest relationship with the biochemical metabolites measured, and was the most predictable physiological measurement. The usefulness of this measurement as a diagnostic still needs to be determined;

- It was not possible to form a generalised model using absolute values at Namche for the biochemical metabolites as explanatory variables;

- Six different *final* models were formed, four generalised models, and two models that could predict the response at one hypoxic exposure. These models varied in the strength of their predictions and used different combinations of biochemical metabolites as explanatory variables;

- Each of these models could be used in either a critical illness environment (to predict how well a patient will adapt if they become hypoxic), or for predicting the adaptation of a mountaineer before ascending to high altitude.

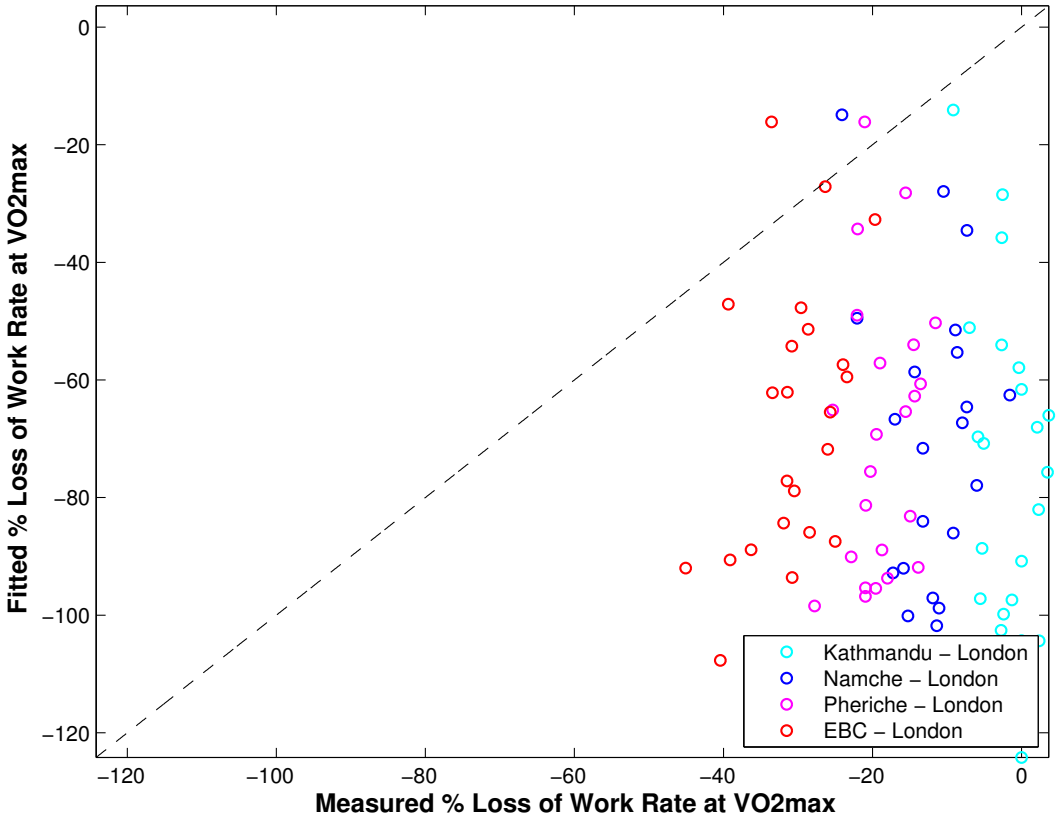


Figure 7.13: Measured vs. fitted values for a MLR for work rate at VO_2max from London levels, using biochemical metabolites at London as explanatory variables, with nitrate and RSNO removed. This model now shows no predictive power over the dependent variable, compared to Figure 7.7, where nitrate and RSNO were included as explanatory variables.

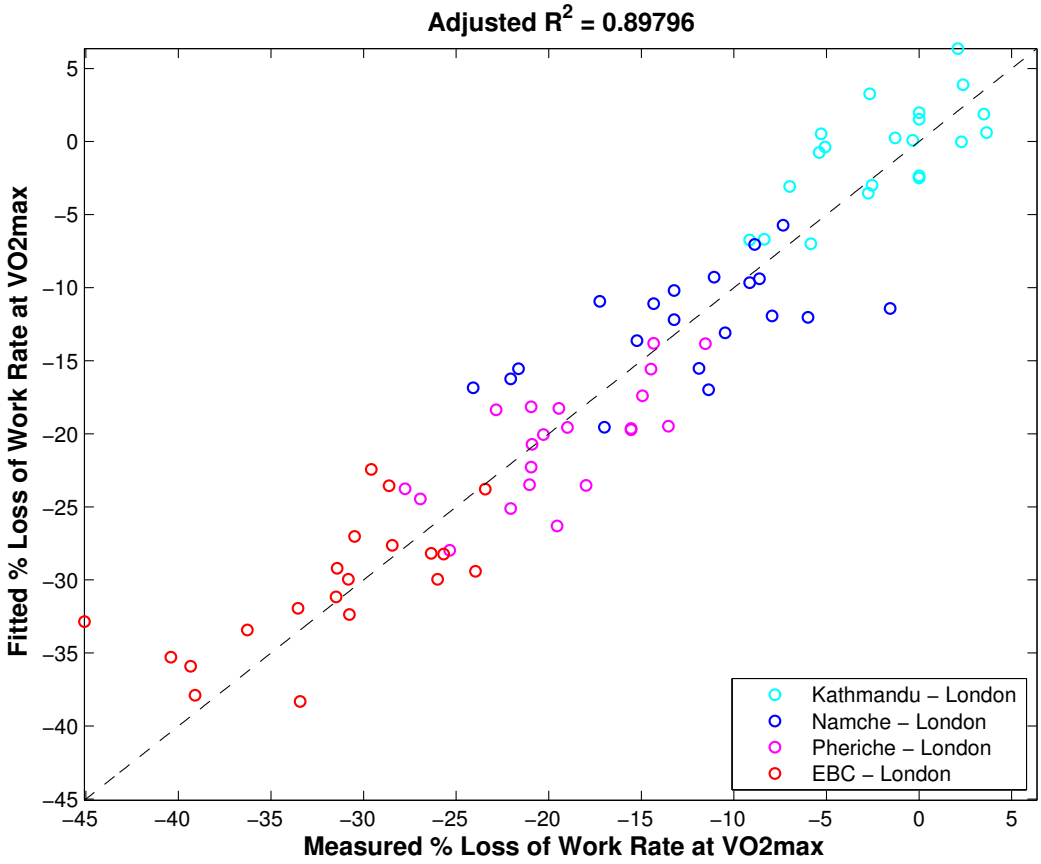


Figure 7.14: Measured vs. fitted values for a MLR for work rate at VO_2max from London levels, using the % difference in biochemical metabolites between Kathmandu and London as explanatory variables, with nitrite removed. This model still shows a strong adjusted R^2 value of 0.898, indicating that the model is describing 89.8% of the variability associated with the observed data. Therefore, it is only describing 0.4% less of the variability in the observed data, than the original model including nitrite, shown in Figure 7.11. There is an even scatter of points either side of the dashed line, indicating that the model is overestimating and underestimating values with the same probability.

The potential applications for these models are discussed further in the next chapter;

- A list of biochemical metabolites used as explanatory variables in this stage of modelling is shown in Table 7.6. Measurement of these metabolites in the 190 trekker plasma samples would allow the validation and training of the models on a larger population size. These are potentially good metabolites to measure in the XE2 study, for further validation of the models reported here.

Explanatory variables used	Model adjusted R^2	Number of explanatory variables	Are any metabolites difficult to measure?
Absolute values at London	0.812	15	Nitrate and RSNO
Absolute values at Kathmandu	0.857	6	No
Kathmandu - London % difference	0.902	8	Nitrite
Namche - London % difference	0.874	10	No

Table 7.5: A comparison of four models formed, predicting work rate at VO_2max levels at various altitudes. *Explanatory variables used* shows the variation of the biochemical metabolites used as explanatory variables in the MLR analysis. The adjusted R^2 value was calculated between the observed data and values fitted from each model. Any metabolites that would be difficult to measure in another lab or within a hospital environment have been highlighted. Ideally, the model will have an adjusted R^2 value close to one, a small number of explanatory variables and no explanatory variables that are difficult to measure.

Biochemical metabolite	Number of MLR used in	How to measure	Easy to measure in a hospital?
Creatinine	4	Bioassay	Yes
VEGF	3	Bio-Plex multiplex assay	Yes
T3	3	Millipore multiplex assay	Yes
GIP	3	Bio-Plex diabetes panel	Yes
cGMP	2	Immunoassay	Yes
8-isoPGF	2	Immunoassay	Yes
EPO	2	Immunoassay	Yes
Resistin	2	Bio-plex diabetes panel	Yes
IL-1ra	2	Bio-Plex	Yes
*Nitrite	1	HPLC	No
*Nitrate	1	HPLC	No
*RSNO	1	Tri-iodide-based chemiluminescence assay	No
HNE	1	ELISA	Yes
IL-8	1	Bio-Plex	Yes
IL-12(p70)	1	Bio-Plex	Yes
IL-13	1	Bio-Plex	Yes
IL-18	1	Bio-Plex	Yes
Adrenaline	1	ELISA	Yes
GLP-1	1	Bio-Plex diabetes panel	Yes
Leptin	1	Bio-Plex diabetes panel	Yes
Visfatin	1	Bio-Plex diabetes panel	Yes
TNF-α	1	Bio-Plex diabetes panel	Yes
Adipsin	1	Bio-Plex diabetes panel	Yes
Insulin	1	Bio-Plex diabetes panel	Yes
Protein Content	1	Assay	Yes

Table 7.6: Biochemical metabolites used as explanatory variables in multiple linear regression analysis for work rate at VO_2max. Most metabolites were measured in a relatively straightforward manner with various assay kits. Measurement of many of the cytokines require a multiplexing assay machine. The metabolites marked with an asterisk (*) show metabolites that would be difficult to measure in another lab environment or hospital, without substantial upfront costs for the equipment, and specialised training.

Chapter 8

Discussion

This chapter brings together the key findings from the project, discusses how the models could have been improved, any further work that can be done on these results, and the applications of the final models developed. It also describes the limitations to the study, and gives proposals for the follow-up study, XE2, in 2013.

8.1 Data analysis and model building

Several stages of data analysis and modelling have been carried out during this thesis project, to gain an appreciation of the dataset and make the most out of the modelling performed. Chapter 4 described the assessment of the diary data, which contained a small number of simple physiological measurements, to determine their day-to-day reliability, and identify measurements that may be useful for modelling. The results from this chapter could be taken further, by the inclusion of the most reliable measurements as additional explanatory variables within modelling efforts.

Chapter 5 described the assessment of the main core dataset, to identify possible patterns and relationships within the different measurements available. This analysis was used to identify the physiological measurements that were potentially the most appropriate as dependent variables, and the biochemical metabolites that were potentially the most appropriate as explanatory variables.

Chapter 6 described the initial stages of modelling, looking at the prediction of several physiological measurements identified in Chapter 5 with a combination of one or more biochemical metabolites, across all different altitudes available. These methods identified physiological measurements that were predictable with the explanatory variables available, and biochemical metabolites that were used as explanatory variables in the MLR. Metabolites from the NO and ROS pathways (as

detailed in Sections 1.4.1 and 1.4.2) were not as dominant in the modelling as anticipated, however, there were several biochemical metabolites that were present in multiple models formed.

Chapter 7 described the final *targeted* stage of modelling, looking at predicting the difference in an explanatory variable at a certain altitude or hypoxic exposure, compared to sea level. Six different models were formed using different combinations of biochemical metabolites as explanatory variables, as described in Section 7.1. Several biochemical metabolites were present as explanatory variables in multiple models formed. This resulted in the formation of a list of biochemical metabolites to measure in the trekker plasma samples, detailed in Table 7.6.

8.2 Applications of the fitted models

The models formed in Chapter 7 are capable of predicting the difference in work rate at VO_2max in a range of altitudes or hypoxic exposures, compared to sea level. Work rate at VO_2max was used as the main dependent variable, as it was the most predictable from the explanatory variables available. Differences in work rate at a certain altitude (level of hypoxia) compared to sea level (normoxia) were used as a proxy for hypoxia adaptation, with individuals showing a small difference compared to sea level considered to be adapting better to the lack of oxygen available compared to individuals who show a larger difference.

The models produced could be used in different situations, depending on the condition of the patient, and the information available. Two other models were also produced, looking at predicting the percentage loss of oxygen consumption at VO_2max (normalised for body weight) between Everest Base Camp and London, and the percentage loss of work rate at VO_2max between Namche and London. These models were more specific, and only allowed the prediction of oxygen consumption or work rate at a high level of hypoxic stress.
There were three different *types* of model produced, as follows;

- The *London model* and *Kathmandu model*, produced using absolute values of the biochemical metabolites at London or Kathmandu as explanatory variables, to predict the percentage change in work rate at VO_2max for several hypoxic exposures/altitudes, as described in Sections 7.4.1 and 7.4.2;
- *Two Namche models*, produced using absolute values of the biochemical metabolites at Namche (3500m) as explanatory variables, to predict the percentage change in oxygen consumption at VO_2max (normalised for body weight) be-

tween Everest Base Camp and London, and work rate at VO_2max between Namche and London, as described in Section 7.4.3;

- The *Kathmandu - London model* and *Namche - London model*, produced using the percentage difference of the biochemical metabolites between Kathmandu and London or Namche and London as explanatory variables, to predict the percentage change in work rate at VO_2max for several hypoxic exposures/altitudes, as described in Sections 7.4.4 and 7.4.5.

Potential applications for each of these models are described below. The choice of which model would be appropriate to use depends on the status of the patient and the information available. Figure 8.1 shows a flowchart that could be used for critically ill patients to decide which model would be most appropriate.

8.2.1 London model

The *London model* was refined in detail, as it was considered the most versatile of the models produced. It uses absolute values for the biochemical metabolites at London as explanatory variables, along with an oxygen saturation value to calculate the percentage loss of work rate at VO_2max at four different hypoxic levels. The model has a good predictive power, with an adjusted R^2 value of 0.812 - indicating that the model is describing 81.2% of the variability associated with the observed data. Figure 7.7 shows the observed values plotted against the values fitted for the model.

This model could be used in a critical illness environment by taking a blood sample immediately after a patient arrives in the ICU, and the results could be used to predict whether the patient will have any problems adapting if they become hypoxic. This model could also be used to predict how well mountaineers will adapt to extreme hypoxic conditions during a climb, by taking a blood sample at a sea-level testing centre. This model would be the easiest to validate during the XE2 trek, by taking blood samples during sea level testing, measuring the 15 required metabolites (shown in Section 7.4.1), predicting the individual's performance, and then comparing this to the actual performance recorded at altitude. However, this model contains the highest number of metabolites out of all of the models formed, and also contains two metabolites that are potentially difficult to measure in a critical illness environment - nitrate and RSNO, which require specialised equipment and training to measure, as described in Section 7.5.2. Removal of these metabolites from the London model removes all of the predictive power, and are therefore vital

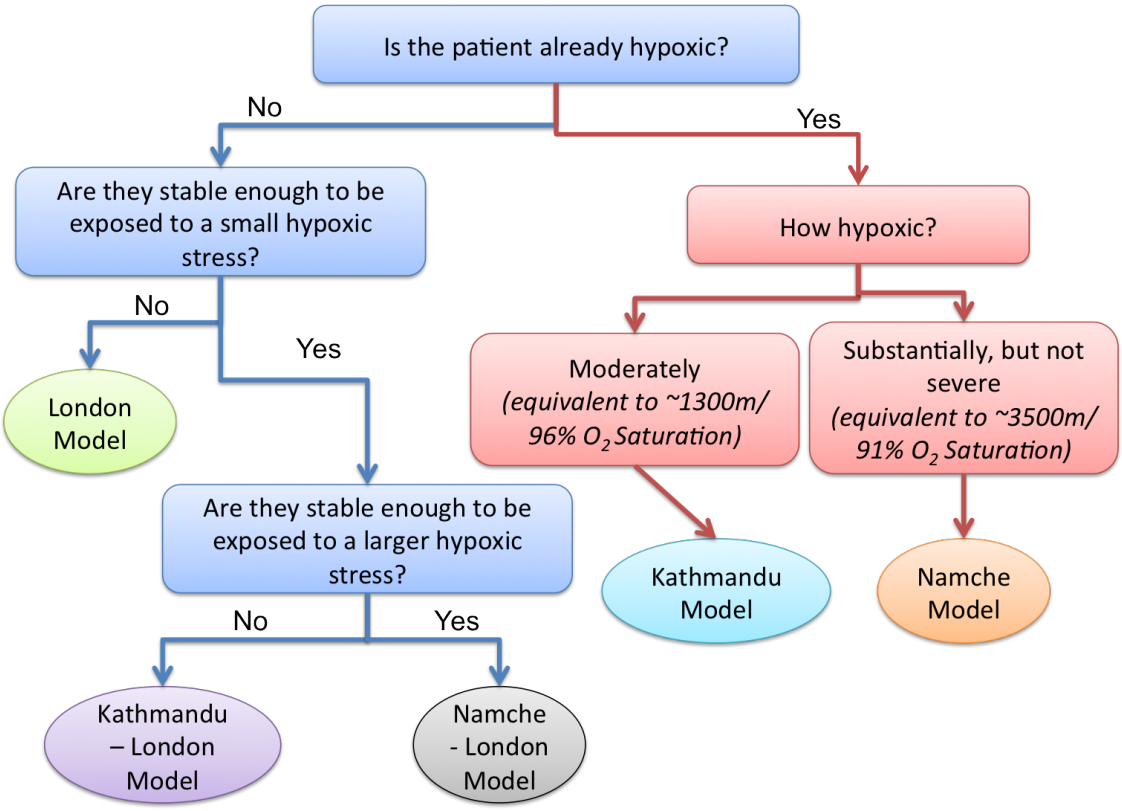


Figure 8.1: A flowchart providing guidance as to which of the predictive models would be most suitable for a critically ill patient.

for the model. This limits how useful this model will be, and would only be useful in situations where it would be possible to measure plasma nitrate and RSNO levels.

8.2.2 Kathmandu model

This model uses absolute values for the biochemical metabolites at a moderate level of hypoxic exposure (similar to an exposure to Kathmandu at 1300m, around 96% blood oxygen saturation) as explanatory variables. This is combined with an oxygen saturation value to predict the percentage loss of work rate at VO_2max at four different hypoxic levels. The model has a high predictive power, with an adjusted R^2 value of 0.857, indicating that it is explaining 85.7% of the variability associated with the observed data. Figure 7.9 shows the observed values plotted against the values fitted for the model.

This model would be suitable for critically ill patients who enter the ICU already slightly hypoxic, and would allow the doctor to ascertain how well the patient will adapt to further hypoxic exposure. This particular model would be suited to use in a hospital environment, as all of the explanatory variables are easy to measure in simple lab assays, as described in Section 7.5. This model contains six explanatory variables, which is the smallest number for any of the models formed.

This model could also be used for mountaineers, where a blood sample could be taken at an altitude similar to Kathmandu at 1300m, and be used to predict how well the individual will cope with the ascent. This particular model would be a good target for further refinement, to assess the importance of each of the explanatory variables within the model.

8.2.3 Namche models

Two separate Namche models were produced, one capable of predicting the percentage loss for oxygen consumption at VO_2max (normalised for body weight) between Everest Base Camp and London, the other capable of predicting the percentage loss of work rate at VO_2max between Namche and London. Oxygen consumption was identified alongside work rate as a good potential proxy for hypoxia adaptation, as described in Section 7.2. Both of these models used absolute values of the biochemical metabolites at Namche (3500m) as explanatory variables. A generalised model was not produced for this particular explanatory variable combination, as it was not possible to produce a model with enough predictive power from the explanatory variables available, as described in Section 7.4.3. Therefore, these models do not include the effect of oxygen saturation (which is included in all of the other models

produced), and are solely based on the absolute biochemical values at Namche as explanatory variables.

The oxygen consumption model shows a very strong predictive power, with an adjusted R^2 value of 0.996, indicating that it is explaining 99.6% of the variability associated with the dependent variable. Figure 7.10a shows the observed values plotted against the values fitted for the model. The work rate model shows a lower predictive power, with an adjusted R^2 value of 0.691, indicating that it is explaining 69.1% of the variability associated with the dependent variable. Figure 7.10b shows the observed values plotted against the values fitted for the model. These models have a very limited use, as it is only possible to predict how well an individual will adapt to a certain hypoxic exposure, which is quite high in both cases. However, the high predictive power of the oxygen consumption model means that the model could potentially be applied in the case of an individual who is already very hypoxic, to see how they will respond if exposed to extreme hypoxia. For example, this model could perhaps be applied before a hypoxic patient was to undergo surgery or receive a treatment that would increase their hypoxic state, to see if they will have any difficulties coping with the higher level of hypoxia.

8.2.4 Kathmandu-London model

This model uses the percentage difference in biochemical metabolites between Kathmandu and London to predict the percentage loss of work rate at VO_2max over a range of hypoxic exposure/altitudes. This model has very good predictive power, with an adjusted R^2 value of 0.902, indicting that the model is explaining 90.2% of the variability associated with the observed data. Figure 7.11 shows the observed values plotted against the values fitted for the model. This model includes 8 explanatory variables, including nitrite, which requires specialist equipment and training to measure. The removal of nitrite from the model reduces the adjusted R^2 value to 0.898, and may allow the wider use of this model, as described in Section 7.5.2. This model has the highest adjusted R^2 value out of any of the generalised models produced.

This model would be suitable for critically ill patents who are stable enough to be exposed to a small amount of hypoxic stress. When the patient first arrives at the ICU a blood sample would be taken, and the individual would then be exposed to a reduced amount of oxygen via an oxygen mask, equivalent to an altitude of 1300m. After exposure, a new blood sample would be taken, and the percentage change in 7 biochemical metabolites would be used to predict how well that individual will adapt to further hypoxic exposure. This particular model could also be used for

mountaineering, with individuals having blood samples taken at sea level before they travel, and then after they have arrived at a higher altitude of around 1300m. The individual could also be exposed to an altitude of 1300m in a hypoxic chamber (the same exposure they have during the flight to Kathmandu). The percentage difference between these two measures could then be used to predict how well that individual would cope with ascent to high and extreme altitude. This model is only useful if an individual is able to be exposed to a small amount of hypoxia, which should not cause too much stress to the individual. There is also an ethical consideration here, as it is not always possible or justifiable to expose all patients to hypoxic stress, when they are already seriously ill.

8.2.5 Namche-London model

This model uses the percentage difference in biochemical metabolites between Namche and London as explanatory variables, in combination with an oxygen saturation measure, to predict the percentage loss of work rate at VO_2max over a range of hypoxic exposures. This model has good predictive power, with an adjusted R^2 value of 0.875, which indicates that it is explaining 87.5% of the variability associated with the observed data. Figure 7.12 shows the observed values plotted against the values fitted for the model. This model uses 10 explanatory variables, which are all relatively easy to measure in a clinical environment. However, this model requires the individual to be exposed to quite a high level of hypoxia, and requires a sea-level measurement. This model may be most suitable for critically ill patients who had a blood sample taken when they first arrived at the hospital, and are becoming quite severely hypoxic (i.e. in a peri-operative setting). An additional blood sample could then be taken, and the biochemical measurements compared to sea level to predict how well that individual will respond to increased hypoxic exposure. This model may also be of use as a hypoxic-stress test for climbers who may be more susceptible to developing AMS symptoms in the field.

8.2.6 Combined test

If one or more of these models proves to be useful in a clinical environment, then it may be possible to produce a *combined assay*, which would test all of the required biochemical metabolites in a single blood sample (similar to the multiplexing capabilities of the Bio-Plex). A blood sample would be taken by a member of staff, measured in the hospital lab, and the results transferred to a hand-held device, which would take the information and calculate the predicted adaptability for the patient

via one or more models. This information would be displayed in an easy-to-use format on the device, to allow the swift transition from blood sample to prediction for patients in a critical illness environment, where time is always a limiting factor.

8.2.7 Explanatory variables in multiple models

There were several biochemical metabolites that were used in multiple models. It was expected that metabolites from the NO and ROS pathways would feature in the models formed, due to the known links with hypoxia, as described in Section 1.4.1.1 and 1.4.2. However, metabolites that were used in multiple models included very traditional measurements such as protein content, osmolality and creatinine. Measures such as protein content and osmolality were originally used in this project as controls. Creatinine was used as a measure of kidney function, however, many groups now favour Cystatin C over creatinine as it is more *specific*, but also more expensive to measure than creatinine. It is interesting that reasonably simple measures would play such an important part in the analysis, however, the underlying reasons are currently unknown. It may be that less specific metabolites show the integrated response of multiple body functions compared to more specific metabolites.

8.2.8 Other applications

The models formed could also be used in the area of sports medicine, to predict how well an individual will perform (during higher physiological stress or hypoxic training) at a higher altitude. This would improve the safety of the *train high, sleep low* method of training, where athletes train at high altitude to improve their performance at sea level, whilst sleeping at a lower altitude to maintain their blood oxygen saturation. These models could allow the assessment of how well individuals will perform at different altitudes, to advise which altitude would be best for a particular individual to train at, to get the most out of the training without becoming too severely hypoxic.

8.3 Extension and modifications of the modelling approach

There are several ways in which the modelling undertaken during this project could be expanded to improve on the initial models formed. These include:

1. Looking at producing a model using the difference between Namche - Kathmandu for the explanatory variables, for patients who are already moderately

hypoxic, and are stable enough to be exposed to a higher level of hypoxia;

2. Looking at further refinement of all of the models produced, to assess the impact of removing each variable, with the aim of *stream-lining* each model;

3. Only the strongest models were refined for each set of explanatory variables, due to time limitations and to increase the likelihood of meaningful results. It is possible that the refinement of one of the other models produced for each set of explanatory variables may have more predictive power than the models reported here;

4. The possibility of log-transforming specific explanatory variables, to reduce the impact of observations with high leverage, as described in Section 6.4.2;

5. The inclusion of simple physiological measurements as additional explanatory variables may help to explain more of the variability associated with the dependent variable of interest. These would include measurements that are easily acquired and in most cases already available for critically ill patients, and are known to be *reliable* due to the diary data analysis;

6. Targeted MLR on other physiological measurements of interest identified during model exploration, such as physiological measurements identified in the diary data analysis as showing differences for ill individuals. These models may be less powerful, but more biologically useful;

7. Assessment of non-linear responses, which would require additional altitude-points;

These are all relatively straight-forward extensions to the analysis performed, however, these were not possible to include as a part of this thesis due to time constraints.

8.3.1 Validation and testing

The validity of any model formed is based on the *reliability* of the data it is constructed from. If the data is unreliable, then any model formed from this data will also be unreliable. Assessing the impact of outlying values requires an understanding of the biological metabolites being assessed. Ideally, the values obtained for the CXE samples would be compared to other studies, to determine whether or not the values were within the *normal biological range*, or were truly erroneous. However, observed values for biochemical metabolites vary widely between studies, and can

depend on the technique used to measure the samples. Additionally, the nature of the CXE study is that the individuals were exposed to high levels of hypoxic and environmental stresses. Therefore, what may seem an erroneous datapoint for normal sea level samples, may be within an expected response to exposure to extreme hypoxia. Many of these measurements have never been acquired from individuals exposed to such conditions before, making them *unique*, and therefore very difficult to formally compare to values obtained from other studies.

Ideally, the model would be cross-validated using an independent data set, however, a second data set was not available for this analysis. The original dataset could have been split, with the model being based on one half of the data and verified with the second half. However, due to the small sample size, it was decided to use all of the data available in model building, in order to produce the most accurate model possible. The next stage to this analysis will be to measure the biochemical metabolites identified in Table 7.6 in the *trekker* plasma samples. This will allow the validation of the models in a much larger sample size of 190 compared to the 24 that the models were originally based on. The model can also be validated in the follow-up XE2 study.

8.4 Limitations

8.4.1 The design of CXE 2007

CXE 2007 was planned and conducted by the CXE team, without any plan to measure the range of biochemical metabolites that have been assessed within this project. The CXE team did not set up the collaboration with the Feelisch lab until a year after the expedition had taken place, and so it was not possible for us to have any input into the design of the study, including the timing and number of samples taken, which is the main limitation to the modelling performed. Whilst this is not ideal, it does not mean that the data acquired from the biochemical analysis of plasma samples is not valid. Xtreme Alps 2010 was a follow-up expedition designed and conducted in direct collaboration with the Feelisch lab. This expedition incorporated intervention studies with control groups (such as supplementary nitrate consumption), and allowed the acquisition of more directly applicable data. Similar additions are being considered for the large direct follow-up study to CXE 2007, denoted XE2, due to take place in 2013.

8.4.2 Performance at altitude

This project is based on the assumption that *performance at altitude* in healthy individuals can be used as a proxy for hypoxia adaptation, and been used to generate models capable of assessing the potential response to hypoxia in critically ill patients. There may be other measures that are more suitable as a measure of hypoxia adaptation, however, this assumption forms the basis of the modelling for this project. If this assumption does not hold, then the models will still be applicable to mountaineers, to predict how well they will perform physically at altitude. However, the application to critically ill patients would be more limited. The models formed need to be *calibrated* on data from critically ill patients, to test the assumptions made, and assess whether the models can be adapted for a critical illness environment.

8.4.3 Self-selection

The core team members were a group of highly selected individuals. This means that their fitness is well above the average for the general population, and they had previous experience at altitude, which can aid in faster acclimatisation [McArdle et al., 2007]. The trekker data contains information for 190 individuals, who have had much less experience at altitude and may not be as physically fit as those from the core group. However, this group is still self-selecting, as it only contains individuals who had an interest in the expedition and who considered themselves fit enough to participate. It is expected that the trekker group of individuals will show responses that will be closer to critically ill individuals, however, there may still be large differences between the groups. It is important to understand this limitation when assessing the results, and making comparisons to the *normal population*.

8.4.4 Translation of results

As the core individuals were a self-selected group, the training and validation of the models for the trekker data will be vital in producing a model that is more applicable to the population as a whole. These refined models should also be more applicable to a critical illness environment than the original models formed, as they are based on a *less* selected group of individuals. The models will then need to be re-trained and re-validated once again to see how applicable they are to critical illness environment. It may be the case that the models produced here are very good at predicting the responses for healthy climbers, but are not applicable to critically ill patients. However, if hypoxia is an overriding theme in critical illness, as it is assumed to be by the CXE team, then the models produced should *hold* to a certain

degree when applied to critically ill patients, but further work is needed to calibrate the results of this study to a critical illness environment.

8.4.5 The role of other environmental factors

During the expedition, the team would have been exposed to multiple environmental stresses. The main stress of interest to this study is hypoxia, however, other factors such as high UV exposure, low environmental temperatures and the physical exertion required to ascend would all have a pronounced effect on the biochemistry and physiology of the participants as they ascended Mount Everest. Critically ill patients are similarly exposed to multiple stresses such as hypoxia, inflammation and infection, but the nature of these stresses is different. The aim of CXE 2007 was to study healthy volunteers exposed to extreme environments, to increase our understanding of the complex response pathways that occur within critically ill patients. However, the overlapping of these response pathways also adds an extra layer of complication to our understanding of the adaptation process.

The stresses that critically ill patients are exposed to are similar to those experienced by the CXE participants, however, they would not be directly comparable. In addition, critically ill patients vary in their stress exposure. We do not know whether some of the changes seen in the participants are due to low oxygen levels, high UV levels, strenuous exercise, or some other factor. It is important to understand that this is both an asset and a limitation of this study. Additional studies involving the assessment of such factors in isolation would allow the modelling to account for the factors that cannot be changed during the expedition. When analysing any results it is important to be aware of this problem, and of the effects it may have on any inferences made from the data.

8.5 Suggestions for XE2

1. The main limitation to this modelling analysis has been the number of altitude/hypoxic stress points. The models produced assessed the change between London and Everest Base Camp week one, which meant that only four data points were available for model building. More levels of hypoxic exposure would allow more accurate modelling of changes that occurred during the expedition, improve the accuracy of the model and improve the application to environments such as critical illness. It would also allow the analysis of non-linear relationships within the dataset, which is not realistic to do using only four levels of hypoxic exposure;

2. The acquisition of whole blood samples would allow the analysis of additional metabolites in white or red blood cells, which are not present in the plasma;

3. The inclusion of intervention studies, similar to those conducted during Xtreme Alps, which looked at the role of supplementary nitrate during the expedition, and allow the analysis of this particular metabolite in the adaptation to hypoxia during the expedition;

4. Oxygen intervention studies, would allow the assessment of the role supplementary oxygen can play during an expedition such as CXE (i.e. does supplementary oxygen during the ascent help, hinder or have no effect on the individuals?)

8.5.1 Controls

Scientific controls allow for the comparison of different hypotheses, and aid in validating any conclusions that are drawn from the results of an experiment. It is more difficult to incorporate suitable controls into some experiments than others, due to the logistics or cost of some experiments. It is important within scientific experiments to control as many variables as possible, to allow the assessment of whether one attribute is responsible for the changes seen during an experiment.

The inclusion of additional controls into a future expedition such as XE2 would increase the amount of information that could be gained from the experiments. Additional controls might include:

1. **Hypoxic chambers**: Simulating an ascent to high altitude using an identical ascent profile in a hypoxic chamber would allow the assessment of the hypoxic response to a change in oxygen availability in the air, whilst minimising other potential confounding factors such as UV, temperature and physical exertion during a climb. The same individual could be assessed during the ascent and then at a later date in the chamber (once the individual had sufficient time to lose acclimatisation), to allow the direct comparison of results obtained;

2. **Additional oxygen**: A subset of individuals ascending could ascend using oxygen masks for the entire expedition, so that they are solely exposed to all *other* stresses encountered during the ascent. A subset of these tank-carrying individuals would also need to breathe ambient air without their knowledge, to test for the effects of utilising a mask system whilst ascending. However, it would not be possible to send the same individual on the ascent twice, both with and without an oxygen mask. Therefore, participants could be *paired*

(one individual with a mask, and one without) based on their fitness and previous experience, to allow the most direct comparison as possible.

Such additional controls would allow a more refined assessment of the biochemical and physiological changes associated with the hypoxic response, and the other stresses induced by the expedition itself. Such controls would only be possible with a small number of individuals, due to the high costs of running a hypoxic chamber, and the logistical issues associated with using oxygen tanks during the ascent. Additional sherpas would also be required to carry the oxygen bottles during the ascent, so that the individuals are not exercising much more than non-oxygen wearing participants by carrying their own oxygen tanks during the ascent.

The extra information that would be gained from these additional controls would have to be weighed against the additional costs and logistics that would be required to incorporate them. These controls would undoubtedly give extremely valuable data, however, whether they would be possible to do in large enough numbers to make comparisons statistically significant is also unclear.

8.6 Conclusions and outlook

Current tests in medicine use very specific measures, which can be expensive, and may not necessarily be *better* in a model than a seemingly similar metabolite. My modelling results support that the use of 'simple' metabolites, which integrate several systems including skeletal, cardiac and pulmonary performance, may be preferable for modelling compared to selective measures of specific organs. The cost and ease in which a particular metabolite can be measured also play a role in determining which metabolites are the most *useful* in modelling.

My results show that sometimes it is best to apply a simple method to a dataset, to see what the data can tell us before using complex methodology which requires a large number of assumptions. The results can sometimes be surprising! It was initially thought that due to the complex nature of the responses seen by these individuals during the expedition, it was unlikely that linear relationships would be present, which seemed the case when looking at simple linear regression in Section 6.2. However, the model fits from the MLR are surprisingly good, and show some potentially valuable applications. The metabolites used within the models as explanatory variables would not have been predicted by knowledge of the biochemical pathways alone, and shows the power of correctly-applied statistical analysis of biological data. These results can now be used to inform the analysis of the trekker plasma samples, to allow the validation and re-training of the models formed on a

larger sample size. Figure 8.2 shows the cyclic nature of the complete analysis of this data, highlighting the strength of a systems biology approach to looking at this type of dataset. This type of approach is perhaps reminiscent of the words of one of the greatest British mountaineers of all time:

In 1923, George Mallory climbed Mount Everest. When asked "Why climb Mount Everest?" he replied "Because it's there."

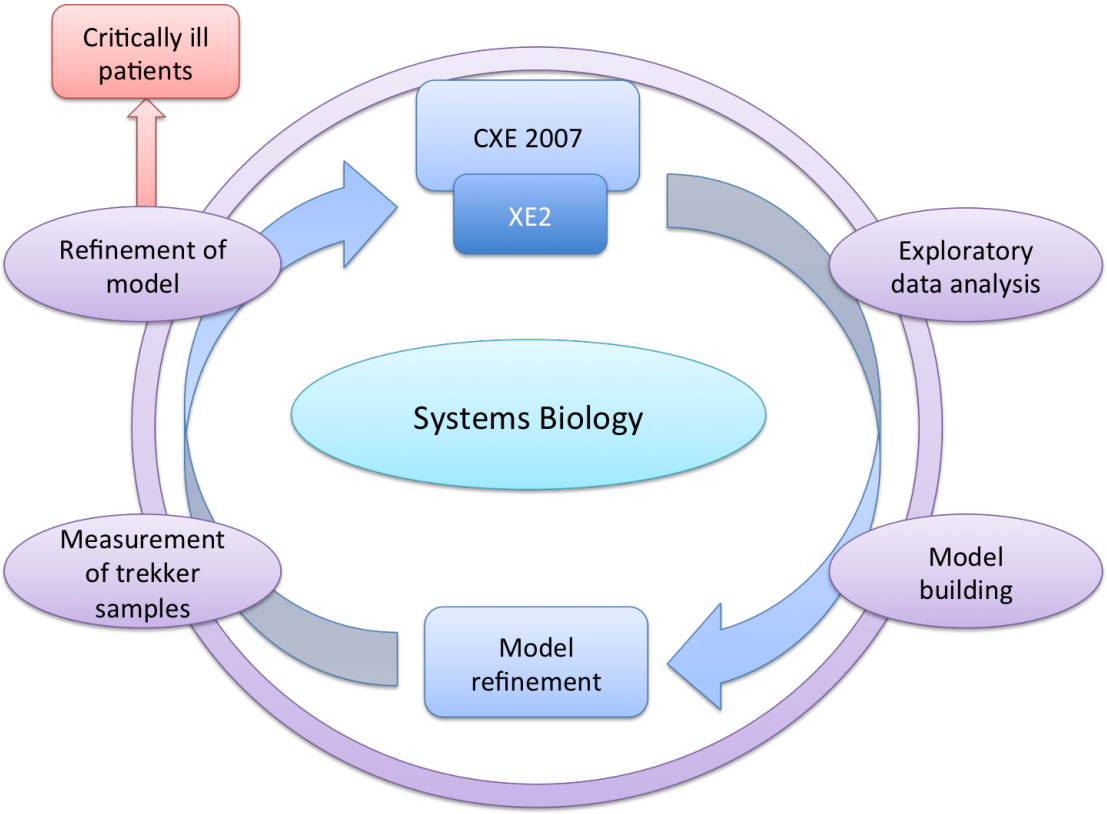


Figure 8.2: The Systems Biology approach used during this thesis project - data collection from CXE 2007, exploratory data analysis, model building, model analysis and refinement, measurement of trekker samples based on information from the modelling and refinement of the model based on the trekker data. This model would then be used to inform XE2 and be calibrated for use for critically ill patients. It was only possible to get to the stage of model refinement in this thesis project, and the next steps would be to measure the trekker plasma samples, and use the information to validate the models reported .

Chapter 9

Appendices

9.1 2-way ANOVA results

	ANOVA (Altitude)		ANOVA (Individual)	
Variable	F-value	p-value	F-value	p-value
Nitrite (mM)	22.07	• 8.6E-13	2.25	• 3.5E-03
Nitrate (mM)	1.18	3.2E-01	1.14	3.2E-01
Total NOx (mM)	1.21	3.1E-01	1.14	3.2E-01
RSNO (nM)	18.01	• 5.9E-11	2.04	• 9.1E-03
RNNO (nM)	11.85	• 8.3E-08	2.88	• 1.9E-04
Total RxNO (nM)	18.19	• 4.9E-11	2.90	• 1.7E-04
cGMP (pmol/mL)	9.21	• 2.7E-06	2.65	• 5.4E-04
8-isoPGF (ng/mL)	7.96	• 1.5E-05	1.49	9.6E-02
HNE (mg/mL)	4.76	• 1.7E-03	3.18	• 7.6E-05
GSH (micromolar)	15.40	• 9.1E-08	1.09	3.8E-01
GSSG (micromolar)	6.89	• 4.2E-04	0.94	5.5E-01
Total Glutathione (micromolar)	3.01	• 3.6E-02	0.62	9.0E-01
GSH to GSSG ratio	8.83	• 5.4E-05	1.37	1.6E-01
IL-1beta (pg/mL)	1.03	4.0E-01	9.92	• 5.3E-11
IL-1ra (pg/mL)	3.70	• 7.7E-03	1.86	• 2.1E-02
IL-6 (pg/mL)	0.30	8.7E-01	6.73	• 1.4E-11
IL-8 (pg/mL)	3.12	• 1.9E-02	1.01	4.6E-01
IL-10 (pg/mL)	1.18	3.5E-01	1.01	5.0E-01
IL-12(p70) (pg/mL)	8.38	• 8.3E-06	12.85	• 9.5E-20

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9.1 – continued from previous page

	ANOVA (Altitude)		ANOVA (Individual)	
Variable	F-value	p-value	F-value	p-value
IL-13 (pg/mL)	9.47	• 1.9E-06	4.38	• 1.9E-07
IL-18 (pg/mL)	8.82	• 4.5E-06	18.34	• 4.7E-25
MIF (pg/mL)	11.05	• 2.3E-07	3.37	• 1.8E-05
Eotaxin (pg/mL)	4.39	• 2.7E-03	11.44	• 4.0E-18
TNFalpha (pg/mL)	3.50	• 1.1E-02	8.38	• 4.1E-14
VEGF (pg/mL)	9.86	• 1.1E-06	6.07	• 1.7E-10
CRP (ng/mL)	2.90	• 2.6E-02	1.23	2.4E-01
Adrenaline (ng/mL)	24.69	• 6.7E-14	0.89	6.1E-01
Noradrenaline (ng/mL)	5.57	• 4.6E-04	1.61	5.9E-02
T3 (pg/mL)	5.05	• 1.0E-03	11.66	• 2.1E-18
T4 (pg/mL)	1.68	1.6E-01	7.89	• 2.1E-13
C-Peptide (pg/mL)	4.46	• 2.4E-03	2.61	• 6.4E-04
GIP (pg/mL)	1.42	2.3E-01	1.95	• 1.4E-02
Ghrelin (pg/mL)	1.65	1.7E-01	15.47	• 1.9E-22
Glucagon (pg/mL)	10.70	• 3.7E-07	7.27	• 1.9E-12
GLP-1 (pg/mL)	5.59	• 4.5E-04	8.64	• 1.7E-14
Insulin (pg/mL)	8.21	• 1.0E-05	8.30	• 5.3E-14
Leptin (pg/mL)	8.82	• 4.5E-06	9.89	• 3.4E-16
PAI-1 (pg/mL)	13.83	• 7.5E-09	1.95	• 1.4E-02
Resistin (pg/mL)	9.25	• 2.5E-06	4.39	• 1.8E-07
Visfatin (pg/mL)	0.50	7.4E-01	9.98	• 2.6E-16
Adiponectin (pg/mL)	6.75	• 8.3E-05	13.94	• 6.4E-21
Adipsin (pg/mL)	28.31	• 2.5E-15	3.89	• 1.7E-06
EPO (mlU/mL)	6.26	• 1.7E-04	3.63	• 5.6E-06
ET-1 (pg/mL)	1.79	1.4E-01	34.19	• 2.2E-35
Glucose (mM)	4.78	• 1.5E-03	3.18	• 4.4E-05
Cystatin C (ng/mL)	28.37	• 2.6E-15	2.40	• 1.8E-03
Creatinine (mg/dl)	6.86	• 7.1E-05	6.15	• 1.3E-10
Lactate (mM)	5.97	• 2.7E-04	1.87	• 2.1E-02
Osmolality (mOsmo/kg)	1.57	1.9E-01	1.48	9.8E-02
Protein Content (mg/mL)	3.87	• 6.0E-03	1.52	8.5E-02
Bicarbonate (mmol/L)	35.86	• 4.9E-18	1.89	• 1.8E-02
HSP-70 (ng/mL)	1.02	4.0E-01	7.14	• 3.0E-12

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	ANOVA (Altitude)		ANOVA (Individual)	
Variable	F-value	p-value	F-value	p-value
Barometric Pressure	95623.60	• 9.4E-161	1.71	• 3.9E-02
Ambient temperature	40.31	• 3.1E-19	0.94	5.4E-01
Economy	9.83	• 1.2E-06	2.81	• 2.7E-04
Maximum voluntary ventilation	27.34	• 7.4E-15	23.90	• 6.6E-29
Forced expiratory volume in 1 second	2.16	8.0E-02	59.65	• 1.1E-44
Haemoglobin	60.25	• 6.9E-25	3.92	• 1.5E-06
Blood oxygen content	1.86	1.2E-01	4.55	• 9.0E-08
Haematocrit	57.79	• 2.7E-24	5.60	• 1.1E-09
Resting oxygen consumption	5.18	• 8.5E-04	10.49	• 1.1E-16
Resting oxygen consumption (/kg)	7.24	• 4.3E-05	4.99	• 1.7E-08
Resting respiratory exchange ratio	7.13	• 5.0E-05	2.64	• 5.9E-04
Resting heart rate	24.58	• 1.0E-13	13.88	• 1.8E-20
Resting minute ventilation	46.64	• 4.5E-21	7.21	• 3.5E-12
Resting carbon dioxide production	5.32	• 6.8E-04	9.47	• 2.2E-15
Respiratory equivalent for oxygen at rest	76.03	• 6.9E-28	4.33	• 2.8E-07
Respiratory equivalent for carbon dioxide at rest	139.01	• 2.1E-37	7.81	• 4.5E-13
Resting oxygen pulse	1.52	2.0E-01	10.72	• 5.8E-17
Resting respiratory rate	15.29	• 1.5E-09	6.65	• 2.7E-11
Resting tidal volume	9.28	• 2.6E-06	5.08	• 1.2E-08
Partial pressure end tidal oxygen	2702.62	• 5.5E-92	7.48	• 1.4E-12
End tidal partial pressure carbon dioxide	319.59	• 6.8E-52	12.99	• 1.5E-19
Work rate at LaT	42.53	• 6.7E-20	6.90	• 1.1E-11
Oxygen consumption at LaT	41.83	• 1.1E-19	7.11	• 5.0E-12

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9.1 – continued from previous page

	ANOVA (Altitude)		ANOVA (Individual)	
Variable	F-value	p-value	F-value	p-value
Oxygen consumption at LaT (/kg)	40.69	• 2.4E-19	4.72	• 5.4E-08
Respiratory exchange ratio at LaT	6.20	• 1.9E-04	3.46	• 1.3E-05
Heart rate at LaT	13.26	• 1.6E-08	6.92	• 9.8E-12
Minute ventilation at LaT	11.66	• 1.2E-07	8.23	• 1.1E-13
Carbon dioxide production at LaT	41.83	• 1.1E-19	6.55	• 3.8E-11
Respiratory equivalent for oxygen at LaT	319.79	• 6.6E-52	8.97	• 1.0E-14
Respiratory equivalent for carbon dioxide at LaT	370.75	• 1.4E-54	11.98	• 1.9E-18
Oxygen pulse at LaT	41.20	• 1.7E-19	25.28	• 1.3E-29
Respiratory rate at LaT	8.01	• 1.5E-05	9.22	• 4.8E-15
Tidal volume at LaT	0.74	5.7E-01	8.44	• 5.5E-14
Partial pressure end tidal oxygen at LaT	2343.54	• 2.9E-89	10.12	• 3.2E-16
End tidal partial pressure carbon dioxide at LaT	706.13	• 1.7E-66	16.65	• 4.1E-23
Work rate at VO_2max	239.68	• 9.3E-47	56.48	• 2.5E-43
Oxygen consumption at VO_2max	159.66	• 9.9E-40	28.72	• 1.1E-31
Oxygen consumption at VO_2max (/kg)	136.57	• 4.1E-37	14.79	• 2.2E-21
Respiratory exchange ratio at VO_2max	18.05	• 6.9E-11	3.24	• 3.7E-05
Heart rate at VO_2max	62.27	• 5.4E-25	9.41	• 2.6E-15
Minute ventilation at VO_2max	13.75	• 9.0E-09	16.59	• 4.6E-23
Carbon dioxide production at VO_2max	221.07	• 2.4E-45	32.56	• 8.8E-34
Respiratory equivalent for oxygen at VO_2max	171.15	• 6.5E-41	6.50	• 4.5E-11

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9.1 – continued from previous page

	ANOVA (Altitude)		ANOVA (Individual)	
Variable	F-value	p-value	F-value	p-value
Respiratory equivalent for carbon dioxide at VO_2max	279.95	• 1.6E-49	11.56	• 5.8E-18
Oxygen pulse at VO_2max	31.21	• 3.1E-16	24.45	• 4.5E-29
Respiratory rate at VO_2max	5.14	• 9.0E-04	10.59	• 8.3E-17
Tidal volume at VO_2max	4.67	• 1.8E-03	40.46	• 1.8E-37
Partial pressure end tidal oxygen at VO_2max	3110.84	• 1.1E-94	8.63	• 3.0E-14
End tidal partial pressure carbon dioxide at VO_2max	231.22	• 4.0E-46	10.24	• 2.3E-16
Systolic blood pressure at rest	0.56	6.9E-01	4.13	• 1.2E-06
Diastolic blood pressure at rest	3.48	• 1.1E-02	3.15	• 8.2E-05
Systolic blood pressure after exercise	0.50	7.3E-01	5.84	• 1.3E-08
Diastolic blood pressure after exercise	1.27	2.9E-01	1.47	1.2E-01
Oxygen cost at LaT	7.26	• 4.2E-05	29.49	• 4.0E-32
Oxygen cost at VO_2max	5.28	• 7.3E-04	24.31	• 5.7E-29
Oxygen saturations at rest	146.30	• 5.2E-38	3.97	• 1.5E-06
Oxygen saturations at LaT	119.36	• 1.0E-29	2.46	• 2.6E-03
Oxygen saturations at VO_2max	132.72	• 1.7E-28	3.87	• 2.3E-05

Table 9.1: Full 2-way ANOVA results (without interactions) looking at the effect of altitude and individual.

9.2 Inter-quartile ranges

	Interquartile ranges				
Variable	London	Kathmandu	Namche	Pheriche	EBC week 1
Nitrite (mM)	0.584	0.392	0.555	0.449	0.461
Nitrate (mM)	0.538	0.440	0.714	0.882	0.824
Total NOx (mM)	0.539	0.436	0.703	0.880	0.818
RSNO (nM)	1.026	0.859	0.782	0.817	0.488
RNNO (nM)	0.625	0.246	0.475	0.340	0.590
Total RxNO (nM)	0.662	0.388	0.429	0.452	0.654
cGMP (pmol/mL)	0.331	0.480	0.395	0.656	0.438
8-isoPGF (ng/mL)	0.476	0.799	0.905	0.427	1.336
HNE (mg/mL)	0.352	0.698	0.262	0.347	0.243
GSH (micromolar)	NaN	0.195	0.330	0.331	0.280
GSSG (micromolar)	NaN	0.817	0.758	0.639	0.811
Total Glutathione (micromolar)	NaN	0.400	0.562	0.453	0.368
GSH to GSSG ratio	NaN	0.914	0.530	0.417	0.762
IL-1beta (pg/mL)	0.217	0.154	1.801	0.869	0.000
IL-1ra (pg/mL)	0.544	0.397	0.816	0.414	0.454
IL-6 (pg/mL)	0.356	0.489	0.279	0.417	0.392
IL-8 (pg/mL)	0.457	0.376	0.667	0.585	0.476
IL-10 (pg/mL)	1.554	0.725	2.729	1.526	1.608
IL-12(p70) (pg/mL)	0.903	0.887	0.648	1.203	0.776
IL-13 (pg/mL)	0.626	0.648	0.451	0.956	0.616
IL-18 (pg/mL)	0.664	0.779	0.627	0.548	0.836
MIF (pg/mL)	0.271	0.351	0.304	0.534	0.708
Eotaxin (pg/mL)	0.710	0.795	0.448	0.605	0.493
TNFalpha (pg/mL)	0.299	0.521	0.303	0.353	0.352
VEGF (pg/mL)	1.069	1.011	0.948	1.171	0.712
CRP (ng/mL)	1.976	2.771	1.809	1.481	2.224
Adrenaline (ng/mL)	1.389	0.724	0.472	0.611	0.531
Noradrenaline (ng/mL)	0.646	0.525	0.400	1.001	1.636
T3 (pg/mL)	0.983	0.505	0.743	0.610	0.634
T4 (pg/mL)	0.490	0.293	0.279	0.281	0.608
C-Peptide (pg/mL)	0.288	0.455	0.352	0.222	0.185

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	Interquartile range				
Variable	London	Kathmandu	Namche	Pheriche	EBC week 1
GIP (pg/mL)	1.024	1.023	0.676	0.825	0.571
Ghrelin (pg/mL)	0.430	0.422	0.304	0.420	0.517
Glucagon (pg/mL)	0.225	0.325	0.266	0.243	0.213
GLP-1 (pg/mL)	0.313	0.518	0.285	0.346	0.329
Insulin (pg/mL)	0.208	0.504	0.394	0.389	0.412
Leptin (pg/mL)	1.212	1.214	0.946	0.786	0.851
PAI-1 (pg/mL)	0.667	0.458	0.248	0.383	0.279
Resistin (pg/mL)	0.558	0.472	0.499	0.371	0.398
Visfatin (pg/mL)	0.842	0.837	0.671	0.646	0.619
Adiponectin (pg/mL)	0.722	0.782	0.858	0.724	1.027
Adipsin (pg/mL)	0.578	0.628	0.371	0.587	0.415
EPO (mlU/mL)	0.399	0.617	0.639	0.568	1.016
ET-1 (pg/mL)	0.955	0.974	0.819	0.663	0.915
Glucose (mM)	0.211	0.242	0.402	0.196	0.298
Cystatin C (ng/mL)	0.242	0.256	0.263	0.276	0.271
Creatinine (mg/dl)	0.163	0.148	0.206	0.205	0.133
Lactate (mM)	0.358	0.499	0.507	0.392	0.389
Osmolality (mOsmo/kg)	0.036	0.036	0.040	0.068	0.064
Protein Content (mg/mL)	0.110	0.084	0.109	0.115	0.102
Bicarbonate (mmol/L)	0.110	0.099	0.130	0.176	0.137
HSP-70 (ng/mL)	0.565	1.025	0.838	0.703	0.912
Groups	0.693	0.693	0.693	0.693	0.693
Gender	0.347	0.347	0.693	0.347	0.000
Weight (Kg)	0.207	0.201	0.192	0.190	0.194
Barometric Pressure	0.006	0.007	0.007	0.006	0.006
Inspired oxygen partial pressure	0.000	0.000	0.000	0.000	0.000
Ambient temperature	0.057	0.077	0.173	0.138	0.975
Economy	0.178	0.099	0.136	0.108	0.170
Maximum voluntary ventilation	0.270	0.254	0.196	0.169	0.232

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	Interquartile range				
Variable	London	Kathmandu	Namche	Pheriche	EBC week 1
Forced expiratory volume in 1 second	0.241	0.223	0.206	0.244	0.191
Exercise ramp wattage	0.405	0.405	0.405	0.405	0.405
Haemoglobin	0.108	0.092	0.078	0.080	0.109
Blood oxygen content	0.098	0.093	0.078	0.087	0.178
Haematocrit	0.080	0.066	0.075	0.073	0.066
Resting oxygen consumption	0.242	0.215	0.313	0.250	0.256
Resting oxygen consumption (/kg)	0.327	0.247	0.226	0.224	0.119
Resting respiratory exchange ratio	0.118	0.118	0.000	0.000	0.100
Resting heart rate	0.226	0.119	0.170	0.187	0.232
Resting minute ventilation	0.199	0.231	0.292	0.253	0.306
Resting carbon dioxide production	0.265	0.233	0.263	0.267	0.252
Respiratory equivalent for oxygen at rest	0.139	0.114	0.158	0.142	0.191
Respiratory equivalent for carbon dioxide at rest	0.163	0.165	0.156	0.140	0.160
Resting oxygen pulse	0.288	0.231	0.352	0.316	0.255
Resting respiratory rate	0.219	0.264	0.218	0.197	0.261
Resting tidal volume	0.154	0.322	0.232	0.224	0.228
Partial pressure end tidal oxygen	0.033	0.047	0.050	0.067	0.046
End tidal partial pressure carbon dioxide	0.152	0.172	0.108	0.109	0.124
Work rate at LaT	0.273	0.236	0.212	0.222	0.206
Oxygen consumption at LaT	0.216	0.181	0.236	0.143	0.163

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	Interquartile range				
Variable	London	Kathmandu	Namche	Pheriche	EBC week 1
Oxygen consumption at LaT (/kg)	0.300	0.155	0.161	0.138	0.162
Respiratory exchange ratio at LaT	0.074	0.063	0.082	0.063	0.070
Heart rate at LaT	0.136	0.142	0.166	0.117	0.149
Minute ventilation at LaT	0.340	0.227	0.333	0.260	0.214
Carbon dioxide production at LaT	0.311	0.175	0.246	0.214	0.148
Respiratory equivalent for oxygen at LaT	0.121	0.159	0.117	0.114	0.149
Respiratory equivalent for carbon dioxide at LaT	0.125	0.113	0.150	0.134	0.175
Oxygen pulse at LaT	0.183	0.198	0.330	0.229	0.318
Respiratory rate at LaT	0.235	0.231	0.260	0.327	0.261
Tidal volume at LaT	0.296	0.265	0.290	0.293	0.259
Partial pressure end tidal oxygen at LaT	0.049	0.075	0.061	0.067	0.070
End tidal partial pressure carbon dioxide at LaT	0.108	0.097	0.107	0.129	0.128
Work rate at VO_2max	0.209	0.202	0.281	0.265	0.221
Oxygen consumption at VO_2max	0.236	0.197	0.305	0.258	0.246
Oxygen consumption at VO_2max (/kg)	0.238	0.176	0.167	0.086	0.155
Respiratory exchange ratio at VO_2max	0.099	0.084	0.083	0.049	0.075
Heart rate at VO_2max	0.065	0.087	0.114	0.090	0.197
Minute ventilation at VO_2max	0.220	0.329	0.416	0.404	0.222

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	Interquartile range				
Variable	London	Kathmandu	Namche	Pheriche	EBC week 1
Carbon dioxide production at VO_2max	0.165	0.169	0.353	0.243	0.249
Respiratory equivalent for oxygen at VO_2max	0.203	0.178	0.137	0.128	0.187
Respiratory equivalent for carbon dioxide at VO_2max	0.211	0.217	0.186	0.091	0.187
Oxygen pulse at VO_2max	0.170	0.260	0.337	0.297	0.335
Respiratory rate at VO_2max	0.355	0.224	0.215	0.302	0.301
Tidal volume at VO_2max	0.288	0.232	0.295	0.361	0.373
Partial pressure end tidal oxygen at VO_2max	0.055	0.058	0.058	0.043	0.045
End tidal partial pressure carbon dioxide at VO_2max	0.204	0.222	0.168	0.090	0.196
Systolic blood pressure at rest	0.225	0.167	0.227	0.150	0.271
Diastolic blood pressure at rest	0.152	0.250	0.165	0.202	0.178
Systolic blood pressure after exercise	0.181	0.263	0.186	0.198	0.257
Diastolic blood pressure after exercise	0.270	0.202	0.151	0.147	0.161
Oxygen cost at LaT	0.210	0.237	0.243	0.251	0.216
Oxygen cost at VO_2max	0.247	0.193	0.218	0.239	0.203
Oxygen saturations at rest	0.020	0.031	0.055	0.058	0.100
Oxygen saturations at LaT	0.031	0.032	0.095	0.074	0.137

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9.2 – continued from previous page

	Interquartile range				
Variable	London	Kathmandu	Namche	Pheriche	EBC week 1
Oxygen saturations at VO_2max	0.021	0.039	0.075	0.101	0.159
Altitude	0.000	0.000	0.000	0.000	0.000
Day	NaN	0.288	0.095	0.069	0.140

Table 9.2: Full interquartile range information for all variables for all altitudes, for the core data.

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